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Khanna, D.R. & Bhutiani, R. (2017): Water Biology. Discovery Publishing House, New Delhi.

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Ph.D. Thesis, Dissertation and Reports

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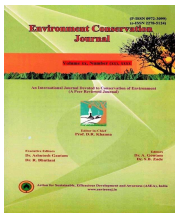

Characterization of the green gram (*Vigna radiata* L.) genotypes through both morphological and biochemical parameters

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ABSTRACT

Eight genotypes of greengram were collected in the present investigation from AICRP on MULLaRP, Bidhan Chandra Krishi Viswavidyalaya (BCKV) and they were characterized with ten quantitative, nineteen qualitative and two biochemical parameters as per the NBPGR descriptor. Grouping based on DUS descriptors indicate the existence of genetic diversity within the genotypes. These eight genotypes were evaluated and characterized for 31 DUS descriptors. However, 21 characters out of 31 characters of DUS descriptors differed significantly indicating a large and exploitable amount of genetic variability for the individual elite improved line profile development for identification and protection. The elite lines are similar for the important plant traits like semi erect and determinate growth habit but the development of erect types is the need of hour and indicates the incorporation of new germplasm for the improvement of this trait in the present material. Genotypes could be easily identified through some unique characters: SML-1822 could be identified amongst genotypes studied here in through its semi-erect growth habit, green stem colour with purple shade, dark green leaf colour, light yellow flower colour and bearing pods below canopy; identification of IPM-512-1 and TMB-37 could be made through seeds with drum shape and dull seed luster respectively; and Pusa Vishal through its leaves with dark green colour along with intermediate pod position and larger seed size. Samrat is having highest amount of protein as well as carbohydrate content among these genotypes. Thus, the DUS descriptor data generated with unique profiles of the elite improved lines can be used for the registration with PPV & FRA and seed purity testing.

Introduction

Vigna radiata L. wilczek which is commonly known as mungbean or greengram is one of the most widely distributed crop species among the six Asiatic *Vigna* species. Essential amino acids especially lysine and tryptophan are mainly found in green gram along with other proteins. It is also having certain other added features compared to other pulses like the crop is relatively drought tolerant and well adapted to a varied soil conditions including light soils and it can thrive well even under limited irrigation. Moreover, it is very well suited to crop rotation and crop mixtures (Uzoh *et al.,* 2019). However, green gram yield advantage is major drawback for this crop that is well below the optimum level. The average yield of mungbean is very low not only in India (425 kg/ha) as well as in entire tropical and subtropical Asia. Other than management factors, the major cause for the low productivity can be described to the inherently low yield potential of the cultivars coupled with susceptibility to diseases. Due to the limited variability prevailed among the parents used for

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hybridization; the success had been very limited in most of the studies (Bordolui *et al.,* 2015). There is always a possibility of improving the crop by incorporating diversified gene present in the germplasm. Sometime stepwise utilization of primary gene pools of this crop can result in tremendous improvement in yield. It is essential to evaluate the available germplasm collections in order to utilize the variability available in the primary gene pool, Hence, this study was taken up to evaluate and characterize available germplasm of green gram using NBPGR descriptors with a view to evaluate the available germplasm using the descriptors and to form the core collection.

Material and Methods

Characterization of greengram genotypes was traditionally carried out by using morpho-agronomic traits. PPV & FRA (Protection of Plant Varieties and Farmers' Rights Authority) has come up with a set of DUS (Distinctiveness, Uniformity and Stability) descriptors for characterization of the lines for their registration and protection. Thus, in the present study, eight genotypes were characterized using PPV&FRA descriptors to know the extent variability present in these genotypes.

The genotypes were collected from AICRP on MULLaRP, BCKV. The laboratory experiment was done in seed testing laboratory and field performance was observed in 'D'-Block Farm, Kalyani, BCKV, West Bengal during 2019 and 2020. Seeds were sown in individual plots following standard agronomic practices and intercultural operations in the plot, with three replications following Randomized Block Design. Spacing was 30 cm between the rows, 10 cm between the plants and 50 cm between the two plots. Each plot was 2m length and 2m breadth. The different morphological and biochemical parameters such as hypocotyl: anthocyanin colouration, growth habit, time of flowering, plant habit, stem colour, stem pubescence, leaf colour, leaf pubescence, leaf shape, flower colour, premature pod colour, pod pubescence, pod position, pod colour at maturity, curvature of pod, seed colour, seed luster, seed shape, seed size, protein content and carbohydrate content were recorded. The different quantitative characters like field emergence (%), plant height at 15 DAS (cm), plant height at first flowering (cm), days to first flowering, number of nodule plant^{-1}, days to 50 % flowering, days to maturity, number of pods plant^{-1}, number of seeds pod^{-1}, and seed yield plant^{-1} (g) were also recorded.

Results and Discussion

The results are discussions are detailed out below:

Characterization through qualitative parameters

Morphological characteristics provide the basic information about the genetic variability among different genotypes. For morphological characterization of such eight genotypes, 19 qualitative characters were recorded. The trait, anthocyanin colouration, was recorded at seedling stage and was noticed in all genotypes. This is the trait which is highly used in breeding programmes for differentiation of genotypes, and also useful in maintenance breeding and Intellectual property protection. Similar exploitation of morphological traits in mungbean was reported by Mukherjee and Pradhan (2002); Khattak *et al.* (2000); Bordolui *et al.* (2006) and Patel *et al.* (2019). The characters, time of flowering, plant habit and stem pubescence, were recorded at 50% flowering stage and variation among the genotypes was not observed. All the genotypes showed early flowering, determinate plant habit and presence of stem pubescence indicating these morphological characters are not useful in characterization of these genotypes. Erect type growth habit was noticed in Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, whileSML-1822 exhibited semi-erect type. All the genotypes were of determinant plant habit. Stem colour was recorded at 50% flowering stage and it varied among the genotypes: for Meha and SML-1822, stem colour was green with purple tinge, while other genotypes were observed with green stem colour.

No variation was observed for leaf shape: all genotypes were of ovate leaf shape. Dark green leaves were observed for Pusa Vishal and SML-1822, while remaining genotypes recorded leaves of green colour. SML-1822 could be identified as the only genotype bearing light yellow flowers, while yellow colour flowers were noticed for all other genotypes. Jain *et al.* (2002) reported the usefulness of flower characteristics in characterization of greengram. Premature pod colour was recorded when pods were fully

Table 1: Characterization of green gram genotypes through qualitative characters.

SN	Characters	Genotypes							
		Pusa Vishal	**PM-11-9**	**IPM-2-3**	**Meha**	**Samrat**	**IPM-512-1**	**TMB-37**	**SML-1822**
1.	Hypocotyl: Anthocyanin colouration	Present	Present	Present	Present	Present	Present	Present	Present
2.	Growth habit	Erect	Erect	Erect	Erect	Erect	Erect	Erect	Semi-erect
3.	Time of flowering	Early	Early	Early	Early	Early	Early	Early	Early
4.	Plant habit	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate
5.	Stem colour	Green	Green	Green	Green with purple	Green	Green	Green	Green with purple
6.	Stem pubescence	Present	Present	Present	Present	Present	Present	Present	Present
7.	Leaf colour	Dark green	Green	Green	Green	Green	Green	Green	Dark green
8.	Leaf pubescence	Present	Present	Present	Present	Present	Present	Present	Present
9.	Leaf shape	Ovate	Ovate	Ovate	Ovate	Ovate	Ovate	Ovate	Ovate
10.	Flower colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Light Yellow
11.	Premature Pod colour	Green	Green	Green	Green	Green	Green	Green	Green
12.	Pod pubescence	Present	Present	Present	Present	Present	Present	Present	Present
13.	Pod position	Intermediate	Above canopy	Intermediate	Above canopy	Above canopy	Above canopy	Above canopy	Not visible
14.	Pod colour at maturity	Black	Black	Black	Black	Black	Black	Black	Black
15.	Curvature of pod	Straight	Straight	Straight	Straight	Straight	Straight	Straight	Straight
16.	Seed colour	Green	Green	Green	Green	Green	Green	Green	Green
17.	Seed luster	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Dull	Shiny
18.	Seed shape	Oval	Oval	Oval	Oval	Oval	Drum	Oval	Oval
19	Seed size	Large	Medium	Medium	Medium	Large	Medium	Large	Medium

Table 2: Grouping of eight green gram genotypes based on DUS descriptors

Morphological characters	Characters	Genotypes
Hypocotyl-Anthocyanin colouration	Absent	None
	Present	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
Time of flowering	Early	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Medium	None
	Late	None
Growth habit	Erect	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37
	Semi-erect	SML-1822
	Spreading	None
Plant habit	Determinate	Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Indeterminate	None
Stem colour	Green	Pusa Vishal, PM-11-9, IPM-2-3, Samrat, IPM-512-1, TMB-37
	Green with purple	Meha,SML-1822
	purple	None
Stem pubescence	Absent	None
	Present	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
Leaf colour	Green	PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37,
	Dark green	Pusa Vishal,SML-1822
Leaf pubescence	Absent	None
	Present	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
Leaf shape	Deltoid	None
	Ovate	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Lanceolate	None
	Cuneate	None
Flower colour	Yellow	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37
	Light yellow	SML-1822
Premature Pod colour	Green	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Green with pigmented suture	None
Pod pubescence	Absent	None
	Present	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
Pod position	Above canopy	PM-11-9, Meha, Samrat, IPM-512-1, TMB-37
	Intermediate	Pusa Vishal, IPM-2-3
	Not visible	SML-1822
Pod colour at maturity	Brown	None
	Black	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
Curvature of pod	Straight	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Curve	None
Seed colour	Yellow	None
	Green	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, TMB-37, SML-1822
	Mottled	None
	Black	None
Seed luster	Shiny	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, IPM-512-1, SML-1822
	Dull	TMB-37
Seed shape	Oval	Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, TMB-37, SML-1822
	Drum	IPM-512-1
Seed size	Small	None
	Medium	PM-11-9, IPM-2-3, Meha, IPM-512-1, SML-1822
	Large	Pusa Vishal,Samrat,TMB-37

developed and all genotypes recorded to bear pods having green colour. Pod pubescence was noticed irrespective of the genotypes. All the genotypes exhibited straight pods but no curvature was noticed at all. During maturity each genotype was observed with black pods. Similar report of straight pods without curvature was reported by Sunil *et al.* (2014) in their study in greengram. Pod position was intermediate in Pusa Vishal and IPM-2-3, but it was below canopy in SML-1822 only, while other genotypes exhibited above canopy pod position.

All the genotypes produced seeds of green seed colour. Seed luster of TMB-37 were dull and the other genotypes exhibited shiny seed luster. For seed shape of the genotypes, Pusa Vishal, PM-11-9, IPM-2-3, Meha, Samrat, TMB-37 and SML-1822 were of oval, while IPM-512-1 only exhibited seeds of drum shape. Seed size (100 seed weight) was

Table 3: Characterization of green gram genotypes through quantitative characters (pooled).

	Field emergence (%)	Plant height at 15 DAS (cm)	Plant height at first flowering (cm)	Days to first flowering	Number of nodule plant^{-1}	Days to 50 % flowering	Days to maturity	Number of pods plant^{-1}	Number of seeds pod^{-1}	Seed Yield plant^{-1} (g)
Pusa Vishal	75.81 (60.52)	12.54	43.23	35.33	9.31	42.67	73.00	27.93	10.45	14.86
PM-11-9	77.73 (61.82)	9.13	34.64	36.33	9.40	44.00	73.67	24.57	10.67	13.33
IPM-2-3	76.09 (60.71)	10.02	35.57	37.67	8.22	44.67	71.00	24.88	10.47	13.27
Meha	79.65 (63.16)	10.44	36.39	36.00	9.69	43.00	74.00	22.89	9.42	10.97
Samrat	78.23 (62.17)	9.26	32.00	38.67	8.64	45.67	71.67	28.53	11.19	16.25
IPM-512-1	79.30 (62.91)	10.19	45.51	38.67	9.93	46.00	71.33	26.46	12.42	16.72
TMB-37	77.66 (61.77)	9.87	37.44	33.67	9.79	42.00	72.33	28.32	12.21	17.62
SML-1822	78.26 (62.18)	9.74	36.63	37.00	10.30	44.67	74.00	27.82	10.54	14.92
SEm(±)	0.135	0.184	0.26	0.398	-	0.496	0.57	0.559	0.153	0.386
LSD (0.05%)	0.413	0.565	0.797	1.22	-	1.519	1.747	1.713	0.468	1.183

(Figures in parenthesis are arc-sin transformed values.)

Table 4: Characterization of green gram genotypes through biochemical characters.

	Protein content (mg g^{-1})	Carbohydrate content (mg g^{-1})
Pusa Vishal	225.254	637.198
PM-11-9	221.232	635.856
IPM-2-3	225.219	636.221
Meha	222.751	636.087
Samrat	225.284	637.416
IPM-512-1	225.263	637.312
TMB-37	225.245	636.871
SML-1822	221.080	635.721
SEm(±)	0.045	0.087
LSD (0.05%)	0.137	0.264

medium for PM-11-9, IPM-2-3, Meha, IPM-512-1, SML-1822, while large seeds were produced by Pusa Vishal, Samrat and TMB-37. Similar reports of exploiting the seed characters' variability in greengram was reported by Venkateswarlu (2001), and Khajudparn and Tantasawat (2011).

Thus, it is clear from both the tables 1 & 2 that genotype(s) could be easily identified through some unique characters: SML-1822 could be identified amongst the eight genotypes studied here in through its semi-erect growth habit, green stem colour with purple shade, dark green leaf colour, light yellow flower colour and bearing pods below canopy; IPM-512-1 and TMB-37 could be identified through seeds with drum shape and dull seed luster respectively among the genotypes; and Pusa Vishal through dark green leaves with intermediate pod position and larger seed size.

Therefore, the present study indicates the importance of morphological characterization using DUS descriptors for the registration, maintenance and protection of genotypes.

Characterization through quantitative parameters

Significant variation was noticed for all the quantitative characters among the genotypes excepting number of nodules plant^{-1}. Highest field emergence was observed for Meha (79.65%) followed by IPM-512-1; while lowest field emergence (75.81%) was recognized for Pusa Vishal. Maximum number of nodule plant^{-1}(10.30) was found for SML-1822, though non-significant, followed by IPM-512-1 and minimum number of nodule plant^{-1} for IPM-2-3 but this trait varied non-significantly among the genotypes. After 15 days of sowing, the highest plant height (12.54 cm) was observed in Pusa Vishal followed by IPM-512-1 and it was lowest in PM-11-9**.** But during first flowering stage, Pusa Vishal and IPM-512-1 interchanged their position i.e., highest was observed for IPM-512-1 followed by Pusa Vishal and at that stage, lowest was observed for Samrat. Minimum days required for 50% flowering (42.00) was observed for TMB-37 preceded by Pusa Vishal; non-significant variation was observed between these two genotypes; but the genotypes varied significantly for this character. Least days were taken for maturity by IPM-2-3 (71.00) preceded by IPM-512-1 and Samrat. These three genotypes performed statistically at par with each other. Highest number of pods plant^{-1} was recorded for Samrat (28.53) followed by TMB-37, while it was lowest for PM-11-9. Seed yield plant^{-1}(g) was maximum for TMB-37 followed by IPM-512-1 and minimum was found in IPM-2-3. These results are similar with the findings of Uddin *et al.* (2010); Dash and Rautaray (2017). Thus, clear variation for these quantitative characters considered here for identification of the genotypes, therefore, could be utilized in a better way for identification of the genotypes, especially for the genotypes occupying lowest and/or highest position for individual character.

Characterization through biochemical parameters

Two biochemical parameters i.e., protein and carbohydrate contents were observed for quantitative characterization of the genotypes. Highest protein was recorded in Samrat followed by IPM-512-1, Pusa Vishal and TMB-37, though these genotypes were statistically at par. Lowest protein was observed in SML-1822**.** But among the genotypes protein content varied significantly. Highest carbohydrate content also was observed in Samrat followed by IPM-512-1, Pusa Vishal and TMB-37; non-significant variation was observed between Samrat and IPM-512-1 and lowest was observed for SML-1822**.** But among the genotypes, this trait varied significantly. Similar type of result was observed by Blessing and Gregory (2010). As a main storage protein mung beans contain higher amounts of protein with globulin and albumin in the seeds (Kirchhoff, 2002). However, the lack of raffinose may be the reason of having smaller amount carbohydrate, resulting in hydrolysis of sucrose to supply energy (Mubarak, 2005).

Conclusion

Genotypes studied in this experiment could be easily identified through some unique characters: SML-1822 could be identified amongst the eight genotypes studied here in through its semi-erect growth habit, green stem colour with purple shade, dark green leaf colour, light yellow flower colour and bearing pods below canopy; identification of IPM-512-1 and TMB-37 could be made through seeds with drum shape and dull seed luster respectively; and Pusa Vishal through its leaves with dark green colour along with intermediate pod position and larger seed size. However, Samrat is having highest amount of protein as well as carbohydrate contents among these genotypes.

Conflict of interest

The authors declare that they have no conflict of interest.

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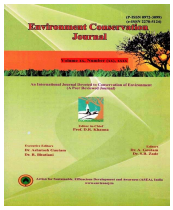

Resistance screening and *in-vitro* efficacy of fungicides for the management of dry root rot of chickpea caused by *Rhizoctonia bataticola*

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ABSTRACT

Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is an emerging threat for chickpea production. It is among one of the chief and common soil borne diseases of chickpea. The present investigation was conducted firstly to identify the resistant source for dry root rot in chickpea and secondly to evaluate the efficacy of different fungicides in inhibiting the growth of *R. bataticola* under *in vitro* conditions. Screening of a set of 50 chickpea entries resulted in identification of three entries namely ICCV 191317, ICCV 191306, and Ujjain 21 as moderately resistant to dry root rot of chickpea. No entry could be identified as completely resistant for dry root rot in chickpea. Further, among the different fungicides tested, pyraclostrobin alone and in combination of Thiophanate methyl completely checked the growth of *R. bataticola* at 100 ppm concentration under *in vitro* conditions. However, another combination product of fungicides namely carboxin + thiram and carbendazim + mancozeb also showed complete inhibition in growth of test pathogen at higher concentration of fungicides i.e. at 300 ppm concentration.The identified moderately resistant genotypes could be a useful resource for development of resistant varieties in chickpea for dry root rot using molecular breeding approaches.

Introduction

Pulses are critical for providing affordable protein to the world's rising human population. In comparison to cereal crops, pulses have fallen behind in terms of genetic development. Nonetheless, in recent years, significant progress has been achieved in utilizing current genomic techniques and breeding approaches that support pulse genetic improvement (Kumar et al., 2021).

Chickpea (*Cicer arietinum* L.) is the most frequently farmed pulse, accounting for 75 % of India's total pulse production (Ali *et al*., 2020). Chickpea seeds have a protein content of 29%, a carbohydrate content of 59 %, a fibre content of 3%, an oil content of 5%, and an ash content of 4%. The therapeutic benefits of malic acid and oxalic acid from leaves are well established (Singh *et al.*, 2020). After the common bean, it is the world's second most important food legume. It is a high-protein crop that also improves soil fertility through biological nitrogen fixation (Zia-Ul-Haq *et al.*, 2007).

Chickpeas are infected by 172 different pathogens, including fungi, bacteria, viruses, and nematodes. Dry root rot caused by *Rhizoctonia bataticola*, wilt

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caused by *Fusarium oxysporum ciceri*, and collor rot caused by *Sclerotium rolfsii* are major soil-borne diseases that inflict serious damage to chickpeas in favourable conditions (Ravichandran *et al*., 2014).

Dry root rot is one of the soil-borne diseases that can cause 10-35 percent yield losses in chickpea production (Pal, 1998). Chickpea dry root rot caused by the nectrotropic fungus *R. bataticola* is becoming a severe danger to global chickpea agriculture (Pandey and Sharma, 2010). It is most severe in Madhya Pradesh's chickpea-growing regions. *R. bataticola* is a polyphagous soil-borne disease that has infected over 500 plant species around the world, resulting in massive economic losses. Despite the fact that the fungus is both seed and soil borne (Dhingra and Sinclair, 1994), soil borne inoculum is more essential in infecting and spreading disease. The fungus is propagated by irrigation water, agricultural practises, and equipment.The stages of pod setting and late flowering are typically when the plant is most vulnerable to dry root rot disease. Plants that have been infected look to have entirely dried out. The most typical symptoms of disease are the destruction of lateral roots and widespread rotting. Yellowing of the leaves is a common indicator of root rot, and these leaves could fall off in two to three days. Within a week, the plant may wilt. In the advanced stages of disease, sclerotial bodies can be seen distributed on the damaged tissues (Singh and Srivastava, 1998).

Looking to the enormous losses imposed by this pathogen, there is a dire need for the control of this pathogen. Although different chemicals and biocontrol agents (Kumar *et al*., 2009; Srivastava *et al*., 2009) have so far been utilized for control of different plant diseases including dry root rot of chickpea but so far limited success have been achieved. Further evaluation of fungicides will certainly open up new avenues for control of this pathogen. Simultaneously, huge genomic resources are now available in different pulses including chickpea (Hiremath *et al*., 2011, 2012; Gujaria *et al*., 2011) which can easily assist in genetic dissection of region harbouring resistance for dry root rot of chickpea. However, to accomplish this trait mapping, identification of donar lines for dry root resistance is a pre-requisite which can be utilized in molecular breeding programmes to incorporate resistance for DRR in elite chickpea lines (Chamarthi *et al*., 2011). Apart from this, use of host plant resistance is not only one of the most feasible eco-friendly approaches for dry root rot management in chickpea which will not only provide immediate solution but also can contribute to identification of source of resistance,likely to be used in molecular breeding programme. Looking to the economic importance of dry root rot of chickpea, the present investigation, was conducted firstly to screen different fungicides against *R. bataticola in-vitro* and secondly to identify resistance source for DRR in chickpea.

Material and Methods

In-vitro evaluation of fungicides against *R. bataticola*

The experiment was conducted *in-vitro* to know inhibitory effect of different fungicides viz. Carboxin+Thiram, Azoxystrobin+Difenoconazole, Thiophanatemethyl+pyraclostrobin, Carbendazim+ Mancozeb, Difenoconazole, Thiophanate methyl and Pyrochlostrobin alone at 100 and 300 ppm using poisoned food technique (Nene and Thapliyal, 1973). Potato dextrose agar (PDA) media was amended with 100 and 300 ppm of the appropriate fungicide, then placed separately in a Petri plate and allowed to solidify. The study used *R. bataticola* cultures that were seven days old and actively developing. Without using fungicides, a fungal disc (5mm diameter) was placed in the middle of the PDA Petri plate and proper control was maintained. The plates were incubated at room temperature (28±2ºC) for seven days. The diameters of colonies measured and the per cent inhibition of growth estimated on the seventh day.

$PI = C – T/C \times 100$

Where

PI = Per cent inhibition

C = Radial growth of pathogen in control plates

T = Radial growth of test pathogen in treatment plates

Resistance screening

A collection of 50 chickpea varieties and advanced breeding lines were employed to screen for resistance using an *in vitro* blotter paper approach (Nene *et al*., 1981). A 5 mm disc of pure culture of a seven-day-old, vigorously developing *R.*

bataticola was transferred to 250 ml flasks with 100 ml Himedia potato dextrose broth for each flask (PDB). The mycelial mats were taken from two such flasks after 7 days of incubation at 25°C, and were added to 100 ml sterilised distilled water in a beaker after proper crushing for 1-2 minutes in the blender. Seeds of various chickpea lines were surface sterilised and sown on plastic trays with sterilised soil + sand (1:1) mixture. Each genotype's ten-day-old seedlings were uprooted in such a way that the root system was not disrupted. These seedlings' root systems were thoroughly cleansed in flowing water before being rinsed in sterilised distilled water. All genotypes (test lines) had their roots immersed in the inoculum kept in a beaker for about 30 seconds, and the excess inoculum was removed by contacting the roots to the beaker's edge. Each test line was given ten seedlings, which were stored separately on two blotter papers (size 45 cm x 25 cm with one fold). The blotter paper was sufficiently saturated with water, and the seedlings were held in such a way that just the cotyledons and roots were covered, leaving the green tops of the seedlings exposed. A check JG 12 seedling was inoculated and kept with each batch of seedlings. The folded blotter papers were stacked in a batch of ten papers in a tray, one on top of the other. These trays were kept in the incubator for 8 days at 35°C. On alternate days, artificial light was provided for 12 hours and the blotter papers were suitably moistened. The seedlings were assessed for dry root rot after 10 days using the scale mentioned in table 1.

Results and Discussion

In vitro evaluation of fungicides against *R. bataticola*

At two distinct concentrations, 100 and 300 ppm, a set of seven fungicides were tested for their fungicidal activity on *R. bataticola* radial growth. When compared to the control, all of the fungicides were observed to suppress the growth of test pathogen to varied degrees. Pyraclostrobin and Thiophanate methyl + Pyraclostrobin were shown to be the most effective and significantly superior to all other fungicides, inhibiting 100% mycelial growth of *R. bataticola* at 100 and 300 ppm, respectively. Further, Carboxin + Thiram and Carbendazim + Mancozeb also showed complete inhibition in growth of test pathogen at higher concentration of fungicides i.e. at 300 ppm concentration. However, at 100 ppm concentration Carboxin + Thiram and Carbendazim + Mancozeb exhibited 87.21 % and 84.24% inhibition. As mentioned in table 2, more inhibitory effect of pyraclostrobin is exhibited then thiophanate methyl because of complete inhibition of test pathogen. In earlier reports also similar findings have been reported by Ravichandran and Hegde, 2017 where carbendazim + Mancozeb, carboxin + thiram were reported as best fungicides against *R. bataticola* with 100 per cent inhibition of *R. bataticola*. The findings of present investigation are in agreement of their findings.

Screening of chickpea lines for identification of resistant source

In total, a set of 50 entries of chickpea consisting of released varieties, advanced breeding lines of different crosses, local checks were evaluated for resistance against dry root rot of chickpea. It was observed that after 10 days of incubation period, no entry showed complete resistance against dry root rot. However, a set of three entries namely ICCV 191317, ICCV 191306, Ujjain 21 exhibited 10.1-20% dry root rot incidence and grouped under the category of moderately resistant entries. A set of 11 entries namely JAKI 9218, JG 226, ICCV-D 201215, JG 12xJG 16-1, ICCV191312, ICCV191305, ICCV191303, JG 14, JG 11, JG 2018-52, C20264 exhibited 20.1-30% dry root rot incidenceand grouped under the category of tolerant. However, 36 entries exhibited 30.1 to 40% dry root rot and grouped under the category of susceptible entries. None of the entry could be grouped under the category of highly susceptible (Table 3).

Under *in vitro* circumstances, Pandey *et al*. (2004) investigated twenty-nine chickpea germplasm accessions, ten cultivars, and eight advanced breeding lines for resistance to dry root rot. Dry root rot resistance was found in one germplasm accession (ICC 14395), a cultivar (ICCV 2), and an advanced breeding line. The other 22 lines were moderately resistant, 19 susceptible, and two highly susceptible lines (BG 212 and ICC 12267) were utilized as controls. Gupta *et al*. (2012) also identified BG 212 as a vulnerable cultivar with 100% mortality, which corroborated the findings of this study. Jagre *et al.* (2018) tested 98 chickpea entries *in vitro* and identified 5 to be disease

Table 1: Rating scale for scoring of dry root rot of chickpea

Rating	Category	Symptoms of DRR	DRR percentage
1	Resistant	No infection on roots	0.0-10.0
2-3	Moderately resistant	On roots very few small lesions	10.1-20.0
4-5	Tolerant	Lesions on roots clear but small, new roots free from infection	20.1-30.0
6-7	Susceptible	Many lesions on roots,Usually new roots free from lesions	30.1-40.0
8-9	Highly susceptible	Roots are infected and completely discoloured	40.1 and above

Table 2: *In vitro* evaluation of fungicides against *R. bataticola* at 100 and 300 ppm

Treatment No.	Treatment details	Average radial growth (mm)	Per cent inhibition	Average radial growth (mm)	Per cent inhibition
		100 ppm		300 ppm	
T_1	Carboxin + Thiram	11.16	87.21	0.00	100.00
T_2	Azoxystrobin + Difenoconazole	10.50	88.44	11.83	86.70
T_3	Thiophanate methyl + pyraclostrobin	0.00	100.00	0.00	100.00
T_4	Carbendazim + Mancozeb	14.00	84.24	0.00	100.00
T_5	Difenoconazole	42.16	52.15	26.00	70.78
T_6	Thiophanate methyl	14.50	83.25	14.83	83.41
T_7	Pyraclostrobin	0.00	100.00	0.00	100.00
T_8	Control	90.00	-	90.00	-
	CD (5%)	2.37		0.83	
	SE(m)±	0.80		0.28	

Table 3: Response of chickpea entries to dry root rot under *in vitro* conditions

SN	Genotypes	Dry Root rot incidence(%)	Number of entries	Reaction of Genotypes
1	No genotypes	0.0-10	0	Resistant
2	ICCV 191317, ICCV 191306, Ujjain 21	10.1-20	3	Moderately Resistant
3	JAKI 9218, JG 226, ICCV-D 201215, JG 12xJG 16-1, ICCV191312, ICCV191305, ICCV191303, JG 14, JG 11, JG 2018-52, C20264	20.1-30	11	Tolerant
4	ICCV191313, Local-check, ICCV191315, ICCV191309, ICCV191316, ICCV191304, Kak2, ICCV191310, ICCV191308, ICCV191307, ICCV191311, ICCV191301, ICCV191318, JG 12xICC06301, JG 12xICC4958, JG 12 x ICC251741, JG 26xICC251741, JG 14xJG 24, JG2017-47, JG2018-53, ICVT-Desi local check, IVTC20247, ICCV-202117, YELLOW TOP, IVT-C-20257, ICCV201212, ICCV201218, ICVTD201214, IVT-MHC-20467, IVT late C-20281, JG 12xICC4959, JG 26xICC251742, JG23 x ICC251742, JG 24, JG 36, JG 12	30.1-40	36	Susceptible
5	No germplasm	40.1 and above	0	Highly susceptible

resistant, with a disease incidence of less than 10%. 42 genotypes, on the other hand, were found to be moderately resistant. The disease's prevalence ranged from 10% to 20%. They are supported by the current investigation's findings. The identified lines in present investigations can not only be directly used under DRR prevalent areas but can also be deployed further in development of mapping populations, identification of QTL for dry root resistance in chickpea which can be utilized in crop improvement programmes.

Conclusion

Looking to the economic losses of dry root rot of chickpea, the findings of present investigation identified two fungicides namely Pyraclostrobin and Thiophanate methyl + Pyraclostrobin which were found completely inhibitory to *R. bataticola* even at 100 ppm concentration. Further, three entries namely ICCV 191317, ICCV 191306, Ujjain 21 grouped under the category of moderately resistant and can not only be utilized in dry root rot prone areas but also can significantly contribute in genetic dissection and development of improved varieties for dry root rot resistance in chickpea using genomic tools and molecular breeding platforms in future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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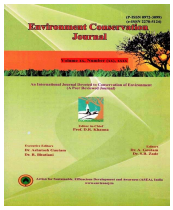

Status of soil and plant micronutrients and their uptake by barley varieties intercropped with *Populus deltoides* plantation

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ABSTRACT

In Agroforestry systems, crops grown in interspaces of tree plantations undergo different kind of interactions with the environment, consequently affecting soil fertility in different ways. In the present study, soil and plants micronutrients and their uptake by five barley varieties (BH 946, BH 959, BH 393, BH 885 and BH 902) grown under poplar plantation as well as sole crop were examined. During this investigation, a significant increase in DTPA (Diethylene triamnine penta acetic acid) extractable micronutrients (Zinc, Copper, Manganese and Iron) was observed at all depths (0-15, 15-30 and 30-45 cm) under poplar plantation than sole crop. Sole crop exhibited higher micronutrient uptake than under poplar plantations. Maximum uptake of soil micronutrients like Zn, Mn and Cu (495.5, 527.06 and 53.8 g ha^{-1}) were recorded in variety BH 946. However, variety BH 959 exhibited minimum uptake of soil micronutrients (401.85, 439.46 and 44.07 g ha^{-1}) during this study.

Introduction

The increasing pressure on the agriculture sector to meet the food requirements of the burgeoning population has led to degradation of the natural resources throughout the world. Moreover, the situation has further aggravated in the highly productive Indo-Gangetic plains of north-western India. Consequently, widespread multi-nutrient deficits have also been documented (Dwivedi *et al.,* 2006; Singh *et al.,* 2015; Shukla and Behera, 2019; Bhardwaj *et al.,* 2020). Therefore, sustainable management of these natural resources is necessary for ensuring livelihoods and environmental protection. Meanwhile, diversifying existing farming systems with suitable region-based agroforestry models has emerged as one of the powerful solutions (Dhyani and Handa, 2013). Agroforestry plays a significant role in protecting the resource base and increasing production capacity and micronutrient availability in arid and semi-arid areas (Dhyani, 2011). Agroforestry is of great importance for North Indian states like Haryana, Punjab and Uttar Pradesh. According to

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FSI (2021) report, Haryana's Forest and tree cover is 6.85 % of its total geographical area. Out of 6.85 %, the forest cover is 3.63 %, and the rest 3.22 % is the tree cover under the agroforestry system. Based on market demand, poplar is one of the most preferred and promoted tree species and the extensive presence of poplar in north India, especially in Haryana, Punjab, Western Uttar Pradesh and tarai regions of Uttrakhand, is an authentication of the broad acceptance of poplar by the farmers (Nandal and Dhillon, 2007). Poplar-based agroforestry systems are more profitable and commercially sustainable than many other crop rotations (Jain and Singh, 2000). Besides tree species, proper selection of understory crops exerts a considerable effect on the performance of the agroforestry system and results in increased productivity, improved soil fertility, foster land resilience, and the quality of resource use (Sharma *et al.,* 2004; Muthuri *et al.,* 2005; Jose, 2009). Poplar-based agroforestry systems serve as a sink and source for the minerals based on the tree-crop combinations. It can maintain and increase plant-available minerals like macronutrients (Bhardwaj *et al.,* 2016; Kumar *et al.,* 2017; Ram *et al.,* 2017; Sirohi and Bhangrwa, 2017) and micronutrients (Sharma *et al.,* 2021), by reducing volatile losses, adopting biological nitrogen fixation, litter and biomass decomposition. However, some researchers have found a substantial decline in nutrient availability in agroforestry systems than sole crops (Chauhan, 2012; Sharma *et al.,* 2012; Sarkar *et al.,* 2017). Moreover, because of its deciduous nature, low shading problem, and sufficient light intensity, poplar-based agroforestry is highly suitable for winter crops, i.e., barley among farmers of Northern India. Barley (*Hordeum vulgare*) belongs to the grass family Poaceae and it is world's fourth most important cereal after wheat, rice, and maize, has become an essential component of the developing countries human diet, including India. It is probably the most widely adapted cereal crop with a strong tolerance for drought, wind and salt. Its ruggedness makes it the only viable rainfed cereal crop under low input and challenging climate in many countries all over the world. It occupied an area of 0.62 million hectares in India, producing 1.59 million tonnes of grain with a productivity of 25.73 q/ha (ICAR-IIWBR, 2020). It is cultivated on 12200 hectares with a production of 44000 tons in Haryana, which ranks second in average productivity (3607 kg/ha) after Punjab (3767 kg/ha). It is used for malt and fermentation, along with its use as food and feed. It reduces blood glucose levels (glycemic index) and blood cholesterol levels in the body. Each 100 g of barley grain comprise 10.6 g protein, 2.1 g fat, 64 g carbohydrates, 50 mg calcium, 3 g crude fibres, 6 mg iron, 31 mg vitamin B1, 0.10 mg vitamin B2 and 50 µg folate. In northern India, farmers cultivate different barley varieties with poplar however, there is a dearth of information regarding micronutrients availability and their uptake in the aforementioned agroforestry system. Considering the above facts, the present experiment was planned to study the interactive effect of barley varieties under poplar (*Populus deltoides*) based agroforestry system on the availability of micronutrients in soil, plant and their uptake by different barley varieties.

Material and Methods

The present study was conducted during the *Rabi* season of 2019-20 in an already established (February, 2015) *Populus deltoides* plantation (5 × 3 m spacing) based agroforestry system at Research area in Department of Forestry, CCS Haryana Agricultural University, Hisar (29^0 10‟ N lat.,75^0 46‟ E long., alt. 215 m mean sea level). A subtropical climate prevails in this area with 350-400 mm average annual rainfall, most of which is received during monsoon (July to September). The temperature ranges from being minimum (0°C) in December and January, to maximum (up to 45°C) in May and June due to hot and sunny days.

In the interspaces of the trees, five barley varieties (BH 393, BH 902, BH 946, BH 885, BH 959) were sown during the first week of November 2019-20 with a row to row distance of 22.5 cm and seed rate of 86.48 kg/ha. following randomized block design with three replications during the *Rabi* season of 2019-20. However, variety BH 885 was sown at a row to row spacing of 18 cm with a seed rate of 98.84 kg/ha. In the nearby field different barley varieties were sown as sole crop (devoid of trees). For field preparation in both the systems (poplar-based agroforestry system and sole crop) two ploughings with disc harrow and one with cultivator followed by planking were given to

prepare a good seed bed for sowing of barley varieties after pre-sowing irrigation. The recommended dose of fertilizers (59.30 kg/ha N and 29.65 kg/ha P) were applied in both the environments. The half amount of nitrogen and whole amount of phosphorus was applied at the time of sowing. The remaining dose of nitrogen through urea was top dressed after 1st irrigation. Three replicates of soil samples were taken randomly from the experimental field (sole crop and under poplar plantation) at different depths (0-15, 15-30 and 30-45 cm), before sowing and after harvesting of barley crop. The samples were air-dried, grounded in a wooden pestle with mortar, passed through a 2 mm stainless steel sieve and stored for further analysis. The pH and EC of the soil were determined in soil: distilled water suspension (1:2). Micronutrient content in grain and straw was determined by di-acid digestion (HNO_3 /$HClO_4$, 4:1, w/v). DTPA extractable micronutrients (Fe, Zn, Cu, Mn) in soil samples were determined with method described by Lindsay and Norvell, 1978 (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA buffer adjusted to pH = 7.3). Statistical data was analyzed using two factor randomized block design.

Results and Discussion

It is evident from the results (Table 1) that the values of zinc varied significantly between different soil depths and environments in both the observations taken before sowing and after harvesting of different barley varieties. Along with an increase in the soil depth, the average value of zinc decreased significantly from the maximum at 0-15 cm to minimum at 30-45 cm in both the observations taken before sowing and after harvesting (0.87 and 0.43 mg/kg, respectively). The average value of copper was significantly higher (Table 1) at the surface layer (0-15 cm) before sowing (0.70 mg/kg) and after harvesting (0.71 mg/kg) of different varieties of barley, while it was significantly lower (0.46 mg/kg) at a soil depth of 30-45 cm. Before sowing of barley varieties, it was observed that the average value of manganese concentration was significantly higher at the surface layer i.e., 0-15 cm (4.21 mg/kg) followed by 15-30 cm (2.89 mg/kg), and it was significantly lower at 30-45 cm (2.26 mg/kg). A similar pattern was observed after harvesting of barley varieties. During this study, it was found that the average iron was significantly higher (Table 1) under poplar-based agroforestry system than that of open conditions (devoid of trees). The maximum concentration of iron was observed at a depth of 0-15 cm (6.81 mg/kg) under poplar based agroforestry system and minimum at a soil depth of 30-45 cm (3.22 mg/kg) under open conditions.

After harvesting of barley varieties, the average Fe concentration was significantly higher at the surface layer i.e., 0-15 cm (6.07 mg/kg) followed by 15-30 cm (5.00 mg/kg), and significantly lower at soil depth of 30-45 cm (4.25 mg/kg). The interaction effect of depth and environment was found significant for Zn and Cu but found non-significant for Mn and Fe. The micronutrients available in poplar-based agroforestry system before sowing and after harvesting of different varieties of barley were significantly higher than the sole crop (devoid of tree). Furthermore, the availability of micronutrients decreased along with an increase in depth, and the maximum amount of micronutrients were available in the surface layer (0-15 cm). It could be possible that the more quantity of micronutrients (Zn, Cu, Mn and Fe) in the surface layer (0-15 cm) is attributed to the presence of more organic matter. Second, via litter fall and root biomass, the tree absorbs nutrients from the deeper layer of soil (30-45 cm) and transfers them to the surface layer. A similar results were also observed earlier by Sarkar *et al.* (2020) and Sharma *et al.* (2021). Additionally, they observed that as a result of tree litter fall and increased C (carbon) input in the form of root biomass, exudates, and above ground biomass under tree plantation, the supply of organic matter increased and stimulated microbial growth, which enhanced micronutrient availability under agroforestry systems. The primary factor influencing the vertical distribution and accumulation of nutrients under different agroforestry systems is nutrient cycling, with human disturbances and leaching as the minor contributors (Jobbage and Jackson, 2001). These results are in agreement with the findings of Campanha *et al.* (2007), Singh and Sharma (2007), Jiang *et al.* (2009) and Khanmirzaei *et al.* (2011). They performed multiple trials in various regions of the globe and concluded that the explanation for these changes is the soil's microclimatological

Table 1: Effect of environment on DTPA extractable zinc, copper, manganese and iron (mg/kg) concentration at different depths before sowing and after harvesting of barley varieties.

Nutrient (mg/kg)	Before harvesting				After harvesting		
	Soil depth (cm)	Under tree	Sole crop	Mean	Under tree	Sole crop	Mean
Zn	0-15	0.94	0.76	0.85	0.96	0.77	0.87
	15-30	0.75	0.54	0.65	0.77	0.55	0.66
	30-45	0.52	0.33	0.43	0.53	0.33	0.43
	Mean	0.74	0.54		0.75	0.55	
	CD at 5 %	Depth = 0.03 Environment = 0.02 Depth x Environment = NS			Depth = 0.03 Environment = 0.02 Depth x Environment = NS		
Cu	0-15	0.74	0.65	0.70	0.76	0.66	0.71
	15-30	0.68	0.57	0.63	0.69	0.57	0.63
	30-45	0.52	0.40	0.46	0.52	0.40	0.46
	Mean	0.65	0.54		0.66	0.54	
	CD at 5 %	Depth = 0.02 Environment = 0.02 Depth x Environment = NS			Depth = 0.03 Environment = 0.02 Depth x Environment = NS		
Mn	0-15	4.80	3.61	4.21	4.88	3.62	4.25
	15-30	3.32	2.45	2.89	3.36	2.44	2.90
	30-45	2.63	1.89	2.26	2.65	1.89	2.27
	Mean	3.58	2.65		3.63	2.65	
	CD at 5 %	Depth = 0.07 Environment = 0.06 Depth x Environment = 0.10			Depth = 0.08 Environment = 0.07 Depth x Environment = 0.12		
Fe	0-15	6.81	5.15	5.98	6.94	5.19	6.07
	15-30	5.83	4.09	4.96	5.88	4.11	5.00
	30-45	5.24	3.22	4.23	5.27	3.22	4.25
	Mean	5.96	4.15		6.03	4.17	
	CD at 5 %	Depth = 0.13 Environment = 0.11 Depth x Environment =0.18			Depth = 0.14 Environment = 0.12 Depth x Environment = NS		

amerlioration as a result of litter fall and organic matter addition. The data presented in Table 2 pertains to Zn, Cu, Mn and Fe content in grain and straw of different barley varieties grown under poplar-based agroforestry system and in open conditions (devoid of trees). Significant variation was observed neither in Zn, Cu, Mn and Fe content in grain and straw among different barley varieties and environments nor the interaction of variety and environment was found significant. It is evident from the Table 3 that the concentration of micronutrients in grain varied significantly among different barley varieties and environments (sole crop and under poplar plantation). However, the interaction effect of variety and environment was found non-significant. Zn and Cu uptake of variety BH 946 were found significantly higher than all the other varieties except BH 393, which was statistically at par. The other varieties were in the following order: BH 902 > BH 885 > BH 959. The uptake of zinc and copper was significantly lower in variety BH 959 (210.92 and 23.23 g/ha, respectively). A slight shuffle in the positions was observed in the uptake of Mn and Fe. The manganese and iron uptake were significantly higher in variety BH 946 (259.18 and 376.46 g/ha, respectively). The other varieties were in the following order: BH 393 > BH 902 > BH 885.

Similar to that of grain, the micronutrient uptake by straw also varied significantly among different varieties as well as in environments. But their interaction effect was found non-significant. The zinc uptake of variety BH 902 (233.15 g/ha) was significantly higher than all the other varieties. The

Table 2: Effect of environment on micronutrients (Zn, Cu, Mn and Fe) content in grain and straw of barley varieties (mg/kg).

Nutrient (mg/kg)	Grain				Straw		
	Variety	Under tree	Sole crop	Mean	Under tree	Sole crop	Mean
Zn	BH 946	65.0	64.0	64.5	36.0	35.0	35.5
	BH 959	64.0	64.0	64.0	35.0	34.0	34.5
	BH 393	65.0	65.0	65.0	35.0	35.0	35.0
	BH 885	65.0	64.0	64.5	35.0	34.0	34.5
	BH 902	66.0	65.0	65.5	38.0	36.0	37.0
	Mean	65.0	64.4		35.8	34.8	
	CD at 5 %	Variety = NS Environment = NS Variety x Environment = NS			Variety = NS Environment = NS Variety x Environment = NS		
Cu	BH 946	7.31	7.22	7.27	3.71	3.61	3.66
	BH 959	7.11	7.00	7.06	3.81	3.72	3.77
	BH 393	7.42	7.31	7.36	3.61	3.50	3.55
	BH 885	7.11	7.08	7.09	3.71	3.60	3.65
	BH 902	7.21	7.11	7.16	3.81	3.71	3.76
	Mean	7.23	7.14		3.73	3.63	
	CD at 5 %	Variety = NS Environment = NS Variety x Environment = NS			Variety = NS Environment = NS Variety x Environment = NS		
Mn	BH 946	59.47	58.88	59.17	44.90	44.31	44.60
	BH 959	57.72	57.13	57.43	45.46	44.89	45.18
	BH 393	58.31	58.30	58.30	44.31	43.73	44.02
	BH 885	57.73	57.70	57.71	44.89	43.71	44.31
	BH 902	58.30	57.72	58.01	45.47	44.88	45.18
	Mean	58.30	57.95		45.01	44.31	
	CD at 5 %	Variety = NS Environment = NS Variety x Environment = NS			Variety = NS Environment = NS Variety x Environment = NS		
Fe	BH 946	87.0	85.0	86.0	278	276	277
	BH 959	84.0	84.0	84.0	276	275	276
	BH 393	85.0	83.0	84.0	272	270	271
	BH 885	85.0	84.0	84.5	274	273	274
	BH 902	86.0	85.0	85.5	280	276	278
	Mean	85.4	84.2		276	274	
	CD at 5 %	Variety = NS Environment = NS Variety x Environment = NS			Variety = NS Environment = NS Variety x Environment = NS		

Table 3. Effect of environment on micronutrient (Zn, Cu, Mn and Fe) uptake (g/ha) by grain and straw of barley varieties.

Micronutrient (g/ha)	Variety	Grain			Straw		
		Under tree	Sole crop	Mean	Under tree	Sole crop	Mean
Zn	BH 946	261.30	303.57	282.44	193.68	232.42	213.05
	BH 959	182.40	239.45	210.92	164.59	217.26	190.92
	BH 393	243.32	290.55	266.93	162.46	212.77	187.62
	BH 885	206.27	257.70	231.98	163.57	212.20	187.88
	BH 902	236.28	284.70	260.49	210.86	255.44	233.15
	Mean	225.91	275.19		179.0	226.0	
	CD at 5 %	Variety = 19.51 Environment = 12.34 Variety x Environment = NS			Variety = 16.57 Environment = 10.48 Variety x Environment = NS		
Cu	BH 946	29.39	34.25	31.82	19.96	23.97	21.97
	BH 959	20.26	26.19	23.23	17.92	23.77	20.85
	BH 393	27.78	32.68	30.23	16.76	21.28	19.02
	BH 885	22.56	28.50	25.53	17.34	22.47	19.91
	BH 902	25.81	31.14	28.48	21.14	26.32	23.73
	Mean	25.16	30.55		18.62	23.56	
	CD at 5 %	Variety = 2.17 Environment = 1.37 Variety x Environment = NS			Variety = 1.71 Environment = 1.03 Variety x Environment = NS		
Mn	BH 946	239.07	279.29	259.18	241.51	294.25	267.88
	BH 959	164.50	213.75	189.13	213.82	286.85	250.34
	BH 393	218.23	260.60	239.42	205.68	265.84	235.76
	BH 885	183.17	232.41	207.79	209.79	272.93	241.36
	BH 902	208.71	252.81	230.76	252.31	318.52	285.42
	Mean	202.74	247.77		224.62	287.68	
	CD at 5 %	Variety = 17.50 Environment = 11.08 Variety x Environment = NS			Variety = 20.71 Environment = 13.10 Variety x Environment = NS		
Fe	BH 946	349.74	403.18	376.46	1495.64	1832.82	1664.23
	BH 959	239.40	314.28	276.84	1297.89	1757.25	1527.57
	BH 393	318.18	371.01	344.60	1262.55	1641.38	1451.97
	BH 885	269.73	338.26	304.00	1280.49	1703.84	1492.17
	BH 902	307.88	372.30	340.09	1553.72	1958.39	1756.06
	Mean	296.99	359.81		1378.06	1778.74	
	CD at 5 %	Variety = 17.50 Environment = 11.08 Variety x Environment = NS			Variety = 127.34 Environment = 80.54 Variety x Environment = NS		

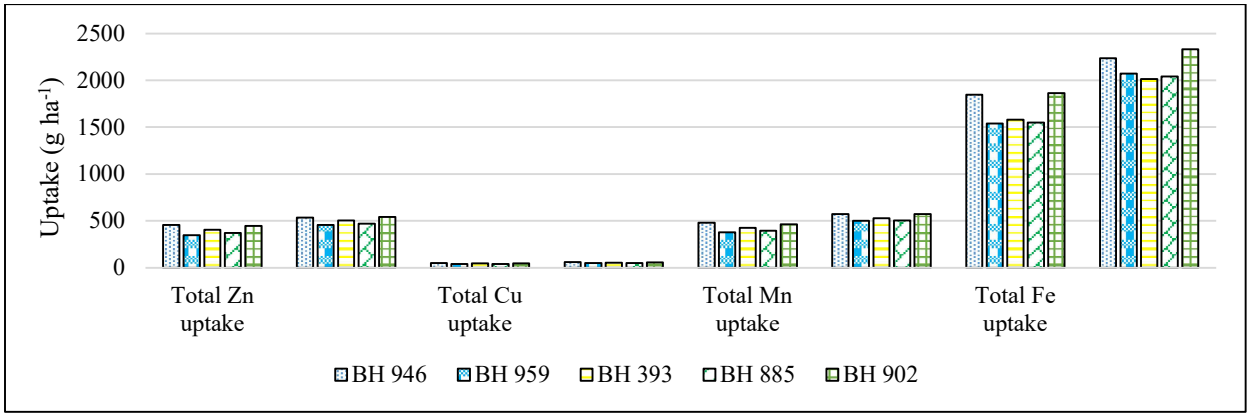

Figure 1: Total micronutrients uptake (g/ha) by five barley varieties.

difference in zinc uptake of varieties BH 959, BH 885 and BH 393 were statistically at par. Variety BH 902 (23.73 g/ha) was significantly higher than all the other varieties in copper uptake. It was closely followed by BH 946 (21.97 g/ha) and BH 959 (20.85 g/ha). A considerable change in positions was observed in manganese and iron uptake. The Mn and Fe uptake of variety BH 902 (285.42 and 1756.06 g/ha, respectively) was significantly higher than all the other varieties except BH 946 (267.88 and 1664.23 g/ha, respectively), which was statistically at par. The total micronutrient (Zn, Cu, Fe and Mn) uptake by barley varieties grown in open conditions (devoid of trees) was significantly higher than grown under poplar plantation. These results are in line with that of Dhillon (1992) and Gill *et al.* (2009) who found that wheat crop nutrient uptake was lower near the eucalypts tree. Thus, the significant reduction in nutrient uptake by barley varieties with poplar plantation than sole crops can be assigned to the intense competition for moisture and nutrients between poplar trees and the barley crop. Additionally, it was noticed that the uptake of total micronutrients (Zn, Cu, Mn and Fe) differed significantly among barley varieties (Fig. 1). On the other hand, the interaction effect of variety and environment was found non-significant. The total uptake of zinc and copper was significantly higher in BH 946 (495.49 and 53.79 g/ha, respectively). It was found to be statistically at par with variety BH 902 (493.64 and 52.21 g/ha, respectively). Variety BH 946 (527.06 g/ha) was observed significantly higher than all the other varieties in total Mn uptake except BH 902 (516.18 g/ha), as it was statistically at par. The maximum iron uptake was observed in variety BH 902 (2096.15 g/ha). It was closely followed by variety BH 946 (2040.69). The other varieties were as follows: BH 959 (1804.41 g/ha), BH 393 (1796.56 g/ha), and it was minimum in variety BH 885 (1796.16 g/ha).

In our experiment, photosynthetic rate, transpiration rate and stomatal conductance were observed maximum in BH 946 and minimum in BH 959 in both the environments and stages (At flag leaf and 10 days after anthesis). Furthermore, it was observed that the photosynthetic rate, transpiration rate and stomatal conductance were significantly higher in sole barley crop than under poplar plantation.

Conclusion

The remarkable change for available micronutrients under *Populus deltoides* based agroforestry system over sole barley crop (devoid of trees) was observed. Micronutrients (Zn, Cu, Mn and Fe) content increased significantly under poplar plantation than sole barley crop at all soil depths. The present study concludes that barley varieties BH 946 and BH 902 could be attributed to having better uptake efficiency of the micronutrients than rest of other varieties. The micronutrients (Zn, Cu, Mn and Fe) uptake by grain was found maximum in BH 946 followed by BH 393, BH 902, BH 885 and minimum in BH 959. The micronutrients uptake by straw was found higher in variety BH 902 than all the other varieties. However, uptake of micronutrients was higher under sole barley than intercropped with poplar-based agroforestry system.

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Conflict of interest

The authors declare that they have no conflict of interest.

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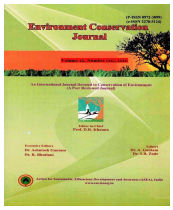

Micro-morphological diversity of rice (*Oryza sativa* L.) as seen under foldscope

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ABSTRACT

Rice being the global grain, its genetic diversity is essential to support farmers' adaptation to climate change for sustainable production. Genetic variability analysis is essential to identify the diverse genotypes and to use them in hybridization programs. Although several advanced molecular techniques are now being used to characterize plants, morphological characterization is always preferred owing to their ease of detection. However, not all morphological traits can be observed through naked eyes. Observing micro-morphological variations requires the help of specialized optical instruments. "Foldscope" is a simple and portable optical instrument, which offers a great opportunity to exploit micro-morphological variations in crop plants. Hence, the current study was aimed at the micro-morphological characterization of rice crop using a foldscope. A total of 24 elite rice genotypes including checks were evaluated using Randomized Complete Block Design during *Kharif* 2018 at Agricultural Research Station Gangavati, to explore their genetic diversity. Five often neglected micro-morphological traits but associated with the traits of economic importance were recorded using 'foldscope' to assess the variability existing among the selected genotypes. The analysis of variance revealed substantial variations across all genotypes for all the characteristics investigated. The traits *viz*., length of leaf serrations, length of hairs on the lemma, and root hair length exhibited higher GCV, PCV, heritability, and GAM most likely because of additive gene effects. So, selection for these traits may be effective. The study also showed that foldscope can be effectively used in agriculture to study micro-morphological diversity between crop genotypes.

Introduction

The rapidly changing climate and the expanding population with the associated threat of food shortage are the major issues affecting the progress of the country. Rice is a predominant food crop in the world (Diwan and Shenoy, 2001). It is the primary source of dietary carbohydrates for billions of people throughout the world (Babu *et al.,* 2012). Rice is the staple meal of around half of the world's rising population, and its demand will continue to climb as the global population grows. By 2030, it is anticipated that we would need to produce 40% more rice to feed the world's 5.0 billion rice

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consumers (Khush, 2005; Tiwari *et al.,* 2020). Being one of the important centers of rice diversity, India harbors vast diversity both at inter- and intra-species levels. Indian rice varieties harbor a huge amount of genetic diversity. Crop morphological variability which forms the basis for their ability to adapt to their changing environment cannot be neglected while studying the diversity among individuals and breeding for better crop plants. However, in recent decades, the trait-based improvement programmes have driven breeders to rely on a small number of parents, resulting in phenotypic similarity and a loss of gene diversity (Singh *et al.,* 2016). In this context, morphological diversity assessment of rice is of prime importance in rice breeding. The germplasms with unique traits can serve as good parents for hybridization programs. But in the quest of characterizing the rice genotypes based on major morphological characters affecting rice yield and quality, some microscopic morphological characters like lemma hairs, root hair length, leaf serrations, *etc* are often neglected. Their role can be known only when they are characterized morphologically first, followed by dissecting their molecular basis.

The root of the plant is indeed a vital organ for nutrition absorption. In nutrient-deficient soils, rice root trait variation is linked to increased nutrient efficiency and crop productivity. In reaction to nutritional deficiency, some rice genotypes lengthen and densify their root hairs, especially when P and K levels are low. Plants with long root hairs can often acquire more P and K and are better suited to nutrient-deficient soils than those with short root hairs. Thus, root hairs play a crucial role in nutrient absorption (Klinsawang *et al.,* 2018).

The major plant physiological processes like Light harvesting, gas exchange, starch buildup, and phytohormone synthesis are all dependent on plant leaves. The morphology of leaf shape, which is predominantly dictated by the size of leaf serrations, has a considerable impact on these processes. Variations in leaf serration features also add to the plant's biodiversity. As a result, the evolution of leaf serration is a fascinating process that has piqued the interest of many experts (Kong *et al.,* 2019). Trichomes, commonly known as hairs or pubescence, are unicellular or multicellular epidermal structures found on the surface of plant organs including leaves, stems, flowers, hulls (glumes), and roots. Trichomes on the leaf surface are vital for plant development and productivity in rice as they contribute to photosynthesis as well as transpiration and respiration processes (Hu *et al.,* 2013). The rice grain is made up of an edible portion called the rice caryopsis that is covered by a coating called the hull (husk). The husk protects the rice caryopsis with its bigger lemma and smaller palea (Roy and Shill, 2020). The hairs on the lemma and palea are known to impact milling and to harbour pathogens. Burkholderia glumae, a bacterial pathogen that causes panicle blight in rice, is one such case (BPBR). Pathogens first appear on the surface of the glumes and then invade the glume hairs and cells on the palea and lemma edges (Li *et al.,* 2017). Foldscope is a low-cost paper microscope that can be used to study various microscopic morphological characters such as leaf trichomes, root hairs, leaf serrations, and lemma hairs. Foldscope being portable and easy to handle, can be taken to fields and microscopic morphological characters can be assessed easily. It has wide range of applications in various fields like food testing and nutrition, sanitary care, environment control, and agricultural applications (Jadhav *et al.,* 2020). There are few reports where foldscope has been used as a powerful tool in the field of agriculture. Maheshwari *et al.* (2018) used Foldscope as an intelligent disease detection tool for Tomato early blight. Sharma and Nischal (2019) have used a foldscope to assess the seed quality in wheat, maize, moong dal, and black dal. Diwan and Chikkanaragund (2019) studied rice root hair length, length, and density of rice lemma, and trichome . Trichome length and density in pigeon pea (*Cajanus cajan* (l.) Millsp) observed by Satish *et al.* (2020).

In the present study, an effort was made to characterize the 24 elite rice genotypes for their neglected yet useful microscopic morphological traits using Foldscope.

Material and Methods

The present investigation was carried out at the Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, College of Agriculture, UAS Raichur, Karnataka. The field investigations were carried out during *Kharif* 2018 at the Agricultural Research Station, Gangavathi, which represents the Tungabhadra command area's

irrigated transplanted rice belt. The research station is located in Karnataka's Northern Dry Zone between 150_15'40" North latitude and 760_31'40" East longitude, at an elevation of 419m above MSL (Mean Sea Level). The experimental material for the current study comprised of 24 rice genotypes which include both early and medium maturing advanced breeding lines. The experiment was conducted in three replications using Randomized Complete Block Design (RCBD). All the genotypes were randomized to minimize errors due to soil heterogeneity. The samples were taken from the experimental plot for recording observations for lemma and leaf traits; however, for recording root hair length, the seeds were taken from the plot and allowed for germination in germination chambers.

Inserting a sample placed on a microscope slide, turning on the LED, and seeing the sample while panning and focusing with the thumbs is all that is required to operate the foldscope. The sample can be examined by holding the foldscope with both hands and positioning the eye near enough to the microlens that the eyebrows contact the paper. Panning is accomplished by positioning the thumbs at opposite ends of the top stage and moving them in tandem, translating both the optics and lighting stages while maintaining the stages aligned. The identical thumb placement is used for focusing, only the thumbs are pulled apart (or pushed together). This produces tension (or compression) along the optics stage, resulting in (or +) deflection of the micro-lens owing to flexure of the sample-mounting stage's supporting structure. Unlike typical microscopes, the foldscope fixes the sample in place as the optics and lighting stages move in unison (Cybulski *et al.,* 2014).

The kit also includes magnets that can be stuck onto the foldscope to attach it to a smartphone which allows taking pictures of the magnification. In the present study, rice root, leaf and grain morphological characters were studied using foldscope. A photograph of the foldscope instrument is shown in Figure S1.

Root hair length

Randomly selected healthy seeds of each genotype were kept for germination in a germination chamber under optimal conditions. After germination and root growth, roots of about 1 mm length were cut and fixed on the glass slide with clear sticky tape. The slide was also fixed with the measuring grid provided with the foldscope tool kit, for easy measurement of root hair length. The prepared slides were observed under the foldscope using LED. Photographs were taken under 3 different microscopic fields for each genotype with the unchanged magnification of the camera.

Length of leaf serrations

The matured leaves of 45-50 days old were collected from randomly selected plants of all the selected genotypes in each replication. A thin section of thread-like leaf margin was dissected out and mounted on the glass slide and fixed with adhesive tape. The serration pattern of leaves was observed by panning and focusing, turning on the LED. Photographs were taken in 3 different microscopic fields of each sample without changing the magnification of the mobile camera.

The density of leaf trichomes

Rice leaf trichome density was calculated using the method published by Maiti *et al* (1980). 45–50 day old matured leaf samples randomly picked from the rice plant were chopped into one square centimetre size and cooked in 20 ml of water in tiny glass vials for 15 minutes in a hot water bath maintained at 85°C. After removing the leaves, the water was drained out and heated for 20 minutes at 80 °C with 20 cc of 96 percent ethanol added. The alcohol was then drained out, and the boiling procedure with ethanol was repeated to thoroughly eliminate the chlorophyll from the leaves. The alcohol was poured out again and 90 % lactic acid was added and cooked at 85°C for 30–45 minutes, until the leaf segments were transparent. The vials were then cooled, and leaf fragments were extracted and mounted on clean slides using adhesive tape to analyse the trichome density with the foldscope. For each sample, the number of trichomes per millimetre area was counted under constant magnification, and trichome density was calculated.

Length and density of hairs on the lemma

Rice seeds of 12–15% moisture content were taken randomly from all the selected 24 genotypes. The seed coat was dissected out by separating the lemma and palea. A small piece of lemma was fixed on the glass slide using adhesive tape. The upper surface of the lemma was observed under the foldscope for length and density of hairs per unit area. Photographs were taken under three different

microscopic fields for each sample without changing the magnification of the camera and the observations were recorded in replications to minimize errors.

Analysis

The photographs of the samples focused under foldscope and captured with the help of a mobile camera with unchanged magnification were fed into the ImageJ imaging software. ImageJ is a Java image processing and analysis software inspired by the National Institute of Health (NIH) and the Laboratory for Optical and Computational Instrumentation (LOCI). The software analyzes the image formats and computes area and pixel value statistics for user-specified choices. It can calculate distances and angles, as well as generate density histograms and line profile charts. ImageJ user-written plugins (Fiji and MBF ImageJ) allow users to tackle practically any problem with the image processing or analysis (Rueden *et al.,* 2017). Length of leaf serrations, lemma hairs, and root hairs were measured using ImageJ software and density of leaf trichomes, lemma hairs per millimeter square area in each microscopic field were counted manually. Windostat version 8.5 software was used to analyse the data.

Results and Discussion

ANOVA for the rice leaf, root, and grain morphological traits studied using foldscope

Analysis of variance (ANOVA) for various morphological characters studied using foldscope revealed that the genotypes under investigation differed significantly for all the traits studied both at 5% and 1% level of significance. This revealed the existence of significant morphological variability among the genotypes included in the study, and the selection for these morphological traits could be effective. Table 1 shows the ANOVA for various morphological traits studied using foldscope; while genetic variability parameters like Vg, Vp, GCV, PCV, heritability, and GAM (Genetic Advance as percent of Mean) for morphological characters studied using foldscope are given in Table 2. Table S1 shows the mean performance of genotypes for various characters studied.

Table 1: ANOVA for rice root, leaf and grain morphological traits studied using foldscope.

Source of variation	DF	RHL	LLS	LTD	LHL	DHL
Treatment	23	8882.29**	5668.91**	0.33**	3549.58**	3.59**
Replication	1	226.70	17.02	0.04	7.75	0.26
Error	23	119.97	184.66	0.11	76.95	0.72
CV		4.72	4.60	12.44	4.70	13.72

(Note: ** significant at 1% level of significance)

Table 2: Genetic variability parameters for rice root, leaf and grain morphological traits studied using foldscope

Sl. No.	Characters	Mean	Range		Variance		CV %		H (%)	GA	GAM
			Min.	Max.	Vg	Vp	GCV	PCV			
1	RHL	232.27	122.87	384.26	4381.16	4501.13	28.50	28.89	97.34	134.52	57.92
2	LLS	295.68	194.12	400.04	2742.13	2926.78	17.71	18.30	93.69	104.41	35.31
3	LTD	3.07	2.39	3.98	0.09	0.24	9.77	15.82	38.12	0.38	12.38
4	LHL	186.52	119.51	272.86	1736.32	1813.27	22.34	22.83	95.76	84.00	45.03
5	DHL	6.17	3.82	9.55	1.44	2.16	19.43	23.79	66.70	2.02	32.73

Abbreviations: DF- Degrees of freedom; **CV-** Coefficient of variation; **Vg-** Genotypic Variance; **Vp**-Phenotypic variance; **GCV & PCV**-Genotypic and Phenotypic coefficients of variation; **H**-heritability in broad sense; **GA-** Genetic advance; **GAM-** Genetic advance as percent of mean. **RHL-**Root hair length in micrometers; **LLS-**length of leaf serrations in micrometers; **LTD-**Leaf trichome density (per mm^2); **LHL-**Length of hairs on lemma in micrometers; **DHL-**Density of hairs on lemma (per mm^2).

Estimation of mean and other genetic parameters for rice leaf, root and grain morphological characters studied using foldscope.

Root hair length

The cylindrical extensions of root epidermal cells are known as root hairs. They vastly increase root diameter as well as root surface area, thus assisting in nutrient acquisition, microbe interactions and plant anchorage (Grierson and Schiefelbein, 2002).

The genotypes showed a mean value of 232.27 micrometers for the trait with the maximum and minimum values of 384.26µm (IET-25497) and 122.87µm (BPT-5204), respectively. The GCV and PCV values were almost equal (28.50 and 28.89) with a minor difference indicating a negligible influence of the environment on the trait. Nestler *et al*. (2016) has earlier reported a maximum root hair length of 395 µm and an average length of 122 µm through *in situ* imaging of rice root hairs in intact soil. The researchers also reported that root hair length remains constant in phosphorus fertilized and unfertilized soil, indicating the negligible influence of the environment on the trait.

The trait has shown higher heritability coupled with high genetic advance (97.34 and 57.92 respectively). It may be due to additive gene action governing the trait. Thus, selection for this trait will be effective for improving nutrient and water uptake efficiency of crop plants. Figure 1 shows the foldscopic view of rice root hairs.

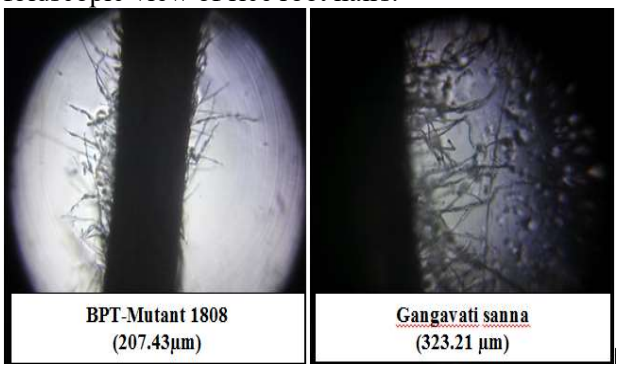

Figure 1: Foldscopic view of rice root hairs.

Length of leaf serrations

The genotypes studied had a wide range of variability for the trait, ranging from 194.12 to 400.04 µm with a mean value of 295.68 µm. The lowest length of leaf serration was recorded in BPT-Mutant 1802 (194.12 µm) and the highest was recorded in SMW-09-32 (400.04 µm). The trait recorded moderate GCV and PCV of 17.71 and 18.30 respectively. Heritability (93.69) and GAM (35.31) were found higher, providing a good scope for selection. Figure 2 shows the photograph of rice leaf serrations captured using foldscope.

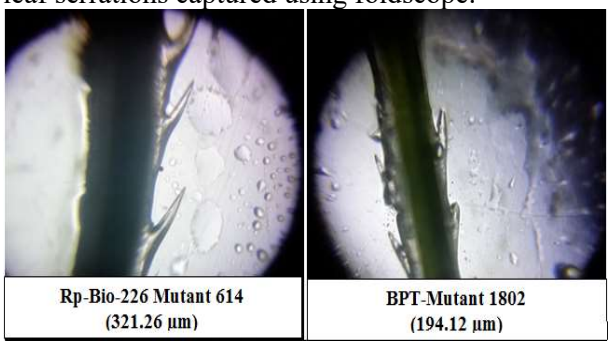

Figure 2: Foldscopic view of rice leaf serrations

Leaf trichome density.

Trichomes on the leaf surface are vital for plant development in rice because they contribute to physiological activities such as photosynthesis, transpiration, respiration, and resistance to biotic and abiotic stress, influencing productivity. (Li *et al.,* 2010).

Hamaoka *et al.* (2017) identified BLANKET LEAF hairy-leaf gene on chromosome 6 of wild *Oryza nivara* which lengthens the macro-hairs. They reported that the hairy-leaf character increases leaf surface temperature and Water Use Efficiency by limiting leaf transpiration. As a result, the characteristic may be advantageous in breeding of rice cultivars that are amenable to water-saving farming practices. In the current study, the density of leaf trichomes ranged from 2.39 (Gangavati sanna) to 3.98 per mm^2 (Rp-Bio-226 Mutant 614) and the mean trichome density was found to be 3.07 mm^2. The results are in agreement with Hu *et al*. (2013) who had reported significant differences in pubescent density of different rice genotypes through microscopic studies. Scanning electron microscopic studies of leaf trichomes by Amsagowri (2017) showed that trichome density of rice accessions differ significantly between accessions ranging from 0.41 to 8.56 per mm^2, and higher trichome density of rice leaves is associated with the lesser ovipositional preference of yellow stem borer thus offering resistance to the pest. Foldscopic study of Satish *et al.* (2020) also

showed the presence of significant variation among the pigeonpea genotypes for pod trichome length and density.

The trait showed low GCV (9.77) coupled with moderate values of PCV (15.82), heritability (38.12) and GAM (12.38). So, the selection based on phenotype may not be effective for the trait as it might be under the control of non-additive gene actions as well. Figure 3 shows the foldscopic view of leaf trichomes in rice genotypes.

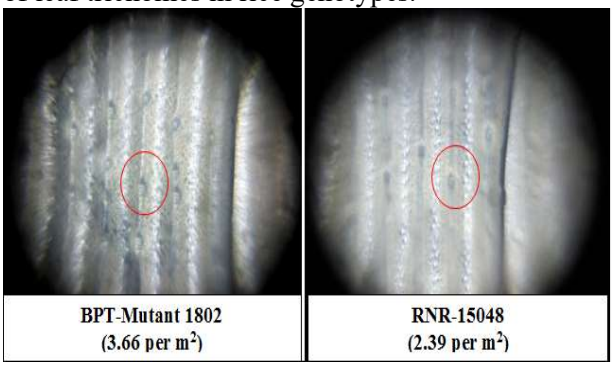

Figure 3: Foldscopic view of rice leaf trichomes

Length of hairs on the lemma

Rice hulls with hairs or pubescence are problematic for transporting. Besides being prickly, they also minimize the weight per unit volume (Hu *et al.,* 2013).The range of lemma hair length varied from 119.51 to 272.86 µm with a mean value of 186.52. The genotype BPT-Mutant 1808 recorded the minimum length of lemma hairs (119.51 µm) whereas IET-25520 showed maximum lemma hair length (272.86 µm). Higher values of GCV (22.34), PCV (22.83), heritability (95.76), and GAM (45.03) were recorded indicating that the trait is governed by additive genes and phenotypic selection is effective. So, the selection of genotypes with short lemma hairs might be rewarding. Li *et al.* (2010) also obtained similar results through scanning electron microscopic studies of rice accessions with the length ranging from 15 to 150 micrometers.

The density of hairs on the lemma

Glabrous leaves and hull are important agronomic traits in rice that facilitates easy harvest and follow-up processes. Glabrous rice varieties are preferred over pubescent ones as they are noted for high yield, resistant to lodging, and superior grain quality (Li *et al.,* 2010). The genotypes with a lesser density of hairs on lemma are preferred for cultivation over hairy types, as glabrous cultivars produce less dust than pubescent ones during processing. (Jodon, 1965). In the current study, the density of lemma hairs ranged from 3.82 to 9.55 per mm^2 with a mean of 6.17 hairs per mm^2 area. The genotype IET-22066 exhibited the lowest lemma hair density (3.82) whereas BPT-Mutant 1808 recorded a higher density of lemma hairs per mm^2 area (9.55). The genotypic coefficient of variation was found moderate (19.43) whereas the PCV, heritability, and GAM was high with the values 23.79, 66.70, and 32.73 respectively; indicating the additive gene actions controlling the trait. Hamaoka *et al*. (2017) had earlier reported non-significant variation of hull pubescence among different rice genotypes through scanning electron microscopic observations. Figure 4 shows the photograph of lemma hairs captured using foldscope.

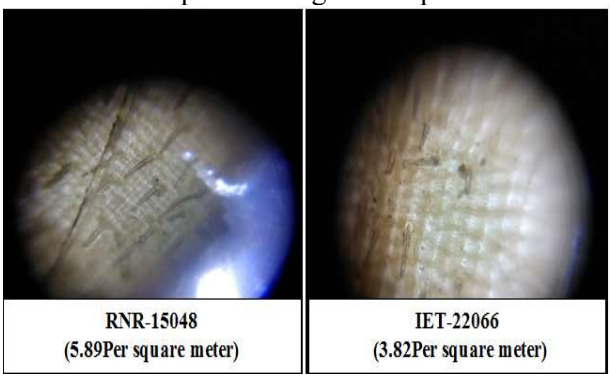

Figure 4: Foldscopic view of rice lemma hairs

The top five genotypes selected based on their performance of various morphological characters studied using foldscope are presented in Table S2, where genotypes are listed in decreasing order of their trait performance for the traits root hair length and leaf trichome density and length of leaf serrations whereas in increasing order for length and density of lemma hairs.

Conclusion

The results of the present investigation helped to identify superior rice genotypes for each micro-morphological trait studied here. These genotypes can be used to improve rice plants for specific morphological traits based on the breeding objective set. The above studied foldscopic traits can also be used as criteria of selection which directly or indirectly affect the acceptance of the varieties by the farmers as well as consumers. The genetics of these traits can be studied in the future

to dissect their linkage behavior and they can be used as morphological markers while breeding for elite cultivars. The results also evidently showed the application of foldscope in agriculture for crop morphological diversity assessment. Being simple, portable, light, and easy to handle, the instrument can be used to assess various agronomically important morphological traits that cannot be neglected while breeding for better crop types.

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Conflict of interest

The authors declare that they have no conflict of interest.

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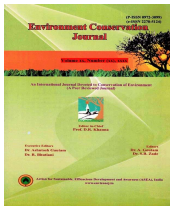

Identification of the race of root knot nematode by differential host test method

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ABSTRACT

Root knot nematode (*Meloidogyne incognita*) cause major damage to the fruit crops, vegetable crops and field crops. Infected plants showed declined symptoms and poor fruit yield also displayed stunting and yellowing symptoms. In order to choose appropriate management control techniques, nematode diagnosis and specimen identification must be accurate, quick and precise. The population of the nematode obtained from the experimental field of Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri were assessed for their host-race status by the differential host test which relies on the combination of resistant and susceptible reactions of six differential hosts to the nematode population. Result revealed that the nematode population infected tobacco (NC 95), pepper (California Wonder), watermelon (Charleston grey), tomato (Rutgers) and unable to reproduce on cotton (Deltapine- 16) and peanut (Florunner) which indicated presence of race 2 of *Meloidogyne incognita*.

Introduction

The root-knot nematode is one of the world's most dangerous plant-parasitic nematodes. Estimating crop losses has already been conducted based on data published by All India Co-ordinated Research Projects on Nematodes in Agriculture over the years, phytonematodes cause 21.3 % crop losses totalling ₹ 102,039.79 million per year; losses in 19 horticultural crops were estimated to be ₹ 50,224.98 million, while losses in 11 field crops were estimated to be ₹ 51,814.81 million Kumar *et al*. (2020). With such a high potential for crop damage, it is critical to monitor and manage root knot nematode population in a field. Because all species of root knot nematode are host dependent, a farmer may be able to rotate a non-host crop if the nematode is recognised at species level. Cotton growers, for example, are frequently infested by *M. incognita*. Cotton to groundnut crop rotation is a frequent crop rotation to reduce root knot nematode population density. *M. arenaria*, on the other hand, thrives in groundnut and can be found in the same field as *M. incognita* Davis and Timper (2000). *Meloidogyne* spp. and its races have long been difficult to identify because of physical similarities, life phases in different habitats, varied host ranges, poorly defined species borders, intraspecific variability, probable hybrid origin and polyploidy Blok and Powers (2009). In general, accurate nematode identification is very essential, especially in the initiation of the research programmes and in the development of control strategies. Major control tactics such as crop rotation and use of resistant cultivars are generally race specific. Therefore, in the process of developing resistant varieties for root knot nematode the exact identity (*i.e*., species and race) of the nematode population being tested must be known. The differential-host test is one of the most commonly used procedures for identifying root knot nematode species. The differential-host test is one of the most commonly used procedures

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for identifying root knot nematode species. Sasser and Triantaphyllou in 1977, introduced this test to distinguish the species and race of four commonly encountered plant-parasitic root-knot nematodes.

Material and Methods

The investigation on the identification of the race was conducted at glasshouse, Post Graduate Institute., Mahatma Phule Krishi Vidyapeeth, Rahuri. during December, 2020 to February, 2021. In the event of a race identification, throughout the experiment, earthen pots with a diameter of 20 cm were used. The earthen pots were cleaned by using water and then disinfected with 4% formaldehyde. Before using these pots for cultivating differential hosts plants, formaldehyde was allowed to evaporate. Medium black to black soil was obtained from experimental field at Instructional Farm of Department Agricultural of Entomology and then mixed in proportion 3:1 with FYM before being steam sterilised for 4 hours in the soil steriliser with boiler at 6.75 kg/cm^2 pressure. Whenever, soil population of nematodes was required, soil sample taken from the feeder root zone of guava from the field and processed by using Cobb's sieving and decanting procedure. The differential host test was used to characterise the host range. The study used six standard crop plant cultivars: tobacco cv. NC 95 (*Nicotiana tabacum* L.); cotton cv. Deltapine- 16 (*Gossypium hirsutum* L.); pepper cv. California Wonder (*Capsicum frutescens* L.); watermelon cv. Charleston Grey (*Citrullus vulgaris* Schrad.); groundnut cv. Florunner (*Arachis hypogaea* L.) and tomato cv. Rutgers (*Solanum lycopersicum* L.). Five to six leaf stage transplanted tomato, tobacco and cotton seedlings were raised from the seeds in pots having 250 g of steam sterilised soil consortium (2 soil: 1 sand) and inoculated with 100 freshly hatched J_2. Each plant cultivar was represented by four replicates. Infected plants (24) were kept at a constant temperature of 25 °C in a glasshouse with 16 hours photoperiod and fertilisers given as needed.

Plants were harvested after 60 days and the roots were cleansed under running tap water and then stained for 2 minutes with trypan blue solution (0.1 g/lit). The root system's root galls and stained egg masses were counted. The gall index was computed on a scale of 1 to 5 based on the number of egg masses produced by each plant. The lines were classified into different categories based on the gall index, as shown in Table 1.

At the end of the experiment, each plant cultivar was categorised as susceptible (+) or resistant (-) depending on whether the average root system egg mass count was higher than 3 or lesser than 3 egg mass. The obtained information was then compared to differential host test reaction chart (Table 2.).

Table 1: Gall Index Scale (Gaur, 2001)

Nematodes gall index	Number of egg masses produced by each plant	Reactions
1	0	Highly Resistant
2	1-10	Resistant
3	11-30	Moderately Resistant
4	31-100	Susceptible
5	>101	Highly Susceptible

Table 2: Differential host test reaction chart (Sasser and Triantaphyllou, 1977)

Meloidogyne species and race	Deltapine 16 (cotton)	NC 95 (tobacco)	California Wonder (pepper)	Charleston grey (watermelon)	Florunner (groundnut)	Rutgers Tomato
M. incognita						
Race-1	-	-	+	+	-	+
Race-2	-	+	+	+	-	+
Race-3	+	-	+	+	-	+
Race-4	+	+	+	+	-	+
M. javanica	-	+	-	+	-	+
M. arenaria						
Race-1	-	+	+	+	+	+
Race-2	-	+	-	+	-	+
M. hapla	-	+	+	-	+	+

Results and Discussion

The population distribution of root-knot nematodes (*M. incognita*) in the experimental field which specified the reactions of root knot nematode population on host differentials is depicted in Table 3. Six host differentials *viz*., cotton, tobacco, pepper, watermelon, groundnut and tomato were used for race identification. The population infected host differentials like tobacco, pepper, watermelon, tomato but were unable to reproduce on cotton and groundnut. The mean gall index recorded was found to be 3, 3, 3 and 4 in tobacco, pepper, watermelon and in tomato, respectively. The average number of egg mass was 22.25, 15.25, 26.00, 31.75 in tobacco, pepper, watermelon and in tomato, respectively. After comparing the observed data with differential host test reaction chart (Table 2.), it is evident that the species and the race of nematode present in the experimental field is race 2 of *Meloidogyne incognita*.These findings are in consonance with the findings of Khan *et al*. (2017) who carried out research on *Meloidogyne incognita* infecting *Passiflora edulis* (passion fruit) on NC host differentials and found that nematode population was impotent to infect and reproduce on cotton and groundnut. However, infected and reproduced on tomato, tobacco and pepper. As a result, race 2 was assigned to the population of *M. incognita*. Similarly, Deuri *et al*. (2016) carried out a study on *M. incognita* populations infecting vegetable crops. The population of *M. incognita* found in Jorhat, Lakhimpur, Sonitpur and Kokrajhar districts of Assam was identified as race 2 based on the response of the differential hosts to *M. incognita*. These findings are in accordance with the research finding.

Results are in line with Hussain *et al.* (2008) who conducted differential host test in the nethouse of the Department of Nematology, Assam Agricultural University, Jorhat to identify the species and races of *Meloidogyne* spp. Five differential hosts *viz.,* cotton, tobacco, pepper, groundnut and tomato were grown separately in pots containing soils collected from different localities. At the termination of the experiment, result showed galls in tobacco, pepper and tomato roots but recorded no gall (gall index 0) in the remaining hosts *i.e*., cotton and peanut which indicated the presence of race 2 of *Meloidogyne incognita* in the area surveyed. Thus, the results are in consonance with the findings.

Table 3: Reaction of root knot nematode in differential host test

Population No.	Cotton (Deltapine 16)		Tobacco (NC95)		Pepper (California wonder)		Watermelon (Charleston grey)		Peanut (Florunner)		Tomato (Rutgers)		Species	Race
	GI	EM	GI	EM	GI	EM	GI	EM	GI	EM	GI	EM		
1	0.0	0.0	3	29	3	16	3	28	0.0	0.0	4	31	*M. incognita*	2
2	0.0	0.0	3	22	3	16	3	24	0.0	0.0	4	31	*M. incognita*	2
3	0.0	0.0	3	20	3	15	3	25	0.0	0.0	4	33	*M. incognita*	2
4	0.0	0.0	3	18	3	14	3	27	0.0	0.0	4	32	*M. incognita*	2
Mean	0.00	0.00	3	22.25	3	15.25	3	26	0.00	0.00	4	31.75		

GI = Gall index, EM = No. of egg masses/plant

Conclusion

Major control tactics such as crop rotation and resistant cultivars are generally race specific. Therefore, in the development of resistant varieties for root knot nematode resistant cultivars the exact identity (*i.e*., species and race) of the nematode population being tasted must be known. In view of the above facts and data, the current investigation on the identification of the race was conducted in the glasshouse, Post Graduate Institute., Mahatma Phule Krishi Vidyapeeth, Rahuri. Differential host test was performed for host range characterization. Six standard crop plant cultivars: tobacco cv. NC

95 (*Nicotiana tabacum* L.); cotton cv. Deltapine- 16 (*Gossypium hirsutum* L.); pepper cv. California Wonder (*Capsicum frutescens* L.); watermelon cv. Charleston Grey (*Citrullus vulgaris* Schrad.); groundnut cv. Florunner (*Arachis hypogaea* L.) and tomato cv. Rutgers (*Solanum lycopersicum* L.) were used for the investigation and inoculated with 100 freshly hatched J_2 of each population. Result revealed that the nematode population infected tobacco (NC 95), pepper (California Wonder), watermelon (Charleston grey), tomato (Rutgers) and unable to reproduce on cotton (Deltapine- 16) and peanut (Florunner) which indicated presence of race 2 of *Meloidogyne incognita*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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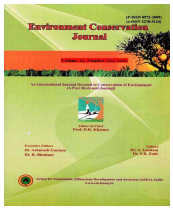

Evaluation of entomopathogenic nematode (EPN) isolate, *Heterorhabditis indica* of Vidarbha region, against the tobacco cutworm, *Spodoptera litura*

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ABSTRACT

The present study on pathogenicity of entomopathogenic nematodes against *Spodoptera litura* in laboratory conditions was undertaken during 2020-21, with the aim to ascertain the effectiveness of entomopathogenic nematodes, against an obnoxious cosmopolitan pest *S. litura.* Experiments were conducted by using entomopathogenic nematode (EPN) isolate *Heterorhabditis indica* (CICR-Guava), on filter paper, against *Galleria mellonella* and *S. litura* at the treatment dose of 10, 20, 30, 40, 60, 80 and 100 IJs/100µl along with control (Sterile distilled water). The results of our study revealed that, EPN isolate *H. indica* (CICR-Guava) caused 100% mortality at the treatment dose of 40 IJs/100µl within 72 h of infection in 5th instar larvae of *G. mellonella* and in case of *S. litura,* 100% mortality was recorded within 72 h of infection at the treatment dose of 100 IJs/100µl in 3rd instar larvae, which was found more susceptible. The median lethal concentration of *H. indica* (CICR-Guava) for 5th instar larvae was 2.29 IJs/100µl. The result of reproductive potential of isolates of entomopathogenic nematodes revealed that the highest yield was obtained from 5th instar larvae of *G. mellonella* at treatment dose of 100 IJs/100µl 278667 IJs per larva. In case of *S. litura,* the highest yield obtained was 152533 IJs. It could be concluded that, there was a positive correlation between nematode treatment concentration, time of exposure and the insect mortality of the tobacco cut worm and multiplication rate of IJs increased with increase of exposure time and size of larvae. This EPN isolate, *H. indica* (CICR-Guava) can be suggested as biocontrol agents for the control of *S. litura* in the Vidarbha region.

Introduction

Pest management in agriculture is a challenging task in the context of increasing agricultural productivity without disturbing the ecological balance and deteriorating the environment. Agrochemicals in agriculture of course are useful for protecting crops against pests and diseases and have played a significant role to boost the agricultural production. However, these chemicals are posing enormous problems like environmental pollution, pesticide resistance, pest resurgence, toxicity hazards, secondary pest outbreaks, destruction of biodiversity of useful natural

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enemies, residues of harmful chemicals in feeds, foods, soil and water, and some social economic and political problems. Failure of chemical insecticides to control insect pests at recommended dosage and problem associated with the use of pesticides made researchers to concentrate on the safer and effective alternative methods that can well fit into the current concept of integrated pest management. However, thirst on biopesticides is increasing due to increasing demand for organic agriculture. The biopesticides take care of crop losses during seed germination, plant growth in the nursery, fruiting phases, post-harvest storage, transport period and loss of man hours and lives. The annual growth in pesticides use is 1-2% and that of biopesticides is 10-25%.

The tobacco cutworm, *Spodoptera litura* (Fab.), is a defoliating and an obnoxious cosmopolitan pest which feeds on more than hundred host plants (Radhakrishnan and Shanmugam, 2017*).* It damages broad leaf plants such as legumes, brassicas, and other economically important crops throughout the year (Park *et al.,* 2001) and causes substantial economic loss. Hatched larvae of first to second instar aggregate at the back of the leaf and feed on the mesophyll, leaving the outline of the leaf veins on the plant. As growth continues, caterpillars eat entire leaves, and even flowers and fruits, causes great loss. Larvae older than third instar hide under the surface of the ground in the daytime and move out for feeding at night. They stay 1-3 cm under the soil surface until pupation (Park *et al.,* 2001). Pupation takes place within the soil near the base of the plants. The current research would help to generate some basic information about the pathogenicity of EPN isolate *H. indica* (CICR-Guava) isolated from local areas against *Spodoptera litura* and their dose *i.e*., at what concentration maximum mortality occurs. It is important to test efficacy of local isolates of nematodes because they are already adapted to specific ecological niches and to some extent, are likely to exert natural biological control to either native or exotic insect pests.

Material and Methods

Collection of nematodes

The entomopathogenic nematode (EPN) isolate *Heterorhabditis indica* (CICR-Guava) was obtained from College of Agriculture, Nagpur, India. This isolate was reconfirmed on the basis of associated bacterium and symptoms caused by the bacteria inside the insect cadaver. The EPN were cultured and multiplied on larvae of *Galleria mellonella* (Wiesner, 1993). The procedure of *In vivo* production of entomopathogenic nematodes was conducted by following the methods described by Poinar (1979) and summarized by Woodring and Kaya (1988).

Collection and rearing of test insect

Larvae of *Spodoptera litura* were collected from infested fields in Nagpur vicinity and reared on castor and cauliflower leaves. Also, laboratory host *Galleria mellonella* was reared on artificial diet in the laboratory.

Multiplication, culturing and Storage of entomopathogenic nematodes

The individual strain was maintained in the laboratory. Pure cultures of indigenous isolates of entomopathogenic nematodes, were prepared and maintained separately in late instar larvae of *G. mellonella*. These pure cultures were used for preparation of different treatment doses/ concentrations for further studies. The infective juveniles of the entomopathogenic nematodes were stored in conical/tissue culture flasks. The double distilled water was used for preparing standard IJs counts. The nematode concentrations were kept in the range of 10,000 IJs/ml of sterile distilled water.

Bioassay against insect pests

In order to know the infectivity and pathogenicity of EPN isolate *Heterorhabditis indica* (CICR-Guava), an experiment was laid down. The EPN isolate was inoculated on different instars of the *G. mellonella* and *S. litura* under similar set of conditions. Infective juveniles (IJs) of the isolate *Heterorhabditis indica* (CICR-Guava), were taken into separate beakers. The serial dilutions were prepared as per the treatments in beakers separately. The infective juveniles count was taken for 100 µl and was repeated for five times. The known IJs were placed in the petri dish lid with the moistened filter paper and in each treatment and replication, five larvae of *Spodoptera litura* of third, fourth, and fifth instar were taken per petri dish. After treatment, the petri dishes were sealed and kept in captivity. After 24 hours the observations for larval mortality in each instar, replication wise and treatment wise were recorded. The observations were taken up to 96 hours at 24

hours interval. Larval mortality data was recorded and larvae were placed on white trap in a petri dish where water was added. In the control larvae were treated with plain distilled water.

Larval mortality was calculated by using the following formula

$$Larval\ mortality\ (\%) = \frac{Number\ of\ larvae\ died}{Total\ number\ of\ larvae} \times 100$$

LC_{50}

The LC_{50} values were calculated as per Finney (1971) using probit analysis with the help of online software (OPSTAT) available on Hissar Agricultural University, Hissar, after computation of corrected percentage mortalities as per Abbott (1925).

Reproduction of EPNs on *Spodoptera litura*

In this experiment, 3rd, 4th and 5th instar larvae of *Spodoptera litura* were exposed to 10, 20, 30, 40, 60, 80 and 100 IJs/100 µl concentration of EPN isolate *Heterorhabditis indica* (CICR-Guava) (Yadav and Lalramliana, 2012) in petri plates. The nematode infected dead larvae were removed from petri plates and transferred individually on to white trap for their emergence from the body of cadaver (White, 1927). Then these petri plates and white traps were observed under stereo zoom binocular microscope for nematode emergence. The nematodes emerged from cadavers moves into surrounding water in the petri dish and this water containing infective juveniles was taken out in a beaker. The suspension taken out was checked for nematode population count, by observing 100 µl suspension under stereo zoom binocular microscope for number of IJs in the droplet. Total count of nematode suspension taken out from each petri plate was noted and total population count was calculated.

Statistical analysis

The data, thus, obtained were statistically analysed by using one factor analysis (CRD) with the help of online software (OPSTAT) available at Hissar Agricultural University, Hissar and depicted in tables under respective subheads.

Results and Discussion

The results depicted in Table 1. revealed that all the treatment concentrations prepared showed significantly high mortality than control against 5th instar larvae of *Galleria mellonella* in laboratory condition. The maximum mortality was observed at the dosage of 100 IJs/100µl *i.e*., 38.33% after 24 h. After 96 h 100% mortality was obtained at 30 IJs/100µl and the same trend was continued for next higher doses.

Table 1: Pathogenicity of EPN isolate *H. indica* (CICR-Guava) against *G. mellonella.*

SN	Treatment concentration	Larval mortality (%) of *G. mellonella*			
		24h	48h	72h	96h
1	10IJs/100µl	15.00 (22.79)	53.33 (46.89)	76.67 (61.12)	93.33 (75.21)
2	20IJs/100µl	16.67 (24.05)	60.00 (50.76)	81.67 (64.66)	96.67 (81.36)
3	30IJs/100µl	21.67 (27.71)	68.33 (55.74)	93.33 (75.21)	100.00 (90.00)
4	40IJs/100µl	23.33 (28.86)	73.33 (58.90)	100.00 (90.00)	100.00 (90.00)
5	60IJs/100µl	31.67 (34.23)	78.33 (62.26)	100.00 (90.00)	100.00 (90.00)
6	80IJs/100µl	36.67 (37.26)	86.67 (68.63)	100.00 (90.00)	100.00 (90.00)
7	100IJs/100µl	38.33 (38.24)	93.33 (75.21)	100.00 (90.00)	100.00 (90.00)
8	Control (distilled sterile water)	0.00 (0.00)	3.33 (8.61)	8.33 (16.59)	13.33 (18.43)
	F Test	sig**	sig**	sig**	sig**
	C.D.@ 5%	3.71	5.97	3.32	5.25
	SE(m)±	1.24	1.97	1.09	1.73
	SE(d)±	1.75	2.79	1.55	2.45
	C.V.(%)	8.66	6.41	2.63	3.83

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

The pathogenicity results of *Heterorhabditis indica* (CICR-Guava) against *Spodoptera litura* demonstrated in Table 2. and clearly indicated that, as the entomopathogenic nematode inoculum's level and time of exposure increased, there was significant increase in mortality of *S. litura*. There was a positive correlation between concentrations and mortality rates. The tested nematode showed the highest mortality at 100 IJ/l00µl concentration. The 100% mortality was obtained at 40 IJs/100µl in 3rd instar larvae, whereas, in case of 4th and 5th instar larvae 100% mortality was obtained at 60 IJs/100µl and 80 IJs/100µl respectively.

Table 2: Pathogenicity of EPN isolate *H. indica* (CICR-Guava) against 3rd, 4th and 5th instar larvae of *Spodoptera litura*

Sr. no.	Treatment concentration	Larval mortality (%) of *S. litura*											
		24h			48h			72h			96h		
		3rd	4th	5th	3rd	4th	5th	3rd	4th	5th	3rd	4th	5th
1	10IJs/100µl	18.33 (25.29)	16.67 (24.03)	13.33 (21.32)	43.33 (41.14)	36.67 (37.24)	31.67 (34.21)	68.33 (55.74)	66.67 (54.72)	63.33 (52.72)	88.33 (70.08)	86.67 (68.63)	83.33 (65.92)
2	20IJs/100µl	21.67 (27.69)	18.33 (25.29)	16.67 (24.03)	48.33 (44.02)	40.00 (39.19)	35.00 (36.22)	73.33 (58.90)	71.67 (57.83)	66.67 (54.72)	93.33 (75.21)	88.33 (70.08)	86.67 (68.63)
3	30IJs/100µl	23.33 (28.84)	21.67 (27.69)	18.33 (25.29)	53.33 (46.89)	45.00 (42.10)	41.67 (40.18)	76.67 (61.12)	75.00 (60.05)	70.00 (56.81)	98.33 (85.68)	93.33 (75.21)	88.33 (70.08)
4	40IJs/100µl	26.67 (31.05)	23.33 (28.84)	21.67 (27.69)	56.67 (48.81)	48.33 (44.02)	45.00 (42.10)	80.00 (63.52)	78.33 (62.26)	73.33 (58.90)	100.00 (90.00)	98.33 (85.68)	93.33 (75.21)
5	60IJs/100µl	33.33 (35.23)	31.67 (34.21)	28.33 (32.12)	65.00 (53.74)	60.00 (50.76)	56.67 (48.81)	88.33 (70.08)	86.67 (68.63)	83.33 (65.92)	100.00 (90.00)	100.00 (90.00)	96.67 (81.36)
6	80IJs/100µl	38.33 (38.22)	36.67 (37.24)	33.33 (35.23)	73.33 (58.90)	71.67 (57.83)	66.67 (54.72)	96.67 (81.36)	93.33 (75.21)	90.00 (71.92)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	100IJs/100µl	41.67 (40.18)	38.33 (38.22)	35.00 (36.22)	83.33 (65.92)	78.33 (62.26)	73.33 (58.90)	100.00 (90.00)	98.33 (85.68)	96.67 (81.36)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
8	Control (distilled sterile water)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.42)	6.67 (14.76)	5.00 (12.91)	11.67 (19.87)	8.33 (16.59)	6.67 (14.75)	13.33 (21.32)	11.67 (19.87)	10.00 (18.43)
	F Test	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**
	C.D.@ 5%	3.07	3.16	3.67	3.28	4.30	3.52	5.92	6.29	6.60	5.47	5.68	5.63
	SE(m)±	1.01	1.04	1.21	1.08	1.42	1.16	1.95	2.08	2.18	1.81	1.88	1.86
	SE(d)±	1.44	1.48	1.71	1.53	2.01	1.64	2.76	2.94	3.08	2.56	2.66	2.63
	C.V.(%)	6.22	6.73	8.32	3.98	5.66	4.92	5.42	5.99	6.62	4.09	4.42	4.61

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

Table 3: Multiplication of *Heterorhabditis indica* (CICR-Guava).

Sr. no.	Treatment concentration	Number of infective juveniles emerged per larva ×10² from 100ml suspension			
		Galleria mellonella	*Spodoptera litura*		
		5th instar	3rd instar	4th instar	5th instar
1	10 IJs/100 µl	1103.33 (33.22)	626.76 (25.05)	960.16 (31.00)	1123.56 (33.53)
2	20 IJs/100 µl	1370 (37.013)	700.76 (26.48)	1037 (32.21)	1175.5 (34.30)
3	30 IJs/100 µl	1606.67 (40.08)	839.56 (28.99)	1106.2 (33.27)	1263.86 (35.56)
4	40 IJs/100 µl	1996.67 (44.68)	932.66 (30.55)	1184.76 (34.43)	1332.93 (36.52)
5	60 IJs/100 µl	2273.33 (47.68)	1010.76 (31.80)	1244.33 (35.28)	1397.7 (37.39)
6	80 IJs/100 µl	2563.33 (50.63)	1085.8 (32.96)	1304.2 (36.12)	1453.76 (38.14)
7	100 IJs/100 µl	2786.67 (52.79)	1147.76 (33.89)	1354.06 (36.81)	1525.33 (39.06)
	F Test	sig**	sig**	sig**	sig**
	C.D.@ 5%	1.69	0.54	0.36	0.30
	SE(m)±	0.55	0.17	0.11	0.10
	SE(d)±	0.78	0.25	0.16	0.14
	C.V.(%)	2.18	1.03	0.60	0.47

(Figures in the bracket are square root transformation; **F test highly significant at 1% level of significance)

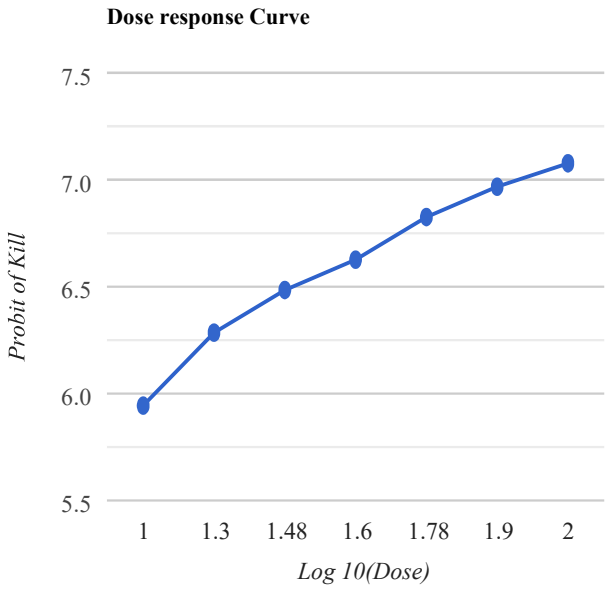

Figure 1: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 3rd instar larvae of *Spodoptera litura.*

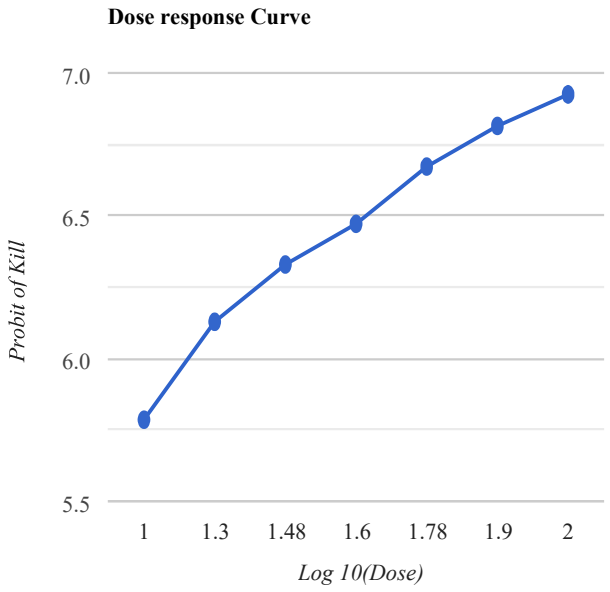

Figure 2: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 4th instar larvae of *Spodoptera litura.*

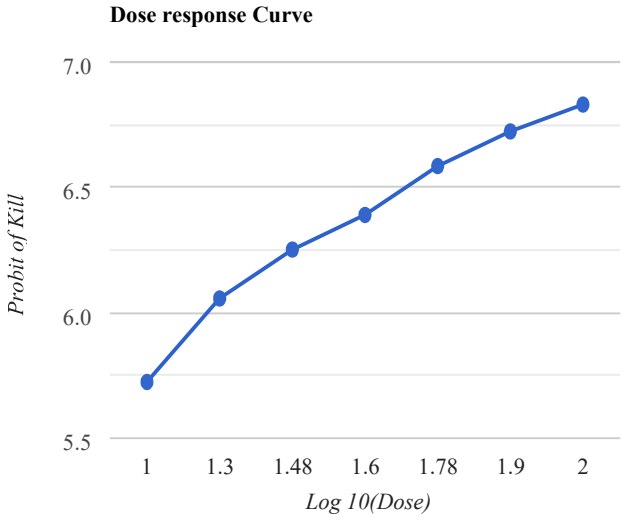

Figure 3: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 5th instar larvae of *Spodoptera litura.*

The results were in confirmation with findings of Atwa and Hassan (2014) who reported that insect mortality was high (60-90%) and low (<45%) at higher and lower nematode concentrations, respectively. Our results were pertinent with findings of Pal *et al.* (2012) and Kamaliya *et al.* (2019). A more or less similar trend was followed by Ganguly *et al.* (2007), Radhakrishnan and Shanmugam (2017), Yuksel and Canhilal (2018).

The results indicated that the third instar larva are more susceptible to *H. indica* than fourth and fifth instar larva of *S. litura* and 100 per cent mortality was obtained at higher inoculum level (100 IJs/larva) which was in line with the findings of Kim *et al*. (2008) and Yan *et al.* (2019), Acharya *et al.* (2020a) and Acharya *et al.* (2020b). Results of present study revealed that, median lethal concentration of *Heterorhabditis indica* (CICR-Guava) required for 50 per cent mortality of 3rd, 4th and 5th instar larvae of *S. litura* were, 1.47 IJs/100 µl, 2.04 IJs/100µl and 2.21 IJs/100µl, respectively (Fig. 1, 2 and 3). The results were in line with findings of Radhakrishnan and Shanmugam (2017). Data in Table 3. clearly indicated that there was significant difference among all treatments with respect to emergence of infective juveniles of entomopathogenic nematode *Heterorhabditis indica* (CICR-Guava) from 5th instar larvae of *Galleria mellonella.* The highest population of infective juveniles 2786.67×10^2 IJs observed from EPN isolate CICR-Guava, when they were infected with dosage of 100 IJs/100µl. Data presented in Table 3. revealed that the number of infective juveniles emerged from cadavers of 3rd, 4th and 5th instar of *Spodoptera litura*. It was observed from the data that the number of infective juveniles emerged increases with the increase of size of larva. The emergence of nematode infective juveniles of *H. indica* from the cadavers of *S. litura* was recorded daily up to the cessation of emergence of infective juveniles. Maximum number of infective juveniles obtained from 3rd instar larva were 1147.76×10^2 IJs at 100 IJs/100µl. In case of 4th instar larvae, maximum number of infective juveniles 1354.06×10^2 IJs obtained when they were inoculated at dosage of 100 IJs/100µl. Similarly, Maximum number of infective juveniles obtained from 5th instar larva were 1525.33×10^2 IJs at 100 IJs/100µl. Similar results were observed by Pal *et al.* (2012), Caccia *et al.* (2014), Holajjer *et al.* (2014) and Dhirta and Khanna (2019).

Conclusion

Based on the results obtained, it can be stated that the tobacco cutworm *S. litura* was found susceptible to the local isolate of entomopathogenic nematodes. As per the data it may be stated that *H. indica* (CICR-Guava) has the ability to kill the insect host within 48-72 h after infection and can be multiplied easily. It could be concluded that the nematode treatment dose, time of exposure and the insect mortality of the tobacco cut worm were positively correlated and multiplication rate of IJs increased with increase of exposure time and size of the larvae. The entomopathogenic nematode isolate *H. indica* (CICR-Guava) can be suggested as biocontrol agent for the control of *S. litura* in the Vidarbha region of Maharashtra.

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Conflict of interest

The authors declare that they have no conflict of interest.

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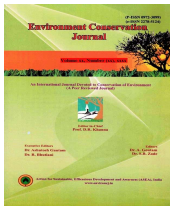

Compatibility studies of *Heterorhabditis indica* with newer insecticides under laboratory condition

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ABSTRACT

Entomopathogenic nematodes (EPNs) have been identified as promising biocontrol agents for controlling economically important insect pests of agricultural and horticultural crops. The compatibility of entomopathogenic nematode *Heterorhabditis indica* with 7 CIB registered insecticides was investigated under laboratory conditions. The effect of these insecticides on nematode survival at recommended concentrations was observed after 12, 24, 48, 72 hours upon direct exposure. EPN *H. indica* was compatible with Imidacloprid 17.8% SL as maximum per cent of live *H. indica* were observed after 72 h of exposure to this insecticide. Similarly, *H. indica* was compatible with Fipronil 5% SC up to 48 h of exposure whereas, less than 70% live EPN were there in Thiamethoxam 25% WG, Diafenthiuron 50% WP and Cypermethrin 25% EC resulting these insecticides to be least compatible. Emamectin benzoate 5% SG and chlorpyriphos 20% EC were incompatible with *H. indica* after 48 h of exposure. The result of this experiment will help in reducing the dependence on chemical insecticides and thus slowing down the development of insecticide resistance and preventing adverse effects on public health and the environment.

Introduction

The aftermath of pesticides on agricultural production has been inescapable. Pesticides have been proved to be harmful to living beings, human health as well as the environment. This is because chemical pesticides are directly linked with the pathogenesis of Parkinson's and Alzheimer's diseases and various disorders of the respiratory and reproductive tracts (Baltazar *et al.,* 2014). In addition to this, oxidative stress is another important mechanism through which many pesticides affect living beings. This oxidative stress leads to DNA damage and later cause malignancies and other disorders (Sabarwal *et al.,* 2018). Excessive pesticide residues on crop produce could have harmful, acute or long-term effects on end-users' health and lead to biomagnification and bioaccumulation. The pesticides enter aquatic organisms or plants through water and later enter food chain to affect other organisms. This has also been observed in earlier case studies on food web components from Zhoushan Fishing Ground, China (Zhou *et al.,* 2018); plankton and fish of Ignacio Ramirez reservoir, Mexico (Favari *et al.,* 2002); organisms of Greenland biota (Vorkamp *et al.,*

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2004); organisms of Antarctic biota (Goerke *et al.,* 2004), etc. Pesticides may be hazardous to non-target species such as bees, wild animals, birds and fish. Most of the pesticides in an application fail to reach the site of action in the target organism. To preclude these detrimental sequels of pesticides, there has been an increased call for a substitute management method, i.e., biological control agents which works quietly in nature.

Entomopathogenic nematodes (EPNs) from the genera *Heterorhabditis* have been identified as promising biocontrol agents for controlling economically important insect pests of a wide range of agricultural and horticultural crops (Ehlers and Hokkanen, 1996; Hazir *et al.,* 2003; Lacey and Georgis, 2012). EPNs are often applied to sites that frequently receives other chemical inputs such as pesticides, fertilizers, herbicides that may mesh with nematodes. It is usually advisable to know if EPNs can be tank-mixed or applied at once with another pesticide to save time and money and become compatible with integrated pest management systems which will reduce the use of chemical pesticides. Therefore, the main aim of the present study was to test the compatibility of CIB registered insecticides with entomopathogenic nematode *Heterorhabditis indica*.

Material and Methods

Treatment details

Multiplication and culturing of EPN

Pure culture of *H. indica* was maintained separately in late instar larvae of *Galleria mellonella* in Entomology Section, College of Agriculture, Nagpur, PDKV, Akola. Initially, measured amounts of suspension with standard count of IJs/100µl from the EPN isolate were taken and larvae of *G. mellonella* were inoculated by direct contact method. Larvae killed by nematodes were placed on white traps for harvesting of nematode population. Emerging infective juvenile stages of nematodes were collected and re-infected to fresh *G. mellonella* larvae and this process of inoculation and re-infection of larvae was repeated until the pure culture of nematode populations with infective juveniles were obtained. This pure culture was used for treatments for further studies.

Table 1. CIB&RC list of label claim insecticides updated on 01.01.2021 (http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides) # Per ha dose is for dilution in 500 litres of water.

Treatment	Trade name	Technical name	Group	a.i.%	Dose (per lit)	Source
1.	Actara®	Thiamethoxam	NN	25 WG	0.2gm	Syngenta India Ltd.
2.	Pegasus®	Diafenthiuron	Thiourea	50 WP	2.5gm	Syngenta India Ltd.
3.	Confidor®	Imidacloprid	NN	17.80 SL	0.1ml	Bayer Crop Science Ltd.
4.	Regent®	Fipronil	Py	5 SC	1.5ml	Bayer Crop Science Ltd.
5.	Proclaim®	Emamectin benzoate	Avermectin	5 SG	0.4gm	Syngenta India Ltd.
6.	Cymbush®	Cypermethrin	SP	25 EC	0.6ml	Syngenta India Ltd.
7.	Excel®	Chlorpyriphos	OP	20 EC	5ml	Moti Insecticides Pvt Ltd.
8.	Control	Distilled water	-	-	-	-

Storage of EPN

The infective juveniles of the EPN isolate *H. indica* were stored in conical/tissue culture flasks in distilled water at room temperature (Songbi and Itamar, 2005). The nematode concentrations were kept in the range of 10,000 IJs/ml of distilled water.

Experimental setup

The present work was carried on the compatibility of *Heterorhabditis indica* with insecticides in the post-graduate laboratory, Entomology Section, College of Agriculture, Nagpur, PDKV, Akola. Stock solution at double the recommended concentration of the pesticide was prepared in distilled water. The suspension of infective juveniles was prepared in distilled water with a concentration of 2000 IJ/ml, and one ml of nematode suspension was transferred to each container. One ml pesticide solution was added to the nematode suspension in each container so that the final pesticide concentration was equal to the recommended concentration. The recommended doses of pesticides were as per the Central Insecticide Board and Registration Committee, Faridabad, Haryana, India. Distilled water without

chemicals was used as a control. There were four replicates in each treatment. The plates were kept at 25 ± 1°C (Alonso, 2018).

Data collection

The mortality of IJs was recorded after 12, 24, 48 and 72 hours. The observations were taken with 100 µl aliquots from each container and observed under the stereo zoom microscope. The observations were recorded for nematode mortality. Straight IJs having no motion and not responding to prodding were counted as dead. The interpretations of observations on the compatibility of nematodes with chemicals were made based on the record of the proportion of nematodes dead stage. The compatibility was classified in to four categories as, highly compatible: 86-100% survivals, compatible: 71-85% survivals, least compatible: 51-70% survivals and incompatible: < 50% survivals.

Statistical analysis

The data obtained were statistically analysed by using one factor analysis (CRD) with the help of OPSTAT software.

Results and Discussion

The result showed that the survival percentage of *H. indica* with different pesticides after 12, 24, 48 and 72 h of exposure were in decreasing trend. As the exposure of time increased, the rate of survival decreased (Table 2).

Observations after 12 h

After 12 h of exposure, among all combinations tested, Thiamethoxam 25% WG showed maximum per cent of survivals (92.85), i.e., highly compatible with *H. indica* followed by Imidacloprid 17.8% SL (90.85%), Fipronil 5% SC (89.35%) and Diafenthiuron 50% WP (88.05%). Cypermethrin 25% EC (84.8%), Chlorpyriphos 20% EC (78.8%) were found relatively less compatible as compared to above-mentioned insecticides. Similarly, Emamectin Benzoate 5% SG showed the least compatibility, having a minimum per cent of survivals (67.60) and were at par with each other in the order mentioned above.

Observations after 24 h

As far as insecticides' effect was concerned, the insecticide Imidacloprid 17.8% SL having per cent survivals (88.05) was highly compatible with *H. indica* and found significantly superior along with the 86.35% survivals at Fipronil 5% SC after 24 h of exposure. The effect of Emamectin Benzoate 5% SG on *H. indica* having 67.3% survivals didn't vary significantly from Chlorpyriphos 20% EC having 65.6% survivals and found least compatible with *H. indica*.

Table 2: Survival percentage of *H. indica* at 12, 24, 48 and 72 hrs of exposure to Thiamethoxam 25% WG (T_1), Diafenthiuron 50% WP (T_2), Imidacloprid 17.8% SL (T_3), Fipronil 5 SC% (T_4), Emamectin Benzoate 5% SG (T_5), Cypermethrin 25% EC (T_6), Chlorpyriphos 20% EC (T_7) and water as control (T_8). Survival (%) = [(Total no. of nematode IJs - No. of died IJs of nematodes) / Total no. of nematode IJs] x 100.

Treatment	12 h	24 h	48 h	72 h
IJs + Thiamethoxam 25%WG	92.85 (75.63)	82.45 (65.23)	59.75 (50.62)	58.75 (50.03)
IJs + Diafenthiuron 50% WP	88.05 (70.00)	82.85 (65.53)	64.30 (53.29)	60.38 (50.99)
IJs + Imidacloprid 17.8% SL	90.85 (72.47)	88.05 (69.77)	86.15 (68.13)	71.80 (57.96)
IJs + Fipronil 5% SC	89.35 (71.04)	86.35 (68.36)	75.60 (60.38)	66.73 (54.76)
IJs + Emamectin benzoate 5% SG	67.60 (55.30)	67.30 (55.21)	27.10 (31.23)	11.85 (20.00)
IJs + Cypermethrin 25% EC	84.80 (67.14)	81.45 (64.62)	58.55 (49.91)	54.78 (47.73)
IJs + Chlorpyriphos 20% EC	78.80 (62.82)	65.60 (54.10)	39.95 (38.99)	8.55 (16.25)
IJs + Distilled water (Control)	100.00 (90.00)	99.40 (85.73)	98.50 (82.93)	97.88 (81.61)
S.E(d)±	2.80	1.92	2.55	2.31
S.E(m)±	1.98	1.36	1.80	1.63
C.D. @5%	5.81	4.00	5.31	4.80
C.V. (%)	5.61	4.12	6.64	6.90

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

Table 3: Summary of compatibility status observed in the present study

Treatment	12hr	24hr	48hr	72hr
IJs + Thiamethoxam 25% WG	Highly compatible	compatible	Least compatible	Least compatible
IJs + Diafenthiuron 50% WP	Highly compatible	compatible	Least compatible	Least compatible
IJs + Imidacloprid 17.8% SL	Highly compatible	Highly compatible	Highly compatible	compatible
IJs + Fipronil 5% SC	Highly compatible	Highly compatible	compatible	Least compatible
IJs + Emamectin benzoate 5% SG	Least compatible	Least compatible	Incompatible	Incompatible
IJs + Cypermethrin 25% EC	compatible	compatible	Least compatible	Least compatible
IJs + Chlorpyriphos 20% EC	compatible	Least compatible	Incompatible	Incompatible

Observations after 48 h

Data about mean per cent survivals presented in Table 2 showed that after 48 h of exposure, maximum per cent survivals were observed in Imidacloprid 17.8% SL followed by Fipronil 5% SC, i.e., 86.15%, 75.6% survivals, respectively. While minimum per cent survivals were recorded in Emamectin Benzoate 5% SG (27.10%) and it was found to be incompatible with *H. indica*.

Observations after 72 h

After 72 h of exposure, the treatment T_3, i.e., Imidacloprid 17.8% SL, having per cent survival 71.80 was compatible with *H. indica* and found significantly superior along with the insecticide Fipronil 5% SC having per cent survival 66.72. Diafenthiuron 50% WP (60.37%), Thiamethoxam 25% WG (58.75%) and Cypermethrin 25% EC (54.75%) were found least compatible with *H. indica* as compare to the insecticides mentioned above and were at par with each other in the given order. Emamectin Benzoate 5% SG and Chlorpyriphos 20% EC were incompatible with *H. indica* showing 11.85% and 8.55% survivals, respectively and were statistically the same. The compatibility status has been summarized in Table 3. Earlier works related to the compatibility of EPN *H. indica* with insecticides are very scant. The present study was supported by an earlier work of Priya and Subramanian (2008), where it has been reported that *H. indica* was compatible with carbofuran, carbosulfan and imidacloprid. Earlier work suggests that neonicotinoid insecticides have fewer adverse effects on nematode survival, pathogenicity, and infectivity (Koppenhöfer *et al.,*2003). Thiamethoxam and imidacloprid belong to the same insecticide group, i.e., neonicotinoid. Thiamethoxam which is moderately toxic is from second generation, whereas imidacloprid which is highly toxic is from first generation neonicotinoid. The mode of action of both these chemicals is the same. But interestingly, the compatibility level of Thiamethoxam decreased in the present study that requires further investigation. Thiamethoxam was also reported compatible with *H. megidis*, *S. feltae* and *S. glasseri*. Organophosphates like monocrotophos, chlorpyrifos have an adverse effect on *S. carpocapsae* and *H. indica* (Chavan *et al.,* 2018). Some reports demonstrated that certain insecticides, particularly organophosphates and carbamates, possess nematocidal properties (Atwa, 1999). Prolonged exposure to some plant protection products can affect the efficiency and reproduction of the nematodes (Negrisoli Jr *et al.,* 2010). Patil et al. (2017) showed that the IJs exposed to Proclaim® recorded 82.71% survival in *H. indica* at 48 h of exposure. However, the mortality of EPN species was less than our present study, which may be due to their lower doses of insecticides and may be related to differences in chemical composition and formulation of the product.

Conclusion

The results of this study increased our knowledge of EPN and insecticide interactions. *H. indica* was found to be compatible with most of the insecticides tested except Emamectin benzoate 5% SG and chlorpyriphos 20% EC. *H. indica* can be successfully included in IPM of economically important crop pests. It may reduce the dependence on chemical insecticides, development of insecticide resistance and adverse effects on public health and the environment. The results of this work expand our knowledge on the compatibility of EPN with registered insecticides for the control of crop pests. Knowledge of the survival per cent with

respect to the used insecticides will be helpful to predict the required application rate of nematodes in IPM programs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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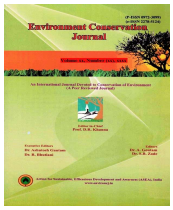

An overview of Covid-19 with special reference to Janapadodhwamsa

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ABSTRACT

Virus, bacteria and fungi are the most common causes for spreading illness in human and in animals. These are the microorganisms and they can cause epidemic and pandemic diseases. World is passing through many viral epidemics affecting respiratory system since last twenty years. It includes SARS-CoV 2002-2003, H_1N_1 Influenza 2009, MERS-CoV 2012 to the recent COVID-2019. COVID-19 is a viral pandemic infection this is air borne illness that is spreading through droplet infection. This virus especially affects the respiratory system by doing immunosuppression in person. In *Ayurveda* there are references of Janapadodhwamsa in Charaka samhita vimansthan. Janapadodhwamsa – is the term coined by Charak which means destruction of population living in same place at the same time because of 4 main reasons i.e. Dushit vayu (Air), Dushit jala (water), Dushit desh (land), Dushit kaal (time) Janapadodhwamsha causes death of individuals in the affected area inflicting huge destruction.

Introduction

Coronavirus is one of the major pathogen that targeted the human respiratory system. At the end of December month in 2019, an onset of a typical pneumonia (COVID-19) started in Wuhan, China (Gorbalenya 2020). Corona virus is zoonotic origin disease it is also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or in general as novel coronavirus, and the disease-associated is being called COVID-19 (Lai *et al.,* 2020; Bhatia, 2020; Bedford *et al.,* 2020). Entire world was challenged with this virus and government had declared lockdowns in their respective countries, states and cities (Fisher *et al*., 2020). In India, national lockdown was announced starting from 25th March 2020. People were informed to stay at home except there will be an emergency. All travel visa were prohibited till 15th April 2020. Peoples, who came back after 15th February 2020, were quarantined for a minimum of 14 days after their arrival in country.The alarming condition arose when the community spread of SARS-CoV-2 majorly impacted the human health, economical conditions and behavioural aspect of the society (Pinto 2020; Ma *et al.,* 2020; Park *et al.,* 2019; Bherwani *et al.,* 2020).The impact of environmental factors is exceedingly related to confirming COVID-19 cases as flu virus spreads rapidly in cold and dry condition and becomes inactive above 30^{o}C (Casanova *et al.,* 2010; Doremalen *et al.,* 2013). The susceptible-exposed-infectious-recovered (SEIR models) has been reported as a successful tool to know more about the pandemic dynamics and to evaluate the impact of environmental and social conditions on the spread of COVID-19 (Chanprasopchai *et al.,* 2017; Kalhori *et al.,* 2019).

Ayurvedic concept related to Covid-19

Ayurveda is the oldest science in India, which plays a vital role in the treatment of any disease. The term

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Ayurveda is derived from two words-*Ayu* and *veda.* As per Shastri (2012) the main aims of Ayurveda are

- *Swasthasya swasthya rakshanam*
- *Aturasya vikara prashamanam*

Ayurveda is considered as pioneer of all medical sciences. Many *Samhitas* are available in Ayurveda which explain about the vyadhis, nidana, laxana, chikitsa etc. Ayurveda described *roga* into two categories (Gupta, 1997).

- *Nija roga*
- *Agantuja roga*

Many communicable diseases, their causes, mode of transmission, prevention and treatment are described in Ayurveda. These communicable diseases described in Ayurveda under Janpadodhwamsa, Aupasargika roga, Sankramika vyadhi.

Natural disaster like endemic, epidemic and pandemic diseases are described in Ayurveda under Janapadodhwasma because having similar sign and symptoms. According to Acharya charaka, any vyadhi which produce due to destruction of large population is known as Janapadodhwamsa. Janapadodhwamsa is derived from two words-Janapad and Dhwamsa. Janapad means nation, community, district, people, persons belonging to a country where Dhwamsa means destruction (Sukumar and Shashirekha, 2018). According to Chakrapani, the famous commentator of Charaka, causes for diseases are mainly of two types (Sharma and Dash, 2018).

1. *Sadharana*-These cause varies from person to person.
2. *Asdharana*-These causes are common to many person like vayu(air), Jala(water), desh(land), kala(time) etc.

Roga are due to asdharana (common) causes are called Janapadodhwamsa.

तासामुपयोगाद्विविध रोग प्रादुर्भावो मरको वा भवेदिति । (सु.सू 6/19)

Acharya Susruta has described the concept of Janapadodhwamsa while discussing Ritucharya (seasonal regimen) under the title Maraka. In these seasonal regimen abnormalities happen due to some providential causes like cold, heat, wind and rain become different from their normal qualities (Shastri, 2012).

रक्षोगणादिभिर्वा विविधैर्भूतसंघेस्तम धर्म मन्यद्वाऽप्यपचारान्तरमुप्लभ्याभिहन्यन्ते। (च.वि.3/22)

Acharya Charaka mentioned that in Janapadodhwamsa, janapad are attacked by raksasas or other micro-organisms due to unrighteousness or other unwholesome act. These acts are also responsible for people getting afflicted with the attack of raksasas. This act is the main cause of the destruction of population by curse (Shastri, 2012). According to Acharya Charaka the epidemics are caused because of dearangement of one or all the four elements shared in-common by the humans like- Vayu (Air), Jala (Water),Desha (Area),and Kala (time) (Tripathi, 2007). Authors have tried to explain the vitiation of air, water, land and time (season) in order of their importance.

Some characteristics of air which is injurious for health-(Sharma 1998)

- Excessive calmness
- Excessive dryness, cold, heat, roughness
- Excessive clashes
- Excessive cyclonic in nature

Some characteristic of water which is injurious for health (Sharma, 1998).

- Abnormality in smell, color, taste and touch
- Excessive stickiness
- Absence of birds
- Reduction of Aquatic animals
- Manifestation of unpleasantness

Some characteristic of land which is harmful for health (Sharma, 1998).

- Abnormality in the natural colour, smell, taste and touch
- Excessive stickiness
- Abundance of wild animals, mosquitoes, flies, rats, owls
- Abundance of smoke in the wind
- Abundance of excessively branched creepers
- Presence of wild cries of birds and dogs
- Presence of excessive crying noise
- Appearance of roughness and coppery, redish and white color in the sun.
- Fierce look and cries in the nature
- Constant agitation and over-flow of water reservoirs
- Frequent occurrence of thunderbolts and earthquakes

Manifestation of the characteristic features contrary to the normal conditions of the various seasons is considered to be harmful (Sharma, 1998). The impairement of these factors responsible for the destruction of countries by epidemics. By nature air, water, land and season are indispensable in their progressive order.

वय्वादीनां यद्वैगुण्यमुत्पघते तस्यमूलमधर्मः तन्मूलं वाऽसत्कर्म पूर्वकृतःतयोर्योनिः प्रज्ञापराध एव। (च.वि.3/20)

According to Acharya charaka, the main reasons for the vitations of these factors is Adharma, Poorvajanama papakarya. The main reasons leading to the same are improper disposal of waste, air pollution, distribution of contaminated water, indulgence in unhealthy and unhealthful activities. *"Pragyaparadha"* (doing mistakes knowingly/ mis-behave) is said to be the basis cause for all epidemics. It is then said to lead "*Adharma" or "Asat-karma*" (Tripathi, 2007). It can also be caused by "*Apavitrata"* (uncleanliness*),Rakshas-gana/Bhutagana* (Micro-organisms). Equal importance is given to mental unstability and "Abhishaapa" as the cause of epidemics (Tripathi, 2007). In Vatakalakaliya adhyaya, Acharya Charaka has considered vikruta vayu as responsible for alterations in normal environment or seasons, earthquake, formation of huge sea waves, and epidemics in animals and humans (Tripathi, 2007)

On the basis of nature of virus, origin of virus and considering the fatality of COVID-19 related illness. It can be considered as Jangam visha janya vyadhi and according to mode of transmission it is similar to Bhootabhisangaj Agantuj Jwar. In Ayurveda, all persons have their own Prakriti which carry on since origin to demise. All person staying in same region doesn't have an similar resistance for an similar disease. The resistance which is responsible to keep a check over intensity and progression of the disease is called as Vyadhishamatva (Immunity) (Patel *et al.,* 2017).

प्रसंगादगात्रसंस्पर्षानिःष्वासात्सहभोजनात्।
सहषय्याऽऽसनाच्चापिवस्त्र्ामाल्यानुलेपनात्।।
कुश्ठं ज्वरष्च षोशश्रच नेत्राभिश्यन्द एव च ।
औपसर्गिक रोगाश्च संक्रामन्ति नरान्नरम्।।

(सु.नि. 5/32-33)

Ayurveda is likely to provide evidence-based medicine for preventive health care and enhance the self-immunity. As Ayurveda described several immunity booster procedures in Dincharya and Riutucharya. A better prevention through Ayurveda approach can be achieved in this pandemic of covid-19 as immunity booster (Dutta and Kaviraj, 2009).

General chikitsa of Janapadodhwamsa

According to Acharya Charaka general chikitsa which are beneficial during Janapadodhwamsa include following activities-

SN	General measures in Janapadodhwasma
1-	Bheshaja prayoga
2-	Panchvidh karma (panchakarma)
3-	Rasayana sevana
4-	Deha vriti
5-	Sadvritta palan

1-**Bheshaja prayog-**During Janapadodhwamsa,we have to administered those medicines which are collected before epidemic.

2-Panchvidh karma-Panchvidh karma are the best therapy for those who are not having identical actions during the previous life and also for those who are not destined to die during the epidemics. Panchvidh karma include these therapies-

- Vamana (Emesis process)
- Virechana (Purgation process)
- Nirhu basti (Aasthapan basti-enema)
- Anuvasana basti (enema)
- Nasya karma (Shirovirechana-nasal medication)

3-Rasayana sevan-Proper administration of Rasayana should be benefical (Rasayananam vividhchupayogah prashashyate).

4-Dehavriti-Physical health of every individual should be maintained through drugs which are collected before the onset of epidemics.

5-Sadvritta palan-According to Acharya charaka implementation of Sadvritta should play a key role for living a healthy life.Sadvritta palan include-

- Satya (Truthfulness)
- Bhut daya (Compassion for living beings)
- Dana (Charity)

- Balee (Sacrifices)
- Devtaarchanam (Prayer to the Gods)
- Sadvrittasya anuvrittischa (Adoption of preventive measures)
- Prasahamo (Maintaining tranquility)
- Guptiraatmanam (Protection of self by chanting mantras etc)
- Hitam Janapadaanaam
- Shivaanaam upasevanama
- Sevanam Brahmacharyasya
- Sevanam Brahmachaarinaam
- Samkatha dharma sastraanaam maharshinaam jitaatmanaam
- Dharmikaihi sattvikaimitayam sahaasyaa vriddha samvataihi

These above therapies should be adopted during epidemics will save the life of individual (Sharma and Dash, 2018).
Acharya Sushruta also given some common treatment plan for all epidemics.These includes (Ghanekar vol. 22.)

- Sthanparityag-Leave the infected place
- Quarantine
- Hom-dhum sevan
- Niyam- cleanliness

Daivyapashray treatment –mantra chanting

As per Tripathi, (1999), Misra vol.169 and Ghanekar vol. 22. some other measures are

- Advice to follow all vyadhikshamatva
- Advice to follow dincharya according to Ayurveda
- Advice to follow dharma
- Advice people to collect food, medicine from unaffected area or before epidemic
- Prohibit vitiation of air,water,land,climate through the use of purification methods
- Dhupana- Some dravya which are use for dhupana process as follows-
  - Tulsi
  - Nimb
  - Nirgundi
  - Ajwain
  - Camphor
- Abhyanga therapy
- Rakshoghna medicine

Role of rasayana in Janapadodhawsma and their immune modulating effect-Rasayana chikitsa is the foremost treatment during this epidemic condition. Rasayana therapy plays a vital role in this epidemic because it gives strength to the body, enrich the dhatu (basic rasa dhatu and further sapta dhatus) and improve immune power (Yadav, 2014). According to Ayurveda, vyadhi is the resultant of imbalanced dosha and dushya which happen in individual who have ksheen vyadhikshamatva. So in this crucial condition of epidemic we should use those dravyas which are useful to improve vyadhikshamatva. Immune modulator are those which gives strength to the immune effector cell (Masihi, 2001). Vyadhikshamatva of every individual is depend upon dhatuposhan and oja. In Rasayana therapy,we have to work upon the Rasa, agni and srotasa level for the healthful longevity.Rasayana generally used in two ways which are as follows-

- As a prophylactic medicine
- As a preventive measures in healthy individual

1-Amalaki (*Embelica officinalis*) Amalaki is considered as a best dravya for Rasayana effect. It is also responsible for sandhaniya karma (improve cell migration and cell binding) and Ayushya (Prolonged cell life) (Sharma, 2009).
Amalaki fruit contains all five rasas except lavana which reduces the all three Doshas and balance all the Dhatus of the body.Amalaki also reduces pitta dosha because of Guru,Ruksha and Sheeta guna and also having Sheet Virya and Madhur Vipaka (Mishra, 2002). According to Acharya Charaka amalaki is the important drug and termed as "Amalaki Vayasthaapanama Shreshthama" (Yadav, 2014).

***2*-Guduchi (*Tinospora cordifolia*)** Guduchi (giloy) is one of the best Rasayana. It is also known as a *"Amrita",jwarari,tantrika,jivantika.Guduchi have tikta and kashaya rasa,guru and snigdha guna,ushno veerya and madhura vipaka.Guduchi* consist a lot of properties. These are as follows-

- Agnideepana
- Balya
- Jwaraghna
- Ama nashaka

Because of these properties Guduchi enhance the killing property of macrophages and also acts in infectious disease (Pandey, 2002; Chunekar and Pandey, 2006; Shankar and Prasad, 1998).

***3*-Haridra (*Curcuma longa*)-**All Ayurvedic literature mentioned several properties of haridra like-Rujahar (reducing pain), Daha hara (reducing burning sensation),Varnya (complexion propellant), Vishodhana (cleansing of the body), kapha pitta shamak (Diwedi, 2008).

According to Acharya charaka haritaki have five rasa except lavan rasa,and having ushna virya.It have several properties like (Sharma and Dash, 2018).

- Doshaanulomini(eliminates the dosas),
- Laghvi(light),
- Depan(stimulates the digestion),
- Pachana(carminative),
- Ayushya paushtiki (promotes longevity and nourishment),
- Sarva roga prashamni (eradicate all diseases)

Haridra act as a immunemodulator because it plays a vital role in the modulation of proliferation and cellular response of many immune cell types (Yue *et al.,* 2010). Haridra is a useful herb in Janapadodhwamsa because of its polysaccharides content which elevates the host defense mechanism.In various pre-clinical and human clinical models immunomodulator activity of polysaccharides and polysaccharides plant products have been demonstrated (Ramberg and Nelson, 2010).

Transmission of SARS-CoV-2-

This virus mainly spread through person to person. When an infected person cough or sneeze, this virus mainly spread through droplets and nasal discharge. Some other ways for the transmission of this virus are as follows (Bedford *et al.,* 2020).

- Through close contact
- Droplets
- Airborne Transmission,
- Surface Transmission
- Fecal-oral

Impact of SARS-CoV-2 On Environment-

The fecal-oral transmission was a matter of concern for the environment. Large population of developing countries was under poverty threshold, so they used open defecation. Hence, detection of SARS-CoV-2 in the human feces was an alarming threat and may cause the drastic consequences for the countries having larger slum areas. Maintaining physical distancing in slum area was the difficult problem because many persons are living in a single room (Coronavirus:2020). Some known methods for cleaning the environmental compartments include a lot of techniques. These techniques are as follows-

- Nitrifying-enriched activated sludge (NAS) approach,
- Microorganisms based approach ,
- Conventional activated sludge (CAS) approach
- These techniques were very beneficial to the environment (Poole, 2020).

Effect of COVID-19 on climate

Temperature, humidity and pH some are the various points for the efficiency. These factors were necessary for the efficiency of the microorganism. The other serious threat for human being was the mutation of microorganisms. Virus has mutated itself into various forms. In January 2021, a new variant of this virus appear in a person of Brazil (Shi *et al.,* 2020; Kalhori *et al.,* 2019).

Pros and cons related to COVID-19

Some positive aspects of COVID-19 are as follows-

- Less noise pollution
- Less air pollution
- Improvement in environment
- Clean rivers
- Improvement in healthcare services
- Improvement in greenhouse gases emission
- Use of traditional medicine

Due to lockdown air quality of entire country was improved. Level of suspended particulate matter also reduced in atmospheric condition (Sharma *et al.,* 2020; ICMR, 2020; Rajkumar, 2020). Some negative aspects of COVID-19 are as follows-

- Anxiety
- Depression
- Unemployment
- Economic loss
- Attacks on COVID warriors

A lot of severe attacks were noticed on COVID warriors. It was a matter of serious concern, so our government had to take some legal action. A bill had passed by the Indian government especially for the protection of COVID warriors attack on COVID warriors (Pedersen *et al.,* 2010; Ali and Alharbi*,* 2020).

Vaccine-The vaccination programme was started in India on January 2021.

- After approval some vaccine was issued by the government .These vaccines are as follows-
- COVAXIN®, developed by Bharat Biotech with the collaboration of Indian Council of Medical Research-National Institute of Virology.
- AstraZeneca's COVID vaccine marketed as Covishield. COVISHIELD, was developed by the University of Oxford and Vaccitech company.

Conclusion

COVID-19 is very challenging pandemic for whole world. Because of mutation property this virus considered as a smart virus. COVID-19 can be considered as Janapadodhwamsa. In this article, a brief insight on ayurvedic concept related to Janapadodhwamsa is described. Viral epidemics spreading now days can be considered as Pranavaha Strotasa dushti with predominant Vata and Kapha Doshas. It affects severely in those with pre-existing respiratory and circulatory co-morbidities. Preventive measures in terms of containing the spread can serve as the best way to combat the epidemic. Ayurveda help to find out the method of preventive and curative management for recent pandemic situation of COVID-19.

Conflict of interest

The authors declare that they have no conflict of interest.

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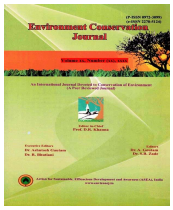

Impact of various irrigation and establishment methods on yield and water use efficiency in rice

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ABSTRACT

The field experiment was executed to evaluate the impact of various irrigation and establishment methods on yield and water use efficiency in rice during *Kharif* 2018. Split plot design was used in the experiment which consists of three irrigation treatments in the main plot and five rice establishment treatments in the sub plot. The results revealed that, higher yield parameters like number of panicles/m^2 (364.83), number of grains per panicle (73.98), number of filled grains per panicle (60.29) were recorded with maintenance of saturation up to panicle initiation (PI) and flooding after PI. Manual transplanting among rice establishment methods recorded significantly higher test weight (25.04 g), grain yield (5253 kg/ha) and harvest index (0.45). Whereas, mechanical transplanting recorded significantly higher number of grains per panicle (74.61) straw yield (7939 kg/ha). Among different irrigation methods, alternate wetting and drying up to PI followed by flooding after PI recorded significantly lower total water usage (94.94 cm) and higher water use efficiency (52.39 kg/ha-cm). Among rice establishment methods, mechanical transplanting recorded significantly lower total water usage (117.81 cm) and higher water use efficiency (48.80 kg/ha-cm). Interaction between alternate wetting and drying up to PI followed by flooding after PI and mechanical transplanting recorded lower total water usage (81.88 cm) and also recorded higher water use efficiency (68.75 ha-cm).

Introduction

Rice (*Oryza sativa* L.) is grown on 164.19 million ha worldwide. China produced 211.86 million metric tonnes of paddy rice in 2020, whereas India produced 178 million metric tonnes (FAOSTAT, 2021). India produces 178 million tonnes of rice in an area of 44 Million ha which constitutes about 35% of area and 40% of production of the food grain in the country. It is a staple food of about 65% of the country's population, which itself indicates its importance in the food security of the country (Kumar *et al.,* 2018). Rice is ranked second in terms of cultivated area. Rice is the primary source of calories for 40% of the world's population (Sain, 2020). Rice crops outnumber all other cereal crops in terms of calorific value. Rice is a rich source of carbohydrate; consisting of protein, fat as well as vitamin B complexes like niacin, riboflavin, and thiamine. The grain of rice constitutes water 12%, starch 75–80%, and protein only 7% with a full complement of amino acids. Its protein, due to its higher lysine concentration (~4%), is highly digestible (93%) with a high biological value (74 percent) and a protein efficiency ratio (2.02%–2.04%). Minerals such as calcium (Ca), magnesium (Mg) and phosphorus (P) together with some traces amount of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are present (Verma *et al.,* 2018).

To meet the rising demand for rice the global rice production needs to increase by 116 million tons by 2035 (Yamano *et al.,* 2016). India's rice demand is

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expected to reach 140 million tonnes by 2025 (Hugar *et al.,* 2009). To meet this goal, rice productivity must increase to 3.3 tonnes ha^{-1} from the current level of 2.2 tonnes ha^{-1} (Anjani *et al.,* 2014).

The proportion of water used for irrigation is predicted to decrease by 10 to 15% during the next two decades (Dhawan, 2017). As a result, the only way to save water for expanding irrigated agriculture is to adopt efficient water usage technology that also conserves soil health, sustainability, and economic stability (Subramaniam *et al.,* 2013). An irrigation management approach known as Alternate Wetting and Drying (AWD) has been discovered to cut down on water use in rice systems (Lampayan *et al.,* 2015). Transplanting and manual weeding become prohibitively expensive due to a lack of irrigation water and a labor shortage during peak seasons. As a result, the global area under transplanted rice has decreased in recent years. As a result, there is a need to investigate alternative crop establishment methods in order to boost rice output (Farooq *et al.,* 2011). This can be achieved by using several rice establishment techniques such as direct seeded rice, broadcasting, mechanical transplanting, and drum seeded rice, among others. Timely planting/seeding is made possible by mechanical transplanting or direct seeded rice (Malik *et al.,* 2019).

Material and Methods

The experiment was conducted at C-Block, Zonal Agricultural Research Station, V. C. Farm, Mandya, University of Agricultural Sciences, Bangalore to study the impact of various irrigation and establishment methods on yield and water use efficiency in rice during *Kharif* 2018. The experimental area was geographically positioned at 76º 82′ East Longitude and 12º 57′ North Latitude with an altitude of 756.80 metre above mean sea level. Considering the nature of factors under study and the convenience of agricultural operation, the experiment was laid out in split plot design, assigning three irrigation management practices *viz*., continuous flooding, maintenance of saturation up to panicle initiation (PI) followed by flooding after PI and Alternate wetting and drying (AWD) with five sub plot rice establishment treatments, *viz*., Drum seeded rice, Broadcasting of sprouted rice, Semi dry rice, Mechanical transplanting and Manual transplanting. The whole field was divided into three blocks each representing a replication.

Irrigation water management

I_1: 5 ± 2 cm of standing water was maintained throughout the crop growth stage.

I_2: The plots were kept under saturated condition (2 cm of water at each irrigation) up to panicle initiation stage. Later, after panicle initiation, the same plots were completely flooded with 5 ± 2 cm standing water.

I_3: The main principle behind this method is to irrigate the plots when hair-line crack appears on the soil surface. The plots were irrigated at 5 to 6 days interval with 5 cm water.

Irrespective of treatments, water was applied to the main plots with the help of PVC pipes and the quantity of water applied was measured at each time by using water meter. Application of water was stopped seven days before harvesting of the crop to facilitate easy harvesting.

Irrigation water

Total water used (cm): The total quantity of irrigation water applied to each treatment was measured using water meters. The effective rainfall received during the crop growth period and the soil moisture contribution was added to the irrigation water and expressed in cm.

Total water = Irrigation water + Effective rainfall + Soil moisture

Table-1: Irrigation water given and total water requirement as influenced by different irrigation methods

Treatments	Effective Rainfall (cm)	Irrigation water given (cm)				Total WR (cm) (WR=ER+IW)
		R_1	R_2	R_3	Mean	
I_1	29.57	153.7	164.9	134.6	151.0	180.66
I_2	29.57	86.89	87.23	78.29	84.13	113.70
I_3	32.07	75.49	62.05	46.60	61.38	93.45

Water Use Efficiency (kg ha-cm^{-1}): Water use efficiency (WUE) is the yield of marketable crop produced per unit of water used (cm). It was worked out by using the following formula and was expressed as kg ha-cm^{-1}.

WUE = Marketable yield (kg ha^{-1}) / total water used (cm)

Water requirement (cm): Total water requirement of the crop is calculated by adding the effective rainfall in the crop growth period and the irrigation water given to each treatment (Table 1).

Results and Discussion

Number of panicles/m^2

Higher number of panicles/m^2 was recorded with maintenance of saturation up to panicle initiation (PI) followed by flooding after PI (364.83) which was on par with continuous flooding (357.50) and alternate wetting and drying up to PI followed by flooding after PI (354.83) as mentioned in Table.2. However, the results were non-significant, there as on behind lesser number of panicles/m^2 in alternate wetting and drying may be due to the unfavorable condition created with drying of rhizosphere during panicle tillering period and panicle initiation stage. These results are in conformity with reports of Hemlata (2015).

Among establishment methods, broadcasting of sprouted rice recorded more number of panicles/m^2 (428.33) and was significantly superior over rest of the methods (313.33 to 367.22). This might be due to the synchronization in maximum tillering period and optimum nutrient supply as confirmed by Sowmyalatha (2015).

Number of grains per panicle

Maintenance of saturation up to panicle initiation (PI) followed by flooding after PI recorded significantly higher number of grains per panicle (73.98) than alternate wetting and drying up to PI followed by flooding after PI (69.11) and continuous flooding (65.94) (Table.2). Lower number of total grains per panicle recorded in continuous flooding indicates that the depth of water can be reduced to greater extent without disturbing crop performance with respect to total number of grains per panicle. The results were in conformity with Kishor (2016).

Among establishment methods, higher number of grains per panicle (74.61) was with mechanical transplanting method followed by manual transplanting (71.89) and was significantly higher as compared to rest of the methods (62.61 to 70.51). Alternate wetting and drying up to PI followed by flooding after PI in mechanical transplanting recorded higher number of grains per panicle (86.35) which was closely followed by continuous flooding in broadcasting of sprouted rice (85.87) and were significantly superior over rest of the interactions (56.43 to 77.12). Higher number of total grains per panicle recorded by mechanical transplanting might be due to the availability of more space within the panicle to accommodate the individual seeds resulting in well-developed seeds (Sowmyalatha, 2015).

Number of filled grains per panicle

Number of filled grains per panicle is tabulated in Table.2. Among establishment methods drum seeded rice recorded significantly higher number of filled grains per panicle (66.08) over rest of the methods (51.02 to 59.86). Lower number of filled grains per panicle was recorded by semi dry method (51.02) might be due to excessive growth during vegetative phase which led to nitrogen dilution in reproductive phase, which in turn reduced spikelet differentiation and hence reduced number of filled grains per panicle. This was in accordance with the findings of Saharawat *et al.* (2010).

Among interactions, maintenance of saturation up to panicle initiation (PI) followed by flooding after PI in drum seeded rice recorded significantly higher number of filled grains per panicle (76.48) as compared to rest of the interactions (44.37 to 69.28). This may be due to the early establishment of crop and early grain filling period which coincided with the nutrient supply and hence higher uptake of available resources like nutrients, moisture etc. The results are also in conformity with findings of Sanjay *et al.* (2018).

Test weight (g)

Continuous flooding recorded significantly higher test weight (24.51 g) as compared to other methods (24.10 to 24.11) as mentioned in Table.2. Different irrigation methods had significant effect on test weight. The possible reason may be due to the effect of irrigation methods on number of grains per panicle and per cent chaffyness and this result is in confirmation with the study of Shantappa (2014). Manual transplanting among establishment methods recorded significantly higher test weight (25.04 g) over rest of the methods (23.05 to 24.55 g). Among interactions higher test weight (25.19 g) was recorded in alternate wetting and drying up to

Table 2: Yield parameters as influenced by irrigation management practices and establishment methods in rice

Treatments	Number of panicles/m^2	No. of grains per panicle	No. of filled grains per panicle	Test weight (g)
Irrigation methods (I)				
I_1: Continuous flooding	357.50	65.94	56.60	24.51
I_2: Maintenance of saturation up to panicle initiation (PI)followed by flooding after PI	364.83	73.98	60.29	24.11
I_3: Alternate wetting and drying up to PI followed by flooding 3±2cm	354.83	69.11	56.90	24.10
S.Em+	3.83	0.87	0.96	0.08
CD (p= 0.05)	NS	3.42	NS	0.32
Rice establishment methods (E)				
E_1: Drum seeded rice	320.28	70.51	66.08	24.55
E_2: Broadcasting of sprouted rice	428.33	66.93	53.81	24.34
E_3: Semi dryrice	366.11	62.61	51.02	24.22
E_4: Mechanical transplanting	367.22	74.61	58.86	23.05
E_5: Manual transplanting	313.33	71.89	59.86	25.04
S.Em+	3.88	1.30	1.02	0.05
CD (p= 0.05)	11.32	3.80	2.98	0.14
Interaction				
I_1E_1	376.67	74.88	67.52	24.64
I_1E_2	407.50	85.87	60.85	24.73
I_1E_3	343.33	60.96	52.11	24.70
I_1E_4	370.00	62.99	48.75	23.42
I_1E_5	290.00	63.41	53.76	25.08
I_2E_1	274.17	74.45	76.48	24.37
I_2E_2	440.83	58.51	56.21	24.12
I_2E_3	402.50	61.44	50.35	24.60
I_2E_4	366.67	74.51	58.56	22.61
I_2E_5	340.00	77.12	59.84	24.84
I_3E_1	310.00	62.19	54.24	24.63
I_3E_2	436.67	56.43	44.37	24.17
I_3E_3	352.50	65.44	50.61	23.36
I_3E_4	365.00	86.35	69.28	23.13
I_3E_5	310.00	75.15	65.97	25.19
S.Em+	7.12	2.20	1.85	0.11
CD (p= 0.05)	19.61	6.57	5.16	0.24

PI followed by flooding after PI with manual transplanting and was on par with continuous flooding with manual transplanting method (25.08 g) which were significantly superior over other interactions (22.61 to 24.73 g). In alternate wetting and drying, aeration was found to reduce the above and below ground competition for water, solar radiation and nutrients. In continuous flooding, plants did not experience any kind of stress during grain filling period. In case of manual transplanting, the transplanted seedlings absorbed moisture, nutrients and solar radiation more effectively hence more photosynthate was accumulated in grains. Similar result was reported by Rajesh and Thanunathan (2003).

Grain yield (kg/ha)

Data tabulated in Table.3 shows that the interaction of alternate wetting and drying up to PI followed by flooding after PI and manual transplanting produced a higher grain yield (5745 kg/ha) and was statistically similar to interactions of alternate wetting and drying up to PI followed by flooding

after PI with mechanical transplanting (5613 kg/ha), maintenance of saturation up to PI followed by flooding after PI with manual transplanting (5202 kg/ha), and continuous flooding with semi-dry rice (5189 kg/ha). The former therapy, on the other hand, was significantly superior to the remainder of the interactions (4093 to 5104 kg/ha). It could be because seedlings were planted before the third phyllochron, resulting in faster crop establishment and a longer tillering period, resulting in greater yield characteristics and thus yield in transplanted rice. The early establishment, growth, and development of the crop, as well as irrigation methods, may be responsible for the increase in grain production in semi-dry and drum seeded rice. Shantappa's conclusions are supported by these results (2014).

Straw yield (Kg/ha)

Mechanical transplanting produced a much greater straw production (7939 kg/ha) than the other establishment methods (5685 to 6509 kg/ha)(Table.3). In comparison to the rest of the interactions, the interaction between alternate soaking and drying up to PI followed by flooding after PI and mechanical transplanting produced significantly greater straw production (9569 kg/ha) (5430 to 7191 kg/ha). The increased absorption of solar radiation in the plant tissues, which increased the rate of photosynthesis and hence the accumulation of more photosynthates in the above ground biomass and creation of more dry matter, could be the reason. These findings are consistent with Satyanarayana and Babu's (2004) and Shantappa's findings (2014).

Harvest index

Significantly higher harvest index (0.45) was recorded in manual transplanting followed by semi dry rice and drum seeded rice (0.44) as compared to other establishment methods (0.40 to 0.42) as mentioned in Table.3. This might be due to the higher grain yield obtained by the former treatments. Similar result was reported by Shantappa (2014).

Total water used (cm)

Total water consumed is tabulated in Table.3 and it depicts that the continuous flooding (186.79 cm) was substantially higher than maintenance of saturation up to PI followed by flooding after PI (118.63 cm) or alternate wetting and drying up to PI followed by flooding after PI (118.63 cm) (94.94 cm). Due to the restriction of seepage and deep percolation losses by irrigating once every 5 to 6 days when hair line cracks appear on the soil surface and maintaining water level up to saturation, the maximum irrigation water saving was observed under alternate wetting and drying up to PI followed by flooding after PI followed by saturation method. Shantappa's observations are supported by the findings (2014). Drum seeded rice used the most total water (145.31 cm), followed by sprouted rice broadcasting (144.20 cm), and was much better than the other establishing methods (117.81 to 131.76 cm).In comparison to the remainder of the interactions, the interaction between continuous flooding and establishment methods like drum seeded rice and broadcasting of sprouted rice used significantly more total water (196.09 and 196.09 cm, respectively) (81.88 to 184.09 cm). The interplay of alternate wetting and drying up to PI, followed by flooding after PI, and mechanical transplanting, however, resulted in lower overall water usage (81.88 cm).

Water use efficiency (kg/ha-cm)

Among methods of irrigation, higher water use efficiency (52.39 kg/ha-cm) was recorded in alternate wetting and drying up to PI followed by flooding after PI method as compared to maintenance of saturation up to PI followed by flooding after PI (41.43 kg/ha-cm) and continuous flooding (26.42 kg/ha-cm) and is represented in Table.3. Water use efficiency was significantly higher in mechanical transplanting (48.80 kg/ha-cm) closely followed by manual transplanting (44.38 kg/ha-cm) as compared to rest of the establishment methods (30.98 to 41.94 kg/ha-cm).

Among interactions, interaction between alternate wetting and drying up to PI followed by flooding after PI and mechanical transplanting recorded higher water use efficiency (68.55 kg/ha-cm) over rest of the interactions (23.12 to 61.10 kg/ha-cm).

The higher water use efficiency was mainly attributed to the increased grain yield besides water saving by reducing seepage and percolation losses in mechanical transplanting when alternate wetting and drying up to PI followed by flooding after PI was followed (Bouman and Tuong, 2001, Elamathi *et al*. 2012 and Pandian, 2012).

Table 3: Grain yield, straw yield, harvest index, total water used and water use efficiency (WUE) as influenced by irrigation management practices and establishment methods in rice.

Treatments	Grain yield (kg/ha)	Straw yield (kg/ha)	Harvest Index	Total water used (cm)	WUE (kg/ha-cm)
Irrigation methods (I)					
I_1: Continuous flooding	4916	6121	0.45	186.79	26.32
I_2: Maintenance of saturation up to panicle initiation (PI)followed by flooding after PI	4828	6389	0.43	118.63	41.43
I_3: Alternate wetting and drying up to PI followed by flooding 3±2cm	4849	6954	0.41	94.94	52.39
S.Em±	190	264	0.01	4.03	2.03
CD (p= 0.05)	NS	NS	NS	15.78	7.95
Rice establishment methods (E)					
E_1: Drum seeded rice	4749	5976	0.44	145.31	34.31
E_2: Broadcasting of sprouted rice	4197	5685	0.42	144.20	30.98
E_3: Semi dry rice	4953	6331	0.44	128.20	41.94
E_4: Mechanical transplanting	5171	7939	0.40	117.81	48.80
E_5: Manual transplanting	5253	6509	0.45	131.76	44.38
S.Em±	121	180	0.01	1.47	1.36
CD (p= 0.05)	354	525	0.03	4.29	3.96
Interaction					
I_1E_1	4932	6279	0.44	196.09	25.13
I_1E_2	4544	6159	0.42	196.09	23.12
I_1E_3	5189	5548	0.49	184.09	28.33
I_1E_4	5104	7191	0.42	171.59	29.68
I_1E_5	4811	5430	0.47	186.09	25.85
I_2E_1	5137	5502	0.48	134.80	38.10
I_2E_2	3953	5433	0.42	134.80	29.32
I_2E_3	5050	7004	0.42	110.80	45.57
I_2E_4	4797	7057	0.40	99.97	47.96
I_2E_5	5202	6949	0.43	112.80	46.19
I_3E_1	4177	6148	0.40	105.04	39.70
I_3E_2	4093	5464	0.43	101.71	40.49
I_3E_3	4619	6442	0.42	89.71	51.92
I_3E_4	5613	9569	0.37	81.88	68.75
I_3E_5	5745	7148	0.45	96.38	61.10
S.Em±	267	385	0.02	4.63	2.92
CD (p= 0.05)	614	910	NS	7.44	6.87

Conclusion

Alternate wetting and drying up to PI followed by flooding after PI method of irrigation up to panicle initiation followed by flooding 3 ± 2 cm among the irrigation methods, mechanical transplanting or semidry method among rice establishment methods and Alternate wetting and drying up to PI followed by flooding after PI method of irrigation in mechanical transplanting and manual transplanting among interactions can be recommended due to higher yield parameters, grain yield, straw yield and WUE.

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Conflict of interest

The authors declare that they have no conflict of interest.

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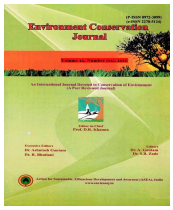

Zinc and Iron fortification through enriched organics and foliar nutrition on growth, yield and economics of foxtail millet (*Setaria italica*

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ABSTRACT

An experiment was conducted to study the impacts of zinc and iron enriched organic manures on growth and yield of foxtail millet in an Organic block. Results revealed that significantly higher grain and stover yield, net returns and Benefit cost ratio (1262 kg/ha, 4210 kg/ha, ₹ 27185 /ha and 2.83 respectively) were recorded with application of Zn and Fe enriched compost along with foliar spray of panchagavya and was found on par with the treatment receiving Zn and Fe enriched vermicompost along with foliar spray of $ZnSO_4$ and $FeSO_4$ (0.5 %) at 30 DAS (1232 kg /ha, 4195 kg /ha,₹ 25522 /ha and 2.62 respectively) and Zn and Fe enriched vermicompost along with foliar spray of panchagavya (3%) at 30 and 45 DAS (1137 kg /ha, 4092 kg /ha, ₹ 21897 /ha and 2.31 respectively). These treatments also showed similar effects with respect to growth and yield parameters contributing for the higher yield and monetary benefits.

Introduction

Malnutrition is a prime fitness problem specifically in less evolved international locations. Millet, called nutrient-dense cereals, attracts greater awareness on research in the areas of bio-fortification and to be able to feed the developing population international with high quality meals, multiplied yields are wished. The adoption of recent technology without thinking about the soil heath condition, a good way to increase crop manufacturing has brought about a fast depletion of vitamins and soil fertility. Among micronutrients, Zn and Fe are highly depleted nutrients that cause terrible crop yields. negative agricultural zinc with terrible zinc (much less than 30%) is considered to be ubiquitous inside the international. because of the ongoing erosion of soil fertility, zinc deficiency in Indian soil is expected to upward push from 42 per cent in 1970 to 63 percent by 2025 (Singh, 2011). Decreased growth, abnormal brain growth, accelerated hazard of infectious diseases which includes pneumonia and diarrhea, and unfavorable beginning consequences in pregnant ladies are all related to zinc deficiency (Black *et al.,* 2008). Abnormal iron metabolism is one of the most common human sicknesses, accompanying with medical symptoms which includes anemia, iron overdose and neurodegenerative diseases (Nazanin *et al.*, 2014). Consistent with iron (Fe) deficiency data in India, 79 percent of preschool youngsters and fifty six percent of women have anemia (Krishnaswamy, 2009) because 1970, India's iron supplementation program has did not deal with iron

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deficiency (Anand *et al*., 2014).Foxtail millet is fairly drought tolerant. It can be planted as a short-term catch crop as a solitary crop or as an intercrop due to its rapid growth. It is a very rich source of various micro and macro nutrients, vitamins as well as minerals. studies display that one hundred grams of foxtail millet grains comprise 12.3 percent protein, 60.9 gram of carbohydrate, 4.3 gram of fat, 3.5 gram of ash, 8 gram of crude fiber, 31 milli gram of calcium, 4.9 milligram of iron, 0.31 milligram of phosphorus, 9.2 milligram of copper, 21.4 milligram of zinc, 0.27 milligram of potassium, 0.01 milligram of sodium, 0.13 milligram of magnesium, 21.9 milligram of manganese, 31 milligram of vitamin E, 0.99 milligram of thiamin, 0.099 milligram of riboflavin, 3.70 milligram of niacin, and 351 kilocalories of energy (Anonymous, 2017). Due to its growth in barren soils with little enter, the yield price of this plant does now not change below rain-fed situations, and the potential yield is yet to be decided. The loss of product is in particular due to low soil fertility, which may be stored inside the organic device for a long time. The plant responds nicely to natural rely due to its low nutrient requirement. Enrichment the use of natural manure, which acts as natural chelates, found economically viable technique. Addition of micronutrients to natural manures, notably zinc and iron, along with increasing decomposition rate, it improves the quality of grain.The process of boosting the natural content of bioavailable nutrients in agricultural plants is referred to as biofortification. Fundamental bio-enriched meals might not offer excessive degrees of minerals and nutrients as nutritional dietary supplements or fortified ingredients, however they can boom micronutrient consumption in terrible dietary supplements on a day by day basis, and therefore complement present remedies. (Prasenjit *et al*., 2016). within the case of biodiversity, gronomic bio-fortification is achieved through the use of natural matter and / or by means of making use of micronutrient fertilizer to the soil and / or by spraying directly on leaves. Biologically enriching with micronutrients along with improving the quality of organics it also enhances quality of grain (developing quantity of micronutrients, minerals, and vitamins), as well as yields and economic returns. (Savithri *et al.,* 1999, Anil kumar and Kubsad, 2017, Patil *et al.,* 2016, Nikhil and Salakinkop, 2017).

Material and Methods

During the 2018 Kharif, field research was carried out at the Main Agricultural Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka, India, located between 16° 12 ' North latitude and 77° 20' East longitude at an altitude of 389 meters above sea level and falls within Karnataka's North Eastern Dry Zone. The study used a randomized block design with ten treatments replicated thrice. Zn and Fe rich compost and vermicompost application with $ZnSO_4$ @ 15 kg/ha and $FeSO_4$ @ 10 kg/ha alone and 3.0% panchagavya spray at 30 and 45 DAS and 0.5 percent $ZnSO_4$ and $FeSO_4$ each in 30 DAS, and compost alone was a treatment option. Enriched manures are applied in comparable to 100% recommended dose. Variety, SiA-2644 high in Fe and medium in Zn content was chosen for study. Natural manure (compost and vermicompost) is enriched with micronutrients such as $ZnSO_4$ and $FeSO_4$ in a recommended dose of $ZnSO_4$ @ 15 kg/ha and $FeSO_4$ @ 10 kg/ha and the manure is allowed to ferment for a month by spraying water regularly and mixing content 2 to 3 times a day. Fermented manure is applied during sowing as per treatment Nutrient composition of compost, enriched compost, vermicompost and enriched vermicompost were 0.77 % N, 0.68 % P_2O_5, 1.01 % K_2O, 75.4 ppm Zn and 1465 ppm Fe ; 1.12 % N, 0.72 % P_2O_5, 1.05 % K_2O, 77.6 ppm Zn and 1500 ppm Fe; 1.57 % N, 0.79 % P_2O_5, 1.22 % K_2O, 84 ppm Zn and 1247.3 ppm Fe and 1.68 % N, 0.82 % P_2O_5, 1.25 % K_2O, 90 ppm Zn and 1290 ppm Fe respectively. Test site was consist of deep black soil with slightly alkaline (pH 7.71), Electrical conductivity (Ec) of 0.22 dSm^{-1} , medium organic carbon content (0.6 percent), low N (137.2 kilogram per hectare), high phosphorus and high potassium (74.70 and 752.5 kilogram per hectare), low Zn (0.58 parts per million), and high Fe (13.91). On August 4, 2018, brushing at a distance of 4 cm in shallow furrows was used for sowing.

Results and Discussion

Growth conditions: The plant's vegetative and reproductive growth are vital in realizing the crop's potential output. Height of the plant and tillers

plant^{-1} at harvest (Table 1) was significantly higher with application Zn and Fe enriched compost, comparable to 100 % recommended dose of nitrogen + foliar sprays of 3.0 % panchagavya (122.73 and 2.27 at harvest, respectively) and followed by Zn and Fe enriched vermicompost, comparable to 100 % recommended dose of nitrogen + foliar spray of 0.5 % $ZnSO_4$ and $FeSO_4$ each and Zinc and iron enriched vermicompost along with foliar spray of 3.0 percent panchagavya over other treatments. Plant height improvements owing to Zn and Fe enhanced organics and foliar sprays might be linked to good crop nutrition, which resulted in optimal growth and these results were in line with Shilpa (2011) reported that growth parameters like plant height (53.33 cm at tillering stage, 64.67 cm at panicle initiation stage and 89.00 cm at harvest stage), number of panicles (288 per m^2) were significantly increased in the treatment of 100 % NPK + FYM + 1 % FYM fortified $ZnSO_4$ + 1 % FYM fortified $FeS0_4$ in rice, Manjunatha *et al.* (2013), observed that among the different enriched compost treatments, application of enriched compost @ 6 t/ha + 100 per cent recommended NPK in sunflower produced significantly higher plant height and number of leaves (158.6 cm and 16.9) as compared to 100 per cent recommended NPK + FYM @ 6 t/ha and compost alone, Mahantesh (2016) and Meena and Fathima (2017).

At harvest, Zinc and iron enriched compost + panchagavya application had a comparatively greater leaf area index (0.91) ,and was on par with, Zn and Fe enriched vermicompost along with foliar spray of $ZnSO_4$ and $FeSO_4$ (0.87) and Zn and Fe enriched vermicompost comparable to 100 percent recommended dose of nitrogen + foliar spray of $ZnSO_4$ and $FeSO_4$ (0.87) and Zn and Fe enriched vermicompost (0.87). The compost application equivalent to 100 percent RDN resulted in a much lower leaf area index (0.82), which was comparable to the other manurial treatments. The improvement in leaf area index was attributed to a significant increase in assimilatory surface area, which resulted in higher photosynthetic accumulation. This could possibly be credited to pile up the nutritional accessibility for plants to uptake, that could have resulted surge in the cell growth and a greater assimilatory surface. These findings were in accord with Shilpa (2011) and Anilkumar and Kubsad (2017), revealed that soil application of RDF + enriched FYM (*i.e*. 50 kg FYM /ha + 3.75 kg $ZnSO_4$ and $FeSO_4$ /ha) to sorghum recorded significantly higher gross returns (Rs. 211924 /ha), net returns (Rs. 85702 /ha) and BC ratio (3.50) over control.

Zn and Fe enriched compost along with foliar spray of panchagavya appliction resulted in more dry matter accumulation in different plant parts at different growth stages (leaves, stem, and reproductive parts), but it was on par with the application of Zn and Fe enriched vermicompost + foliar spray of $ZnSO_4$ and $FeSO_4$ and Zn and Fe enriched vermicompost. When compared to the other treatments, compost application equivalent to 100 percent recommended dose of nitrogen resulted in much lower dry matter buildup in leaves, stems, and reproductive regions. (Fig 2)

At different growth stages (Table 2), significantly higher total dry matter production was noticed with application of Zn and Fe enriched compost along with foliar spray of panchagavya and on par results with application of Zn and Fe enriched vermicompost along with foliar spray of $ZnSO_4$ and $FeSO_4$ and Zn and Fe enriched vermicompost + foliar spray of panchagavya which were superior over compost application alone and remaining treatments at different growth stages. Individual plant elements such as leaves, stems, and reproductive parts accumulate more dry matter in various treatments, results in higher overall dry matter output. These results were conforming to Senthil *et al*. (2004), opined from his experimental results that zinc and iron enriched coir pith (1 t/ha) in curcumin recorded significantly higher leaf dry matter, stem dry matter and rhizome yield of turmeric (770 kg/ha, 543 kg/ha and 28 t/ha) over control and other treatments, however the same treatment was found on par with zinc and iron enriched with FYM (1 t/ha). Mahantesh (2016), and Patil *et al*. (2017), opined that significantly higher maize grain (60.9 q/ha) and straw yield (62.57 q/ ha) was recorded with application of 7.5 t of maize residue compost enriched with 15 kg $ZnSO_4$ on account of significantly higher test weight (31.51 g), grain yield plant^{-1} (134.7 g/ plant) and total dry matter production (326.38 g/plant).

Table 1 : Growth parameters of foxtail millet as influenced by agronomic fortification through organic nutrient management practices

Treatments	Plant height (cm)			Number of tillers per plant at harvest	LAI		
	30 DAS	60 DAS	At harvest		30 DAS	60 DAS	At harvest
T_1: Compost*	18.15	101.00	106.00	2.00	0.47	1.30	0.82
T_2: Enriched compost** with Zn and Fe	19.97	106.33	112.93	2.07	0.52	1.37	0.84
T_3: Enriched vermicompost*** with Zn and Fe	21.37	113.33	115.53	2.13	0.54	1.39	0.86
T_4: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$	20.78	111.13	114.40	2.07	0.52	1.38	0.86
T_5: Enriched compost** with Zn and Fe +Foliar spray of Panchagavya	22.44	120.60	122.73	2.27	0.63	1.49	0.91
T_6: Enriched vermicompost*** with Zn and Fe +Foliar spray of Panchagavya	21.88	114.53	120.13	2.23	0.56	1.40	0.87
T_7: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of Panchagavya	18.29	102.47	106.20	2.07	0.50	1.35	0.83
T_8: Enriched compost** + Foliar spray of $ZnSO_4$ and $FeSO_4$	21.67	114.07	115.60	2.20	0.54	1.39	0.86
T_9: Enriched vermicompost*** + Foliar spray of $ZnSO_4$ and $FeSO_4$	22.42	118.27	122.33	2.27	0.57	1.41	0.87
T_{10}: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of $ZnSO_4$ and $FeSO_4$	21.10	111.60	115.20	2.10	0.52	1.38	0.86
S. Em±	0.94	2.38	2.97	0.06	0.02	0.03	0.01
C. D. at 5%	2.79	7.06	8.84	0.17	0.06	0.09	0.04

Table 2 : Growth parameters of foxtail millet as influenced by agronomic fortification through organic nutrient management practices

Treatments	DMP (g/plant)			Rate of DMP (g/plant/day)	
	30 DAS	60 DAS	At harvest	31 DAS to 60 DAS	61 DAS to harvest
T_1: Compost*	0.28	3.59	6.19	0.11	0.08
T_2: Enriched compost** with Zn and Fe	0.38	4.07	7.52	0.12	0.11
T_3: Enriched vermicompost*** with Zn and Fe	0.45	4.49	8.21	0.13	0.12
T_4: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$	0.41	4.22	7.64	0.13	0.11
T_5: Enriched compost** with Zn and Fe +Foliar spray of Panchagavya	0.49	5.32	9.57	0.16	0.13
T_6: Enriched vermicompost*** with Zn and Fe +Foliar spray of Panchagavya	0.45	4.73	8.43	0.14	0.12
T_7: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of Panchagavya	0.36	3.74	7.31	0.11	0.11
T_8: Enriched compost** +Foliar spray of $ZnSO_4$ and $FeSO_4$	0.45	4.54	8.29	0.14	0.12
T_9: Enriched vermicompost*** + Foliar spray of $ZnSO_4$ and $FeSO_4$	0.48	4.89	8.93	0.15	0.13
T_{10}: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of $ZnSO_4$ and $FeSO_4$	0.43	4.41	7.93	0.13	0.11
S. Em±	0.03	0.21	0.33	0.01	0.01
C. D. at 5%	0.08	0.62	0.98	0.02	0.03

Table 3 : Yield and yield parameters of foxtail millet as influenced by agronomic fortification through organic nutrient management practices

Treatments	Ear head length (cm)	Ear head weight (g)	Grain yield plant^{-1} (g)	1000 seed weight (g)	Grain yield (kg/ha)	Stover yield (kg/ha)	Harvest index
T_1: Compost*	14.53	3.57	3.28	2.83	695	3080	0.18
T_2: Enriched compost** with Zn and Fe	15.47	4.29	3.57	3.03	837	3704	0.18
T_3: Enriched vermicompost*** with Zn and Fe	16.07	4.49	4.21	3.21	917	3918	0.19
T_4: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$	15.87	4.37	3.90	3.04	869	3794	0.19
T_5: Enriched compost** with Zn and Fe +Foliar spray of Panchagavya	16.53	5.36	4.88	3.47	1262	4210	0.23
T_6: Enriched vermicompost*** with Zn and Fe +Foliar spray of Panchagavya	16.27	5.12	4.47	3.24	1137	4092	0.22
T_7: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of Panchagavya	15.87	4.22	3.38	3.01	795	3523	0.18
T_8: Enriched compost** + Foliar spray of $ZnSO_4$ and $FeSO_4$	16.13	4.96	4.35	3.21	951	4065	0.19
T_9: Enriched vermicompost*** + Foliar spray of $ZnSO_4$ and $FeSO_4$	16.33	5.18	4.70	3.32	1232	4195	0.23
T_{10}: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of $ZnSO_4$ and $FeSO_4$	15.93	4.38	3.92	3.19	889	3898	0.19
S. Em±	0.37	0.30	0.32	0.15	83	175	0.01
C. D. at 5%	NS	0.89	0.95	NS	246	521	NS

Table 4: Economics of foxtail millet as influenced by agronomic fortification through organic nutrient management practices

Treatments	Cost of cultivation (Rs./ha)	Gross returns (Rs./ha)	Net returns (Rs./ha)	Benefit Cost ratio
T_1: Compost*	12578	25070	12492	1.99
T_2: Enriched compost** with Zn and Fe	13578	30177	16599	2.22
T_3: Enriched vermicompost*** with Zn and Fe	15450	32716	17266	2.12
T_4: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$	13578	31210	17632	2.30
T_5: Enriched compost** with Zn and Fe +Foliar spray of Panchagavya	14878	42063	27185	2.83
T_6: Enriched vermicompost*** with Zn and Fe +Foliar spray of Panchagavya	16750	38647	21897	2.31
T_7: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ +Foliar spray of Panchagavya	14878	28679	13801	1.93
T_8: Enriched compost** + Foliar spray of $ZnSO_4$ and $FeSO_4$	13903	33943	20040	2.44
T_9: Enriched vermicompost*** + Foliar spray of $ZnSO_4$ and $FeSO_4$	15775	41297	25522	2.62
T_{10}: Compost* + Soil application of $ZnSO_4$ and $FeSO_4$ + Foliar spray of $ZnSO_4$ and $FeSO_4$	15775	31974	16199	2.03
S. Em±	-	2224	2224	0.15
C. D. at 5%	-	6608	6608	0.45

***Compost equivalent to 100 % RDN in T_1, T_4, T_7 and T_{10}**
****Enriched compost equivalent to 100 % RDN in T_2, T_5 and T_8**
*****Enriched vermicompost equivalent to 100 % RDN in T_3, T_6 and T_9**
$ZnSO_4$ @ 15 kg/ha and $FeSO_4$ @ 10 kg ha^{-1} application to soil
DAS: Days after sowing

0.5 % $ZnSO_4$ and 0.5 % $FeSO_4$ @ 30 DAS and 3.0 % of Panchagavya at 30 and 45 DAS foliar application

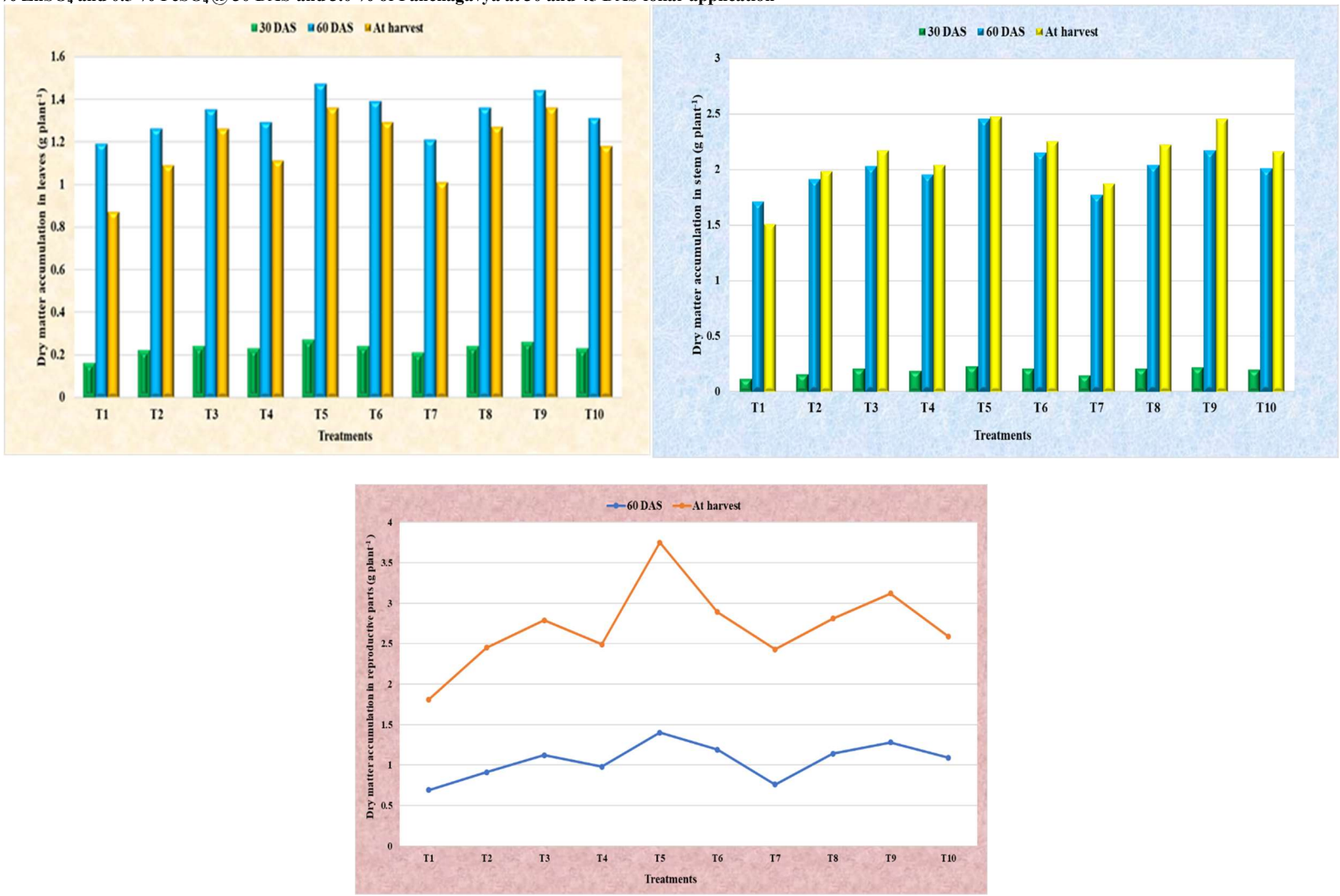

Fig. 1: Dry matter accumulation in leaves, stem and reproductive parts (g/plant) at different growth stages of foxtail millet as influenced by agronomic fortification through organic nutrient management practices

Rate of dry matter production is higher with Zn and Fe enriched compost, along with foliar spray of three percent panchagavya application, this is due to increase in balanced and continuous supply nutrients resulted in higher photosynthesis and translocation of photosynthates to the different parts of the plant. These findings were in agreement with Latha and Sharanappa (2014) opined that application of enriched biodigested liquid organic manure (EBDLM) at 25 kg N equivalent ha^{-1} + 3 sprays of panchagavya at 3% resulted in significantly higher pod yield and kernel yields (2.34 and 1.78 t/ha respectively) and the yield attributes like number of pods/plant and shelling outturn (47.2 and 76.0% respectively) of groundnut and also resulted in higher onion bulb yield (37.78 t/ha) and yield parameters like bulb diameter (6.10 cm), bulb length (5.52 cm) and bulb size index (33.7) in the groundnut-onion cropping system. and Pradeep and Sharanappa (2014) revealed that application of enriched bio digested liquid manure at 125 kg N equivalent/ha + 3 sprays of panchagavya (3 %) to chilli crop resulted in significantly higher plant height, branches per plant, leaf area index, leaf area duration, total dry matter production and dry fruit yield as compared to control.

Yield parameters: Different agronomic fortification procedures had a considerable impact on foxtail millet grain output (Table 3). Application of Zn and Fe enriched compost with foliar spray of panchagavya (3 %) @ 30 and 45 DAS resulted in significantly higher grain yield (1262 kg/ha) and on par with the treatment receiving Zn and Fe enriched vermicompost comparable to 100 percent recommended dose of nitrogen + foliar spray of $ZnSO_4$ and $FeSO_4$ (0.5 % each) (1232 kg/ha) and Zn and Fe enriched vermicompost + foliar spray of panchagavya @ 30 and 45 DAS (1137 kg/ha). These treatments outperformed compost equivalent to 100 percent RDN, which produced lower grain yields (695 kg/ha). With Zinc and iron enriched compost along panchagavya spray, Zinc and iron enriched vermicompost with foliar spray of $ZnSO_4$ and $FeSO_4$ and Zinc and iron enriched vermicompost + 0.5 percent panchagavya spray, yield increases were 45.0, 43.6, and 38.9%, respectively, over compost alone.The high grain production of these treatments *i.e.,* the use of zinc and iron rich in organics in the soil, may be due to improved availability of zinc and iron in the soil by limiting their fixation and precipitation, which can improve their efficiency. Foliar spray of panchagavya as a growth promoter increases the efficiency of plant by creating a grater source to immerse the energy in the plant and increase nutrient absorption. In addition, the yield is increased by these treatments, namely, the combined use of compost and vermicompost as well as foliar nutrition such as panchagavya or zinc and iron nutrition compared to application of compost or vermicompost alone, without enrichment and foliar nutrition may be due to better metabolism and photosynthates production by the plant as well as enlargement of and cell division. Furthermore, the rise in yield attributes in both treatments could be attributed to the continued provision of organically chelated iron and zinc, in addition to accessible NPK nutrients to the crop. Assimilation of photosynthates and translocation from the source (leaves) to the sink (ear) all require iron and zinc. Therefore, the use of Zinc and iron through the use of enriched organics with foliar spray of panchagavya or $ZnSO_4$ and $FeSO_4$ may be the best way to manage micronutrient pressure to increase crop yields. Latha *et al.* (2001), Tolessa *et al*. (2001), Elnaz *et al.* (2016), Anilkumar and Kubsad (2017), Ananda and Kalyanamurthy (2017), Nikhil and Salakinkop (2017), and Vipen *et al.* (2017) have shown that spraying with foliar fertilizers or liquid organics increases the yield.

Increased yield characteristics such as ear head weight (5.36 g) and yield of grain per plant (4.88 g) may also be linked to the treatment of Zinc and irone enriched manures (compost) with spray for panchagavya. These results are compared with those obtained with enriched vermicompost + $ZnSO_4$ and $FeSO_4$ sprays, as well as improved vermicompost + panchagavya sprays (Table 3). The data on the ear head length of the foxtail millet did not differ significantly from the use of Zn and Fe enriched manures. However the use of Zinc and iron enriched manure (compost) + panchagavya spray (16.53 cm) recorded a much higher earhead length compared to other treatments. While composting alone indicates the length of the small earhead of the foxtail millet (14.53 cm). Increased ear length with organic manures + panchagavya spray may be due to the abundant availability of appropriate growth resources. Data on test weight

(weight of 1000 seeds) not differ significantly between treatments.

Enriched compost with Zinc and iron application + foliar spray of panchagavya (4210 kg/ha) application had a significance in increased stover yield, which was on par with Zinc and iron enriched vermicompost along with foliar spray of $ZnSO_4$ and $FeSO_4$ (4195 kg/ha), Zinc and iron enriched vermicompost with foliar spray of panchagavya (4092 kg/ha) and Zn and Fe enriched compost with foliar (4065 kg/ha). While significantly lower stover yield was noticed with compost alone (3080 kg/ha). Differences in growth factors such as total dry matter production and accumulation in different plant parts such as leaves and stem were blamed for differences in stover output. These reports were in consonance with Yadav *et al*. (2011), Hamaad *et al.* (2012) concluded that increase in plant growth and Zn concentration was higher when plants were grown with Zn enriched FYM compared to $ZnSO_4$ or Zn-EDTA application alone, indicating the positive role of organic matter in increasing grain yield and grain Zn concentration The maximum Zn concentration in rice grains (13.9 mg/kg) and straw (19.1 mg/kg) was seen and Choudhary *et al.* (2013) concluded that foliar application of panchagavya + leaf extract of neem recorded significantly higher number of nodules, number of pods per plant, pod weight per plant, pod yield, haulm yield and harvest index in ground nut as compared to other treatments. Panchagavya + leaf extracts of neem recorded significantly higher 100 kernels weight, shelling percent, nutrient uptake of N and P, oil content over other sources.Economics: Application of zinc and iron enriched compost along with foliar spray of panchagavya (Rs. 27185 /ha and 2.83 respectively) surpassed all other treatments except Zn and Fe enriched vermicompost with foliar spray of $ZnSO_4$ and $FeSO_4$ (Rs. 25522 /ha and 2.62 respectively) and Zn and Fe enriched vermicompost with foliar spray of panchagavya (Rs. 21897 /ha and 2.62 respectively) and lowest is with compost equivalent to 100% RDN (Rs. 12492 /ha and 1.99 respectively) (Table 4). This could be due to higher yield of grain and stover in comparison to the rest of the treatments. Naveen (2009), Patil *et al.* (2012) and Bandiwaddar and Patil (2015) found that the use of enriched organic manures with foliar sprays of nutrients or liquid organic manures resulted in greater net monetary returns and BC ratio.

Conclusion

Micronutrient fertilizer in the form of enriched organic manures not only enriches plants with the trace elements, but also preserves the health of the soil to produce healthy and quality grain because the quality of food, which is the basis of human health, ultimately determined by the soil quality which is raised upon it so, under the organic production system by application of zinc and iron enriched manure (compost) + 3 percent foliar spray of panchagavya @ 30 and 45 DAS, or Zn and Fe enriched vermicompost + foliar spray of 0.5 percent $ZnSO_4$ and $FeSO_4$ each @ 30 DAS, or zinc and iron enriched vermicompost + foliar spray of 3% panchagavya @ 30 and 45 DAS is best recommendation for sustainable growth and yield.

Conflict of interest

The authors declare that they have no conflict of interest.

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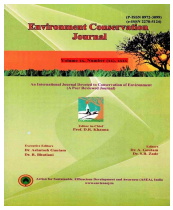

Lactic acid bacteria as an adjunct starter culture in the development of metabiotic functional black pearl grapes beverage

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ABSTRACT

Black pearl Grapes are highly nutritious and one of the richest sources of polyphenols, but due to being delicate with very high loss at harvest and during distribution, is not consumed adequately. This study intended to develop functional lactic acid starter culture based fermented grapes beverage, in order to improve the quality and stability of this low pH fruit and to develop a fermented non-dairy beverage. Results showed that grapes blend was an excellent matrix for LAB growth with more than 9.38 $\log_{10}$ CFUml^{-1} of viability at the end of fermentation. LAB fermentation affectedly enhanced the total polyphenols and flavonoids content. Likewise, antioxidants capacities based on DPPH and FRAP activity were considerably increased correlating with each other, impacting the color and sensory properties of the grapes beverage. This way, the lactic acid fermentation can be considered as an appropriate tool for developing black pearl grapes based novel bio-intervention with enhanced antioxidants, polyphenols and flavonoids with anti-proliferative activity and antagonistic efficacy against recurring food borne pathogen in this post-antibiotic era.

Introduction

The appetite of nutraceutical enriched, functional fermented plant based food and beverages are on the upsurge apart from traditional milk based products. Demand for non-dairy functional fermented beverages is growing in parallel with the on-going trend of veganism and increasing health problems associated like lactose-intolerance, allergy to milk proteins or high cholesterol content. Fermented foods with plant origin have been evaluated as vectors for administration of functional Lactic Acid Bacterial (LAB) cultures following the proficiency of the production of vegetable and fruit based fermented products (Soccol *et al.,* 2010; Yoon *et al.,* 2006). Fruits and vegetables are excellent matrices rich in carbohydrates, polyphenols, vitamins, minerals, dietary fibres and antioxidants, providing a suitable growth substrate for LAB in parallel with a strong health appeal. Lactic acid fermentation, using pure functional LAB, as one of the appropriate approach for the utilisation of functional potential of plant based matrices, improving the bioactivity, bioavailability of phytochemicals and augments the plant matrices with functional bacterial secondary metabolites that are able to exert putative benefits on human health (Filannino *et al.,* 2018). Intestinal microbiome, rightly called "our second genome" comprises of trillions of mini life forms and their genetic material and is tightly regulated by the mysterious cross-talk between gut microbiome and intestinal epithelial cells. Dysbiosis of the gut microbiota therefore leads to various intestinal and extra-intestinal ailments such as colitis, inflammatory bowel disease, colon cancer and metabolic syndrome. Both clinically and experimentally, it has been observed that regaining

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the gut balance via oral supplementation of the fermented fruits and vegetables beverages is becoming increasingly popular for various gastrointestinal diseases, reducing risk of certain cancers and cardiovascular diseases in this post antibiotic era.

Black pearl grapes being rich in phenols particularly quercitin (12-15 mg/kg), kaempferol (2 mg/kg), myricetin (4.5 mg/kg), catechins (19 mg/kg) and coumaric acid (1-3 mg/kg) (Gil, 2000) and impressive antioxidative properties, correlated with the polyphenols and anthocyanins (ACNs) reconciles its usage as suitable substrate for lactic acid fermentation. Moreover, the ability of the phytochemicals (flavonoids specially resveratrol) in inducing human protective enzymes and the protective effects against cardiovascular diseases, cancers, and other age-related diseases (Yao *et al.,* 2004) substantiates the functionality of grape juice. As Grapes are not available in winter and are very delicate with very high loss at harvest and during distribution. Bio preservation through lactic acid fermentation of grapes harvested in summer can be a great prospect for consumers to acquire the benefits of nutraceutically enriched grapes throughout the year.

Despite, an ideal source, the survivability of the LAB in fruit-based matrix is complex and is influenced by the fermentation bio-process conditions, such as fermentation temperature, pH, medium composition and bacterial species. As pH has a strong influence on the LAB growth, keeping this point, the products were developed either via fortification (without fermentation) or by fermentation using a single lactic acid bacteria strain. LAB must survive and retain their functional properties during the entire bio-processing and storage and the formulated product must contain at least 10^6cfu/ml of the living functional lactic acid bacteria at the time of consumption. Therefore, reckoning the value of simple biotechnological fermentation technique and wish to prolong the shelf- life of grapes juice, pure culture application of functional LAB could be valuable in augmenting its availability and functionality.

In this context, the role of microorganisms in fermented grapes beverage health promoting properties was studied with particular attention to the metabolic contribution by LAB in the development of functional grapes beverage and to comprehend the potential beneficial effect and bioactive properties of metabiotic functional grapes beverage, its antioxidant capacity, the spectrum of functional bacteria, in relation to the chemical composition. The specific aim of this study was to investigate substrate metabolism, antagonistic (against disease causing pathogens) and bacterial composition of grapes beverage fermented via selected consortium of ten lactic acid bacteria (which have been evaluated for compatibility and individual growth in the beverage) as a basis for explicating the mechanisms underlying its functional properties. This attempt was made to develop lactic acid starter cultures based fermented grapes beverage manifesting high antioxidant activity, nutraceutical properties of the Black Pearl Grapes and bioactive compounds (secondary metabolites) of lactic acid bacterial fermentation with the beneficial functional properties of the bacteria.

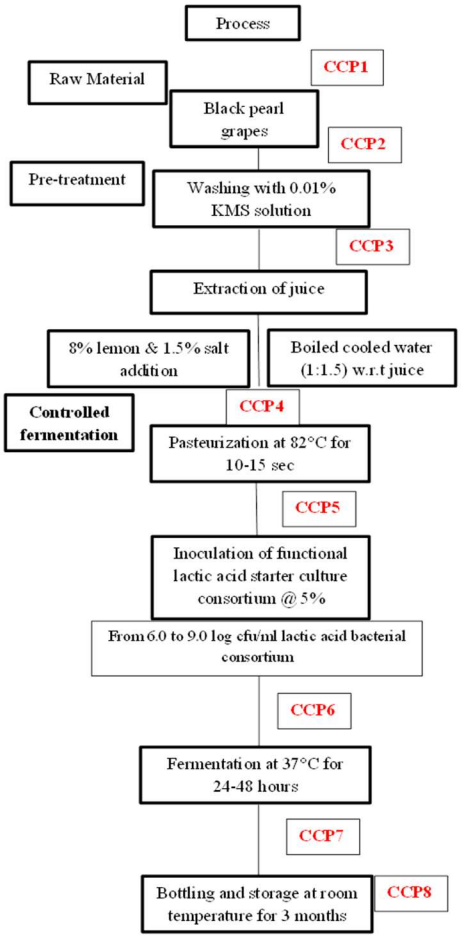
Material and Methods

Plant Material

Black pearl grapes [*Vitis vinifers* var. NS6] were acquired from Department of Fruit and Vegetable Science, PAU, Ludhiana, India along with lemons (var. *PAU Baramasi*). The nearly ripened grapes and other plant materials were harvested manually and washed properly with sodium hypochlorite solution to remove any surface microbial load. Afterwards, the substrates were refrigerated before beverage formulation.

Starter culture activation

Ten strains belonging to different species of lactic acid bacteria - LAB1 (*Pediococcus lolii* MH752471), LAB2 (*Lactobacillus plantarum*,), LAB3 (*P. acidilactici strain* 5560), LAB4 and LAB5 (*Enterococcus sp.*), LAB6 (*P. acidilactici* MK028218), LAB7 (*P. acidilactici* strain 8613), LAB8 (*P. acidilactici*), LAB9 (*P. acidilactici*) and LAB10 (*P. pentosaceous* strain L16)] were singly used for the fermentation. The bacterial stock cultures were stored frozen (-20°C) in MRS (Mann Rogassa Sharpe) with glycerol (20%) and by means of double passage on MRS were reactivated when required. For preparation and activation of functional starter culture for fermentation, grapes juice was extracted to which equal volume of sterilised water, tested for potability using BWTK (*Sahota et al*., 2010), was added. The diluted juice



Hazard	Preventive measures
[1]Microbiological contamination Pathogenic spoilage	Use only high quality chemical free appropriate plant matrices Sort out any damaged or rotten fruits Tested using BFTK
[2]Biological cross contamination	Boiled potable water (tested using BWTK) with 0.01%KMS kill vegetative pathogens
	Clean & sanitised equipment prevent cross contamination
[3]Biological: pathogenic, fungal, mold contamination cross contamination	Clean & sanitised utensils and equipment Keep containers covered with clean & sanitised porous cloth (cheese cloth) Prevent overhead contamination
[4]Biological: mold or wild culture cross contamination	Adequate heating procedure Cooling parameters also need to be monitored
[5]Biological: Spore-formers may be heat shocked and germinate	Dry heat sterilization at 120-180°C, The addition of active fermentation culture will outcompete spore-formers to prevent growth
[6]Biological: mold or wild culture cross contamination	Use of active @5-10 % starter culture will outcompete wild culture growth
[7]Biological: Pathogens, mold or wild culture growth Chemical: Lactic acid can leach metal	Ferment in a safe, non-metallic food grade fermenter Keeping environment should be also be free from contamination
[8]Biological safety and shelf life stability	Store in sterile glass bottles Prevent overhead contamination during filling of the bottles

Figure 1: HACCP plan for bio-process of lactic acid fermented Grapes beverage
***CCP-Critical control point, HACCP-Hazard Analysis Critical Control Point**

was pasteurised at 82°C for 15-20 min and further cooled to room temperature of 28± 2°C. After cooling, the grapes juice was inoculated with the consortium of functional lactic acid starter culture and incubated at 37°C for 24 h for activation.

Beverage formulation and fermentation

Prior to the beverage formulation, refrigerated grapes were thawed at 4° C for 8 h and juice was extracted. The optimized bioprocess parameters using Response Surface Methodology (data not shown here), comprised of grapes blend [grapes juice (100ml); lemon juice (8% v/v)]; dilution ratio (1:1.5 with boiled cooled water), and condiment (salt) concentration (1.2%) for osmotic stable decoction in the fermented grapes beverage and then pasteurization at 82ºC for 10-15 sec and (5% v/v) consortia of ten potential lactic acid bacterial strains as functional starter culture (3.8×10^7 log CFU/ml) for controlled fermentation at 37ºC for 28h. Thus, changes in active culture counts, phytochemical composition and antioxidant activity were evaluated fortnightly throughout the fermentation process every 4-h interval and control was kept without lactic acid fermentation. The final product was tested for retention of functional LA Bacteria, titrable acidity, ºbrix, shelf stability, nutraceutical enrichment and sensory evaluation on nine point hedonic scale.

Measurement of fermentation characteristics

Microbial growth

Total plate count method was used to determine the bacterial growth during the fermentation process.

Concisely, every four hours samples were drawn from the formulated beverage and LAB count with antioxidant capacity, phenols and flavonoids concentration was determined.

pH, total titrable acidity (TTA) and acidification kinetics

pH was estimated using a digital pH meter and TTA defined as quantity of lactic acid (g) per 100 mL was determined by taking (10 ml) homogenized sample with 50 mL of distilled water, titrated with 0.1 M NaOH using (0.1%) phenolphthalein as an indicator.

The acidification kinetics was modelled according to the Gompertz equation:

$$y = k + A \exp \{- \exp[(Vmax e/A) (\gamma - t) + 1]\}$$

where, y is the acidification value at time t expressed as dpH/dt (units of pH/h); k is the initial level of the dependent variable to be modelled (pH units); A is the difference in pH (units) between inoculation and the stationary phase (dpH); Vmax is the maximum acidification rate expressed as dpH/h; t is the time and k the length of the lag phase expressed in hours.

Phytochemical concentration assay

Total Polyphenols concentration (TPC)

The Folin-Ciocalteu method explained by Aydın and Mammadov, (2017) was used for the determination of total polyphenols. A 2 mL freshly prepared (1:1 v/v) Folin-Ciocalteu reagent was added to 100 µL FGB samples after which 2 ml $NaCo_3$ was subsequently added, vortexed for 1 min and incubated at room temperature for 30 min and absorbance was recorded at 760 nm using UV spectrophotometer. Total polyphenols were expressed as milligram of Gallic acid equivalent per 100ml of FGB.

Total Flavonoid concentration (TFC)

The total flavonoids were estimated using colorimetric method adopted by Kwaw *et al*. (2017). 300 µL of sodium nitrite (50g/l) with 4 mL dw was added to one ml FGB, vortexed for 1 min and let stand for 5 min. Then one ml of $AlCl_3$ (100 g/l) was further added, vortexed and let stand again for another 5 min. Afterward, 2 mL of 1M sodium hydroxide and 2.4mL of dw were added and the resultant mixture was incubated at room temperature for 2 minutes with intermittent shaking. The absorbance was read at 510 nm using UV spectrophotometer after 10 min. The TFC was expressed as milligram of Quercetin equivalents per 100ml FGB.

Antioxidant activity assay

DPPH radical scavenging activity

Radical (%) scavenging activity of the grapes blend against stable DPPH free radicals was determined by method described by Zhang and Xu (2015). The free radicals in the DPPH solution absorbs light at 517 nm and as a result of scavenging free radicals in the presence of samples the decreasing absorbance was measured as antioxidant activity. A 0.6mM of DPPH stock solution was prepared from DPPH crystals (0.0238g) dissolved in 95% ethanol. After 30 min of incubation, the absorbance of the negative (A_0) control was measured with 0.5ml ethanol and 2.5ml of the DPPH solution. However, the effect of the blend color was excluded by measuring the absorbance of a mixture of 0.5ml different sample concentrations with 2.5ml of ethanol and measured as (A_2). 2.5ml of DPPH solution was mixed with 0.5 ml of blend at different concentration (50, 100, 150, 200 and 250µg/ml) and the absorbance was measured as A1. The percentage inhibition was calculated using the expression in Eq. (1).

$$\textbf{Scavenging activity, DPPH (\%)} = (1 - [A_1 - A_2])/A_0 \times 100\%$$

Ferric reducing antioxidant power assay

The method explained by Suarez *et al.* (2010) was adopted for estimation of ferric reducing antioxidant power. The working FRAP reagent was prepared freshly by mixing 5.0 ml of TPTZ (10 mM in 40 mM Hydrochloric acid), 5.0 ml of Ferric chloride (20 mM), and 50 ml Sodium acetate buffer (300 mM, pH 3.6). The FRAP reagent (3.6 ml) was first maintained at 37°C and then mixed with 0.4 ml of each sample and mixed vigorously, incubated for 4 at 37°C min and then absorbance was measured at 593 nm. The FRAP anti-oxidant activity was expressed as mM Ferrous sulphate equivalents using standard curve prepared by using known concentration of Ferrous sulphate solution (Ferrous sulphate 1 mM= 1.51 mg/10 ml).

Color assessment

The study used the Konica Minolta colorimeter (CR-410, Konica Minolta Inc. Japan) to quantify the color characteristics (L*, a* and b*) and the index of hue angle (H°) was calculated as $\tan^{-1}(b/a^*)$ with chroma (C) as $(a^{*2} + b^{*2})^{1/2}$ and total color difference (ΔE) calculated as $[(L_o-L)^2 + (a_o-$

$a)^2 + (b_o-b)^2$] ½ (Fazaeli, Hojjatpanah, and Emam-Djomeh., 2013).

Antagonistic Activity of formulated metabiotic grapes beverage

The antagonistic activity of the metabiotic grapes beverage was assessed using strains *Staphylococcus aureus* MTCC3906, *Listeria monocytogenes* MTCC657, *Klebsiella pneumoniae* MTCC109, *Escherichia coli* MTCC443 and *Aeromonas hydrophila* MTCC173 was examined using agar well diffusion. The test pathogens containing 2×10^7 cfu/mL were seeded on molten Muller Hilton agar plates and wells were bore on seeded plates after solidification. The beverage samples along with erythromycin as a positive control were introduced into the wells and first incubated for 60 min at 4 °C, allowing the test material to diffuse in the agar, and then incubated for 18h at 37°C and clear zones were measured.

Human sensory evaluation

The sensory evaluation was carried out according to the method of Kwaw and Sackey (2013). Briefly, a panel of both male and female members (25-45 age group) comprising of staff, students of the Department of Microbiology were offered with coded samples. Each panel evaluated samples (10 ml) for color, flavor, taste, aroma, bouqet, astringency and overall acceptability using a 9-point hedonic according to the ISO8586-1 (1993) sensory analysis guidelines and assessments were recorded on the designed sensory analysis form.

Statistical Analysis

All the microbial, physic-chemical analyses were performed in triplicate and expressed as mean ± standard deviation (SD) and analysed using SPSS (version 16.0). The significance of difference was tested by one-way ANOVA and Tukeys post hoc test was performed for post comparisons. Results with $p\leq0.05$ were considered to be statistically significant.

Results and Discussion

The microbial growth kinetics, pH, TTA and acidification kinetics

During the fermentation process, the growth pattern of the Lactic Acid Bacteria gives important information regarding the properties of on-going fermentation. The fermentation kinetics for Lactic Acid Bacterial consortium is presented in Figure (2a). The differences between the pre-culture and the fermentation medium might have induced nutritional stress and there resulted into decreased microbial growth rate during initial stages of fermentation process (first 2h). This stage was clearly defined for the Lactic Acid Bacterial during which minor metabolic activity is observed and the bacterial culture attempts to acclimatise with the new fermentation conditions. An exponential growth of the lactic acid bacteria in the grapes beverage after 2h up to 28 h was experiential, reaching the count of 9.44 log10 CFU/ml, which relates to the normal growth curve of microbes and were higher than the minimum (10^6) recommended value of viable LAB in fermented product. The elevated viable cell counts advocates the supply of sufficient nutrients and favourable conditions for the exponential growth of functional Lactic Acid Bacterial consortium.

The Figure (2b) shows the results for pH and TTA dynamics during the fermentation of grapes blend using lactic acid bacterial at 37ºC for 28 h. The pH values decreased from 4.32 (initial) to 2.64 (28h). The initial TTA value was (0.448) and increased to (0.63) after 28 h of fermentation (Figure 2b). The highest value of ΔpH (1.683) and V_{max} (0.089) occurred on the 28^{th} hour and 16^{th} hour, respectively (Table 1), representing maximum growth rate of fermentation at the 16^{th} hour during which the LAB grows exponentially. Similar increases were found during lactic acid fermentation of rice-based beverage (Gosh *et al.,* 2015). The variation in pH and Titrable acidity, with the microbial growth during the fermentation bio-process, suggests the usage of sugars present in the blend by Lactic Acid Bacteria to produce lactic acid.

Antioxidant capacity and active phytochemical concentration during lactic acid fermentation of Grapes blend

Total antioxidant activity is a unique parameter widely used in phytomedicine for the determination of scavenging activities of bioactive formulations. DPPH-SA (1- Diphenyl -2- picryl hydrazyl-radical scavenging activities) and FRAP (Ferric Reducing Antioxidant Power) assay are among the most widely accepted methods for determining the antioxidant capacity of the food and beverages. The changes in antioxidant capacity during 28 h

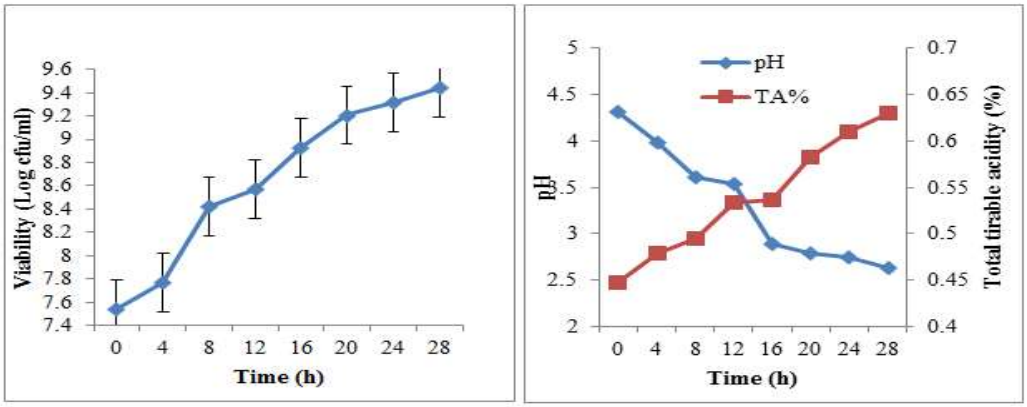


Figure 2: **Lactic acid bacterial viability (A), pH and total titrable acidity (B) during lactic acid fermentation of the Grapes beverage.**

Table 1: Acidification kinetics of lactic acid bacterial consortium during 28 h of Grapes beverage fermentation.

Fermentation time (h)	Acidification kinetics	
	ΔpH (pH units)	Vmax (ΔpH/h)
0	0^{g}	0^{f}
4	0.336 ± 0.01^{f}	0.084 ± 0.003^{b}
8	0.709 ± 0.010^{e}	0.088 ± 0.001^{a}
12	0.783 ± 0.03^{d}	0.065 ± 0.002^{d}
16	1.43 ± 0.015^{c}	0.089 ± 0.001^{a}
20	1.53 ± 0.01^{b}	0.076 ± 0.001^{c}
24	1.576 ± 0.03^{b}	0.0656 ± 0.001^{d}
28	1.683 ± 0.015^{a}	0.060 ± 001^{e}

Table 2: Antioxidant activity and active phytochemical composition of lactic acid fermented Grapes beverage during 28h of fermentation

Antioxidant activity			Phytochemical ingredients	
Fermentation time (hours)	**DPPH-SA (%)**	**FRAP (μM of ferrous sulphate equi.)**	**TPC (mg/100ml)**	**TFC (mg/100ml)**
0	58.51 ± 0.59^{d}	64.92 ± 0.04^{e}	35.16 ± 0.71^{e}	33.08 ± 0.11^{d}
4	60.85 ± 0.41^{cd}	73.26 ± 0.03^{d}	45.11 ± 0.21^{d}	39.65 ± 0.51^{c}
12	63.77 ± 0.79^{c}	81.9 ± 0.01^{c}	48.86 ± 0.54^{c}	42.27 ± 0.35^{b}
20	67.82 ± 0.67^{b}	98.2 ± 0.07^{b}	52.36 ± 0.35^{b}	44.41 ± 0.39^{a}
28	69.70 ± 0.77^{a}	100.3 ± 0.01^{a}	52.29 ± 0.32^{a}	46.96 ± 0.06^{a}

DPPH-SA- 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, FRAP-ferric reducing antioxidant power, TPC-total phenolic content, TFC-total flavonoid content

FRAP quantified as- μM Ferrous sulphate equivalent antioxidant capacity

Data expressed as mean ± standard deviation.

Means that do not share the same letter are significantly different at $P \leq 0.05$

fermentation of the grapes blend are shown in Table 2. The free radical scavenging activity increased during the 28 h fermentation and then remained stable during storage the maximum value of 69.70 %SA was found after 28 hours. The drift in FRAP activity was characterized by an increment during 28 hour of fermentation. It reached its highest value (100.3 μM of ferrous sulphate

equivalents) at 28th hour of fermentation. Torres *et al.* (2015) has correlated the increased profile of phenols and flavonoids in fermented fruits with antioxidant capacity. The total phenolics (TPC), total flavonoids (TFC) changes during the fermentation process have also been determined (Table 2). The total polyphenols in the grapes based beverage was 52.29 mg quercetin equivalent per100ml of beverage former to fermentation (28 hours). The variation in TPC content was in conjunction with fermentation process as initially it increased rapidly while more stable lately. It increased during the fermentation period and then remains stable in synchrony during the fermentation phase. The study by Ng *et al.* (2011) exhibited an increase in the total phenolic concentration of the plant substrates after fermentation hence, an experiential increment in the antioxidant activity. It has been also proposed by Chu and Chen (2006) that LABs transformation and depolymerisation of plant compounds could account for the increased content of phenolic compounds during the fermentation process.

The TFC content of grapes beverage varied in similar manner as TPC. It enhanced as the fermentation progresses (28 hours) and remained stable. The maximum value of TFC was 46.96 mg Gallic acid equivalents per 100ml, whereas the TFC level at 0 h was 33.08 mg/100ml. The lactic acid bacterial enzymes and acids could have facilitated the release of flavonoids from their complex compounds and make them more available in the fermenting medium (Katina *et al.,* 2007). Therefore, lactic acid bacterial metabolism can increase the flavonoids during fermentation of grapes beverage and resulted in a high-quality nutritionally enriched fermented beverage.

Effect of lactic acid fermentation on color characteristics of the Grapes beverage

The effect of fermentation on color characteristics (L*, a* and b*) of FGB were determined along with total color difference (ΔE). The observations (Table 3) revealed a decrease in L*and b* and an increment in a*of the fermented sample compared to the control. Pereira *et al.* (2011) observed a lightness (L*) reduction in cashew apple juice due to the higher turbidity caused by bacterial growth and hence, the decrease in brightness. Grapes beverage color is attributed to the tannins, alkaloids, flavonoids and phenols and increase in a* could be due to the increase in phytochemical concentration among the FGB compared to control (Table 2).

Antimicrobial activity of lactic acid fermented grapes beverage against selected food grade spoilage microorganisms

In the present study the efficacy of FGB fermented using LAB consortium at the rate 5%w/v (10^8 CFU/ml), was evaluated against microorganisms responsible for causing food infection and food intoxication (Table-4). The microorganisms selected were *Staphylococcus aureus* MTCC3906, *Listeria monocytogenes* MTCC657, *Klebsiella pneumoniae* MTCC109, *Escherichia coli* MTCC443 and *Aeromonas hydrophila* MTCC173. The inhibition zones were compared with Erythromycin (positive control) after 24 hours of incubation at 37ºC.

The antimicrobial effect against *Escherichia coli* was observed with 15.0±2.6mm and 10±2.3mm zone of inhibition with fermented and unfermented samples respectively, followed by *Listeria monocytogenes* (13mm), *Staphylococcus aureus* (11mm). Strains of *Klebsiella pneumonia* and *Aeromonas hydrophila* displayed inhibition zones (9.0mm and 5.0mm respectively) only with fermented sample. The results are in concordance with earlier studies reported by *Mantzourani et al.,* 2019 where Cranberry juice fermented with potentially isolated probiotic *Lactobacillus paracasei* K5 exhibited greater antimicrobial efficacy against *Enterobacter faecalis* and *Staphylococcus aureus* as compared to unfermented juice with zone of inhibition ranging from 10.75-15.45mm. This antagonistic response is believed to be derived from organic acids, bacteriocins, antimicrobial peptides and hydrogen peroxide action on the cell membrane of bacteria, playing role in maintain the membrane potential while inhibiting the active transport (Parvez *et al.,* 2006; Vasconcel*os et al.,* 2018; Muhammed *et al.,* 2018).

Sensory assessment of lactic acid fermented Grapes beverage

Sensory assessment (Figure 3) showed that NFGB and the FGB were above six (like) on the 9 points hedonic for all the sensory attributes. The FGB was

Table 3: Colorimetric properties of lactic-acid fermented Grapes beverage

Sample	L*	a*	b*	H°	C*	ΔE
NFGB	16.63±0.37	3.5±0.47	6.76±0.35	62.90±0.85	7.61±0.44	-
FGB	6.6±0.11 *	7.66±0.32 *	7.56±0.05 *	44.30±0.73 *	10.73±0.20 *	10.89±0.35

Data expressed as mean ± standard deviation
NFGB-non-fermented grapes beverage, FGB-lactic acid fermented grapes beverage
L*- Lightness, a*- Redness, b*- Yellowness, C*- Chroma, H°- Hue angle, ΔE- color difference
Means with different superscripted letter (a,b) on the same column shows significant difference at P≤0.05

Table 4: Inhibition zones for lactic acid fermented grapes beverage (1000mg/ml+5%w/v consortium) against selected food grade spoilage microorganisms

Isolate	Accession number	Zone of inhibition (mm)		
		Erythromycin (15mcg)	NFGB (10ul)	FGB (10ul)
Staphylococcus aureus	MTCC3906	15.0±2.6	7.0±0.8	12.0±1.2
Listeria monocytogenes	MTCC657	14.0±2.1	11±1.4	11±2.0
Klebsiella pneumoniae	MTCC109	18.0±3.0	ND	9.0±1.0
Escherichia coli	MTCC443	10.0±1.4	9.5±2.3	14±2.5
Aeromonas hydrophila	MTCC1739	8.0±0.3	ND	6.0±0.8

***Values reperesented as mean±SD**
*** Antibiotic disk: HIMEDIA Laboratories pvt. Ltd (HX023-1PK)**
***Incubation for 24h at 37°C**
***ND-not detected**

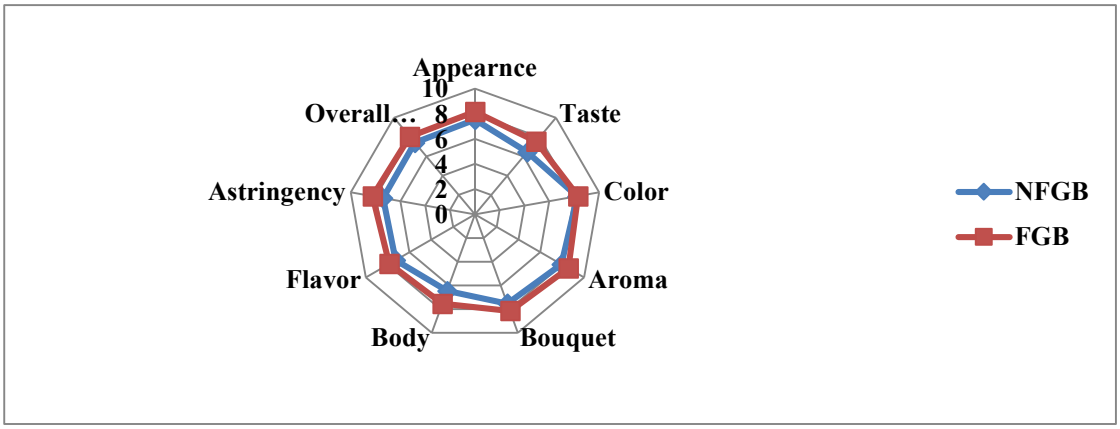


Figure 3: Radar plot for sensory assessment of non-fermented Grapes beverage and fermented Grapes beverage
NFGB-non-fermented grapes beverage, FGB-fermented grapes beverage
Asterisk indicates significant difference at P ≤ 0.05.

significantly ($p<0.05$) better in astringency, flavor, taste and overall acceptability compared to the NFGB (Figure 3). The higher color score for FGB compared to the NFGB could be accredited to its enhanced content of phytochemicals (Table 2). Though high concentrations of nutraceuticals and phytochemicals are accountable for the bitterness and acidic taste in various products (Sun-Waterhouse and Wadhwa, 2013), the high lactic acid content of FGB might have lessened its bitterness and astringency (Milkulic-Petkovsek *et al.,* 2012). As previously reported by Sun *et al.* (2016) lactic acid strains can considerably transform the aroma characters of fermented beverages with the ability to produce diverse enzymes. But FGB and NFGB have no significant difference ($p>0.05$) in terms of aroma, color and body having almost alike sensorial scores (Figure 3). The observations also showed that appearance and bouqet were significantly different ($p<0.05$) between the FGB and NFGB having a higher score for FGB. This could be due to the secondary metabolites and chemical changes and lactic acid contents of the FGB. The overall acceptability

score by the panel showed that FGB was much more preferred as compared to NFGB (Figure 3).

Conclusion

Lactic acid fermentation is commonly applied on milk, however, owing to the lactose intolerance and milk-resistant proteins related with milk consumption, is an immediate need to develop fruit and vegetable-based fermented beverages. Lactic acid fermentation can also be applied for preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables and fruits and an ideal medium for the administration of probiotic lactic acid bacteria. We found grape juice as a reliable/favourable substrate supporting all nutritional requirements for potential growth of Lactic Acid Bacteria (LAB). Lactic acid bacterial fermentation enhanced the antioxidant capacity by altering the phenolic content in the grape blend. The refinement of polyphenols along with the increase in flavonoids and total phenols participates in the elaboration of grape juice offering optimised nutritional profile. Enrichment with polyphenolic compounds, flavonoids limits the risk of innumerable chronic diseases related with oxidative stress along with the metabolites released during fermentation are able to exert biological activities more pertinent to human health in reverence to their parent phenolic compounds with bioaccumulation property inside the endothelial cells retaining an intracellular antioxidant activity. Beyond the basic nutritional properties the fermented beverage can possess anti-diabetic, anti-inflammatory and anti-cancer properties with an impact on reducing obesity, firmly correlated with antioxidant activity, flavonoids and the occurrence of other phytochemicals in fermented grapes beverage which can be further tested. Hence, the resulting fermented grapes beverage was nutraceutically enriched, healthy functional food alternative providing functional lactic acid bacteria with potential probiotic properties, which might be an appropriate and effective fermentation medium providing desired characteristics even during storage time.

Conflict of interest

The authors declare that they have no conflict of interest.

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Production of a Potentially Symbiotic Pomegranate Beverage by Fermentation with *Lactobacillus plantarum*

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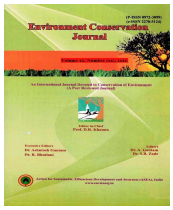

Effect of *Pseudomonas fluorescens* in manganese uptake by chickpea (*Cicer arietinum* L.) cultivars infected by root-knot nematodes (*Meloidogyne incognita*)

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Root-knot nematode

ABSTRACT

Chickpea (*Cicer arientinum* L.) is one of the most dominant pulse crops in India, which contributes 38 percent of the area and 50 percent production of pulses compare to the total pulse production of India. Chickpea contains protein-2.1%, carbohydrates-61.5%, and fat-4.5% and more iron, calcium and niacin content. The main constrain of chickpea production due to parasitic nematodes (*Meloidogyne incognita*) is about 14% of total global production in annual yield loss. *Pseudomonas fluorescens* is a bacterial bio-agent that can help in nematode suppression in chickpea plants. This experiment was conducted to experience the differences, if any, in manganese content concerning chickpea inoculated with *M. incognita* with a combination of *Pseudomonas fluorescens* as a bioagent, where different treatments of nematode, bacteria, and chemicals are used sustaining the enhancement of disease resistance in chickpea cultivars RSG 974, GG 5, GNG 2144. The total manganese content of chickpea variety GNG 2144 was found highest in treatment, where only bacteria (*P. fluorescens*) was inoculated, *i.e.*, 6.44 mg/100g of a root, followed by GG 5, *i.e.*, 5.63 mg/100g of root and RSG 974 was, *i.e.*, 4.14 mg/100g of root respectively. Application of *Pseudomonas fluorescence* combined or alone gradually increased the manganese concentration in roots of chickpea plants *i.e.*, RSG 974 (4.14 mg/100g), GG 5(5.63 mg/100g), GNG 2144 (6.44 mg/100g) compared to the health check.

Introduction

Among various species, root-knot nematodes, mainly *Meloidogyne incognita* are causing major economic damage in chickpea (*Cicer arietinum* L.). Nematodes mainly attack as a primary pathogen and aggravator, causing damage to roots by reducing the functionality of roots, leading to nutrient deficiency and water stress in plants and resulting in poor plant growth and less yield. By growing resistant crops which comes under ecologically based management with a very good cultural practices are the cheapest, safest and practically feasible management tactics for plant disease causing nematodes till date. However, breeding for resistance to such nematodes has various problems originating from the *C. arietinum* cultigens (Zwart *et al.,* 2019). The metabolic process of the host is being altered by plant-parasitic nematodes like *M incognita,* by using biochemical, cellular, or physiological parameters. Due to root-knot nematodes, there is occurrence of

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devasting morphological and physiological variations in the host plants (Williamson and Gleason, 2003). These nematodes infect the plants, resulting in the discoloration of leaves showing yellowing symptoms and slow plant growth. Broad host range minimizes the chance of crop rotation as a management to plant parasitic nematodes.

Therefore, an alternative source to avoid the losses is growing resistant cultivars (Howell and Krusberg, 1966), which can also be done by inducing some bioagents to the plants. *Pseudomonas fluorescens* is a bacterial bio-agent grows well with a simple nutritional requirement (Palleroni 1984). The ability to adapt well in soil *P. fluorescens* is easy to induce, and survival chances are significant in soil. Chief among all biocontrol agents suppress the roots and plant diseases that bacterial and fungal pathogens have caused due to the production of antibiotics and siderophores. (O'Sullivan & O'Gara 1992; Wei *et al.,*1996; Hoffland *et al.,*1996). Hass and Defago (2005) stated that *P. fluorescens* shows competitive behavior to the disease-causing pathogens by rapid rhizospheric colonization, is one of the major factors in disease control. Physiological variations and biochemical alterations due to nematode invasion are occurred because of the disturbed metabolism in the affected plants, deciding the hosts to become susceptible or tolerant to check the nematode's attack (Krusberg 1963). Some progress regarding host plant interaction and biochemical mechanism has also been made to understand the matter by several workers in the recent past (Ganguly and Dasgupta 1983; Mohanty *et al.* 1995; Howell and Krusberg, 1966). Considering these matters and the necessity, the present research was carried out to find the differences and know the details of the current matter, if any, in manganese content about chickpea inoculated with *M. incognita* in combination of *Pseudomonas fluorescens* as a bioagent.

Material and Methods

Cultivars of chickpea were sown in 15 cm diameter earthen pots filled with steam-sterilized soil. A week after germination, seven treatments with four replications to each chickpea varieties RSG 974, GG 5, and GNG 2144 were done.

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2),
T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot,
T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot)
T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot)
T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time
T_6- Carbofuran 3G @ 2.5kg ai/ha,
T_7- Control

Harvesting of inoculated plants side by healthy plants were done after 45 days after planting. The roots after harvesting were kept separately for chemical analysis. Estimation of micronutrient 'Mn' in roots was done by mineral acids like diacid (HNO_3 - $HClO_4$) digestion (Jackson, 1973; Gahoonia *et al.,* 2007). After standardizing the AAS, the digested sample was introduced to AAS for Mn analysis, with respecting standards.

$$(Mn)\ mg/100\ g\ dry\ weight = \frac{AASR \times 50}{Sample\ \mathrm{wt(g)} \times 10}$$

Results and Discussion

Estimation of manganese contents in variety RSG 974 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety RSG 974 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, i.e., 4.14 mg/100gm of root with a percent increase of 35.74% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e*., 4.08 mg/100mg with a percent increase of 33.61% respectively. These findings were found quite similar to findings by Sathya *et al.* (2016), where they experimented 19 isolates of plant growth-promoting actinobacteria and concluded there is significant (p\0.05) increase of minerals compared to untreated check in all the isolates and that for also Mn (18–35 %), that indicates the benefits of bio agents as they supress the intake of nutrients to other harmful microorganisms. Furthermore, an increase is recorded in all nematode combinations (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) simultaneously

Table 1: Manganese concentration in various treatments of chickpea variety RSG 974, GG-5, GNG-2144

Treatments	RSG 974		GG-5		GNG-2144	
	Root	Change over control (%)	Root	Change over control (%)	Root	Change over control (%)
T_1	3.23	5.90	3.91	8.91	4.48	14.16
T_2	4.14	35.74	5.63	56.82	6.44	64.29
T_3	3.36	10.16	4.11	14.35	4.73	20.73
T_4	3.74	22.62	4.66	29.87	5.28	34.69
T_5	3.51	15.08	4.57	27.16	5.17	31.89
T_6	4.08	33.61	5.44	51.46	5.96	52.04
T_7	3.05		3.29		3.92	-
SE(m)±	0.02		0.03		0.04	
CD (0.05)	0.06		0.09		0.11	

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2); T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot; T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot); T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot); T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time; T_6- Carbofuran 3G @ 2.5kg ai/ha and T_7- Control.

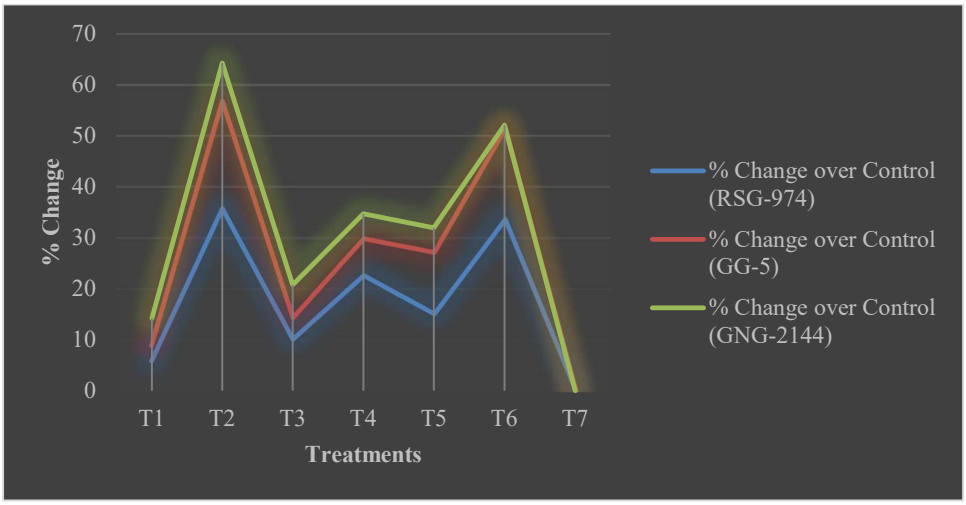

Figure 1: Percent change over control in manganese concentration (T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2); T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot; T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot); T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot); T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time; T_6- Carbofuran 3G @ 2.5kg ai/ha and T_7- Control).

or one after another. Among combinations, treatment-4 (nematode inoculated one week prior to *P. fluorescens*) was recorded as a higher amount of manganese content, *i.e*., 3.74mg/100mg of roots with a percent increase of 22.62% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e*., 3.51 mg/100mg (15.08%) and treatment-3, where (*P.fluorescens* inoculated one week prior to *M. incognita*) *i.e*., 3.36 mg/100mg (10.16%) respectively. Not only for pulse crop also for fruit melon promotion of Mn, N, Zn, and P absorption was noticed due to *Pseudomonas fluorescens* Martínez *et al.* 2019.The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, *i.e*., 3.23 mg/100mg of the root of variety RSG 974 (Table 1; Figure 1) with a low increase in percentage 5.9% over the control. Mobility of Mn enhanced by *Pseudomonas*

fluorescens, in sunflower grown on vineyard soils. However, mainly Root-knot nematode plants create galls in roots that block the xylem and phloem vessels in the case of infected nematode plants. Giant cells were induced by juveniles of *Meloidogyne incognita* near the head in vascular strands and xylem as well as phloem were affected by exhibiting abnormalities in their shapes (Robab 2010), by inhibiting the mobilization of nutrients from roots from shooting, storing nutrients molecules in the root portion happened, which is the main reason for enhancing Mn content in roots of chickpea plants, which disobeys the above statement of mobility by Randriamamonjy *et al.* 2021.

Estimation of manganese contents in variety GG 5 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety GG 5 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, *i.e*., 5.63 mg/100gm of root with a percent increase of 56.82% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e*., 5.44 mg/100mg with a percent increase of 51.46% respectively. An increase is recorded in all combinations of nematode (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) simultaneously or one after another. Among combinations, treatment-4 (nematode inoculated one week prior to *P. fluorescens*) was recorded as a higher amount of manganese content, *i.e*., 4.66 mg/100mg of roots with a percent increase of 29.87% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e*., 4.57 mg/100mg (27.16%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *M.incognita*) *i.e*., 4.11mg/100mg (14.35%) respectively. The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, i.e., 3.91 mg/100mg of the root of variety GG 5 (Table 2) with a low increase in percentage 8.91% over the control. Mohanty *et al*. (1999) reported that the inoculation of *R. reniformis* in cowpea roots reduced micro nutrients *viz*., Zn, Cu, Fe, Mn in inoculated plants over the control. Concentration of iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were significantly increased in only *P. fluorescens* inoculated roots over control. These statements corroborate our fimdings where nematode infecting the chickpea plant showed a slight increase in Mn content.

Estimation of manganese contents in variety GNG 2144 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety GNG 2144 (Table 1) was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, *i.e*., 6.44 mg/100gm of root with a percent increase of 64.29% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e*., 5.96 mg/100mg with a percent increase of 52.04% respectively. The chemical application was also reasonably practical, although cost-effective, and disobeyed the law of soil sustainability because of residual effects of the chemical. At the same time, *Pseudomonas fluorescens* treated chickpea plant has the highest increase than all treatments. Hence plant growth-promoting bacteria enhanced macro and micronutrients to varying degrees compared to healthy plants, and in the case of Mn, an increase was witnessed in chickpea variety PBG5 compared to PBG1treated with plant-growth promoting bacteria *Pseudomonas citronellis* (PC), *Pseudomonas* spp. RA6, *Serratia* spp. S2, *Serratia marcescens* CDP13, and *Frateuria aurantia* (Symbion-K) by Dogra *et al.* 2019. The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, *i.e*., 4.48 mg/100mg of the root of variety GNG 2144, with a low increase in the percentage of 14.16% over the control. An increase is recorded in all combinations of nematode (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) simultaneously or one after another. Among combinations, treatment-4 (nematode inoculated one week prior to *P. fluorescens*) was recorded as a higher amount of manganese content, *i.e*., 5.28 mg/100mg of roots with a percent increase of 34.69% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e*., 5.17mg/100mg (31.89%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) *i.e*., 4.73mg/100mg (20.73%) respectively. In this experiment, *Pseudomonas fluorescens* gives a positive result of increasing the Mn content in the roots of the

chickpea plant. Likewise, bacteria, fungi can also be used as a bioagent like *Pseudomonas fluorescens,* and similar results, *i.e*., there was an increased amount of Mn content was seen where bioagents were applied in cluster bean infected with *Meloidogyne incognita* and VAM fungi (Rao and Tarafdar 1993), wheat (Suri *et al.,* 2011) and a common leafy vegetable *Ipomoea aquatica* (Halder *et al.* 2015).

Conclusion

Alterations of enzymatic activity, redistribution, uptake, and nutrients use like N, P, Fe, Ca and Mg are hampered by heavily concentrated plant tissues with Manganese. Due to manganese severity, the reduction of stomatal conductance, and deterioration of pigments in leaf were seen in different plants. Higher Mn concentration enhanced the production of ROS (reactive oxygen species), with elevated peroxidase activity in plant cells, which has a potential role in the disease resistance mechanism in plants. Mn content was found more in GNG 2144 and GG 5 than that of tolerance one RSG 974 among three chickpea cultivars, and *P. fluorescens* has the leading role in increasing Mn content in roots chickpea plants.

Conflict of interest

The authors declare that they have no conflict of interest.

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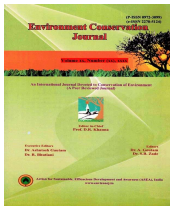

Cold plasma technology – An overview of basics and principle

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ABSTRACT

Thermal processing can produce non-enzymatic browning, protein denaturation, flavor alterations, and vitamin loss in food products. A cold plasma treatment, which is non-thermal, is the greatest option for preserving food products, keeping bioactive ingredients, and prolonging shelf life. It is used for brief treatment durations at moderate temperatures. The review's goal is to discuss cold plasma procedures, parameters, and processes for microbial and enzyme inactivation. It also discusses the numerous uses in the dairy business as well as their impact on quality factors. The cold plasma technique shows an excellent performance in the elimination of spoilage microorganisms and maintaining the quality characteristics of food products.

Introduction

Milk is a nutritious liquid food that contains fatty acids, good quality protein, lactose, and micronutrients such as minerals and vitamins. Various health benefits are associated with the consumption of milk and milk products. Milk in diet can fulfill the deficiency of essential nutrients like calcium, vitamin D, vitamin K, phosphorous, and magnesium. It helps to maintain your body healthy and strong. Although the nutritious quality of milk makes it more susceptible to microbial attack, that is why it is needed to preserve the milk. Consumption of raw milk is having no risk at the nutritional level but it could have a detrimental effect on health by consuming potential pathogens with it. Pathogen like Campylobacter spp., *Salmonella spp*., *E.coli* O157:H7, *Y.enterocolitica*, *L. monocytogenes*, and *S. aureus* are mostly the reason behind the health hazard caused by milk (De Buyser *et al.,* 2001; Gillespie *et al.,* 2003; Lee and Middleton, 2003; Oliver *et al.,* 2009). The risk of consuming pathogen with milk is reduced or even eliminated with the help of thermal treatment. Pasteurization, thermization, sterilization, and Ultra high treatment (UHT) are thermal methods that have different time-temperature combination by which different level of bactericidal effect is achieved. UHT and Sterilization are known to kill almost all vegetative and sporulating pathogenic microorganisms including spores of *B. cereus* (Lindstrom *et al.,* 2010). At the same time, any heat processing has the potential to alter the nutritional and sensory properties of food. UHT has been reviewed to show the degrading effect on milk nutrition. Protein, vitamin, and to some extent lactose are lost in UHT processing. Also, ultra heat-treated milk is not suitable for the production of several products like cheese and yogurt. To preserve the nutritional loss made by heat method, non-thermal preservation techniques for decontamination of milk came into frame. One of such emerging non-thermal preservation techniques is cold plasma technology. A plasma state is attained when the gas molecule reaches a certain energy level and gets ionized. The energy for ionizing the gas molecule could be from any source. It could be electrical, thermal, microwave which ultimately causes elevating the kinetic energy of electrons within gas molecules. The increase in kinetic energy causes a collision which results in the formation of ions, radiation of

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different wavelengths and radicals, there are various methods for the generation of plasma such as dielectric barrier discharge (DBD), corona discharge, gliding arc discharge, and radiofrequency plasma(RFP) (Conrads and Schmidt, 2000). Plasma can be classified based on temperature as thermal plasma and non-thermal plasma. In thermal plasma temperature of an electron is almost equal to heavy particle temperature which is approx. 10000 K (Tendero *et al.,* 2006). The non-thermal plasma in the context of temperature, the density of ions, and energy level is said as a non-equilibrium plasma or cold plasma. Nowadays this technique is seeking more attention as it can show a decontamination effect on relatively low temperature and at atmospheric pressure (Roth *et al.,* 2007). Cold plasma is produced at 30-60 °C under low or atmospheric pressure (Thirumdas *et al.,* 2015) Its low-temperature operation makes it applicable in the food industry for heat-sensitive food (Tolouie *et al.,* 2018). In the medical industry, it has been utilized for surface disinfection of devices that are heat sensitive. In packaging industry also uses this technique for sterilization of package surfaces (Pankaj *et al.,* 2014). Also, it has been reported that bacterial spores can be eliminated with better efficiency with plasma technology than the thermal process which is generally used in food industries. In the dairy industry, milk and its products require sterilization to make the product microbiologically safe, to enhance the shelf life by elimination of deteriorative micro-organism but these methods consist of severe thermal operation which interferes with the nutritional quality of the end product. This review is aimed to collect and share knowledge regarding the work done with cold plasma technology on food products.

Cold plasma generation

There are various methods of achieving cold plasma. This requires energy to generate and maintain a plasma state, it could be thermal light or electrical energy. Generally, electrical energy is utilized for plasma generation. Various methods for the generation of cold plasma are discussed below.

Dielectric barrier discharge method.

It utilizes a dielectric barrier for plasma generation. DBD constitutes two metal electrodes, between these two, a high potential difference is maintained (Table 1). The frequency for the operation ranges between 0.05 to 500 kHz for different pressure conditions (Zhang *et al.,* 2017). In DBD arrangement either one or both, the electrode is covered with a dielectric material. The dielectric material collects the charge into it and prevents it from igniting, which cause retardation in potential difference. In these dielectric barrier discharge setup, charge is self pulsed which does not allow the current to discharge to such level that causes arcing. The plasma produced by DBD method contains large number of filament but it is found in very random manner hence the plasma produced is non uniform. The productivity of plasma by DBD method depends upon factor such as, gas employed, gap between electrodes and level of voltage maintained for operation (Ehlbeck *et al.,* 2010). Advantages with DBD method is that, plasma can be produced with wide variety of gas, flow of gas could be low or no flow is required, and also different shape of electrode could be used. It is good source for large surface application (Phan *et al.,* 2017). Its disadvantage involve voltage requirement of at least 10 kV for the operation

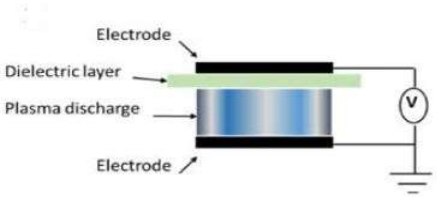
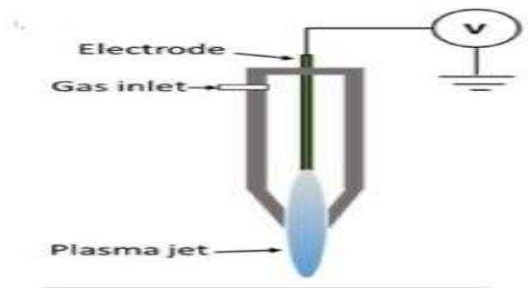
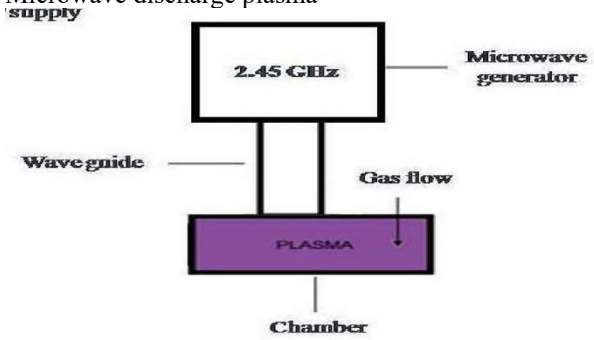
Atmospheric plasma jet

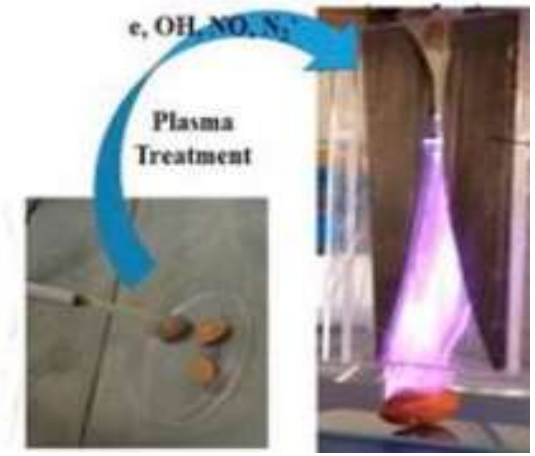
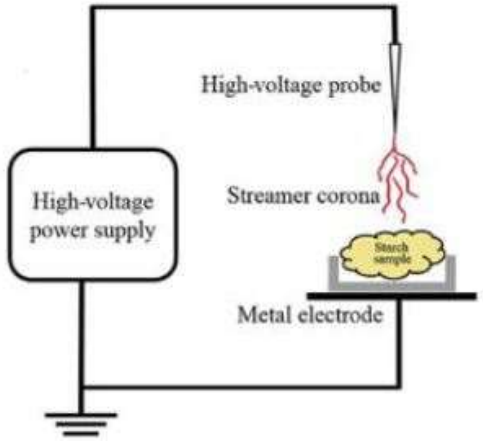
In plasma jet there is two electrodes arranged in concentric manner. The gas is passed between these two electrodes. The electrode at outer side is grounded and inner electrode is applied with high frequency (at radio frequency range commonly at 13.56MHz) and high voltage (100-250 V), these cause the ionization of passing gas. The field produced by radio frequency excite the free electron which produces plasma products (free radicals, excited atoms and molecule) by inelastic collision (Misra *et al.,* 2016). At few millimetres subjected sample is placed the gas plasma impact it through a nozzle. In this process noble gas like helium and argon are used. Flow of gas for so needed to maintain at very high rate (> 10 slm), which make it very expensive process. Its advantage lies in its potential to penetrate in narrow spaces and its direct applicability (Weltmann *et al.,* 2008).

Gliding arc discharge method

Gliding arc discharge method involves plasma generation within a large reactor which constitutes two or more metallic electrode kept at high potential difference of 9kV (Table 1). Gas is forced

Table 1: Different methods of cold plasma generation.

Method	Configuration	Parameter	Application	Used for	Reference
Dielectric barrier discharge  (Pankaj *et al.,* 2017)	Two electrode kept at high potential difference. Dielectric material is placed between these two electrodes	Frequency 0.05-500 KHz power consumption 10 - 100 W Gas pressure 10^4-10^6 Pa Gap distance 0.1 mm-several centimetre	Orange juice Cold-smoked salmon Tomatoes Strawberries Sliced Cheese Cheddar cheese	Ideal for large surfaces application	(Shimizu *et al.,* 2018) (Segat *et al.,* 2016) (Pankaj *et al.,* 2017) (Zhang *et al.,* 2017) (Saragapani *et al.,* 2017)
 Plasma jet (Pankaj *et al.,* 2017)	Two coaxial electrode central electrode is excited at radio frequency Outer electrode grounded	Radio frequency 13.56 MHz Gas flow rate >10 slm*	Mango melon skin	Applicable where penetration and direct application is require.	(Pankaj *et al.,* 2017) (Weltmann *et al.,* 2008)
Microwave discharge plasma 	Electrode free Microwave driven (Microwave generally produced by magnetron)	Frequency range-300MHz – 10 GHz (generally produced at 2.45 Ghz)	lamb's lettuce carrot apple strawberry	Suitable for surface modification	(Tolouie *et al.,* 2017) (Schnabel *et al.,* 2015)

Gliding arc discharge  (Khalili *et al.* 2018)	Knife edged two electrode made up of steel . Curved at the bottom	Thickness of electrode - 2 mm, length - 80 mm, width - 20 mm gap distance - 6 mm. gas flow rate - 4 lit/min frequency - 20 kHz voltage 8 -14 kV sample distance – 1.6 cm	Almonds Red apples	Irrespective of shape and size of product.	(Khalili *et al.,* 2018) (Niemira and Sites, 2008)
Corona discharge  (Wu *et al.,* 2018	Two electrode - pin to plate electrode Corona like discharge	Pressure- Atmospheric pressure Frequency	Raw Milk and UHT milk	It is applicable for small space with non-uniform distribution . Best for food application.	(Wu *et al.,* 2018) (Gurol *et al.,* 2012)

into the gap between the electrodes. Plume like plasma is created by broken and blown arc which is formed at small gap between the electrodes and the process continues by immediate formation of fresh arc for every cycle (Moreau *et al.,* 2008). Gliding arc plasma offers plasma action for products irrespective of their shape and size, as it uses knife shape electrode which is curved at the bottom. The gap between the electrodes is adjustable. Greater extent of thermal non equilibrium is assured by gliding arc plasma generation method. This plasma generation method offers high electron density and high electron temperature its outcome. The gliding arc plasma generation can be utilized for surface treatment of bulky materials. It also permits rapid processing. All these make gliding arc method potentially available selective chemical processes with good efficiency. Outcome of this process is extremely dependent on temperature of both, electrode and discharge.

Microwave discharge plasma

Plasma can be generated without using electrode through microwave. Microwave-driven discharge uses electromagnetic wave produced by magnetron generally at 2.45 GHz Frequency (Tolouie *et al.,* 2017). The electron present in gas gets energized by absorbing the microwave. Kinetic energy of electrons increased which leads to inelastic collision and ionization of gases ultimately (Schlüter and Fröhling, 2014). The plasma through microwave can be obtained at frequency of 300 MHz–10 GHz. Microwave plasma is one of the most suitable plasma to be used for surface modification as its ions having low energy (Kusano, 2009).

Corona discharge

Corona discharge is method that consist two sets of non - uniform electrodes that are fitted with a high-voltage gas ionisation system (Li *et al.,* 2004). Corona is limited emission which can be ignited on application of strong electric field at atmospheric pressure. Mainly the discharge of these setup is around the edges (sharp or pointed) of electrode, where electric field is high enough to excite the electron to level of ionization energy of gas (Phan *et al.,* 2017). Advantage of corona discharge is, it does not require complicated equipments can be produced with simple device also it is not expensive operation. But the disadvantage with it is applicable for small space with non-uniform distribution.

Parameters of cold plasma technology

The effectiveness of cold plasma process depends upon several factors, of which can be divided into equipment, process products parameter. All of these influence final result of the process. The parameters within the equipment such as plasma generation method, electrode characteristics, distance between electrode effects the final outcome of the process. During plasma generation certain parameter are needed to be controlled or maintained are called process parameter like mode of plasma application, gas composition, flow rate of gas, electrical power , frequency, voltage, relative humidity, mode of exposure of plasma, and time duration for which it is exposed. The product parameter involves composition of raw material, physical state of food, initial microbial concentration, shape, and structure.

Source of plasma

There are many generation sources for plasma-like dielectric barrier discharge (DBD) microwave discharge, radio frequency plasma, etc. Plasma generated through different methods has different characteristics. DBD is used for large surface applications (Phan *et al.,* 2017), the atmospheric pressure plasma jet is used where penetration is required. In addition, the electrode material used and the distance between them result in changes in the plasma generated. Lesser the distance greater will be the efficiency of the treatment (Moreau *et al.,* 2008).

Direct or indirect mode of application

The effectiveness of the operation depends upon mode of application of plasma. There are two ways of exposing plasma direct and indirect. Direct plasma application depicts more short-lived reactive species in its component which is having a life span of millisecond and might be involved in damaging interaction with cells (Misra and Jo, 2017). The indirect mode means plasma is generated in a separated chamber also known as remote or afterglow plasma. Indirect plasma involves more of longer living reactive species such as nitrogen and oxygen reactive species (Surowsky *et al.,* 2015). The amount of heat transferred and charged particles received in the sample is

comparatively less concerning the direct method (Misra *et al.,* 2011). The direct exposure of plasma is more efficient than indirect exposure also the indirect plasma generator is difficult to build and operate (Niemira, 2012).

Gas and their flow rate

The selection of gas for the operation is important as its composition is correlated with the generation of active species. Initially, Nobel gases were used for the plasma treatment because of their high thermal conductivity and ultraviolet ray emission, also it require low power for operation at atmospheric pressure. But nobel gas is expensive which opens the path for utilizing air and gaseous mixture to be used in the plasma generation process. The more the presence of oxygen and nitrogen in the operating gas mix the greater will be the amount of reactive oxygen and nitrogen species in the discharge, which ultimately affects decontamination efficiency (Misra and Jo, 2017). Gas flow rate on the other hand assures the delivery of reactive species into the target sample within their life span. Rapid flow rate enhances the rate of carrying short-lived reactive species into sample, assure enough mass transfer and collision rate for decontamination (Zhang *et al.,* 2017).

Power input & treatment time

The non thermal plasma is generally generated by utilizing electrical energy for increasing the energy of gas to reach the plasma state. The controlled input is required in the process for efficient output. Parameter such as frequency, voltage, power are positively correlated with the microbial reduction rate but with the use of high electrical power other food quality parameter might get disturbed, hence controlled input is required (Liao *et al.,* 2017). Another parameter that is needed to consider is treatment time. The time for which plasma product and sample remain in contact have direct influence on the microbial reduction rate (Nishime *et al.,* 2017).

Relative humidity

In decontamination process through plasma, relative humidity play essential role. Studies show that presence of water vapour enhances the generation of reactive species in the plasma product but excess of it may lead to dilution effect in the product. The generation of peroxyl group and OH group increased due to the added water vapour decomposition which thereby boosts the antimicrobial effect of the process (Guo *et al.,* 2015; Liao *et al.,* 2017).

Parameters related to food

The parameter other than process parameter which influences the effectiveness of non thermal plasma process is of food or sample itself. The chemical composition of food such as in food with high fat composition, reactive oxygen species will lead to oxidation (Saragapani *et al.,* 2017). The activity of plasma depends also upon whether food is in solid or liquid medium. In solid food, its activity is restricted to surface only but in liquid sample penetration into the sample is possible (Surowsky *et al.,* 2015). The penetratibilty and success of plasma action depend also upon physicochemical characteristics, moisture content and porosity of solid food whereas in liquid food volume of it coming into contact with plasma is important instead of penetration (Surowsky *et al.,* 2016). Other than these, the initial concentration and the type of microorganism into the sample also influence the process. Micro-organism which is in vegetative form and in exponential phase is more susceptible for destruction than those in stationary phase and sporulated form. Efficiency of microbe inactivation of plasma process is reduced with higher initial concentration of micro-organism. Inactivation of gram negative bacteria are more efficient than gram positive bacteria, hence the type of bacterial culture present in sample also affect the process. (Liao *et al.,* 2017). Difficulties caused by the irregular shape and structure of food for the treatment, as it provide site for growth or hide the micro-organism where the plasma is not able to reach (Misra and Jo, 2017).

Working mechanism of plasma for microbial inactivation

Relatively recent non-thermal technology cold plasma, which effectively inactivates microorganisms such as bacteria, their spores, biofilms and fungi. The inactivation of microorganism is done by plasma through activating three fundamental pathways. Those three possible-pathway are etching of surface of cell by the action of reactive species developed during the generation of plasma, degradation of genetic content and compound volatilization, inherent desorption by UV photon (Laroussi, 2005). The operating pressure, level of plasma discharge, plasma sources configuration are the decisive factor

for maximum possible contribution of UV radiation in cell death process (Misra and Jo, 2017). Chemical bond inside the micro-organism is broken and volatile compound such as CO and CHx, due to irradiation by UV also it may cause desorption within the cell (Schlüter and Fröhling, 2014). Etching of cell surface refers to inability of healing an injury not sufficiently fast which leads to cell death (Fig. 1). The injury caused due to cell surface interaction with energetic radical ion and reactive species. The activities of plasma as microbial destruction tool involve the interaction between plasma products and cell. Application of plasma activate many agents and chemical products such as free radicals (OH & NO) , reactive oxygen species (ROS), Reactive nitrogen species (RNS,) radiation of high energy, UV radiations, fluctuating electric field and charged particle all these act synergistically and make it impossible for pathogen to survive against such condition (Ehlbeck *et al.,* 2010). RNS and ROS of plasma effect directly on the outer membrane of cells. Availability of water effect plasma action the most, as it has been found on comparing with plasma action on moist cell and dry cell, maximum effect were detected in moist cell (Dobrynin *et al.,* 2009). It has been reported by Wiseman and Halliwell that on application of plasma, reactive oxygen species is formed directly in the region of DNA inside cell nucleus (Wiseman and Halliwell, 1996). The reactive species of plasma damage the deoxyribonucleic acid (DNA) by forming malondialdehyde (MDA) in microbial cell which is involved in DNA adduct formation (Dobrynin *et al.,* 2009). ROS leads to oxidation of amino and nucleic acid and also converts membrane lipid to unsaturated fatty acid peroxide as it found at surface of cell membrane it is most susceptible for reaction. (Liao *et al.,* 2017). Due to this reaction leakage of macromolecule associated with this lipid is occurred (Schlüter and Fröhling, 2014). On application of plasma, micro-organism experiences intense bombardment. OH and NO (Plasma radicals) get absorbed at surface of micro-organism and form compound like CO_2 and H_2O, which are volatile. These compounds induce surface defect, which cannot be healed by cell, results in death of cell. Also the membrane of cell is revealed to extreme electric field which creates electrostatic tension and that can induce rupture (Misra and Jo, 2017). The formation of pores helps in liberation of inner fluid and inhibit micro-organism healing activity, simultaneously the active species get their way into cell which can cause cell damage by destructing DNA, protein and other component inside the cell (Phan *et al.,* 2017). Higher membrane permeability resulted due to formation of pores, which directly affect the pH regulation of the cell, with acidification induced by humid plasma air. But this parameter is inessential factor for microbial inactivation as many microbes have buffer capacity due to cytoplasmic protein and very dense cytoplasm which maintain pH within cell (Moreau *et al.,* 2008). In any food there are two type micro-organisms present for which all these preservation techniques are required. One which deteriorates the end food product quality by various microbial actions and the other is which affect the health of one who consumes it. They are commonly known as spoilage micro-organism and pathogen respectively. In milk and milk product spoilage organism are generally psychrotropic bacteria such as bacterias of *Bacilli*, *Micrococci*, *Staphylococci species* and pathogen such as *Listeria monocytogenus*, *Bacillus subtilis* , *E.Coli* O157:H7, *Salmonella spp.* etc are checked for quality assurance. Preservation technique for milk and milk product are designed targeting these micro-organisms. Different research work has been done to check the efficiency of cold plasma treatment in reduction of microbial count of these micro-organisms. Table 2 show the list of different microorganism (which is also generally found in milk) inoculated on various substrate for study of capability of non thermal plasma in decontamination of specific microbes inactivated through cold plasma treatment. Cold plasma technique has been successfully studied for log reduction of microbes in the different substrate.

Working mechanism of plasma for enzyme inactivation

Defects in foods are not only caused by microorganism, the enzyme activities also lead to production of some off flavour, browning, vitamin loss etc. In order to make food preserved not only elimination of spoilage micro-organism is taken into consideration but also the enzyme residual activities should be considered (Mastwijk and Groot, 2010). Enzyme in milk and milk products

Table 2: Different microorganism inoculated on various substrates

Micro-organism	substrate	Plasma treatment	Log reduction /time	Reference
Bacillus cereus	-	DBD (3.5 W)	1.0 log	Bayrer *et al.,* 2020
B. coagulans	-	DBD (3.5 W)	3.3 log	
Listeria monocytogenes.	Sliced Ham	APP	1.73log /120 s up	Lee *et al.,* 2011
	Sliced cheese	APP	More than 8 log / 120 s	
Salmonella spp. Typhimurium KCTC 1925	Bacon	APP	1.7 log / 90 s	Kim *et al.,* 2011
Campylobacter jejuni, E.coli	Chicken skin	Pulsed gas plasma discharge	8 log /24 s up	Noriega *et al.,* 2011
S. aureus, E.coli, C. Albicans	Orange juice	DBD	5 log / 25s	Shi *et al.,* 2011
***E. coli* O157:H7**	Apple surface	NTAP	1.79log/ 15 min	Calvo *et al.,* 2020

***APP (atmospheric pressure plasma); CAPJ (cold atmospheric plasma jet); DBD (Dielectric barrier discharge); NTAP (Non thermal atmospheric plasma)**

degrade lipids, carbohydrates, and protein by their various actions which result in changes in texture, pH, flavour, colour etc. Some of those activities are desired and some are not desired in the milk and milk products. Enzymes such as lipase which hydrolyze fat and give rancid defect, some proteases that produce bitter flavour by degrading milk protein etc, are not acceptable and suitable for consumption as well as manufacturing of other milk products. Inactivation of these enzymes can be done with non thermal plasma. The mechanism for inactivation depend upon, interaction between reactive species and enzyme component, amount of reactive species produced, power of discharge, structure of enzyme etc. The inactivation of enzyme is mainly due to damage of specific bond or alteration of chemical structure due to action of reactive species. Which if left undamaged or unaltered, will produce secondary structure for catalysis of the chemical reaction (Misra *et al.,* 2016). Enzyme in milk are produced either by bacteria or it find its way into milk from blood of bovine. They perform specific function in the milk most of them are heat sensitive and get inactivated with certain temperature. Various research works has been successfully done for inactivating enzymes with atmospheric plasma application. In a study researchers have been deteriorated the immobilized lysozyme enzyme with plasma produced by mixture of oxygen and nitrogen gas. They hypothesized the destruction is due to the damage of the active site of enzymes by O_2 and N_2 plasma reactive species (Bernard *et al.,* 2006).

Alkaline phosphatase is one of the enzymes generally found in milk, which is used as parameter to check efficiency of pasteurization (Rankine *et al.,* 2010). In a research work alkaline phosphatase is subjected to DBD plasma treatment of 5 sec to 5 min in a discrete voltage of 40- 60 KV. It resulted in significant decrease in activity of alkaline phosphatase (Segat *et al.,* 2016). Different enzymes were studied for inactivation through plasma treatment. Table 3 enlists various enzymes that are also found in milk and treatment provided for their inactivation.

Application in dairy and food industry

Recently the effect of dielectric barrier discharge plasma on different MAP packaged ready to eat Ham was stidied where, L. monocytogenes has been reduced to >2 log with, 20% O_2 + 40% CO_2 + 40% N_2, MAP composition. Initial cell population on the surface of ham was 8 log CFU/cm^2. The reduction in microbial count was irrespective of formulation ham (Yadav *et al.,* 2020). In other study on the Korean rice inoculated with, *E. coli*, *S. typhimurium*, *Listeria monocytogenes*, and *Penicillium chrysogenum,* the rice was treated with plasma activated water the cell population reduced by E. coli 2.01–2.03 log CFU/g, S. Typhimurium 2.08–2.12 log CFU/g, *Listeria monocytogenes* 1.98–2.17 log CFU/g *Penicillium chrysogenum* ~2 log CFU/g (Han *et al.,* 2020). Whereas in the barley grain cheddar cheese DBD based atmospheric cold plasma effects germination parameter, the best result found with the 6 min treatment time (Feizollahi *et al.,* 2020).

Table 3: Various enzymes inactivated using cold plasma technology

Enzyme	Plasma generation method	Surface	Plasma treatement	Gas used	Result	Reference
Lysozyme	Microwave plasma	Enzyme immobilised on polystyrene 96 well plate	(Power: 300 W ,Frequency: 915) Time : 0 - >800 sec	Nitrogen + oxygen	destruction and desorption of Significant protein	Bernard, *et al.,* (2006)
Lysozyme	Low pressure inductively coupled plasma	Si wafers, glass slides, gold and polystyrene plates	Pressure: 10 Pa; RF power: 200 W	Ar, Ar+N2, Ar+O2 mixtures	Ar+O2 was most effective in etching the enzyme deposits	Kylian *et al.,* (2008)
Lipase (from *Candida rugosa*)	Radio-Frequency (RF) atmospher icpressure glow discharge (APGD)	Stainless steel	Power: 180 W RF Flow rate: 10 L/min; Temperature: <57 °C; Operation time: 0-50 s	Helium	Activity of the lipase increased significantly after 1 min; Enhanced activity attributed to change in secondary and tertiary structures of protein	Li *et al.,* (2011)
α-chymotrypsin	Cold plasma jet	Aqueous solution (Buffer)	Frequency: 60 Hz; Operation time: 5 min	Air	Secondary structure changes; β-strands decrease	Attri *et al.,* (2012)
Alkaline phosphatase	Dielectric Barrier Discharge	Buffered solution	Voltage: 40-60 kV; Frequency: 50 Hz; Operation time: 0-5 min;	Air	Voltage and time dependent inactivation; Inactivation follows a sigmoidal logistic function	Segat *et al*., (2016)

Various research works has been performed to check applicability of non thermal plasma process in dairy industry as a mean of preservation technique. The inactivation ability of the cold plasma technique is checked by inoculating strains of micro-organism that are generally found in milk and milk products and is evaluated for inactivation through various plasma methods. Gurol *et al.,* (2012) in their experiment inoculated *Escherichia coli* ATCC 25922 in milk with different fat content with an objective to check capability of low temperature plasma in killing *E. Coli*. Corona discharge plasma for this experiment. The system consist of two tungsten electrode, one is rotating above with the help of dc motor, the other one is dipped into milk, plasma is generated between the milk and upper electrode tip. Power of 9 kV AC is supplied in the system. The temperature

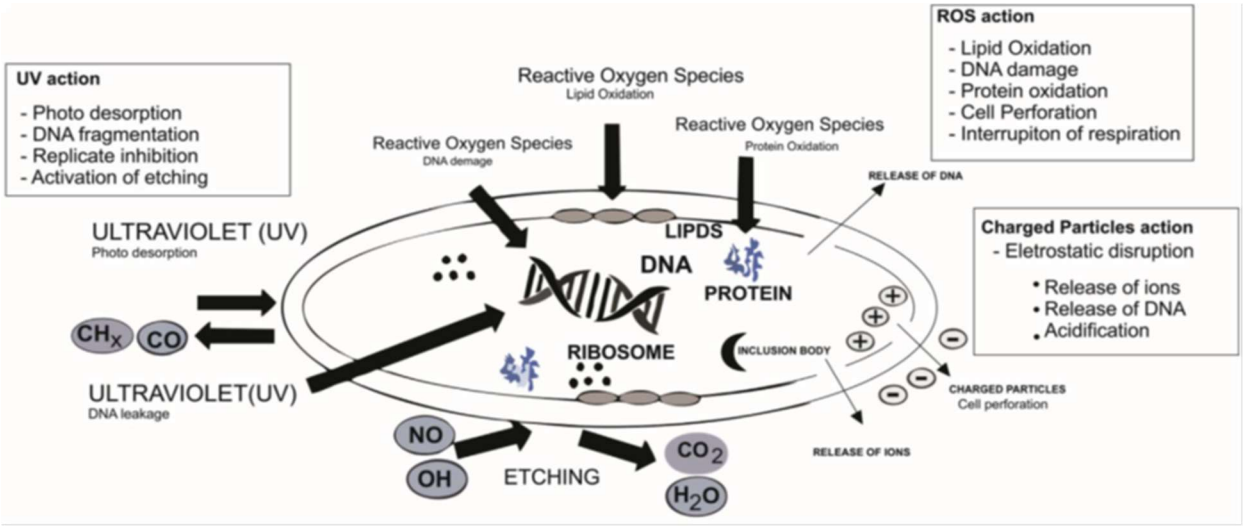


Figure 1: Overview of cold plasma mechanisms involved in microbial inactivation. Adapted from Schlüter and Fröhling, 2014.

during the process was maintained below 35°C. The time dependent effect of plasma process is checked at time interval of 0, 3, 6, 9, 12, 15, and 20 min. They observed that there is significant reduction of 54 percent in E.coli population in just after 3 min. There is no remarkable difference in the result due to distinct fat content of the sample. Initial count was 7.78 log CFU/mL which has been reduced to 3.63 log CFU/mL after 20 min of treatment. After one week no viable cell is detected and it last as such for 6 week storage period. Also other quality parameter is evaluated such as pH and color properties of the sample are not significantly altered by the treatment. In another experiment, kim *et al.* (2015) in addition *E.coli* two other pathogen namely *L.monocytogenes* and *Salmonella typhimurium* is inoculated into the milk. To eliminate microbes dielectric barrier discharge (DBD) method was chosen. The setup for encapsulated DBD plasma generation involves a parallel-piped rectangular container of plastic inside which sample was kept. 250W power is provided to the system and voltage at 15 kHz bipolar waveform is given to one electrode and the other one was grounded. Treatment of plasma is given for 5 and 10 min. Initially the aerobic bacterial count was 0.98 log CFU/mL after treatment no viable cell were observed on both 5 and 10 min treated sample. The load of 6.28, 6.43, and 6.21 log CFU/mL was calculated of *E. coli*, *L. monocytogenes*, and *S. Typhimurium* on milk after treatment of DBD plasma, microbial count of pathogens was reduced to 2.43, 2.40, and 2.46 respectively. Researcher also observed that the milk pH decreased and L*(lightness) and b*(yellow/blue coordinate) values of hunter color for milk increased were as a*(red/green coordinate) value decreased after 10 min of encapsulated DBD plasma treatment.

The evaluation of non thermal plasma technology for decontamination effect is not just limited to milk but researcher performed experiment to evaluate the effect of plasma into solid milk product such as cheese. In a research study by Lee *et al.,* (2012), UV sterilized cheese slices were inoculated with *Escherichia coli* and *Staphylococcus aureus* strain. DBD plasma for treatment is generated at 3.5 kVpp and a bipolar low-frequency voltage is maintained at 50 kHz. Helium and a mixture of helium and oxygen gas are used to improve the inactivation effect. Plasma treatment was given to the sample for 1, 5, 10, and 15 min. Other quality parameters such as color parameters and sensory characteristics were evaluated. Significant log reduction in the microbial count of E.coli ranging from 0.09 to 1.47 has been observed with the helium and 0.05-1.98 log with the Helium oxygen mixture gas .S. Aureus reduced logarithmically 0.05- 0.45 log and 0.08- 0.91 log with helium and mixture of helium-oxygen gas respectively. Whereas a considerable increase in b* value and decline in L* value were observed in the study. The researcher also discovered that cheese slices got damaged after 10 and 15 min of the

treatment and there is a remarkable reduction in the sensory attribute of the cheese slices which includes flavor, odor, and acceptability. Experiment outcomes show that with the addition of oxygen there is an increase in pathogen inactivation ability although the effect was limited.

Cheese slice were also evaluated for inactivation of pathogen through encapsulated dielectric barrier discharge. Slices were inoculated with *E coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. Encapsulated DBD plasma is generated in plastic rectangular container though electrical power of 250 W and voltage at frequency 15 kHz. No viable cell was detected after for 90 s, 60 s, and 10 min respectively for pathogen. After treatment of 10 min no visible damage is evident on the cheese slice. Logarithmic reduction in microbial count of *E coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* 2.88, 3.11, and 2.26 were observed respectively after 15 min of plasma treatment. The results show that pathogens were successfully reduced and inactivated by the DBD plasma system in cheese slices (Yong *et al.,* 2015). In another study cheddar cheese slice was inoculated with pathogen strain *Escherichia coli* O157:H7), *Salmonella typhimurium*, and *Listeria monocytogenes* with DBD plasma (flexible thin layer) with input 100 W power, the voltage at 15 kHz frequency for 0, 2.5, 5and 10 min of duration. After 10 min of treatment notable microbial count reduction of 3.2, 2.1, and 5.8 logs CFU/g has been observed for pathogen strain respectively (Yong *et al.,* 2015).

Recently the effect of cold plasma has been analyzed on tofu by treating it with plasma-activated water, the polyphenol retained was 80 % of the initial content also, the gumminess reduced up to 32% and immediate softening has occurred in the tofu (Frías *et al.,* 2020).

Cold plasma application on packaging material

Packaging material protects the food from physical damage, contamination also restricts the movement of moisture, gas from both sides (Zhang *et al.,* 2020). Packaging material remains in direct contact with the food. That is why the pre-packing sterilization of packaging materials is needed (Ganesan *et al.,* 2021; Peng *et al.,* 2019). Cold plasma processes involve surface treatment hence used in sanitization of packaging material surface (Banu *et al.,* 2012). Plastic bottles, films and lids can sterilized using non thermal plasma as it offers rapid and safe processing without unfavourably altering the characteristics of material and without any residue leaving behind. In recent years, cold plasma has been employed to improve the functionality and interfacial properties of biopolymers (Bahrami *et al.,* 2020).

There are two types of cold plasma treatment for sterilising food packaging materials: direct treatment and indirect treatment. Materials of interest are placed into the plasma discharge region and sterilised by exposure to active species created within the plasma region, as well as high-energy photons such as UV radiation, during direct treatment. Indirect treatment, on the other hand, places the materials to be sanitised outside of the plasma discharge region, allowing some of the active species departing the plasma region to coat the material's surface, similar to surface modification treatments (Peng *et al.,* 2019). The effect of cold plasma is lethal to bacterial cells, resulting in the death of microorganisms (Corradini, 2020); nevertheless, the ionised gas created by plasma is not lethal to fresh produce cells or tissues. This plasma characteristic inhibits mutant cells from forming following treatment, providing some useful information on the effects of plasma treatment on biological materials (Gavahian and Khaneghah, 2019). A plasma's ability to transport energy is determined by its chemical composition and temperature (Hosseini *et al.,* 2020). So, plasma has also been utilised in the packaging industry to modify polymer structure in order to acquire desired qualities in packaging materials (Pankaj *et al.,* 2014). Both Gram-positive and Gram-negative bacteria are inactivated differently by cold atmospheric plasma (CAP), according to studies. The major goal of using CAP is to protect minimally processed goods (such as fruits and vegetables) from microbial and chemical contamination (Sarangapani *et al.,* 2017; Ganesan *et al.,* 2021).At limited exposure times, CAP treatment lowers the total bacterial count in fresh fruits (Rana *et al.,* 2020) and meat products (Kulawik *et al.,* 2018). Cold plasma decontamination, preservation, and sterilisation of

food products is an innovative and extremely dependable technology without affecting the food or the package's properties (Peng *et al.,* 2019) Because it is a dry, ideal for in-line processing, easy to produce and control, chemical-free, and waste-free method, it can be considered a viable technique for improving the attributes of edible films and packaging material (Bahrami *et al.,* 2020).

The non thermal plasma as name suggest operate at low temperatures, that make it acceptable for treatment of heat sensitive packaging materials such as polythene, polycarbonate etc. Surface of food packaging polymer should be more hydrophobic and surface energies should be low (Vesel and Mozetic, 2012). Deilmann *et al.,* (2008) sterilized polyethylene terephthalate bottles by projecting microwave plasma gas mixture into the bottle. For so they had developed a plasma reactor setup which offers three dimensional movement of plasma gas into bottle. Mixture of hydrogen, nitrogen and oxygen had been used in the process. Microbial reduction of 105 and 104 CFU for Bacillus atrophaeus and Aspergillus niger respectively, has been resulted from microwave plasma treatment of fewer than 5 seconds (Deilmann *et al.,* 2008). Inactivation of E. coli K12, P. Aeruginosa, S. Aureus from the surface of polypropylene by glow discharge plasma has been reported by Gadri *et al.,* (2000). Also, sterilization of packaging material such as polystyrene (PS) films, PET, multi-layer packages like PET/polyvinylidene chloride (PVDC)/polyethylene (PE) through plasma has been reported by Muranyi *et al.,* (2010). Various studies have been done to study the changes caused by the treatment of plasma on packaging materials such as polypropylene, low-density polyethylene, High-density polyethylene terephthalate. Studies revealed that plasma treatment changes in properties such as an increase in weight, roughness, crystallinity, and decrease in contact angle, wettability, aging effect for different packaging materials (Thirumdas *et al.,* 2015).

Conclusion

The non-thermal plasma has proven its potential in the decontamination or microbial load reduction for various food materials including milk. Results of various researches revealed that cold plasma technology can deal with pathogens and spoilage bacteria. Not only bacteria it is possible to inactivate the residual activities of enzymes in foods utilizing this technology. This review paper converses various research work and studies done on milk and milk products revealing the fact that with Nonthermal plasma or cold plasma process decontamination is done, in less amount of time without reaching high temperature. But the application of this technology is not only restricted to product sanitization or decontamination. Although there are certain limitations with this process and the need for a lot of research work in standardizing the process for the desired outcome without disturbing the quality parameter of food.

Conflict of interest

The authors declare that they have no conflict of interest.

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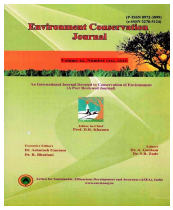

Efficacy of oral probiotics on morphometric measurements and their allometric relationships in Asian elephants

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ABSTRACT

An experiment was undertaken on 18 Asian elephants to study the effect of oral probiotics on body measurements for two months. Simultaneously, the efficacy of existing prediction equations and allometric relationship of heart girth-body weight (BW), height-forefoot circumference (FFC) and height-body weight were also observed. The animals were divided into three groups, with six each. The experimental probiotics; *Lactobacillus acidophilus* and *Saccharomyces cerevisiae,* were supplemented @ 1 gm 1 × 10^9 cfu/gm for every 50 kg BW/day to the elephants of LACTO (T_2) and SAC (T_3) groups, respectively, whereas no probiotic was given to the control group. Heart girth was measured four times, on days 0, 20, 50 and 60 of the experiment to determine BW. Other morphometric estimations, like length, height, hind girth, and FFC were documented once, at the end of study. The data of heart girth and body weight revealed non-significant effect of the treatment. Irrespective of probiotics treatment, allometric parameters such as heart girth-body weight and height-FFC showed an isometric relationship whereas, the height-body weight relationship wasn't found to yield an equivalent accuracy. The equations involving heart girth and FFC were observed to be most authentic to calculate BW and height, respectively.

Introduction

Several studies have revealed about microbiota's indispensable role in disease control, homeostasis and health promotion (Alayande *et al.,* 2020). Predominantly, dose and duration of the treatment as well as the microbial strains, are among the vital components impacting the competence of probiotics (De Cesare *et al.,* 2017). To the investigators' information, no such research trials have been performed on endangered elephants yet.

The body sizes usually vary in correlation with another related variant by way of exponential scaling, called as allometry (Anzai *et al.,* 2017). The aspect of allometric relationships could be profitably used in various ecological studies, which may involve ageing wild elephants or estimating biomass of the population. Precise computation of body weight (BW) is advantageous for the assessment of well-being, nutritional condition, feeding program, chemical immobilization and medication for the treatment (Kanchanapangka *et al.,* 2007). Nonetheless, it is impracticable to weigh enormous sized elephant due to tremendous BW. Weighing the earth' largest living animal is a challenging work, as it needs a distinctive training plan, proficient drivers and appropriate scales. Hence, the only approach to calculate their BW is by applying prediction equations based on definite body variables (Sukumar *et al.,* 1988; Hile *et al.,* 1997). Therefore, the study was conducted with the objectives to assess the effect of probiotics feeding on morphometric measurements and it also examined the efficacy of existing prediction equations as well as their allometric relationships in Asian elephants.

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Material and Methods

The experiment was organized with the prior permission of the Additional Principal Chief Conservator of Forest and Chief Wildlife Warden, Government of Rajasthan, Jaipur (India). The study protocol was duly approved by the Institute Animal Ethics Committee (PGIVER/IAEC/I9-05) and performed in accordance with relevant guidelines and regulations for care and management during the experiment (MoEF and CC, 2008).

Selection of experimental animals

Eighteen healthy, captive adult female Asian elephants with alike BW (3495 ± 133.34 kg) were divided into three groups of six elephants each. The average age composition in T_1, T_2 and T_3 groups was 44, 42.50 and 48 years, respectively. The group wise details of experimental elephants are given in Table 2. All the elephants were housed in a hygienic and well ventilated individual enclosure, with a separate feeding arrangement.

Experimental feeding

The experiment was planned for 60 days, in which, ten days adaptation period was observed and then elephants were placed on three dietary experimental feeds for 50 days of digestibility trial. During the digestibility trial, experimental probiotics *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were administered @ 1 gm 1×10^9 cfu /gm for every 50 kg BW per day orally along with basal feed to all the experimental elephants of LACTO (T_2) and SAC (T_3) groups, respectively. The group T_1 was CONT group (control) received no probiotic.

Morphometric measurements

Measurements of the heart girth were recorded randomly on days 0, 20, 50, and 60 of the experiment to estimate body weight (Figure 1). Other morphometric estimations, like length, height, hind girth, and FFC were recorded at the end of the experiment before feeding and watering. The chest circumference around the thoracic cavity behind the elbow was considered as girth of the animals. It was measured with care taken to ensure it was not affected by inhalation by the elephant. The length was measured between the base points of the trunk along the curvature of the back to the base point of the tail. The straight-line interval between the rod and the earth was measured as height (Figure 2). The circumference of hind girth was measured in front of the wing of the ilium. The FFC was measured at the widest point of the right forefoot, including nails, at the level of sole. Body weight was calculated as per Hile *et al.* (1997), applying the following formula:

Body weight (kg) = 18.0 × Heart girth (cm)-3336

Height-body weight relationship was calculated as per Sukumar *et al*. (1988), applying the following formula:

Body weight (kg) = $\{(0.06 \text{ height in cm}) - 0.335\}^3$

Height-FFC relationship was calculated as per Sukumar *et al*. (1988), applying the following formula:

Height (cm) = 2.03 FFC

Statistical analysis

All the statistical analysis of data was performed using SPSS 16. The difference among groups was calculated by one way ANOVA. The significant effects of different means were compared by Duncan's Multiple Range Test. Significance was defined at $P < 0.05$. All the values represent mean ± standard errors of the mean (Snedecor and Cochran, 2004).

Results and Discussion

In the present study, the heart girth and body weight were recorded as an ancillary observation to ascertain the effect of feeding probiotics on elephants' physical health. The accomplishment of morphometric variables could be measure of the animal's nutritional condition and considered as an index of an animal's health. The results of heart girth and body weight, as shown in Table 1, revealed non-significant effect of the treatment. Non significant differences regarding the heart girth and body weight were recorded in the Asian elephants of different groups. The body weights recorded at different periods showed more or less similar results. In agreement to this, no significant differences were also observed in the weight changes in probiotics supplemented captive cheetahs (Koeppel, 2004), rats (Hamad *et al.,* 2009); horses (Agazzi *et al.,* 2011); dogs (Marelli *et al.,* 2020). In contrast to the present study, karimi *et al*. (2013) reported a significant reduction in weight gain in animal models. Whereas, significant increase ($P < 0.05$) in the body weight was recorded in the *Saccharomyces cerevisiae* fed rabbits (El-Badawi, 2018; Ahmad *et al.,* 2019).

Table 1: Average values of heart girths and body weights in the Asian elephants

Period	T_1	T_2	T_3	Overall	P-value
Heart Girth (cm)					
0 day	375.17± 12.18	378.83 ± 10.82	384.50 ± 16.90	379.50 ± 7.41	0.887
20 days	376.50 ± 11.37	379.33 ± 10.91	385.67 ± 16.23	380.50 ± 7.14	0.880
50 days	378.33 ± 10.49	378.83 ± 10.52	388.17 ± 15.71	381.78 ± 6.86	0.823
60 days	379.33 ± 10.45	378.33 ± 10.70	388.83 ± 15.35	382.17 ± 6.81	0.806
Body weight* (kg)					
0 day	3417 ± 219.25	3483 ± 194.78	3585 ± 304.12	3495 ± 133.34	0.887
20 days	3441 ± 204.69	3492 ± 196.28	3606 ± 292.19	3513 ± 128.59	0.880
50 days	3474 ± 188.82	3483 ± 189.32	3651 ± 282.79	3536 ± 123.45	0.823
60 days	3492 ± 188.08	3474 ± 192.67	3663 ± 276.30	3543 ± 122.55	0.806

* **calculated body weight as per Hile *et al.* (1997)'s prediction formula**

Table 2: Details of the morphometric measurements of the Asian elephants.

Name of elephant	Reg. no.	Age (yrs)	Heart girth (cm)	BW* (kg)	Actual BW (kg)	Length (cm)	Height (cm)	Hind girth (cm)	FFC (cm)	Height/ FFC ratio
Jaimala	11	41	353	3018	3088	645	230	410	114	2.02
Rajrani	20	56	352	3000	2994	663	255	405	118	2.16
Phoolwanti	116	30	385	3594	3520	725	253	448	128	1.98
Jhomati	53	48	402	3900	3881	798	236	434	130	1.82
Chameli	123	44	370	3324	3341	702	241	415	126	1.91
Jaytara	92	45	414	4116	4066	750	269	449	134	2.01
Laxmi	125	47	352	3000	3089	709	248	405	122.5	2.02
Laxmi	130	52	350	2964	3103	738	258	408	122	2.12
Anno	93	48	365	3234	3268	715	251	418	119	2.11
Tami	109	35	391	3702	3779	730	248	429	121	2.05
Gomati	81	33	400	3864	3791	751	256	443	128	2.00
Shobha	96	40	412	4080	3998	773	270	457	135	2.00
Bhogwati	30	49	333	2658	2722	660	221	375	120	1.84
Champa	105	33	362	3180	3239	682	228	412	120	1.90
Rangoli	43	44	402	3900	3812	790	260	465	124	2.10
Majani	55	50	390	3684	3742	727	253	441	123	2.06
Champakali	52	50	404	3936	3852	799	282	468	132	2.14
Chanchal	9	62	442	4620	4440	780	274	453	134	2.05
Overall Mean ± SEM		44.83± 0.45	382.17 ± 6.81	3543 ± 122.55	3540.28 ± 104.23	729.83 ± 11.24	251.83 ± 3.86	429.72 ± 5.93	125.03 ± 1.45	2.02 ± 0.002

* **calculated body weight as per Hile *et al.* (1997)'s prediction formula**
FFC- forefoot circumference

The possible reason for not achieving the desired result can be viability of strains and short duration of the intervention. Mechanisms by which probiotics might regulate body weight have not been clearly understood. Irrespective of probiotics treatment provided, the study also examined the efficacy of existing prediction equations for estimating weight and height, as shown in Table 2. The predicted body weights were found to be more or less similar to the actual weights of the elephants. The height was observed as twice the FFC measured at the sole. The height/FFC ratio was observed to be 2·02. The predicting approaches based on morphometric measurements have been applied in many species like black rhino, elephants, and zebu cattle with the aims of nutritional formulation, herd management, and medication in circumstances where factual weighing is not feasible (Freeman and King, 1969; Sreekumar and Nirmalan, 1989; Lesosky *et al.,* 2012). Heart girth has been proved to be authentic

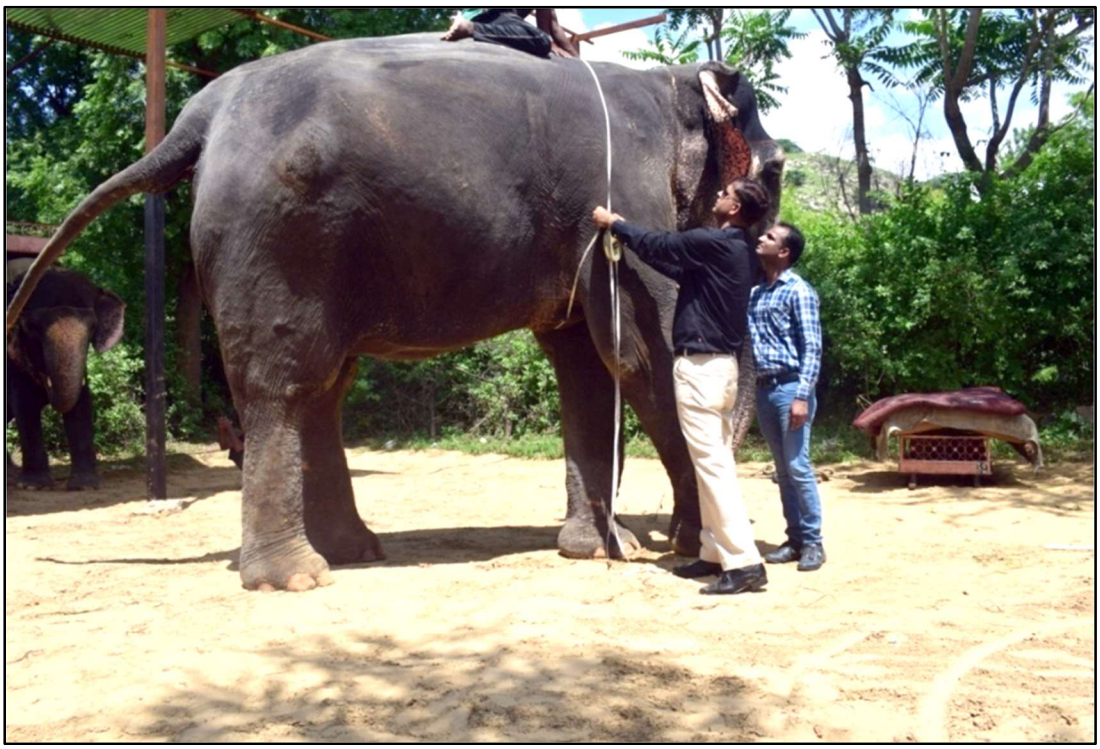

Figure 1: Measurement of heart girth

Figure 2: Measurement of height

predictor of body weight (Hile *et al.,* 1997; Lesosky *et al.,* 2012). In the present experiment, the predicted body weights were found to be more or less similar to the actual weights of the elephants which confirm the existing prediction equation formulated by Hile *et al*. (1997) whereas, the results were not in agreement with Sukumar *et al*. (1988). In addition to this, the data of predicted height was found as twice the forefoot circumference measured at the sole, which also confirm the existing prediction equation for height (Sukumar *et al.,* 1988). Allometric parameters such as heart girth-body weight and height-FFC relationship showed a proportionate isometric relationship in all the elephants, as shown in Table 2, which coincides with the observations of Hile *et al*. (1997) and Sukumar *et al*. (1988), respectively. Contrary to this, the height-body weight relationship was not found to yield an equivalent accuracy in elephants (Hanks, 1972; Sukumar *et al.,* 1988).

Conclusion

No statistical difference due to probiotics treatment regarding the body measurements was observed. Though, the body weights and height of elephants can be authentically estimated from several morphometric measurements. The predicted body weights were found to be more or less similar to the actual weights as well as height was found as twice to FFC which confirm the existing prediction equations. The heart girth and FFC were indicating an isometric relationship with body weights and height of elephant, respectively. The height- body weights relationship was not found to yield an equivalent accuracy in elephants. The results may have significant importance for size estimation of large wild animals in the field, as well as for management in captivity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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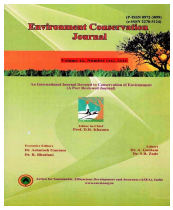

Yield and economic response of *Rabi* maize (*Zea mays* L.) to different mulching and nutrient management

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ABSTRACT

An experiment was conducted during *Rabi* season of 2019-20 with the objective of evaluating the effect of mulching and nutrient management practices on growth, yield and economics of maize (*Zea mays* L.) at Balindi Research Complex, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India on clay loam soil. The experiment was laid out in Randomized Block Design with nine treatment (T) combinations such as T_1- Live mulch (*Trifolium alexandrium*)+50% recommended dose of fertilizer (RDF) [120:60:40 kg /ha N, P_2O_5 and K_2O], T_2- Live Mulch+75% RDF, T_3- Live Mulch+100% RDF, T_4- Straw Mulch (rice straw)+ 50 % RDF, T_5- Straw Mulch+75% RDF, T_6- Straw Mulch+100% RDF, T_7- No Mulch+50% RDF ,T_8- No Mulch+75% RDF and T_9- No Mulch+100 % RDF, replicated thrice. Experimental results revealed that different mulching and nutrient levels exerted significant influence on growth, yield, net return and benefit-cost ratio (B:C). Application of straw mulch+100% RDF (T_6) resulted in the highest plant height (164.57 cm), grain yield (5.28 tonnes /ha), stover yield (7.65 tonnes/ha) and B: C (2.16), however, treatment T_7 recorded the lowest grain and stover yield. So, the integrated application of straw mulch along with 100% RDF could be recommended for better yield and higher profit of *Rabi* maize. Integration of organic mulch might be useful for long-term soil health benefits for the nutrient exhaustive maize crop.

Introduction

Maize is an important cereal crop ranking third after wheat and rice in area and production globally (Olaniyan, 2015). It is also familiar as the "Miracle Crop" or "Queen of Cereals" due to its high productivity potential among the cereal crops. In India, maize is grown on area of 9.47 M ha with production of 28.72 Mt and productivity of 3.03 tonnes/ha (Anonymous, 2017). The predominant *Rabi* maize growing states are Andhra Pradesh (45.5%), Bihar (20.1%), Tamil Nadu (9.3%), Karnataka (8.5%), Maharashtra (7.7%) and West Bengal (5.3%) (Anonymous, 2012). Rice being the major staple food of India which mainly comes from the winter paddy grown in *Kharif* season. So, it would be a great opportunity to promote maize cultivation in other seasons such as *Rabi*. *Rabi* season maize yielded invariably higher (>5 tonnes/ha) than *Kharif* season maize (2-2.5tonnes/ha) due to long duration of growth period and least infestation of pests and diseases. In recent years, significant changes have occurred in maize production and utilization due to increasing commercial orientation of this crop and rising demand for diversified end users, especially for feed and industrial uses. A sizable number of districts (110 districts), have potential for growing winter maize in the *Rabi* maize growing states. But, low temperature during early growth period, imbalanced use of fertilizers, low input use efficiency and high cost of cultivation are the prime

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factors of low yield of maize during the *Rabi* season. The continuous increase in the cost of chemical fertilizers has forced the farmers to resort to imbalanced nutrition of crops and thus reduction in crop yields in India. Due to nutrient based subsidies by the Government on fertilizer, farmers are convinced to apply higher doses of nitrogen containing fertilizer particularly urea. At this critical juncture, optimising nutrient use to sustain crop production without affecting soil health and protection of environment from pollution is highly required. Maize being a heavy feeder as well as nutrient exhaustive crop requires more nutrient compared to other cultivated cereals. Thus, optimizing nutrient dose for the crop will help to obtain a good yield with economic advantage. Reducing the cost of fertilizers not only have an economic advantage but also helps in conservation of soil by reducing the harmful effect caused by the excessive fertilizer use. Mulching is one of the best agronomic strategies which can be easily utilized at farmer's level for increasing input use efficiency and optimizing crop yield. Mulching practices protect the soil surface from direct radiation of the sun there by reduces evaporation, improves soil moisture content and also controls weed infestation. It maintains or improves the physical, chemical and biological properties of soil by protecting it from direct impact of raindrops, promotes soil microbial activity, soil organic content, soil aggregate formation and greatly reduced soil runoff as well as wind erosion (Liang *et al.,* 2002). It has also been reported to decrease diurnal soil temperature variations (Dahiya *et al.,* 2007). Therefore, keeping above facts in mind the present study was planned and carried out to evaluate the effects of mulching and nutrient management on crop performance and economics of *Rabi* maize.

Material and Methods

The field experiment was conducted at Balindi Research Complex, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal during *Rabi* season of 2019-20 (96′ N latitude, 88° 53′ E longitude, 9.75 m MSL). It lies in the New Alluvial Zone of West Bengal and the region is characterized by tropical and semi-humid climate with annual rainfall about 1350 mm. Experimental field had fairly levelled medium-upland topography with good drainage system and clay loam soil having slightly alkaline pH (7.2) with medium fertility status. The experiment was laid out in Randomized Block Design with 3 replications, comprising of 9 treatment combinations; T_1- Live mulch (*Trifolium alexandrium*) +50% recommended dose of fertilizer (RDF), T_2- Live Mulch+75% RDF, T_3- Live Mulch+100% RDF, T_4- Straw Mulch+ 50 % RDF, T_5- Straw Mulch+75% RDF, T_6-Straw Mulch+100% RDF, T_7- No Mulch+50% RDF, T_8- No Mulch+75% RDF and T_9- No Mulch+100 % RDF. The recommended dose of fertilizer (RDF) is 120:60:40 kg/ha N, P_2O_5 and K_2O. Maize cultivar 'VMH-45' was sown on 2nd fortnight of November, 2019 with a spacing of 60cm × 20cm in 6m × 4m plots with a seed rate of 20 kg/ha by dibbling. For Fertilizer management a recommended dose of 120: 60: 40 kg/ha N: P_2O_5: K_2O for *Rabi* maize was applied as per treatments in the form of Urea, Diammonium Phosphate and Muriate of Potash respectively. 1/3rd nitrogen of the full dose of P_2O_5 and K_2O were applied as basal by broadcasting and rest 2/3rd dose of nitrogen was top dressed by band application at knee high and tasseling stage of the maize crop in equal doses. In plots with live mulch treatments, berseem was sown in the inter row spaces of maize. Straw mulch was applied at 7 DAS in the inter-row spaces in the respective plots.

The growth attributes viz., plant height (cm), leaf area index (LAI), dry matter/plant and crop growth rate (CGR) were recorded at different growth stages from five randomly tagged plants in each plot. Yield attributes *viz.,* cob length (cm), cob girth of cob (cm) recorded at harvest. Yield of grain and straw (kg/ha) were recorded after harvest from net plot. Shelling (%) and Harvest Index (HI) (%) were computed as

$$Shelling\ \% = \frac{Grain\ yield\ (q/ha)}{Whole\ cob\ weight\ (q/ha)} \times 100$$

$$HI = \frac{Economic\ yield\ (kg/ha)}{Biological\ yield\ (kg/ha)} x\ 100$$

Data recorded from various observations were statistically analyzed by adopting appropriate method of "Analysis of Variance". The significance of the treatment effect was judged by 'F' test (Variance ratio) and difference of the treatments

mean was tested using critical difference (C. D.) at 5% level of probability (Sahu, 2013).

Results and Discussion

Mulching and nutrient management significantly affected plant heights, LAI at 90 DAS and CGR at 60-90 DAS (Table-1). Long stature plants (164.57 cm) were produced at 90 DAS from T_6 (Straw Mulch+100% RDF) treatment, which was statistically at par with T_3 (Live Mulch + 100% RDF) treated plots with average plant height (160.32 cm). The increase in plant height with 100 % RDF doses might be attributed to increased length of the internodes due to more cell division and cell elongation which in turn resulted in higher plant height. Highest LAI (3.70 at 90 DAS) was obtained in T_6 treatment, where straw mulch along with 100% RDF was applied, which was statistically similar to T_3 and T_9 treatment. Better soil moisture conserved under mulches with higher moisture reserve noticed under straw mulch which helped in better utilization of fertilizers and moisture which resulted in higher leaf area index in maize due to larger leaf size and broader expansion of leaf blades. This corroborates the findings of Ravindranath *et al*. (1974) and Ahmed (1989). At 90 DAS highest dry matter accumulation was found in the plants of T_6 treatment (Table-1) whereas lowest dry matter accumulation (63.32 g/plant) was recorded in T_7 treatment. The possible reason may be that straw mulches created favourable soil temperature and soil moisture conditions which encouraged higher nutrient use efficiency, that in turn, increased the dry matter accumulation in plants, reported by Khan and Pervej (2010) and Jena *et al*. (2014). Among all the date of observations taken, maximum CGR (15.53 g/m^2 /day) was recorded at 60-90 DAS at elevated 100% RDF level along with straw mulching (T_6 treatment) which was statistically similar to T_3 and T_9 treatment. The reason for superior CGR in T_6 treatment may be due to synergistic effect of mulching and 100 % recommended dose of fertilizer that resulted in increased availability and absorption of nutrients through production of growth promoting substances and greater accumulation of dry matter another possible reason of the improvement in the crop growth of maize under mulching could also be due to moisture conservation for long time and the suppression of weeds, thus competition for available resources was greatly reduced. These results are in accordance with the findings of Pramanik (1999) and Sawant (1992). Different level of fertilizer and mulching did not significantly influence the length of cob and cob girth (Table-1), however maximum cob length (15.30 cm) was recorded in T_3 (Live Mulch+100% RDF) treatment. Highest cob girth (13.86 cm) was obtained from T_6 treatment (Straw Mulch+100% RDF). The mean data revealed that cob length and girth increase with increased fertilizer levels along with mulching. This might be due to moisture conservation and reduction of weed infestation which influenced higher utilization of resources during cob formation stage. These finding are in agreement with Lal (1995) and Avval and Khan (2000).

The highest grain (5.28 tonnes/ha) and stover yield (7.65 tonnes/ha) were obtained from T_6 treatment (Table-2). Significant increase in the grain yield of maize under these treatments was due to significant increase in yield components like number of grains per cob, cob length, cob girth and hundred grain weights (seed index). This might be due to moisture conservation and reduction of weed infestation which helped in utilization of the resources from the soil in a better way. These finding are in agreement with Bhatt *et al.* (2004). The mean data also revealed that 100 % RDF and straw mulching significantly improve stover yield due to the combined effect of fertilizer and along with favourable and adequate moisture available to the crop around the root zone due to presence of mulch. Combined application of N, P_2O_5 and K_2O is beneficial in significantly increasing the dry matter yield of maize as observed by Prasad (1981), Ravindranath *et al*. (1974), Singh and Singh (1984) and Karki *et al*. (2005). Harvest index (%) and shelling % of maize significantly varied among the treatments. Treatment T_6 exhibited the maximum harvest index (37.49 %), which was statistically at par, with T_3 treatment. Higher values of harvest index indicated greater partitioning of photosynthates and other essential elements towards reproductive development and economic yield. The findings were supported by Khan and Pervej (2010). The treatment T_6 recorded maximum shelling percentage (85.09 %) which was statistically similar with treatments T_3 and T_9. which is in close conformity with the findings of Singh and Singh (1984). Results revealed that net

Table 1: Effect of different mulching and nutrient management on plant height, LAI, dry matter accumulation (DMA), CGR, cob length and girth in *Rabi* maize.

Treatments	Plant height at 90 DAS (cm)	LAI at 90 DAS	DMA at 90 DAS (g/plant)	CGR at 60-90 DAS (g/m^2 /day)	Length of cob (cm)	Girth of cob (cm)
T_1 (Live Mulch+ 50 % RDF)	132.83	1.65	64.92	12.29	12.33	12.54
T_2 (Live Mulch+ 75% RDF)	143.20	2.63	80.62	15.07	13.49	13.08
T_3 (Live Mulch+ 100% RDF)	160.32	3.37	92.22	15.37	15.30	13.00
T_4 (Straw Mulch+50 % RDF)	138.43	2.00	69.23	13.14	12.85	13.32
T_5 (Straw Mulch+75% RDF)	147.70	2.95	80.37	15.11	14.30	13.08
T_6 (Straw Mulch+100% RDF)	164.57	3.70	95.21	15.53	15.23	13.86
T_7 (No Mulch+ 50 % RDF)	126.77	1.45	63.32	12.61	12.22	12.51
T_8 (No Mulch+75% RDF)	141.37	2.25	77.77	14.49	12.93	13.09
T_9 (No Mulch+100% RDF)	152.17	3.01	89.15	15.15	13.77	13.75
SEm (±)	1.51	0.28	0.52	0.58	0.94	0.46
CD(*p*=0.05)	4.33	0.84	1.57	1.76	NS	NS

Table 2: Effect of different mulching and nutrient management on grain yield, stover yield, harvest index, shelling percentage and economics of *Rabi* maize.

Treatments	Grain yield (tonnes/ha)	Stover yield (tonnes/ha)	Harvest Index (%)	Shelling (%)	Net Return (Rs/ha)	B:C
T_1 (Live Mulch+ 50 % RDF)	2.81	6.16	28.33	81.53	29439.40*	1.63
T_2 (Live Mulch+ 75% RDF)	4.25	6.75	36.21	83.47	50609.70*	2.05
T_3 (Live Mulch+ 100% RDF)	5.02	7.47	37.29	84.77	55907.89*	2.13
T_4 (Straw Mulch+50 % RDF)	3.40	6.33	32.54	82.99	18385.40	1.53
T_5 (Straw Mulch+75% RDF)	4.52	7.06	36.66	84.01	33749.42	1.93
T_6 (Straw Mulch+100% RDF)	5.28	7.65	37.49	85.09	43869.44	2.16
T_7 (No Mulch+ 50 % RDF)	2.53	5.82	27.40	79.42	6997.92	1.21
T_8 (No Mulch+75% RDF)	3.76	6.54	34.19	83.10	24104.94	1.70
T_9 (No Mulch+100% RDF)	4.64	7.34	36.24	84.07	35989.96	2.00
SEm (±)	0.05	0.03	0.31	0.48	993.04	0.001
CD(*p*=0.05)	0.14	0.09	0.94	1.44	3,002.78	0.002

***Includes return obtained from selling berseem foliage**

return and B: C ratio was also influenced by Mulching and nutrient management significantly (Table2). Maximum net return (55907.89/- /ha) was obtained in T_3 treatment (Live Mulch+100% RDF) due to additional returns obtained from selling the berseem cuttings as an opportunity cost. But the B: C ratio for T_3 treatment (Live Mulch+100% RDF) was (2.13) which is lower than T_6 (Straw Mulch+100% RDF) treatment (2.16), the lower B:C obtained from T_3 treatment is due to higher cost of cultivation which included a high cost of labour for the purpose of cutting the berseem foliage and selling of cuttings at regular interval as well as additional cost of the berseem seeds for the intercropping. The results were confirmed by Bhatnagar *et al*. (1994).

Conclusion

Considering the findings as summarized above, it can be concluded that the integrated application of straw mulch along with 100% RDF can be recommended for better grain yield and higher profit of *Rabi* maize. The integration of organic mulching such as straw mulch may bring long-term benefits to soil health for cultivating the nutrient exhaustive maize crop.

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Conflict of interest

The authors declare that they have no conflict of interest.

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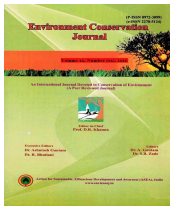

Morphological, biochemical and SSR marker based genetic diversity and identification of trait-specific accessions in exotic germplasm collection of tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Characterization and evaluation of genetic base of exotic collections of germplasm hastens the process of crop breeding. Exotic collections of 25 tomato germplasm accessions along with a local check 'Vaibhav' were characterized at morphological, biochemical and DNA marker level in the University of Agricultural Sciences, Bangalore. Both morphometric and biochemical trait data divided the accessions into five clusters by model-based K-means cluster analysis. Accessions EC-620481 and EC-620554 were found highly diverse and promising to broaden the genetic base of breeding stocks in tomato. SSR marker based genetic parameter estimates inferred lower genetic differences at marker loci. However, UPGMA classification displayed similar kind of diversity as exhibited at morphometric level. Traits specific accessions identified have potential to accelerate trait specific breeding for economically important traits. This investigation resulted in the identification of such potential accessions for their use in commercial tomato breeding.

Introduction

Among the members of nightshade family Solanaceae, tomato is the major vegetable crop in the world (Rothan*et al.*, 2019). The advanced plant breeding activities narrowed down the genetic base of available tomato breeding lines due to repeated selections (Miller and Tanksley, 1990). Introducing new variation into available breeding lines in tomato is need of the hour to broaden the genetic base. Inclusion of exotic variation into tomato breeding programs is necessary to introduce new gene combinations (Bergougnoux, 2014). Assessment of genetic diversity of such exotic germplasm accessions provides an insight about its value (Rick and Chetelat, 1995). Characterization based on morphological trait expression is most commonly employed for assessing genetic differences among individuals in a population (Anilkumar *et al*., 2017). In complement to it, the biochemical products produced during different stages of plant development also serve the purpose. Nevertheless, expression of these morphological and biochemical characters are highly influenced by environment and hinders the estimates of genetic diversity (Brunlop and Finckh, 2010). However, DNA markers are crop non-stage specific and environmentally neutral complement to the morphological characters in diversity study (Milevska *et al.,* 2011). Supplementing morphological character based diversity with biochemical and DNA marker data assure breeders to strategically select appropriate genotype for breeding programs (Herraiz *et al.,* 2015). The

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objective of present investigation is to examine genetic diversity among exotic collection of tomato germplasm accessions at morphological, biochemical and SSR marker loci.

Material and Methods

The genetic material for the investigation consisted of 25 exotic germplasm collections obtained from National Bureau of Plant Genetic Resources (NBPGR), Regional station, Rajendranagar, Hyderabad, India and a local check variety 'Vaibhav' released by University of Agricultural Sciences, Bangalore (Table-1). The genotypes were evaluated at experimental plots of Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore. The experiment was laid out in randomized complete block design with two replications. Four weeks old seedlings from nursery trays were transplanted to field maintaining a spacing of 75cm between rows and 45cm between plants. To ensure a healthy crop, the recommended tomato management practices like spacing and regular watering were followed.

Table1: List of exotic collection of tomato accessions used in the study.

Sl. No	Accessions	Sl. No	Accessions
1	EC-620437	**14**	EC-620553
2	EC-620438	**15**	EC-620554
3	EC-620456	**16**	EC-620557
4	EC-620460	**17**	EC-620560
5	EC-620472	**18**	EC-620563
6	EC-620474	**19**	EC-620567
7	EC-620481	**20**	EC-620568
8	EC-620521	**21**	EC-614997
9	EC-620543	**22**	EC-614998
10	EC-620544	**23**	EC-620343
11	EC-620545	**24**	EC-620394
12	EC-620546	**25**	EC-632946
13	EC-620550	**26**	Vaibhav (Check)

Ten randomly tagged plants were considered for recording observations on 11 morphometric traits. The traits recorded were days to flowering, days to flowering to fruit set, plant height at 65 days, number of branches, fruits per cluster, fruits per plant, fruit length, fruit width, fruit weight, number of locules and plant yield. Apart from these, qualitative trait data were also recorded on flower color, fruit color and growth habit of experimental material (Table 2 and Figure 1). The data on biochemical parameters *viz*., lycopene content (Ranganna, 1976); total soluble solids (TSS) and ascorbic acid content (Johnson, 1948) were recorded. TSS was recorded from five randomly selected fruitsfrom all accessions by squeezing the juice on Erma hand refractometer (0-32° Brix) atroom temperature and mean was worked out.

The genomic DNA of 26 genotypes was extracted from young and healthy leaves using CTAB method (Doyle and Doyle, 1987). A total of 42 SSR markers (Table 3)were used to differentiate 26 genotypes at SSR marker loci. The size variations of amplicons produced at SSR priming regions in the genomic DNA were scored as different alleles at each SSR loci and subjected to analysis.

Table 2: Qualitative characters in different tomato accessions.

SN	Accessions	Growth type	Flower color	Fruit color
1	EC-620437	Indeterminate	Yellow	Orange red
2	EC-620438	Indeterminate	Yellow	Deep red
3	EC-620456	Indeterminate	Yellow	Light yellow
4	EC-620460	Indeterminate	Yellow	Orange red
5	EC-620472	Indeterminate	Yellow	Deep red
6	EC-620474	Determinate	Yellow	Orange red
7	EC-620481	Indeterminate	Yellow	Deep red
8	EC-620521	Indeterminate	Yellow	Light yellow
9	EC-620543	Determinate	Yellow	Orange red
10	EC-620544	Indeterminate	Yellow	Deep red
11	EC-620545	Indeterminate	Yellow	Light yellow
12	EC-620546	Indeterminate	Yellow	Orange red
13	EC-620550	Indeterminate	Yellow	Light yellow
14	EC-620553	Determinate	Yellow	Light yellow
15	EC-620554	Determinate	Yellow	Deep red
16	EC-620557	Determinate	Yellow	Orange red
17	EC-620560	Indeterminate	Yellow	Light yellow
18	EC-620563	Indeterminate	Yellow	Orange red
19	EC-620567	Determinate	Yellow	Orange red
20	EC-620568	Indeterminate	Yellow	Light yellow
21	EC-614997	Determinate	Yellow	Light yellow
22	EC-614998	Indeterminate	Yellow	Light yellow
23	EC-620343	Indeterminate	Yellow	Orange red
24	EC-620394	Determinate	Yellow	Orange red
25	EC-632946	Indeterminate	Yellow	Light yellow
26	Vaibhav	Indeterminate	Yellow	Deep red

Statistical analysis

The mean data on 11 morphological characters recorded over two years was subjected to Levene's

test (Levene, 1960) of variance using SPSS V.16 software. The test results assured pooling of data from two years as their variances did not differ significantly. The pooled data was subjected to K-means cluster analysis using RStudio version 1.2.133 (RStudio team, 2019). Trait specific accessions were identified for each morphological traits based on comparative performance of accessions with check. Among the 42 SSR markers used, 23 were found polymorphic across the germplasm collection. Powermarker V3.25 (Liu and Muse, 2005) was used for estimation of various population genetic parameters such as polymorphic information content (PIC), major allele frequency and gene diversity. The same data was also subjected to UPGMA classification using DARwin 6 software to classify the accessions.

Results and Discussion

The highest plant height was recorded in EC-620563 and EC-620546 (126.25 and 124.25 cm). The number of branches per plant were higher in EC-614998 (63.50) followed by EC-620546 (62.00). Among twenty five tomato accessions studied, seventeen were indeterminate type and eight accessions were determinate type. There was no significant difference in the number of days taken for flowering and it ranged from 31.00 to 36.00. There was significant variation among the accessions for the individual fruit weight. The higher fruit weight was recorded in EC-620998 (104g) and the lower fruit weight was recorded in EC-620554 (33g). Three accessions recorded higher number of fruits per plant *viz* EC-620546, EC-620568, and EC-614997 (70.00 to 72.25 fruits/plant) whereas the check variety Vaibhav recorded around 46.75 fruits/plant which was on par with the average mean. The number of fruits per cluster varied from 4.50 to 12.50, the higher number was recorded in EC-620557 and EC-620568 followed by EC-620553 as compared to Vaibhav with 5.50 fruits per cluster. The total fruit yield per plant varied from 1.43kg to 6.30kg/plant. The higheryields were obtained in EC-614997 (6.30kg) followed by EC-620560 (6.14kg). However the average mean yield per plant was 3.59kg. Similar type of variability with respect to yield parameters was reported by Pradeep Kumar *et al*. (2001); Kaushik *et al*. (2011) and Reddy *et al*. (2013). The growth type of determinate (17) and indeterminate (8) were observed in the accessions. Flower color in all the accessions was yellow. Fruit color varied from deep red to light yellow. Study conducted by Hussain *et al.* (2021) also indicated that the PCV and GCV were found to be higher for number of fruits plant-1, average fruit weight (g), fruit yield hectare-1 (q), and pericarp thickness (mm), indicating greater phenotypic and genotypic variability among the accessions. Significantly higher lycopene content was observed in two accessions viz., EC620521 (5.47mg/100g) and EC-620550 (5.21mg/100g). Higher TSS was observed in EC-632946 (6.95^0Brix) followed by EC-620557 and EC-620550 (6.15^0Brix and 6.05^0Brix). The ascorbic acid levels were very high in some of the accessions. It varied from 8.76mg/100g to 55mg/100g. Similar reports on the variations in the lycopene content, TSS and ascorbic acid content are reported by Bose *et al.* (2002); Collins and Veazie (2006) and Panthee *et al.* (2012). Hussain *et al.* (2021) reported that the PCV and GCV for total Soluble Solids (^{0}Brix) ranged from 6.86 to 48.78 for reducing sugar (%).

The pooled mean values of 11 morphological characters subjected to K-means clustering divided 26 accessions into five different clusters. Among five clusters, cluster four was included highest number of accessions and cluster two was found solitary with only accession EC-620481 inferring its distinctness from other accessions (Fig. 2). The heat map of Euclidian genetic distance among 26 accessions clearly depicted the differences among themselves (Fig.3). The estimate of genetic distance for accession EC-620554 (15) and EC-620481 (7) were higher from any other accessions under the study inferring the genetic worth of these accessions. Genotypes with higher genetic distances can be best utilized for developing heterotic hybrids in practical plant breeding schemes (Anilkumar and Lohithaswa, 2018).

Based on three biochemical traits, genotypes were grouped into five clusters (Fig. 4), while the composition of these clusters differed with those based on morphometric traits. However, the results from biochemical data paved the path for selection of genotypes in breeding programs targeted to improve quality. Observed gene diversity and Polymorphic information content (PIC) value

Table 3: List of SSR markers used in the study.

Marker	Sequence	Expected product size (bp)
SSR111	F: TTCTTCCCTTCCATCAGTTCT	190
	R: TTTGCTGCTATACTGCTGACA	
SSR134	F: CCCTCTTGCCTAAACATCCA	175
	R: CGTTGCGAATTCAGATTAGTTG	
SSR146	F: TATGGCCATGGCTGAACC	220
	R: CGAACGCCACCACTATACCT	
SSR248	F: GCATTCGCTGTAGCTCGTTT	270
	R: GGGAGCTTCATCATAGTAACG	
SSR310	F: GCGATGAGGATGACATTGAG	175
	R: TTTACAGGCTGTCGCTTCCT	
SSR318	F: GCAGAGGATATTGCATTCGC	180
	R: CAAACCGAACTCATCAAGGG	
TOM49	F: AAGAAACTTTTTGAATGTTGC	190
	R: ATTACAATTTAGAGAGTCAAGG	
TOM144	F: CTGTTTACTTCAAGAAGGCTG	180
	R: ACTTTAACTTTATTATTGCGACG	
TOM152	F: ATTCAAGGAACTTTTAGCTCC	190
	R: TGCATTAAGGTTCATAAATGA	
TOM184	F: CAACCCCTCTCCTATTCT	180
	R: CTGCTTTGTCGAGTTTGAA	
TOM210	F: CGTTGGATTACTGAGAGGTTTA	205
	R: ACAAAAATTCACCCACATCG	
TOM236	F: GTTTTTTCAACATCAAAGAGCT	200
	R: GGATAGGTTTCGTTAGTGAACT	
TGS0385	F: ATGCCAAAAAGTGATCAGGG	163
	R: GGGACAAACGTGTAACACACA	
TGS2259	F: ACGCAAGCTGAAGCCATAAT	205
	R: GTCTCCCTGCTGCTTACTGC	
Eaat001	F: GATGGACACCCTTCAATTTATGGT	136
	R: TCCAAGTATCAGGCACACCAGC	
LEaat002	F: GCGAAGAAGATGAGTCTAGAGCATAG	106
	R: CTCTCTCCCATGAGTTCTCCTCTTC	
LEaat003	F: CTTGAGGTGGAAATATGAACAC	189
	R: AAGCAGGTGATGTTGATGAG	
LEaat006	F: GCCACGTAGTCATGATATACATAG	174
	R: GCCTCGGACAATGAATTG	
LEaat007	F: CAACAGCATAGTGGAGGAGG	100
	R: TACATTTCTCTCTCTCCCATGAG	
LEaat008	F: GAGTCAACAGCATAGTGGAGGAGG	178
	R: CGTCGCAATTCTCAGGCATG	
LEat006	F: CATAATCACAAGCTTCTTTCGCCA	166
	R: CATATCCGCTCGTTTCGTTATGTAAT	
LEat012	F: CGGCAAAGGGACTCGAATTG	110
	R: GTGGCGGAGTAGAAACCTTAGGA	
LEat013	F: ATCACAAGCTTCTTTCGCCACA	163
	R: ACCCATATCCGCTCGTTTCG	
LEat018	F: CGGCGTATTCAAACTCTTGG	120
	R: GCGGACCTTTGTTTTGGTAA	
LEat020	F: ACTGCCTCTCTTCAAAGATAAAGC	212
	R: ACGGAAAGTTCTCTCAAAGGAGTTG	
LEga003	F: TTCGGTTTATTCTGCCAACC	241
	R: GCCTGTAGGATTTTCGCCTA	
LEga005	F: TTGGCCTAATCCTTTGTCAT	314
	R: AACAATGTGACGTCTTATAAGGG	
LEga006	F: CCGTCCAGAAGACGATGTAA	248
	R: CAAAGTCTTGCCAACAATCC	

Marker	Sequence	Expected product size (bp)
LEga007	F: CCTTGCAGTTGAGGTGAATT	193
	R: TCAAGCACCTACAATCAATCA	
LEgata002	F: TTGGTAATTTATGTTCGGGA	344
	R: TTGAGCCAATTGATTAATAAGTT	
LEta002	F: GCCTCCCACAACAATCATCTATACA	190
	R: TCCTCCGTACTTTGATCATCTTGTT	
LEta003	F: GCTCTGTCCTTACAAATGATACCTCC	111
	R: CAATGCTGGGACAGAAGATTTAATG	
LEta006	F: CCCTCTTGCCTAAACATCC	167
	R: TCTACTCGTTGCGAATTCAG	
LEta007	F: GCCGTTCTTGGTGGATTAG	291
	R: CCTCCTTTCGTGTCTTTGTC	
LEta015	F: ATATGCATGGACAAATCTTGAGGG	107
	R: CTCGCGCATCAAATTAATGTATCAG	
LEta016	F: AGGTTGATGAAAGCTAAATCTGGC	174
	R: CAACCACCAATGTTCATTACAAGAC	
LEta020	F: AACGGTGGAAACTATTGAAAGG	275
	R: CACCACCAAACCCATCGTC	
LEtaa002	F: TGAGAGAGATCAACCAACTCC	133
	R: ACTACTCCTGCCTCTCTATATCC	
LEtca001	F: TGCATGGCAACATTAAAGTC	176
	R: CGTGGATGCAACTTCATTG	
SSR45	F: TGTATCCTGGTGGACCAATG	260
	R: TCCAAGTATCAGGCACACCA	
SSR96	F: GGGTTATCAATGATGCAATGG	210
	R: CCTTTATGTCAGCCGGTGTT	
SSR104	F: TTCCATTTGAATTCCAACCC	220
	R: CCCACTGCACATCAACTGAC	

Figure 1: Variation in number of locules as high as 10 (EC-620481) to as low as 2 (EC-620456).

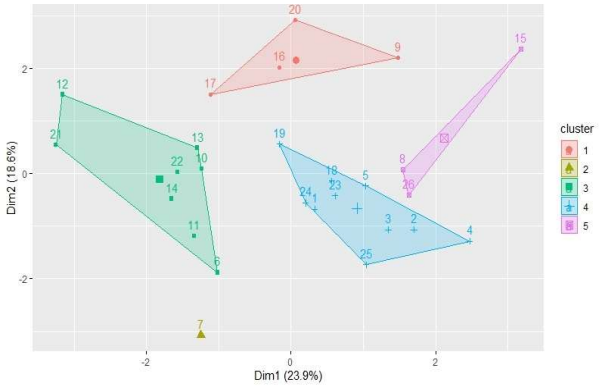

Figure 2: Grouping of genotypes using K-means cluster analysis based on 11 morphological characters.

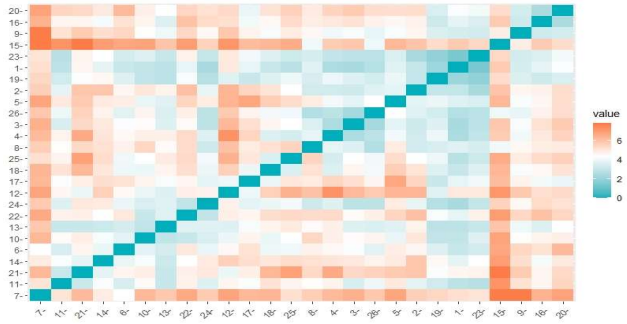

Figure 3: Heat map representing Euclidian genetic distance between germplasm accessions based on 11 morphological characters.

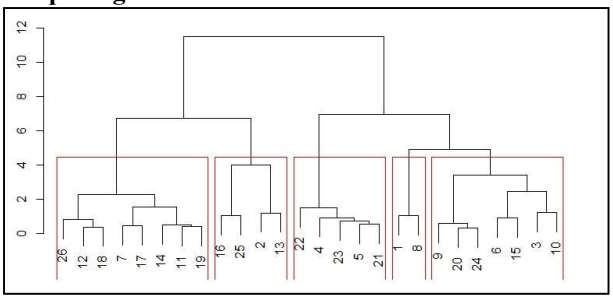

Figure 4: Clustering of genotypes based on data recorded on three important biochemical parameters.

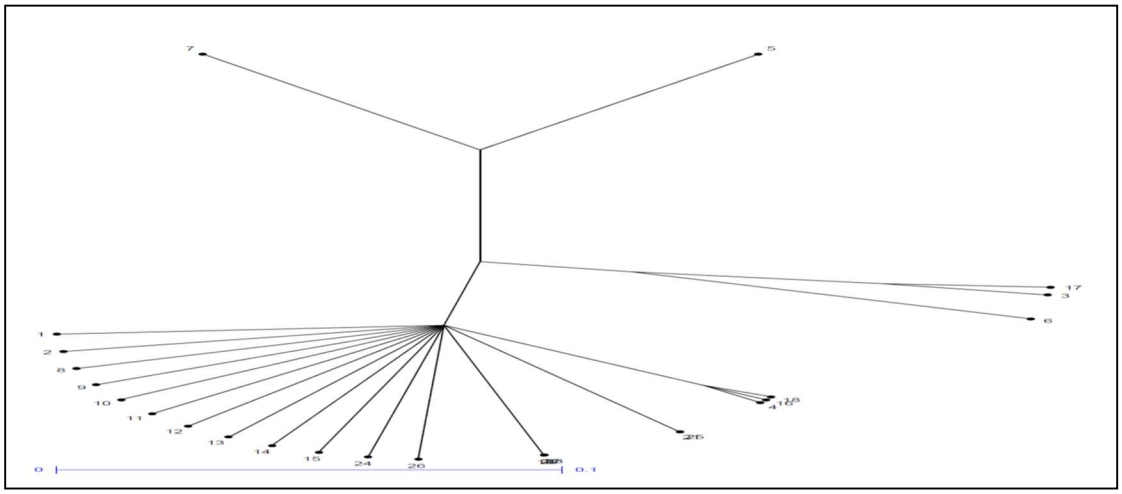

Figure 5: Tree diagram depicting SSR marker based UPGMA classification of germplasm accessions.

Table 4: List of 23 polymorphic markers and their major allele frequency, gene diversity, polymorphic information and content (PIC).

Marker name	Major Allele Frequency	Allele No.	Gene Diversity	PIC
SSR111	0.81	2.00	0.30	0.26
SSR134	0.94	1.50	0.10	0.09
SSR146	1.00	1.00	0.00	0.00
SSR318	1.00	1.00	0.00	0.00
TOM49	0.96	1.50	0.07	0.07
TOM144	0.92	1.50	0.13	0.11
TOM152	1.00	1.00	0.00	0.00
TOM184	0.81	2.00	0.28	0.23
TGS0385	0.85	1.50	0.21	0.17
Eaat001	0.98	1.50	0.04	0.04
LEaat002	1.00	1.00	0.00	0.00
LEat013	0.96	1.50	0.07	0.07
LEat020	1.00	1.00	0.00	0.00
LEga003	0.85	1.50	0.21	0.17
LEga005	0.94	1.50	0.10	0.09
LEta006	0.75	2.00	0.34	0.27
LEta015	0.75	1.50	0.25	0.19
LEta016	1.00	1.00	0.00	0.00
LEta020	0.87	1.50	0.20	0.16
SSR45	0.85	1.50	0.21	0.17
SSR96	0.65	2.00	0.45	0.35
SSR104	0.75	2.00	0.36	0.29
LEaat007	0.81	2.00	0.31	0.26

Table 5: Promising trait specific accessions in exotic collection of tomato germplasm.

Traits	Selection criteria	Range	Vaibhav (Check)	Accessions
Days to flowering	Earliness	62-73	69	EC-620474, EC-620546, EC-614997, EC-620472, EC-620544, EC-620550, EC-620553, EC-620481, EC-620521, EC-620545, EC-620567, EC-614998, EC-620438 and EC-620394
Flowering to fruit set	Earliness	6-12	10	All the accessions except EC-614998 and EC-620554
Plant height at 65 days	High	124-252 cm	185 cm	EC-620563, EC-620546, EC-620544, EC-620474, EC-632946, EC-620560, EC-620543, EC-620550, EC-620557, EC-620437, EC-620567, EC-614998, EC-620553 and EC-620343
Number of branches (primary, secondary and tertiary)	Medium	42-125	46	EC-620456, EC-620460 and EC-620472
Fruits per cluster	High	9-12.5	11	EC-614998, EC-620438, EC-620474, EC-620521, EC-620437, EC-620550, EC-620554, EC-620560, EC-620567, EC-614997, EC-620343, EC-632946, EC-620460, EC-620544, EC-620481, EC-620472, EC-620543, EC-620563, EC-620553, EC-620557 and EC-620568
Fruits per plant	High	32-170	94	EC-620554, EC-620546, EC-620568, EC-614997, EC-620543, EC-620521, EC-620560, EC-620557, EC-620550, EC-620567, EC-620343 and EC-620545
Fruit length	High	8.24 – 13.82 cm	10.48 cm	EC-620543, EC-614997, EC-620472, EC-614998, EC-620544, EC-620545, EC-620553, EC-620550, EC-620568, EC-620557, EC-620456, EC-620437, EC-620546, EC-620343, EC-620567, EC-620460, EC-620438, EC-620394, EC-632946, EC-620474 and EC-620560
Fruit width	High	8.52-12.90 cm	9.82 cm	EC-620481, EC-620550, EC-614997, EC-614998, EC-620546, EC-620553, EC-620545, EC-620544, EC-620472, EC-620474, EC-620568, EC-620343, EC-620456, EC-620437, EC-620567, EC-620560, EC-620557, EC-632946, EC-620394 and EC-620438
Fruit weight	High	66-208 g	144.20 g	EC-614998, EC-614997, EC-620553, EC-620563, EC-620394, EC-620560, EC-620545, EC-620481, EC-620474, EC-620460, EC-620456, EC-620437, EC-620544 and EC-620521
Number of locules	Fewer	2-10	7	EC-620456, EC-620472, EC-620543, EC-620460, EC-620394, EC-620554, EC-620567, EC-620438, EC-620544, EC-620560, EC-620563, EC-620568, EC-614998, EC-620521, EC-620550 and EC-620557
Plant yield	High	2.88-12.61 Kg	6.58 kg	EC-614997, EC-620560, EC-620546, EC-620557, EC-620553, EC-620568, EC-620550, EC-620545, EC-620521, EC-614998, EC-620567, EC-620554 and EC-620481

inferred the lower differences between accessions at SSR marker loci (Table 4). However, tree constructed based on SSR amplicon data using UPGMA approach provided the visibility of diversity among accessions (Fig. 5). The accessionsEC-620481 (7) and EC-620472 (5) formed solitary branches away from other accessions, proving its distinctness from other accessions. However, accession EC-620472 (5) was in cluster four under morphometric cluster analysis. This might be due to differences at genomic regions that amplified at SSR loci. These results indicated the significant differences at genetic level which can be exploited by strategic breeding programs. Brake *et al.* (2021) in their study, among the genotypes screened for variability using ISSR markers observed the lowest genetic similarity value (0.46) was found between landraces Jo964 and Jo955, while the highest (0.94) was obtained between landraces Jo983 and 29. The accessions were compared with check variety to identify some accessions which are superior to check variety with respect to 11 morphological traits (Table 5). Such accessions which surpassed local check variety for given trait were considered as trait-specific accessions. The germplasm accessions identified for specific traits can be potentially used for trait specific breeding activity without further evaluation, saving breeder's time and resources.

Conclusion

It is concluded that this investigation resulted in the identification of specific traits which are economically important, and are having potential to be utilised in commercial tomato breeding.

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Conflict of interest

The authors declare that they have no conflict of interest.

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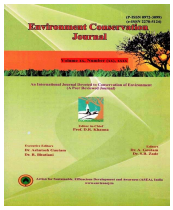

Agronomic practices for sustainable diseases management in rice: A review

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ABSTRACT

Rice is globally the most important food crop and there is a dire need to feed the ever-increasing population by improving its productivity. It has been realised that diseases are the major impediment towards enhancing the productivity of this crop. Despite the advent of modern effective disease control measures such as use of chemicals, bioagents and resistant varieties; agronomic practices still play a vital role in disease management in rice. Optimum use of different agro-techniques can be exploited for efficient control of various devastating diseases like rice blast, sheath blight, bakanae and many more by providing a favourable environment to better crop survival. Besides, appropriate selection of a variety, use of quality seed, method of establishment, planting time, nutrient, water and weed management practices can be well exploited to control various diseases. This manuscript entails to review the work pertaining to use of agronomic practices for exploiting the potential of crop environment interaction through reduced disease infection and to bridge the yield gap for ensuring sustained food security.

Introduction

Rice serves as the principal food crop for about 50% of the global population (Sain, 2020) and contributes to 50% of the calorie intake of the total population (Singh *et al.,* 2012). Globally rice crop occupies an area of about 160 mha with 495.07 mt of production and productivity of 4.54 t/ha. In India rice is grown over an area of about 43 mha with the production of about 112.9 mt (Pathak *et al.,* 2020). To sustain and meet the food and nutritional requirement of India, the requirement of rice is expected to go up to about 156 mt by 2030 (ICAR, 2010) with the 3 mt annual incremental rate in the current rice production (Dass *et al.,* 2016). Rice is cultivated over a wide range of environments owing to its wide range of adaptability, such as irrigated uplands, irrigated low land and rainfed uplands (Choudhary and Suri, 2014; Kaur *et al.,* 2015; Younas *et al.,* 2020). Though the productivity level varies among diverse growing environments, there are considerable constraints which hamper rice productivity. Among these, disease incidence is prominent, which alone reduces the yield by 21-

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51% (Arya and Chander, 2012; Wangsawang *et al.,* 2019). The rice crop is infected by number of diseases right from the time seed is incubated, establishment of nursery and in the main field from transplanting up to the maturity expressing different types of symptoms (Figure 1). These diseases are seed, soil as well as air born with the variability in extent of damage (Table 1). Generally chemical measure is resorted for the control of these diseases; however, chemicals are posing serious threat to environment and human health. Additionally, resistant pathogenic strains have also been reported (Ohtani and Takeuchi, 2013; Sorbara and Pamer, 2019). Thus, to safe guard the environment and food security, the use of chemicals should be minimized (Damalas and Eleftherohorinos, 2011; Shah and Wu, 2019) and alternatives should be looked at. Hence it becomes imperative to control the disease by adopting those agronomic measures that serve dual purpose of controlling the disease and harness better yield without harming the environment. Manipulations in agronomic practices *viz*., sowing methods, time of sowing, nutrient management, water management and control of weeds have proven very effective in reducing the disease parameters of rice (Bhat *et al.,* 2013; Asibi *et al.,* 2019). These agro techniques have proven effective in controlling these diseases successfully by interrupting disease cycle of pathogen at any stage of crop by creating congenial environment to the crop. Response of rice diseases to agronomic manipulations has been discussed under the following headings.

Table 1: Extent of yield loss caused by different diseases in rice

Disease	Yield loss (%)	References
Rice blast	5-100	Dubey (1995); Musiime *et al.,* (2005); Wilson & Talbot (2009)
Bacterial leaf blight	2-74	Reddy *et al.,* (1978); Rajarajeswari & Muralidharan (2006)
Sheath blight	10-30	Xue-Wen *et al.,* (2008)
Brown spot	4-52	Chakrabarti (2001); Savaryet *et al.,* (2006); Barnwal *et al.,* (2013)
Rice false smut	0.2-49	Singh *et al.,* (1992); Biswas (2001)
Sheath rot	14-85	Sakthivel (2001); Singh & Raju (2012)
Bakanae	3-95	Hajra *et al.,* (1994); Singh & Sunder (2012)

Selection of variety

Selection of suitable variety for a particular agro-ecology is the most efficient and economical method to combat the diseases besides obtaining the higher yield. With the rice crop existing in three varietal types flourishing under appropriate agro-ecology. As Indica type of rice is suited to tropical areas of the world and these varieties of rice fail to mature at higher altitude of ≥ 1850 meters above mean sea level (amsl), while as Japonica varieties mature early in the plain belts and succumbs to blast disease. Thus, the varietal selection process in rice is an important step in reducing production risks caused by diseases. The varietal selection should be based on grain quality, maturity, fertilizer response, plant growth characteristics and resistance against major diseases. Varietal resistance is primary element of integrated disease management and is most acceptable method for farmers. For sustaining rice productivity, host resistance is an attractive and significant tool for controlling diseases in rice as it requires no additional cost to farmers and is also environmentally safe (Mew, 1991; Angeles-Shim *et al.,* 2020). However, due to the wide adoption of modern high yielding rice varieties, the traditional rice production system has been transformed into monoculture system which predisposes the varieties to loss the resistance over time. Thus, in addition to resistant varieties, agronomic practises play a vital role for disease management in rice. For example, panicle blast can be controlled by inter-planting rice varieties (Zhu *et al.,* 2000); infection can be escaped by using early maturing genotypes as compared to late maturing genotypes (Singh and Khan, 1989); avoiding use of semi-dwarf and photoperiod-insensitive cultivars as these are susceptible to sheath rot disease (Bigirimana *et al.,* 2015). In addition to these, multilines with different resistance genes can be used to control blast (Koizumi, 2001). Amongst virus diseases, rice tungro is found to be most destructive in rice growing areas. For this, BR8 and BRRI dhan37 has

Figure 1. Symptoms of major rice diseases: a) Rice blast b) Sheath blight c) Brown spot, d) False smut f) Bakanae g) Sheath rot

been found to have high recovery ability among upland and rainfed lowland areas, respectively (Khatun *et al.,* 2017). Recently, Rashid *et al.* (2019) have found Basmati 515 mm less prone as compared to Super Basmati.

Quality seed

Selection of quality seed is important determinant for crop health and the first step for obtaining healthy and robust seedlings for successful rice cultivation. More than thirty diseases of rice can be transmitted on or inside rice grains. Seed treatment is sound assurance against a number of diseases that damage early stands, reduce yields, and grain quality. Initially the seeds should be immersed in 2.5% salt solution for removing the chaffy, half-filled and diseased seeds, acting as potential carrier for seed borne diseases. Number of seed borne bacterial as well as fungal diseases have been reported to occur at nursery stage. Seed rot and

seedling blight are complex disease caused by several seed-borne and soil-borne fungi and some bacteria including species of *Cochiobolus, Curvularia, Fusarium, Rhizoctonia*, *Sclerotium*, *Burkholderia glumae* and *B. plantarii* (Cartwright and Lee, 2001). Moreover, the major diseases of rice such as rice blast, sheath rot, brown spot, bakanae and bacterial leaf blight, are seed borne diseases. Thus, in order to produce high-quality nursery plants, it becomes essentially important to manage these diseases at seedling stage (Adhikari *et al.,* 2013) and for this, seed treatments with hot water and fungicides can prove to be effective against these pathogens. By immersing the seed material in hot water (60 °C) for 10 min has been relatively found effective in controlling several seed borne diseases in rice (Hayasaka *et al.,* 2001). More specifically, seed immersion in hot water (60 °C) for a period of 10-15 minutes was observed to kill *B. plantarii* effectively (Eguchi *et al.,* 2000), while soaking of seeds in water for 12 hours followed by exposure to hot water at 53°C eradicates the seed borne inoculum of *Xanthomonas oryzae* pv. *oryzae.* Since rice blast fungus is externally seed borne, hence seed treatment with fungicides or hot water eradicates the fungal inoculum from the seeds. Hot water treatment at 50 °C for 15 minutes was found effective in reducing rice blast incidence and severity (Faruq *et al.,* 2015; Hashim *et al.,* 2019). Similarly, for bakanae disease (*Gibberalla fujikuroi*) which causes yield losses of about 3.7-50% (Webster and Gunnell, 1992) can be controlled by fungicides (Ahangar *et al.,* 2012). Further, it has been reported that soaking of seeds into hot water at 60 °C for 10 minutes before sowing can significantly reduce the seed infections and bakanae incidence in nursery and fields conditions (Miyasaka *et al.,* 2000; Yamashita *et al.,* 2000).

Establishment method

A number of diseases have been observed to infect rice seedlings in the nursery stage, mostly being seed and soil borne. Most prevalent ones being seedling blight, or damping off and bakanae. Seedling blight has been observed to be more prevalent in early sown crop due to low temperature and damp soil. In addition, seedling blight and seed rot diseases are wide spread in soils with monoculture of rice, heavy rice residue, and minimum or reduced tillage. As compared to dry-seeded fields, water-seeded fields have more seedling diseases. In addition to these, planting depth also played an important role in disease severity. The management practice which tends to delay the emergence should be avoided for the favour of reducing seedling blight. Similarly, the type of blight in which cottony white mold growth is observed at the basal portion has been reported to be controlled by flooding the soil immediately after the visibility of symptoms. Moisture content in the nursery at the time of uprooting has been reported to influence the incidence of bakanae, a seed borne disease in nature. Sunder *et al.* (2014) observed significantly less incidence, where the nursery was uprooted in standing water as against the low water content in the nursery at the uprooting time.

Rice crop being established by different methods (direct seeding and transplanting) influencing the microclimate of the crop due to their varied canopy structure owing to the distance between the neighbouring plants which in turn influences the overall health of the crop. In direct seeded rice, planting density determined by number of plants per unit area tends to provide more shade and probably more humid microclimate in the canopy leading to more leaf-to-leaf contacts which ultimately favours rice disease epidemics (Savary *et al.,* 1998). Zhao *et al.* (2007) reported that high seed rate in rice harbours more disease infection by creating congenial environment for pathogen. Hence, the spatial distance between the plants plays a very crucial role in the dissemination of diseases which is highly influenced by the method of establishment. In direct seeded rice where there are more number of plants per unit area but the aggregation at plant level is slightly less as against transplanted rice, where there is more aggregation within each hill. This spatial difference between the plants is the reason for more incidence of disease in transplanted rice in comparison to direct seeded rice (Willocquet *et al.,* 2000). However, disease severity and incidence of blast (*Pyricularia oryzae*) were found to be less in transplanting compared to broadcasting method in rice (Reza *et al.,* 2011; Thoudam and Chhetry, 2017). With wider spacing of 22.5 cm in direct seeded rice, Rashid *et al.* (2019) observed maximum panicle length, kernels

per panicle, paddy yield and harvest index with less incidence of bacterial leaf blight. Higher disease incidence and severity was reported in direct seeding than transplanted method by Iwuagwu *et al.* (2017) which then attributed to poor spacing and high population density conjointly producing high humidity suitable for fungal disease to perpetuate. Less diseases incidence has been reported in SRI system of rice cultivation against conventional method (Pathak *et al.,* 2012). Saremi and Farrokhi (2004) reported that transplanted rice plants display more symptoms of bakanae than those grown from broadcast seeds. On the contrary Kaur *et al.* (2015) reported sheath blight incidence did not vary significantly among different establishment methods.

Furthermore, variation in the crop geometry by altering the row spacing at the same planting density may influence the yield and incidence of sheath rot (*Sarocladium oryzae*) and false smut (*Ustilaginoidea virens*) in the rice crop (Kewat *et al.,* 2002). An optimum row spacing which has witnessed to reduced disease incidence is evident from the fact that sheath blight requires close foliar contact. Decrease in the incidence of sheath blight in skipped row planting (2:1) was observed by Rautaray (2007) owing to discontinuity in canopy, thus restricting the spread of disease. Skip row planting also proved to be beneficial in restricting the spread of sheath blight and sheath rot by restricting the spread of insect vectors *viz*., green leaf hoppers, *Nephotettix nigropictus and N. virescens,* and zigzag leaf hoppers, *Recilia dorsalis*). Closer spacing of 10 × 20 cm showed increase in the severity of bacterial leaf blight than at wider spacing (Meah, 1987). Shengfu *et al.* (2002) recorded less incidence of rice sheath blight was less when planted at 33.3 cm x 33.3 cm (58.4%) spacing or 40 cm x 40 cm (54.6%) spacing under SRI than that with traditional cultivation (70%). Similar results of more disease incidence at closer spacing of 15 cm x 15 cm in comparison to wider spacing of 20 cm x 25 cm was reported by Iwuagwu *et al.* (2017).

Planting time

Planting date-based strategies have been used successfully for management of various diseases, as it helps the plants by making better use of favourable environment at each growth stage favouring the escape from severe infection phase. Optimum sowing time helps in the controlling of tungro virus disease (Manwan, 1987). The sheath blight which is a very serious disease of rice has been observed to become more severe with increase in age of the plant. Minimum incidence of sheath blight was recorded, when rice was sown on July 15th (early sowing) as compared to on 30th June (Pal *et al.,* 2016). It has been reported that sheath blight resistance can be enhanced by square method of transplantation and sparse planting along with higher grain yields (Yang *et al.,* 2008). Sparse planting not only lowers the sheath blight occurrence but also enhanced the lodging resistance in rice (Sugiyama *et al.,* 2007). Blast infection in rice is a devastating disease which can cause up to 80% yield loss, depending up on factors like inoculum pressure, crop growth stage at infection, prevailing climatic conditions, varietal susceptibility and cultural practices (Groth, 2006; Prabhu *et al.,* 2006). In Sulawesi province of Indonesia, lowest leaf blast severity and neck blast incidence were recorded in 4th February transplanted crop than 22nd March and May 16th, due to the presence of less inoculum in early transplanted crop (Nasruddin and Amin, 2013). Iwuagwu *et al.* (2017) evaluated the effect of planting periods (Early June 15th, late June 30th, early July 15th and late July 30th) on disease incidence and severity in rice and observed least disease incidence in early sown crop. Similarly, Naklang *et al.* (1996); Laory *et al.* (2012) and Ahonsi *et al.* (2000) observed that disease incidence accelerates as the planting is done after optimum date. Singh *et al.* (2000) and Jha (2001) demonstrated that brown spot disease causes more damage in late sown rice crop. The infection with *Ustilaginoidea virens* the casual organism of rice false smut is favoured by high relative humidity, late sowing and high soil fertility (Narinder and Singh, 1989; Ahonsi *et al.,* 2000).

Nutrient management

Knowledge of the impact of nutrient management on the interaction of rice and disease serves the basic purpose of acquiring higher productivity (Luong *et al.,* 2003). The ability of plants to resist diseases has been attributed to better nutritional environment (Luong *et al.,* 2003). With this background, the farmers have been using nutrient management as one of the productive measures of disease management (Magdoff *et al.,* 2001).

Nutrients are not only vital for growth and development of the plants but also plays a significant role in the control of diseases (Agrios, 2005). There are a number of essential nutrients required by the plants for their optimum growth, development and resistance against biotic stresses. These nutrients help the plants to overcome the diseases either by improving the growth to optimum level, so that plant can tolerate the infection or by improving its ability to avoid the infection. The rapid growth in turn helps the plant to escape the most susceptible stage. Nutrients can affect the development of disease by influencing the physiology of plant or affecting pathogens or both (Dordas, 2008).

Nitrogen, phosphorus, potassium and silicon have been reported to influence the disease incidence in rice in number of ways depending upon different factors *viz*., form, quantity and interaction between host and pathogen. At higher dose of nitrogen, the proportion of young to mature tissue shifts towards later, which is more susceptible owing to more succulence and less suberin and lignin synthesis. Also, more amino acid accumulation in the leaf surface has been found to promote the germination and subsequent growth of conidia (Robinson and Hodges, 1981). Higher nitrogen rate has been reported to increase the blast severity (Kurschner *et al.,* 1992) by promoting the luxuriant crop growth, which resulted in increased relative humidity and leaf wetness of the crop canopy, thus favouring blast disease. In addition, higher rate of nitrogen is found to decrease the Si content, which then affects disease tolerance in plants. Time of nitrogen application also holds significance in reducing the effect of diseases. Leaf blast incidence and severity were significantly lower when nitrogen was applied in splits than its single dose application at normal or higher rate (Long *et al.,* 2000). Similar results of suppression of blast severity and most other foliage diseases in irrigated rice due to split application of nitrogen have been reported by Koraka (1965), Templeton *et al.* (1970), Amin and Venkataro (1979), Lee and Lee (1980), Bernaux (1981) and Kurschner *et al.* (1992). In contrast, application of full dose of nitrogen at panicle initiation stage in drought-prone upland conditions reduced the leaf and panicle blast as compared to its application at planting stage (Dos Santos, 1986). It is not only quantity and time of application but also the form of nitrogen, which affects the disease incidence. Nitrogen in the form of ammonium has been noticed to decrease the disease in case of *Pyricularia* spp. (Dordas, 2008). A reduced brown spot damage in rice was reported by Giudici *et al.* (1997) with extended period of drainage in mid-July along with late application of N + K and adjusting the planting date so that reproductive and ripening stage are not exposed to too high temperature. Rice false smut can be controlled in soils with low residual N, using low rate of N (Singh *et al.,* 1987).

Phosphorus (P) is the second most essential nutrient involved in metabolic process of the plant as well as pathogen (Dordas, 2008) and is vital for vigorous root formation which helps the plant to escape the disease (Huber and Graham, 1999). P fertilization in rice has been reported to reduce bacterial leaf blight and blast disease (Huber and Graham, 1999). Role of potassium is multifarious in terms of its pivotal role in various metabolic functions of plants and thereof in disease management. Potassium supply prevents disease incidence by initiating the development of thicker epidermal cells outer walls. It was observed that infestation intensity was closely related to the hardening of tissue and stomatal opening pattern (Marschner, 1995). Supply of potassium at sub optimum level is found to increase the accumulation of amides, a nutrient source for invading pathogen (Dordas, 2008) while its optimum level found to increase the resistance against the disease. Use of potassium has been reported to reduce the fungal incidence by 70 %, bacterial by 69 %, insects and mites by 63% and viruses by 41% (Perrenoud, 1990). It is not only sole application of potassium, but its ratio with other nutrients in the soil is also vital. Stem rot (*Helminthosporium sigmoideumas*) has been observed to occur at higher supply of nitrogen with low potassium level. Similarly inverse relationship was observed between the different diseases in rice *viz*, brown leaf spot (*Helminthosporium oryzae*), rice blast (*Piricularia oryzae*) and sheath blight (*Thanatephorus cucumeris*) and the supply of potassium, though the response was highly variable with the type of variety, with higher response imprinted by varieties possessing moderate degree of disease resistance (Hardter, 1997).

Silicon is known to provide the resistance to plants against infection at the minimum concentration of 3-5% at tissue level (Datnoff *et al.,* 1997), particularly in rice which is considered the Si accumulator with the ability to accumulate SiO_2 up to 10% (Liang *et al.,* 2006). It plays an unprecedented role for controlling fungal and bacterial diseases in rice. It seems that silicon helps to modulate lignin biosynthesis in rice by virtue of which it becomes resistant to fungal or bacterial attack (Song *et al.,* 2016). Silica after absorption is deposited in the cells and on cell walls, thus forming a strong barrier to halt the entry of hypha into the host, in addition it also helps in reducing the cell wall degradation enzymes secreted by fungi. Deposition of the Si on the cell walls affected the bacterial leaf blight development in rice (Song *et al.,* 2016). Silicon helps in the augmentation and accumulation of antifungal compounds like flavonoids, diterpenoids and phytoalexins which help in degrading fungal and bacterial cell walls (Brescht *et al.,* 2004). In rice adequate level of silicon has been witnessed to provide the resistance against diseases. Both brown spot disease (*Bipolaris oryzae*) and neck rot (*Pyricularia oryzae*) of rice were found to be severe in rice when the supply of silicon was inadequate (Datnoff *et al.,* 1997, 2001; Santos *et al.,* 2011). Similar results of increased susceptibility of rice to blast and other diseases has been attributed to the inadequate uptake of silicon (Kobayashi *et al.,* 2001; Rodrigues *et al.,* 2001; Massey and Hartley, 2006).

Zn is also known to possess the variable effect on disease incidence and severity in crops. This variable effect can be attributed to its impact on the availability of other nutrients. Zinc is known to reduce the leakage of low molecular weight compounds on account of free radical damage via detoxification of super oxide radicals (Simoglou and Dordas, 2006). Supply of Zn can lead to increase or decrease in disease severity in plants (Duffy, 2007). Moreira *et al.* (2013) observed a positive correlation between the elevated Zn concentration in leaves and its susceptibility to brown spot in rice. Though these effects of elevated levels of Zn were found selective. Increase in the dose of Zn application from 5 to 6 kg resulted in significant increase in disease infection of sheath blight and bacterial leaf blight as against false smut, sheath rot and brown spot (Singh *et al.,* 2012). Furthermore, Khaira disease, a non-pathogenic disorder, which is prevalent in low land condition mostly in Asian countries is found to be well managed by the foliar application of zinc sulphate (Nene, 1966).

Water management

Rice is sensitive to the shortage of water and is the highest water requiring crop in terms of water required to produce an economic product (5000L /kg rice). Irrigation affects both foliar and soil borne diseases (Rotem and Plati, 1969). It was observed that by altering the soil moisture content, disease incidence can be affected via its possible impact on soil/foliage biotic and abiotic processes. Air borne spores of *Pyricularia grisea* help in the spread of blast disease in rice. Maintaining the water level of >10 cm is recommended to curtail the disease (Tacker *et al.,* 2001). Similar results in tropical areas were noted by Lee *et al.* (2003) and IRRI (2010), as they found that blast incidence can be suppressed by flooding the soil as often as possible. Under water deficit condition brown spot (*Bipolaris oryzae*) has been reported to flare up for the lack of moisture. Furthermore, brown spot disease was observed to occur in susceptible plants when moisture was maintained below 50 cent bars. Das and Dath (1997) reported negative effects of crop submergence on the progress and development of sheath blight. However, in another study, it was reported that increase in sheath blight in flooded rice can be due to mobility of soil borne fungus in water (Stevens *et al.,* 2012). The greater incidence of bakanae disease of rice were observed in dry nurseries and summer crop than wet nurseries and spring crop as high temperatures and relatively humidity favours disease incidence.

Weed management

Weeds compete with the crop for both underground as well as above ground resources. Apart from competition, weeds pose greater threat of disease prevalence in the associated crop. This is because most of the weeds serve as alternate hosts for the disease-causing pathogen and also harbour pest vectors that migrate later to the crop. These alternate hosts play a vital role in providing the inoculum during the growing season. Thus, the weeding provides an effective measure for control of diseases through removal of alternate host. Sheath blight which is found to cause 7-50% yield

reduction has been noticed in the fields, densely populated with weeds like *Echinochloa crusgalli*, *Echinochloa colonum* and *Cynodon dactylon.* The control of *Echinochloa* spp., which is alternate host of fungal pathogen leads to the less disease incidence of Sheath blight (Kaur *et al.,* 2015). Frequency of weeding (1st, 2nd and 3rd hand weeding) was found very ideal for avoiding incidence and severity of diseases (Mola *et al.,* 2015). Mohammadi and Amiri (2011) also found significant role of weeding on reducing disease incidence in rice crops. A number of weed species have been found to act as alternate hosts of most important diseases of rice, thereby helps in the development and survival of rice pathogens (Table 2).

Table 2: Important rice diseases and their alternate hosts

Disease	Causal organism	Alternate host	References
Rice blast	*Pyricularia Oryzae (Magnaporthe Grisea)*	*Carolina foxtail*, *Brachiaria mutica, Dinebra retroflexa, Leersia hexandra, Panicum repens*	Jia *et al.,* (2008)
Bacterial leaf blight	*Xanthomonas oryzae* pv. *Oryzae*	*Cyanodon dactylon, Cyperus rotundus, Leersia hexandra, Leersia oryzoides, Panicum repens, Paspalum dictum.*	Goto *et al.,* (1953); Gonzalez *et al.,* (1991); Noda & Yamamoto (2008)
Sheath blight	*Rhizoctonia solani*	*Pennisetumm americanum, Solanum tuberosum, Cynodon dactylon, Digitaria adscendus, Echinochloa crusgalli and Cyperus rotundus, C. difformis, Setaria glauca, Panicum repens, Brachiaria, Commelina oblique and Amaranthus viridis*	Chahal *et al.,* (2003)
Brown spot	*Bipolaris oryzae, Cochilobolus miyabeans*	*Cynodon dactylon, Digitaria sanguinalis, Echinochloa colona, Leersia hexandra, Pennisetum typhoides, Setaria italic, S. glauca, Imperata arundica, Panicum miliare, Saccharum officinarum, Zizania latifolia.*	Ou (1985); Biswal & Mohanty (1995); Sunder *et al.,* (2014)
Rice false smut	*Ustilaginoidea virens*	*Echinochloa crus-galli, Imperata cylindrical, Digitaria marginata*	Shetty and Shetty (1985); Atia (2004)
Sheath rot	*Sarocladium oryzae*	*Cyperus diformis*, *Echinochloa crusgalli*, *Monochoria vaginalis, Cyperus teneriffe, Hyrneachne assamica*, *Leersia hexandra*, *Panicum walense,* O*ryzae rufipogon*	Deka & Phookan (1992); Srinivasachary *et al.,* (2002)
Bakanae	*Fusarium moniliforme (Gibberella fujikuroi)*	*Vigna unguiculata, Panicum miliaceum, Echinochloa oryzoides, Echinochloa crus-galli*	Anderson & Webster (2005); Carter *et al.,* (2008)

Conclusion

In order to meet the future food demand of rapidly growing population, rice production and productivity needs to be increased, besides the rice cultivation should be done in sustainable way to reduce its environmental adversities. Diseases cause an average yield loss of about 15 to 30 percent annually to rice production and the excessive use of pesticides cause water pollution and human health hazards. These yield losses can be reduced significantly by using different agronomic practices on the pretext of congenial environment favoring the crop. However, the use of combination of agronomic practices with careful acquaintance of the crop environment interactions under variable agroecology becomes imperative for attainment of tangible outcome.

Conflict of interest

The authors declare that they have no conflict of interest.

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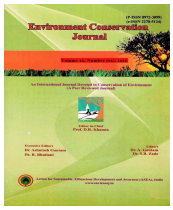

Assessment of heavy metal contamination by multivariate statistical methods from the sediment of Ulhas river estuary, Maharashtra, India

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ABSTRACT

Ulhas River estuary is one of the most significant estuarine systems situated western coast of India. This estuary has been polluted by several point and nonpoint source and therefore, the multivariate statistical methods were used to determine sediments parameter concentrations, their distributions, and their relationship. In the present study, sediment samples were collected from five different stations and analyzed eight heavy metals' concentrations with seven other parameters. The multivariate statistical methods (PCA, nMDS, and ANOSIM) were used to determine sediments parameter concentrations, their distributions, and their relationship. The PCA results showed that the concentrations of N%, H%, S%, C/N, C/H, EC, and OC% were significant contributors to PC1 (36%) while the heavy metals such as Cu, Pb, Cd, Ni, Si, and Hg concentration were major contributor to PC2 (20%). Both PCs are indicated anthropogenic pollutant deposition towards the mouth of the estuary. Other results of nMDS showed a high degree of similarities within the stations such as 2, 3, and 4. Moreover, analysis of similarities (ANOSIM) results also support them at a significant level of 0.01% with a global R-value (0.6). The observed level of heavy metals contamination in the sediment samples was in the order of Cr >Pb >Cu > Ni >Zn > Hg >Si>Cd. Industrial discharges within the catchment area may be the potential source of sediment pollution and warrants immediate targeted actions to protect this vital ecosystem and its biodiversity.

Introduction

Coastal zones are rich in natural resources despite being adversely influenced by human activities and ongoing/projected climate impacts (Goudie, 2006). Along the coastal areas, estuaries are ecologically dynamic and fragile ecosystems with unique physico-chemical and biological features (Rathod, 2005). The surface water chemistry of an estuary (at any point) reflects major influences such as the lithology of the catchment, atmospheric inputs, climatic conditions, and anthropogenic inputs (Vase *et al.,* 2018; Bhutiani *et al.,* 2018; Tyagi *et al.,* 2020). Timely identification and critical quantification of these influences are vital for managing land and water resources within the catchment (Bellos *et al.,* 2005). Derived trace metals from the catchment have been shown to accumulate in estuarine sediment and potentially capable of bioaccumulation in aquatic flora and fauna (Diagomanolin *et al.,* 2004). The ulhas river estuary is important in the Arabian Sea region of India. Not only does it support a biodiversity rich mangrove ecosystem, but also acts as a vital nursery ground for many commercially important fish species, which support the livelihoods and food security of local communities (Rathod and Patil, 2009). Nonetheless, the estuary suffers from various anthropogenic stressors within its catchment (Bhosale, 1991; Mirajkar *et al.,* 1995).

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This is not surprising as the Ulhas river flows through the industrial (electrical, chemical, and textile) zone of the cities of Thane, Ambernath, and Ulhasnagar, which release their mostly untreated effluent directly into the river (Rathod and Patil, 2009), mainly due to the high cost of the available treatment processes (Dotaniya *et al.,* 2016). As a result, the trace/toxic elements in dissolved and particulate form end up in the estuary (Pavoni *et al.,* 2021) and contribute substantially towards its deterioration. Moreover, Thane creek is more vulnerable to oil spills due to heavy ship transportation of crude oil (Sukhdhane *et al.,* 2013). This creek is connected to the Ulhas river estuary; therefore, oil pollution could be contributing to the estuarine pollution. Past studies from the Ulhas river estuary have reported the presence of heavy metals such as mercury (Hg) from water, sediment, and fish species (Sukhdhane *et al.,* 2013; Raut *et al.,* 2019); lead (Pb) and zinc (Zn) in sediment and polychaete (bristle) worms (Zingde, 1999); nickel (Ni) in sediment (Jha *et al.,* 2000); and mercury (Hg) from the hair samples of the fishermen communities residing near the banks of the Ulhas River (Menon and Mahajan, 2012). This is extremely worrying in terms of the health and wellbeing of the local communities dependent on ecosystem services from this estuary. The present study was conducted on the Ulhas River estuary to evaluate current heavy metal patterns, their distribution, relations, with other nutrient parameters of the sediment by multivariate statistical techniques. This was critical to identify the distribution pattern and their loads on this important coastal environment, recommend remedial strategies to protect and conserve the condition and sustainability of this estuarine ecosystem.

Material and Methods

Study area

The study was conducted on the Ulhas river estuary (19°29′ to 19°18′N, 72°88′ to 72°49′E), it is a major estuarine system along the western coast of India. The Ulhas River originates at the western slope of the Sahyadri range in Maharashtra state. It is the largest river of the Konkan coast (a rugged section of India's western coastline) and meets the Arabian Sea. The sampling stations were spread from Ghodbandar (19˚16'N, 72˚59'E) at the very narrow mouth of the river with a sandy bed, to Dongrichouki (19˚18'N, 72˚47'E) with the broad mouth of the estuary with muddy banks and mangrove vegetation (Figure 1).

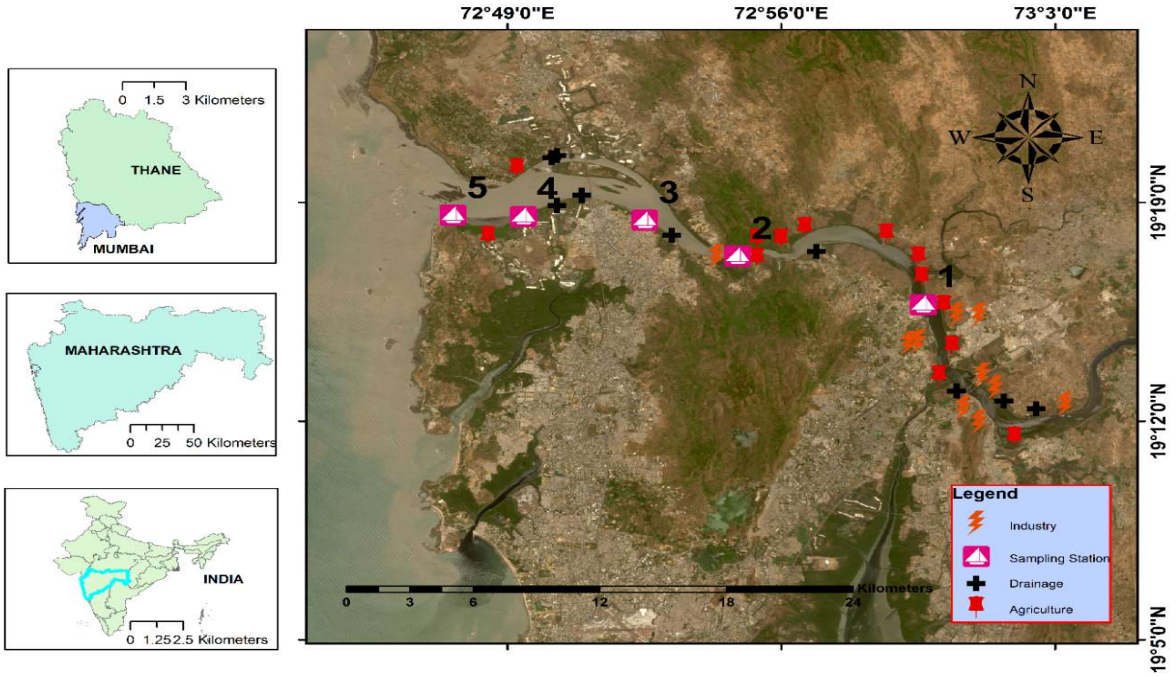

Figure 1: Locations of the study sites (Station 1 – Station 5) on the Ulhas river estuary.

Sample collection and analysis

Sediment samples were collected along the estuary from the selected sites (S1 – S5) monthly during the post-monsoon season (September 2017 to February 2018). The collected sediment samples were air-dried, ground to a fine powder in a glass mortar, and separately stored in new polythene bags. The sediment pH and the electrical conductivity (EC) was measured by portable conductivity meter (Eutech Instruments, India). total nitrogen (N), total sulphur (S), and total hydrogen (H) were analyzed using vario Micro cube CHNS Elemental analyzer, Germany. The available phosphorus (AP) in the sediment samples was estimated according to Olsen's (Olsen *et al.,* 1954) and the oxidizable organic carbon (OC) by Walkley-Black method (Walkley and Black, 1934).The analysis of trace metals was conducted by digesting the collected samples using supra pure concentrated acids in a microwave-based digestion system (microwave 3000, Anton Parr, USA). About 0.5 g of the sediment sample was taken in the microwave digestion vessel, to which 5 ml concentrated nitric acid, 2 ml hydrochloride acid and 1 ml hydrofluoric acid were added. The vessel was capped and heated in the microwave unit at 1200 W to a temperature of 190˚C for 30 minutes at a pressure of 25 bars. The digested sample was diluted to 50 ml and used for the analysis of metals, cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), silicon (Si) and lead (Pb) by atomic absorption spectrophotometer using flame atomization (Analyst 800, Perkin Elmer, USA). The cold vapour atomic absorbance method with flow injection for atomic spectroscopy (FIAS) was used to measure the concentration of mercury (Hg) (APHA, 2005). The analytical method and instrumentation efficiency was verified by the analysis of the reference materials obtained from the National Research Council of Canada for estuarine water (SLEW-3) and lobster hepatopancreas (TORT-2) in five replicates.

Results and Discussion

Nutrient concentration of sediment

The spatial distribution of various characteristics of the sediment values of pH, EC, OC, AP, N, H, S, C/N ratio and C/H ratio are presented in the box plot (Figure 2). Water salinity in the estuary varied from 10 to 30 ‰ during the sampling period, minimum value 10-25 ‰ was reported at stations 1 and 2 during September and October. The station-wise concentration range of EC (0.30 to 4.18 mS/cm) and pH (7.60 to 8.50) of sediment were recorded. The organic carbon contained in the sediment was measured between 0.38 to 2.49%. Higher mean OC% was found in the middle portion of the estuary, and lower mean value at the narrow belt of the estuary (Station 1). Other sediment nutrients like AP were reported between 0.09 to 1.60 g/kg, higher at station 3. The percentage range of N (0.31 to 0.63) and S (0.03 to 0.43) had similar distribution except station 1. This similarity range distribution is also shown in the ratio of C/N (5.57 to 0.85) and C/H (0.49 to 2.27).

Metal concentration of Sediment

The distribution of heavy metals (Cr, Cu, Zn, Pb, Cd, Ni, Si, and Hg) in the sediment represented on the boxplot (Figure 2). The average concentration of metal during the sampling period of the five-station ranged between 31.28 and 872.8 mg/kg for Cr; between 61.73 and 418.20 mg/kg for Pb; between 56.3 and 149 mg/kg for Cu; between 50.93 and 119.70 mg/kg for Zn; between 0.08 and 30 mg/kg for Hg; between 0.98 to 4.73 mg/kg for Si; between 0.14 to 3.46 mg/kg for Cd. Higher concentration levels of metal were observed between the station 3 and 5. Based on the average concentration of the metal, the following sequential order viz., Cr >Pb >Cu > Ni >Zn > Hg >Si>Cd was observed. There is no significant association in the spatial and temporal distribution of the estuary sediment's nutritional parameter. In disparity, metal concentrations in sediment were showed an increasing trend towards the mouth of the estuary and correlated to each other. Therefore, the correlation coefficient for metal concentration in sediment was performed ($p< 0.05$), Among the metal concentration in sediment, Hg has strongly correlated with Cd, Pb and Si ($r^2= 0.93, 0.96$; $p < 0.001$ respectively) whereas Cr has strongly correlation with Ni, Cd and Pb, ($r^2= 0.88, 0.87$; $p< 0.05$) (Figure 3).

Principle component analysis (PCA) and nonmetric multidimensional scaling (nMDS)

Multivariate statistical techniques are constructed by using various methods for viewing and analyzing complex data (Olkin and Sampson, 2001).

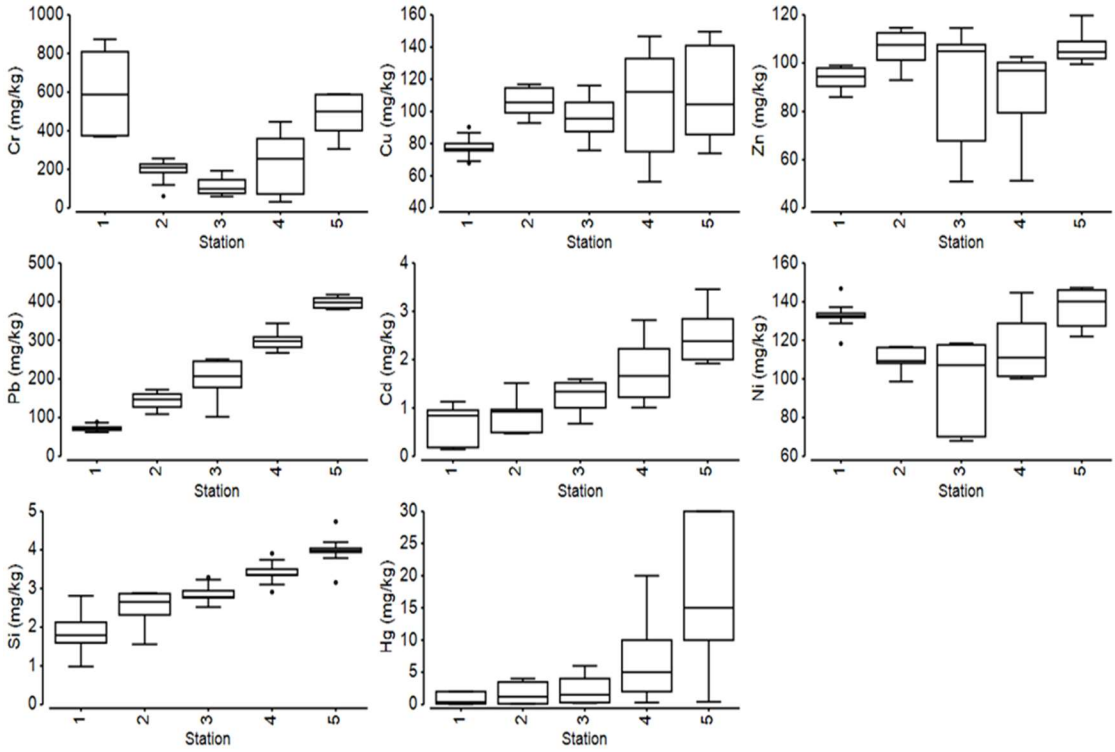

Figure 2: Box plots of nutrients parameter and heavy metals of the Ulhas river estuary sediment (the whisker shows the minimum and maximum values and the line of each plot is the median value).

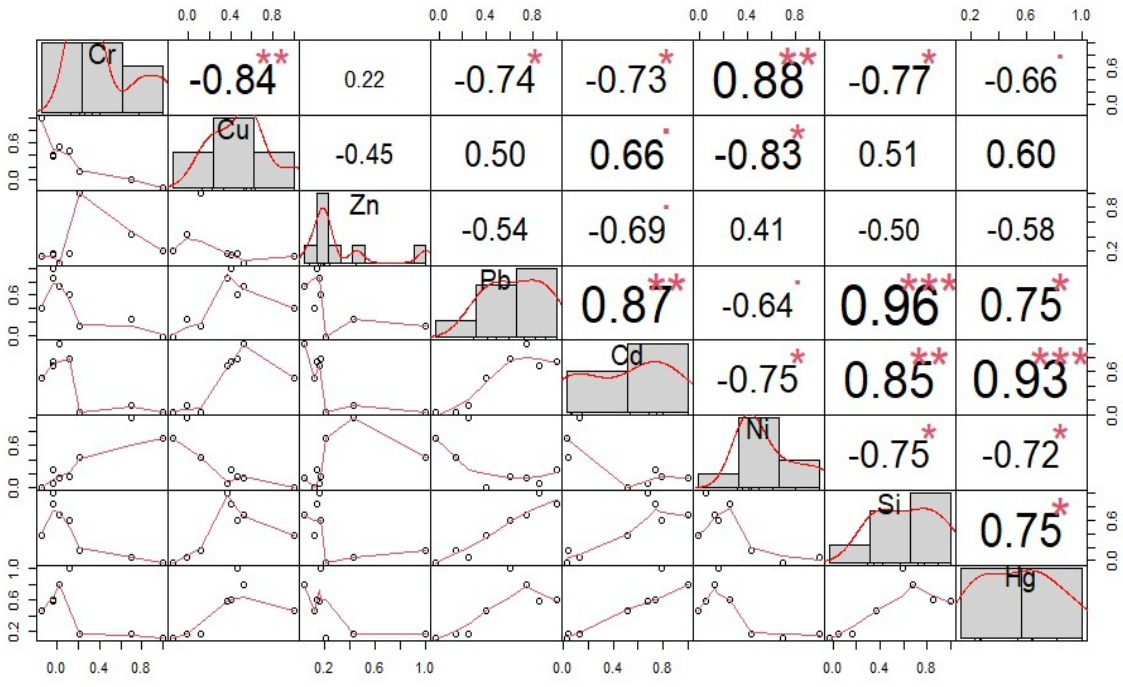

Figure 3: The bivariate scatter plots of heavy metals (Cr, Cu, Zn, Pb, Cd, Ni, Si, and Hg) (bottom) and the value of the correlation plus the significance level (top), symbol: *p*-values (0, 0.001, 0.01, 0.05, 0.1, 1) symbols ("*", "**", "*", ".", " ")**

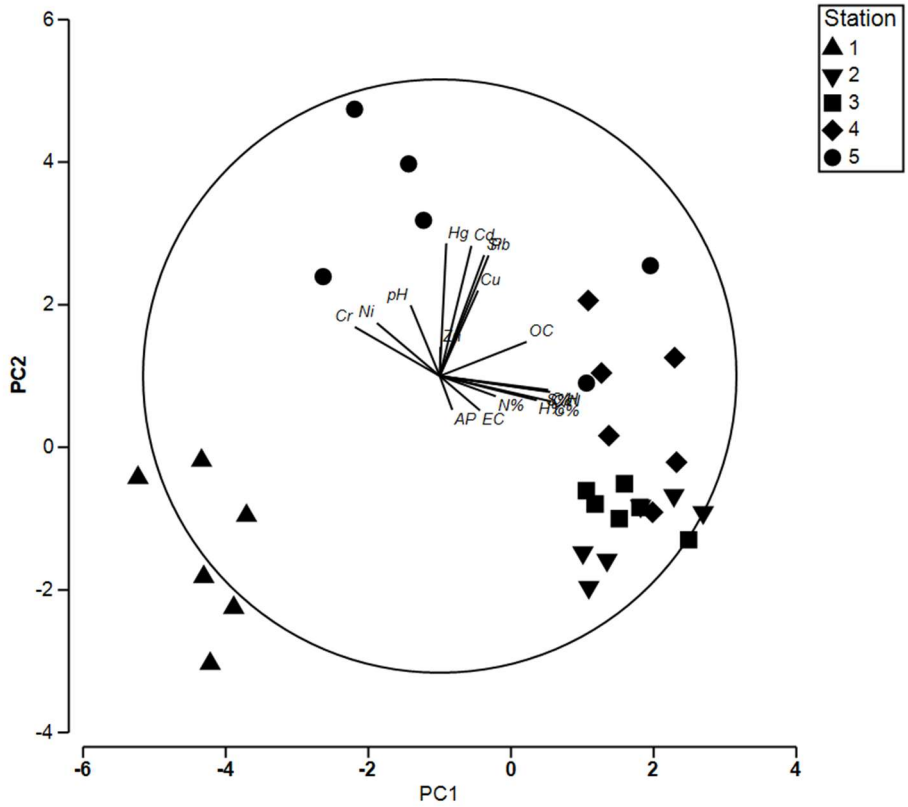

Figure 4: Plots of PC1 vs. PC2 showing all sampling station and sediment parameters.

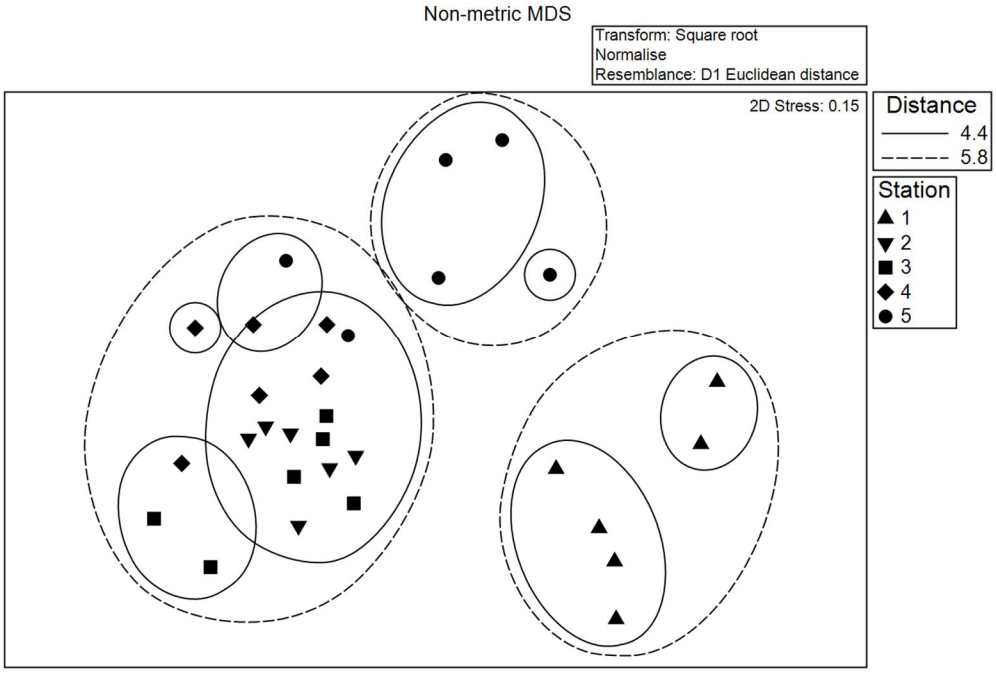

Figure 5: Ordination plot from nMDS (Euclidean distance) showing the relationship between monthly sampling site.

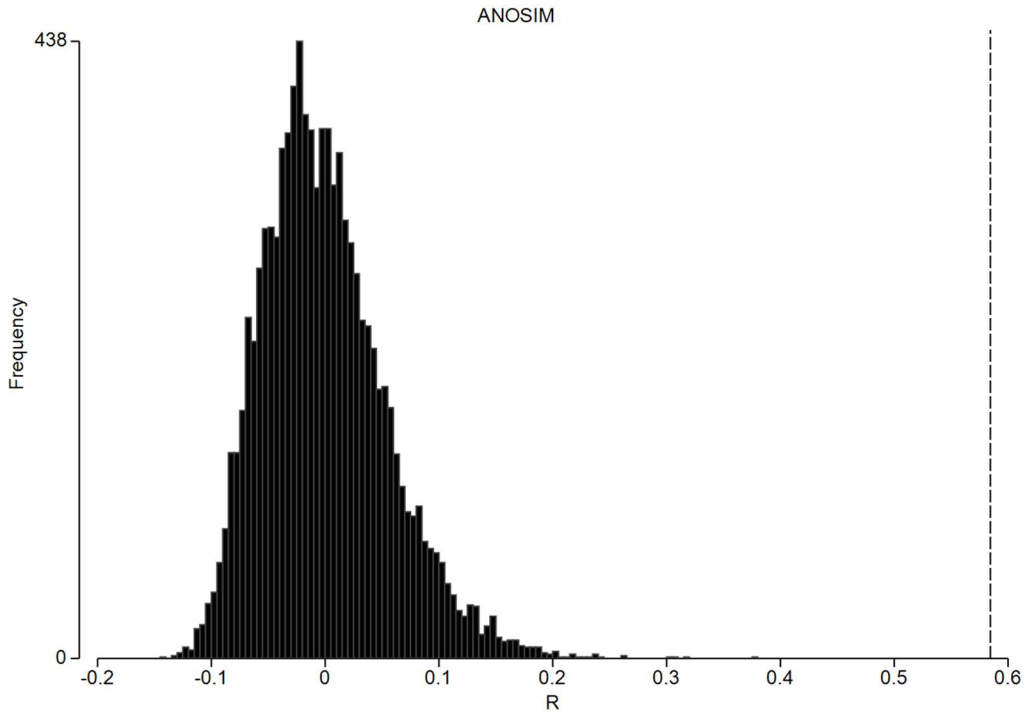

Figure 6: Analysis of similarities (ANOSIM) of the pairwise tests on the sampling stations at significant level of 0.01% with a global R-value (0.6).

Principle component analysis (PCA) and nonmetric multidimensional scaling (nMDS) are two visualized methods that allow us to assume how particular variables are distributed, correlated, and determine their association (Clarke and Gorley, 2015). Therefore, euclidean distance-based PCA and nMDS were calculated and visualized by using PRIMER-e package. PCA estimates the variable's correlation structure by finding a new principal component (PC) that accounts for the variance (or correlation) in a multidimensional data set. These new variables are a linear combination of the original variable (Grimnes and Martinsen, 2015). This method assists us in identifying groups of variables based on the loadings and groups of samples based on the scores (Zakir *et al.,* 2021). Each sample has a score and each component, which shows the sample's location to detect sample patterns, groupings, similarities, or differences (Facchinelli *et al.,* 2001; Han *et al.,* 2006). All selected sediment variables are analyzed by the PCA model (Figure 4). To understand the association of sediment samples from the five areas in six months. This implies normalizing all variables using their mean subtracted and are divided by their standard deviations. The eigenvalues measure the variance accounted for by the corresponding eigenvectors (Jolliffe and Cadima, 2016). From the eigenvalues of the PCA model, the first three PCs are adequate to explain 70% of the variation. The eigenvectors of the sediment parameter vectors lie along the PC1 axis, showing their larger values towards stations 2, 3, and 4 compared to stations 1 and 5. They all have significant positive coefficients in the PC1 but negligible in the PC2. The concentrations of N%, H%, S%, C/N, C/H, EC, and OC% were significant contributors to PC1 (36%) while the heavy metals such as Cu, Pb, Cd, Ni, Si, and Hg concentration were major contributor to PC2 (20%). The nMDS method is used for grouping variables or samples based on resemblance matrix and stress value. The results of nMDS at stress (0.15) showed that monthly sampling stations were clustered into three groups viz., station 1, station 5, and included stations 2, 3, and 4 (Figure 5). The stress value for nMDS indicated a strong representation of the degree of similarity of monthly sampling station

and distributed nutrient concentrations; however, hypothesis test and analysis of similarities (ANOSIM) results also support them at a significant level of 0.01% with a global R-value (0.6) (Figure 6).

Ulhas river estuary has been laid with urban pollution for more than four decades and has been recognized as a hotspot of metal contamination. The present study reports on the spatial distribution of nutrient and metals concentration from the bottom sediment along the estuary. The average depth of the sampling site was varied between 3.8 m to 7.7 m during the low tide period. The minimum depth ranges were reported at the broad mouth of the estuary (Stations 3 to 5), and maximum depth ranges were reported at the narrow mouth (Stations 1 to 2). The sediment texture of the Ulhas river estuary was found sandy loamy with an average of 60% sand, 24% silt, and 14% clay (Raut *et al.,* 2019). Sampling sites 1 and 2 are located adjoining the industrial discharges point of metropolitan cities like Dombiwali, Thane, and Bhayandar. The sediment texture in the sampling site 1 and 2 was sandy (>80%) at the average depth between 5 to 7m. this estuary has carried a large amount of untreated sewage and effluents towards the coastal area. However, the initial station (*i.e*., stations 1 and 2) has been reported less concentration of nutrients and heavy metals; it may be occurred due to the presence of >80% sand in the soil texture. Therefore, the concentration range of sediment parameters and heavy metals was increased from stations 3 to 5. The observed concentrations of heavy metals were higher in sediment samples at the mouth of an estuary, which could be due to nutrients availability and the high sedimentation rate here. The shallow depth of the river mouth has been reported to favor the re-suspension of sediment matters that contain a significant amount of Pb, Cu, Cr, Ni, and Zn; hence, it gets easily scavenged at the sediment-water interface (Dauby *et al.,* 1994). Additionally, automobile exhausts (a significant source of lead pollution) from urban run-off and the fishing boats could be contributing to the high level of lead. The considerable concentration of Si, Cd, and Hg was also reported in the sediment sample. It could be due to domestic discharges, and industrial effluents around the catchment sediments serve as sorbents/concentrators for various inorganic and organic chemicals. Thus, the strong binding affinity of the heavy metals has the potential to result in low concentration in water and high concentration in sediments (Salomons, 1982). The higher concentration of metals also supported during the post-monsoon months might be attributed to the heavy rainfall and subsequent river runoff, bringing much industrial and land-derived material along with domestic, municipal, and agriculture waste (Karthikeyan *et al.,* 2007; Sankar *et al.,* 2010; Ruhela *et al.,* 2019). Moreover, sediment particles moved downstream and were deposited at the river's mouth, thus facilitating the increasing accumulation of Hg, Pb, Cr, and Cd in the sediment. The metals were significantly correlated with each other and moderately correlated with the nutrient composition of the estuarine sediment, suggesting a common origin in the area. The most untreated wastewater discharges from industries into the surrounding drainage (which runs into the estuary) could be responsible for this (Bhosale, 1991; Ram *et al.,* 2003). Therefore, a significant concentration between the metals Cd, Pb, Si, Hg, Cr, and Ni in the sediment is attributed to the spatial distribution in the estuary.

In PCA, eigenvectors of all sediment parameters and heavy metal concentration of PC1 and PC2 lie towards stations 3, 4, and 5 (Figure 4). Factor loadings are classified into three categories i.e., strong (>0.75), moderated (0.75 to 0.5) and weak (0.5 to 0.3) (Liu *et al.,* 2003). PC1 dataset is explain 36% of the total variance and is strongly positive load with EC, OC%, H%, N%, S%, C/N, and C/H ratio. It indicated high nutrient availability toward the mouth of the estuary by the content of silt and clayey texture of soil (Chaston *et al.,* 2008). All the nutrients availability may have come from various sewage discharges from the metropolitan cities of the river catchment area (Singare *et al.,* 2011). PC2 dataset is explain 20% of the variance. It is strongly positively loaded with pH, Cr, Cu, Pb, Cd, Si, and Hg, indicating dispersion of heavy metals. It may originate from industrial effluents and infrastructure activities surrounding the catchment area (Singare *et al.,* 2012). Some nutrient parameters like salinity, pH, OC% are moderately correlated with the heavy metal concentration in the sediment's particles (Najamuddin and Surahman, 2017). PC3 represents

14% of the variance and is positively loaded with EC, AP, Zn, and Ni, indicating anthropogenic sources. Zn is dispersed within the aquatic environment may from high traffic density (tire wear particles) (Callender and Rice, 2000), and Ni is the primary source from the use of liquid and solid fuels, as well as municipal and industrial waste (Genchi *et al.,* 2020). They were settled as sediment at the estuarine bottom, and water currents and tidal fluctuation have carried out the dispersion of heavy metals towards the estuarine mouth (Dias *et al.,* 2013). The non-metric multidimensional scaling (nMDS) method is described the degree of similarity among the sampling station of each month based on sediment parameter concentrations. Moreover, cluster analysis analyzed groups of similarity by using Euclidean distance. Here, the group of station 1 of each month has separated, indicating less concentration of nutrient and heavy metal parameters. This station is situated at a narrow part of the estuary. There was less silt%, clay%, and organic carbon% was recorded; therefore, maybe it was separated from the remaining station (Figure 5). Another cluster of station five is situated at the front of the estuary. More exposure to seawater and salinity at this station may dilute some nutrients and heavy metals parameters like N%, H%, AP%, Cr, and Zn. Therefore, they are reported less in concentration. The third-largest cluster of stations 2, 3, and 4 indicates that the middle portion of estuarine sediment quality is similar (Figure 5). Moreover, in the last month of the post-monsoon season's station, five also support them. In this cluster, the physical properties like depth and soil texture were also recorded similarly in that area.

Conclusion

The gradual increase in heavy metal levels (compared to previous studies in the catchment area) has the potential for bioaccumulation in aquatic species, and eventually in humans, through the food web. Aquatic species such as fish form an important component contributing towards the food and livelihood security of the dependent communities in the catchment area. Therefore, the current findings contribute to the strong information base for the estuary and, more importantly, stress the need for long-term monitoring of pollution sources. The findings also warrant the formulation of targeted policies for establishing, developing, and improving wastewater treatment plants in the riverine catchment area, mangrove plantation on the estuarine margin area and increased attention on the rich biodiversity supported by the ecosystem.

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Conflict of interest

The authors declare that they have no conflict of interest.

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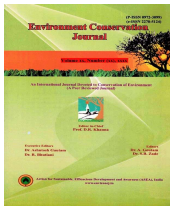

Assessment of water quality using different physicochemical and biological parameters: a case study of Buddha nallah, Punjab, India

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ABSTRACT

For the assessment of physicochemical and microbiological quality of Buddha Nallah the water samples were drawn from 7 different sites and analysed during winter (December 2020) and summer (May 2021) for most probable number, heterotrophic plate count, total coliform, fecal coliform, indicator, emerging pathogens and physicochemical parameters. A strong correlation was found among the indicator organisms (r= 0.504-0.898), while relatively weak or no correlation was found between indicator and emerging pathogens. Moreover, the correlation between indicator and emerging pathogens was found to be heavily dependent on physicochemical parameters. Cluster analysis successfully classified the different polluted sites based on physicochemical and microbiological parameters. The water quality index (WQI) score of all sites was found between 0-25 indicating poor water quality and emergency treatment is required for reuse. Based on present study results, it has been concluded that water of study area is highly polluted and pose serious health risk concerns due to presence of fecal and emerging pathogens in samples.

Introduction

Water pollution is a global threat to both natural diversity and human health. With increase in urbanization / industrialization and to achieve global food security, the use of chemical pesticides, mining and other anthropogenic processes has been increased. These practices result in the deterioration of water bodies and increases morbidity rate (Bhutiani *et al.,* 2021a; Ruhela *et al.,* 2022). Annually about 37.7 million Indian population got affected by water-related illness resulting in $600 million economic cost. It has been estimated that 70 % of surface water in India is unfit for human consumption. Every day about 40 million liters of wastewater enters surface waters, groundwater and other water bodies (Bhutiani *et al.,* 2021b). Water contamination from human waste, agriculture runoffs, and industrial effluent discharges is related to the release of toxic compounds that can stimulate the rapid and excessive growth of microbial pathogens. The risk of contamination increases when waterborne pathogens and nutrients, such as nitrates, phosphorous and nitrogen, are transported from residential, agricultural and industrial areas to natural streams like ponds, lakes, rivers, sea and finally to ocean, causing serious disruption in aquatic biodiversity (Sinha *et al.,* 2016; Ruhela *et al.,* 2021). Assessment of water quality using physicochemical and biological parameters have been studied to protect the biodiversity and to rejuvenate the water resources reported in literature (Jindal and Sharma, 2011; Mavukkandy *et al.,* 2014; Bhutiani *et al.,* 2018; Kaur *et al.,* 2021; Das *et al.,* 2021). Total Coliform (TC) and Fecal Coliform (FC) have been used for assessment of water quality and emergence of waterborne pathogens in water. According to the World Health

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Organization (WHO) and Bureau of Indian Standards (BIS), *Escherichia coli* is widely used as indicator of fecal contamination, as it is highly tolerant to environmental changes and disinfection than viruses, bacteriophages etc. However some reports have shown a weak relation between coliform and waterborne pathogens that leads to uncertainty in quantifying the exposure (Goh *et al.,* 2019).

Ludhiana is a district of the Punjab, India with catchment area 159 kilometer square. It is a fast growing industrial hub of northern India and referred as Indian Manchester by BBC in 2014 (Anonymous, 2014). Until the early 90's, the residents of Ludhiana uses "Buddha dariya" water for drinking and domestic purposes. But due to ongoing continuous discharge of wastewater like industrial effluents, sewage disposal and agricultural runoffs water gets extremely deteriorated and name got changed to "Buddha Nallah" by local people. This polluted water is being used by the farmers for irrigation purposes in this area and cause high metal toxicity in soil which leads to high human health risks (Kaur *et al.,*2021). According to PGIMER Chandigarh and Punjab Pollution Control Board, the villages near the Nallah contain more amounts of calcium, magnesium, mercury, fluoride and heptachlor than the permissible limits in tap and ground water. Several projects have been involved in rejuvenation of water quality of Buddha Nallah like on April 2011 Indian Ministry for Environmental and Forests installed 'In-Situ Bioremediation project' on Buddha Nallah. In 2021, Punjab Government launches 840 crore Buddha Nallah rejuvenation project for domestic wastewater management.

Keeping all these points, this study is focused on assessment of water quality of Buddha Nallah, Ludhiana, India during winter (December 2020) and summer (May 2021) season. The present study is being undertaken with the following objectives: (1) to generate a database of physicochemical and microbiological quality of Buddha Nallah water with season variability and to correlate with pathogenicity; (2) to assess the emergence of indicator and emerging pathogens in water with seasonal 103 variability; (3) to identify the correlation between physicochemical, biological and indicator/emerging pathogens.

Material and Methods

Sampling sites and processing

The Buddha Nallah or Buddha dariya is a natural seasonal water stream that runs through the Malwa region of Punjab, India. It originates from Koom Kalan, Punjab (76˚06′55.30′′E, 30˚92′07.21′′N) and passes through Ludhiana city, where it ultimately drains into the Sutlej River near village Walipur (75˚62′69.02′′E, 30˚97′49.74′′N), District Ludhiana, Punjab, India. Sample sites of Buddha Nallah were selected to represent possible pollution sources for industrial effluent discharges, agricultural runoffs, dairy and domestic wastes. Water samples were collected from 7 sites of Buddha Nallah (Table 1, Figure 1) during the winter and summer. A total of 14 water samples were collected during winter (December 2020) and summer (May 2021) from 7 sites of Buddha Nallah, Ludhiana, India. The water samples were collected from approximately 20cms depth of the water surface in sterile bottles (1L capacity) under aseptic conditions to avoid other bacterial contamination from different locations. The sample bottles were tightly capped and labelled with the site, time, and date of collection and stored at 4˚C during transit to the laboratory. Water sampling, handling, preservation, and processing were done by following standard protocols (WHO, 2008; USEPA, 2012).

Physicochemical analyses

Water samples were analysed for pH, water temperature, electrical conductivity (EC), nitrates, sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}) as standard procedures of American Public Health Association (APHA 2012). Calcium, magnesium, chloride, and nitrate were determined using the titration method, whereas sodium and potassium were analysed through flame spectrometry. Biological oxygen demand (BOD) and dissolved oxygen (DO) were estimated using the Winkler titration method (Winkler 1888) with azide modification followed by five days of incubation in dark conditions at 20˚C under aerobic conditions (for BOD samples).

Total heterotrophic and coliform count

Total Heterotrophic plate count (THPC) includes all the micro-organisms capable of growing in nutrient-rich media.

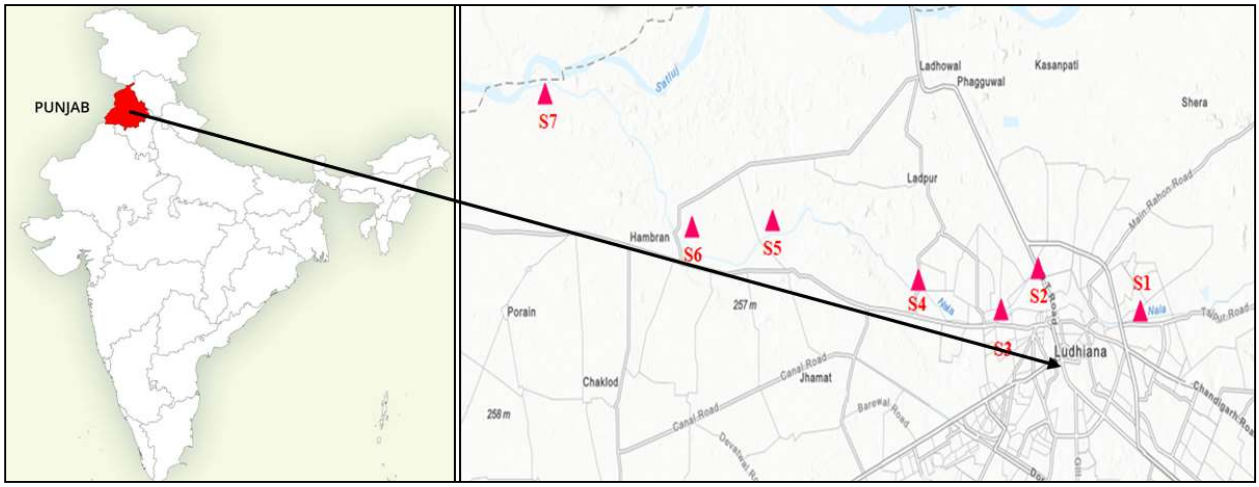

Figure 1: Study area map indicating (A) Punjab location in India map (B) Labeled sampling sites (S1 to S7) of Buddha nallah, Ludhiana, Punjab, India. (*Source (A) from Google images (B) assessed from USEPA (US environment protection agency) site through DWMAP* https://www.epa.gov/sourcewaterprotection.)

Table1: Description of sampling location and pollution sources in Buddha nallah, Ludhiana, India

Sampling sites	Description	Co-ordinates		Waste sources
		Longitude	Latitude	
S1	Tajpur road, near Haibowal Kalan, Ludhiana, India	75˚87′70.45″ E	30˚91′72.29″ N	Domestic, agricultural, dairy and textile waste effluent
S2	GT Road Ladhowal, New Kitchulu Nagar, Ludhiana, India	75˚79′20.31″ E	30˚92′53.33″ N	Domestic, agricultural and dairy
S3	Village Baran Hara, Distt. Ludhiana, India	75˚76′94.73″ E	30˚93′76.93″ N	Domestic and dairy
S4	Village Talwara, Distt. Ludhiana, India	75˚74′90.01″ E	30˚94′22.44″ N	Domestic, dairy and agricultural
S5	Near Malakpur-Nurpur Bet Road, Distt. Ludhiana, India	75˚72′54.39″ E	30˚93′71.08″ N	Domestic and paper mills effluent
S6	Near Hambran Laddowal Road, Distt. Ludhiana, India	75˚68′93.46″ E	30˚93′94.53″ N	Domestic, agricultural and paper mills effluent
S7	Near Village Walipur Kalan where Buddha nallah emerges River Sutlej	75˚65′11.87″ E	30˚97′28.52″ N	Domestic and agricultural

All water samples were analysed for bacterial total heterotrophic plate count consisting of the mixing of aliquots of 1-ml water with nutrient agar (Hi-media) thoroughly and incubation at 37ºC for 24-48h to encourage the growth of bacteria associated with the human fecal flora. The number of bacteria were enumerated and expressed in $\log_{10}$ colony-forming unit per ml ($\log_{10}$ CFU/ml). The presence of coliform in water samples were confirmed through the multiple fermentation tube technique as a most probable number (MPN) index. The MPN tests are statistical methods based on probability dispersion analysis (APHA 1998, APHA 1971). Analysis of total coliforms (TC) and fecal coliform (FC) was carried out by using the membrane filtration method as described Koster *et al*., (2003).

The colonies of bacteria were enumerated and expressed in a $\log_{10}$ colony forming unit per ml ($\log_{10}$ CFU/ml).

Isolation of indicator and emerging pathogens

The enriched samples from positive MPN tubes were cultured for the presence of *E.coli*, Urinary tract infection (UTI) bacteria, *Vibrio spp*., *Salmonella spp*., *Shigella spp*., *Aeromonas spp*., *Listeria spp*., and *Staphylococcus spp*. by following the standard procedures (Koneman *et al.,* 1983). The samples were inoculated on selective media procured from Himedia Pvt. Ltd; Eosin Methyl Blue gar (EMB agar), Thiosulfate Citrate Bile salts Sucrose agar (TCBS agar), Salmonella Shigella agar (SS agar), Aeromonas selective agar supplement with ampicillin, Listeria selective agar with Listeria supplement, Baird parker agar supplement with 1% potassium tellurite followed by incubation at 37˚C for 24-48h. Bacterial pathogens were identified based on phenotypic and biochemical characterization that was further confirmed by using Bergey's Manual of Determinative Bacteriology. The single colony of each pathogen selected from the agar plate and streaked onto freshly prepared nutrient agar slants were stored in trypticase soy broth containing 30% glycerol stock solution at -20˚C.

Water quality index (WQI)

The water quality index (WQI) method is used to evaluate the overall water quality based on physico-chemical and biological parameters for multiple usage purposes. Based on the calculated water quality index (WQI), water samples were categorized into different categories. Lesser the WQI score; more the water pollution level and greater risk to health. The WQI calculated using the following equation (VEA 2011):

$$WQI = \frac{WQI_{pH}}{100}\left[\frac{1}{5}\sum_{a=1}^{5} WQI_a \times \frac{1}{2}\sum_{b=1}^{2} WQI_b \times WQI_c\right]\frac{1}{2}$$

Where WQI_{pH} represents pH; WQI_a for chemical parameters (BOD, DO, nitrates, Na, K, and chlorides); WQI_b for physical parameters (EC and total hardness) and WQI_c for biological parameters (coliform count).

Statistical analysis

All the experiments were completed in triplicate and the data were presented as mean± standard deviation (SD). The Pearson method was selected for the correlation analysis to examine if there was a significant difference between the distributions of microbial population and physicochemical parameters with seasonal variability at different sites. Cluster analysis was used to classify the sampling sites based on physicochemical and microbial quality of water during the winter-summer season. Data was analysed using SPSS software (version 16.0, SPSS Inc.).

Results and Discussion

Water samples were collected along the course of Buddha Nallah and analysed for physicochemical parameters (pH, temperature, EC, BOD, DO, Ca, Mg, Na, K, Nitrate and Chlorides) and microbiological qualities (MPN index, Heterotrophic plate count, Total coliforms, Fecal coliform, indicator and emerging pathogens) during the winter (December 2020) and summer (May 2021) season before second COVID lockdown. So, the seasonal variation should be observed and water quality can be assessed.

Seasonal variation in physicochemical analyses of Buddha Nallah

All physicochemical parameters analysed during different seasons were presented in Table 2. The water samples analysis of Buddha Nallah revealed that in winter, pH of different sites varied from 6.1 to 6.96 and 6.09 to 7.18 during summer season. As high or low pH in water is an indicator of presence of chemical or heavy metal pollution. The maximum value of pH is observed in summer and minimum in winter that attributed to increase or decrease in inorganic and organic pollutants. pH values for sites S1, S3, S6 and S7 were found to be acidic in nature and below the permissible limits (6.5-8.5) prescribed by WHO (2011). Acidic nature of water is an indication of contamination and unsafe for drinking purposes. Matta *et al.*(2017) recorded variation in pH in Ganga River with season variability due to discharge of industrial waste.Water temperature is one of most important characteristics of an aquatic system, as it directly affects the dissolved oxygen (DO) levels, chemical and biological processes in water. With increase in temperature, the solubility of oxygen decreases in water. In Buddha Nallah, water temperature ranged between 26.3˚C (S1) to 28.2˚C (S6) during winter and 33.8˚C (S1) to 36.9˚C (S6) in summer season.

Table 2: Seasonal variation of physicochemical characteristics of Buddha Nallah water samples

Parameters*	S1	S2	S3	S4	S5	S6	S7
Winter Season							
pH	6.21±0.01	6.80±0.02	6.36±0.14	6.96±0.04	6.27±0.06	6.10±0.10	6.25±0.04
Temp (˚C)	26.3±0.20	27.8±0.25	26.6±0.20	27.3±0.80	26.8±0.65	28.2±0.40	27.3±0.25
EC (µS/cm)	3214±4.00	3554±4.00	3426±5.50	2685±2.00	2542±2.00	2274±4.00	1532±1.50
BOD (mg/L)	229.1±1.60	356.1±2.10	122.8±2.75	107.2±2.10	244.4±1.80	56±1.00	76±1.75
DO (mg/L)	BDL	1.31±0.11	BDL	BDL	0.66±0.03	0.98±0.04	4.68±0.40
Nitrate (mg/L)	21.1±1.05	19.4±0.10	16.7±0.95	20.0±0.50	18.2±0.95	11.9±0.40	12.2±0.20
Ca+Mg (mg/L)	241.7±1.70	140.4±2.68	134.9±1.19	346.3±5.07	234.5±1.79	148.8±3.58	215.9±3.36
Na^+ (mg/L)	306.6±4.55	389.2±0.95	276.5±2.00	337.7±5.15	274.2±3.00	322.3±2.95	276.3±0.90
K^+ (mg/L)	27.8±0.55	24.2±1.15	24.8±0.20	23.8±0.35	21.9±0.65	27.9±0.65	23.3±0.90
Choride (mg/L)	208.2±6.75	203.3±4.80	210.1±0.90	130.6±1.65	163±4.00	236±9.00	194.6±3.60
Summer season							
pH	6.03±0.00	7.17±0.01	6.45±0.10	7.18±0.02	6.59±0.03	6.09±0.01	6.15±0.02
Temp (˚C)	33.8±0.70	35.9±0.10	34.7±0.30	35.5±0.55	35.8±0.75	36.9±0.15	35.8±0.25
EC (µS/cm)	3911±0.50	4083±1.00	3932±1.50	3079±1.00	2864±3.50	2239±1.50	1823±3.00
BOD (mg/L)	370.1±0.75	422±0.00	257.6±3.60	213.4±1.20	247±1.00	82.2±2.00	101.8±1.50
DO (mg/L)	BDL	0.81±0.03	BDL	BDL	BDL	BDL	2.27±0.31
Nitrate (mg/L)	34.2±0.05	22.7±0.15	19.5±0.20	26.2±0.10	20.6±0.05	22.1±0.04	14.1±0.03
Ca+Mg (mg/L)	310.5±1.53	235.0±3.16	164.9±3.73	398.3±3.44	258.6±1.72	186.1±2.30	278.3±2.44
Na^+ (mg/L)	341.5±0.55	411±0.80	317.7±20.7	362.5±3.70	313.4±2.60	381.3±1.05	316.9±4.15
K^+ (mg/L)	27.0±0.10	28.9±0.25	34.8±0.18	35.0±0.26	31.6±0.18	34.1±0.60	32.9±0.13
Choride (mg/L)	273.2±1.75	257.3±0.80	219.7±0.25	155.6±3.35	173±4.00	261±6.00	206±5.00

Temp: water temperature; EC: electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K:potassium

The fluctuation in temperature at different sites might be due to discharge of cooling water and heated industrial effluent into water. Similar results were observed by Bhatia *et al.,*(2018), in Buddha Nallah during the study period of 2014.Increase in ion concentration or dissolved solids in water enhance its electrical conductivity (EC). According to WHO (2011) standards, EC value should not exceed 1000µS/cm. In current study, EC value varied from 1532µS/cm (S7) to 3554µS/cm (S2) in winter and 1823µS/cm (S7) to 4083µS/cm (S2) in summer season. Electrical conductivity (EC) was observed to be relatively high in upstream as compared with the downstream sites. These results clearly indicate that water in the present study area contain high amount of dissolved solids and is highly polluted with industrial and domestic discharges (Jindal and Sharma 2011).

Dissolved oxygen (DO) is the most well-established parameter of water quality assessment and essential for the survival for all aquatic life. Moreover, oxygen affects a vast number of water indicators, not only biochemical but also aesthetic ones like odour, clarity and taste. The water samples analyses of Buddha Nallah seem to indicate low level of dissolved oxygen (DO) as compared to standards given by WHO (2011) which ranged from 4-6mg/L. In Buddha Nallah, the DO found to be more in downstream as compared to upstream sites. Majority of sites were observed to have DO below detection level (BDL) during both winter and summer season. This finding revealed that the pollutants in the water consumed a large amount of dissolved oxygen. Biological oxygen demand (BOD) is used as approximate measure of the amount of biochemically degradable organic matter present in sample. Laboratory analyses indicates BOD value varied to 56mg/L (S1)-356mg/L (S2) during winter and 82.2mg/L (S1)- 422mg/L (S2) in summer season. The increase in BOD values in summer season can be due to high microbial activities while decomposing organic matter under aerobic conditions (Yisa and Tijani 2010). Similar results reported by Mena-Rivera *et al.* (2017) in Burio river of Costa rica and Sharma *et al* . (2020) in Yamuna river of India.

Nitrate is oxidized form of dissolved nitrogen and required as main source of nitrogen for aquatic plants. However, in high amount nitrate can be toxic and leads to eutrophication in water bodies. In India, rising nitrate level in water resources is one of most challenging and growing water problems. Irrigation water containing fertilizers is a common source of nitrate contamination. In Buddha Nallah, nitrate content varied from 12.2mg/L (S2) to 21.1mg/L (S1) in winter and 14.1mg/L (S7) to 34.2 mg/L (S1) in summer season. The nitrate content in both seasons was found to be below the permissible limit of 50mg/L as prescribed by WHO (2011) and BIS (2012).The degree of hardness in water is expressed in the terms of calcium (Ca) and magnesium (Mg) content. According to WHO (2011), the permissible limit for Ca and Mg content in water should not exceed above 500mg/L. The concentration of Ca and Mg in Buddha Nallah at different sites during winter and summer season was found to be under prescribed values. Kaur *et al.*(2021) also observed calcium and magnesium contents present in Buddha Nallah within the permissible limits.

Sodium (Na^+) and potassium (K^+) are essential elements for human health. High amount of these elements in drinking water could be a concern for human health. In present study, results showed the presence of high amount of sodium (Na^+) and potassium (K^+) in water samples of both winter and summer season, particularly due to high amount of industrial effluent, domestic waste and agriculture runoff discharges. Chlorides occur in surface and groundwater from anthropogenic processes such as agricultural runoffs, industrial effluent, irrigation drainage and animal feeds. The high amount of chlorides in water increases electrical conductivity (EC) and led to corrosion of metal pipes. All the water samples contain chlorides within permissible limits (WHO 2011) except sites S1, S2 and S6 during the summer season that indicates the human health risks.

Seasonal variation in microbiological quality of Buddha Nallah

The results were analysed seasonally for the portability of water samples based on MPN index. It has been observed that in both seasons winter and summer all the samples were found to be non-potable, attributed to environment factors and industrial waste that favour the survival rate of microorganisms. Total Heterotrophic plate count (THPC) includes the natural micro-biota of water and also organisms derived from different pollutant sources e.g. industrial effluent, sewage mixing and

agricultural runoffs. The maximum microbial load in water samples was recorded in summer as compared to winter season as shown in Fig2a.The highest log value of microbial count, winter and summer was recorded as 8.46±0.01 (S2) and 8.59±0.02 (S5), respectively. The variation in microbial count at sites has been attributed to the change in temperature, availability of nutrient and extent of pollution. Prasad *et al.*(2015) reported variation in microbial count from 1.0 to 3.2×10^3 along east coast of India.

Coliform are often referred to as 'indicator organisms' because it indicate the presence of faecal contamination and enteric pathogens in the water system. The maximum total coliforms and faecal coliform count for water samples were found in summer as compared to winter season (Fig 2b, 2c). All samples were highly contaminated with the coliform and indicate the presence of water-borne pathogens in water. Maximum log value of Total coliform (TC) and Faecal coliform (FC) were ranged between 7.41±0.01 (S5) and 5.41±0.01 (S5), respectively during summer. In winter maximum log value of Total Coliform and Faecal Coliform was recorded as 7.16±0.02 (S7) and 5.15±0.00 (S7), respectively. The results revealed that water of Buddha Nallah for both seasons were equally contaminated with coliform organisms but counts were significantly higher in summer season. The high load of coliform attributed to the heavily populated area along Buddh Nallah and mixing of domestic/sewage wastewater to water.

Seasonal occurrence of indicator and emerging pathogens

According to World Health Organization (WHO) and Bureau of Indian Standards (BIS) standards, no water intended for human consumption shall contain *Escherichia coli* in 100ml of water sample. *E.coli* is an indicator of presence of the faecal contamination and act as surrogate microbe for water quality assessment. The samples were analysed for the occurrence of *E.coli* and relative abundance was recorded during winter and summer season with respect to temperature fluctuation (Fig 3).It has been observed that the incidences of indicator organisms have been detected in all samples. The maximum relative abundance of *E.coli* was 29.3% in winter and 29.1% in summer. The results suggested that seasonal variation doesn't affect the prevalence of *E.coli* in Buddha Nallah. In recent years emerging pathogens have arisen as a major public health concern. Emerging pathogens include urinary infection bacteria (UTI), *Vibrio cholera,V. parahaemolyticus, Aeromonas hydrophilia, Salmonella entrica, Shigella flexneri., Listeria monocytogenes and Staphylococcus aureus.* In present study, all emerging pathogens shared more than 1% relative abundance in different sites of Buddha nallah are presented in Fig 3.The results from Figure 3 shows that UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica,Listeria monocytogenes and Staphylococcus aureus* were detected in the both seasons, while the distribution *Shigella flexneri* varied greatly. In summer, there were 6 dominant emerging pathogens detected in the water samples. The maximum relative abundance of UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica,Listeria monocytogenes and Staphylococcus aureus* were found to be 31.1%, 14.1%, 12.4%, 13.6%, 14% and 11.2%, respectively. None of the samples were contaminated with *S. flexneri* during the summer season. In winter season, 7 emerging pathogens were analyzed in water samples. There was no significant difference in the pathogen abundance for the two seasons except for the *S. flexneri* which had significantly higher abundance in winter season. The maximum abundance of UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica, Shigella flexneri, Listeria monocytogenes and Staphylococcus aureus* in winter was recorded as 31.9%, 15.1%, 13%, 12.9%, 6.14%, 14.8% and 11.5%, respectively.

Correlation between microbial indicators, pathogens and physicochemical parameters

Correlation analysis was conducted with Pearson coefficient method. The correlation examined (i) between physiochemical parameters (Table 3); (ii) between microbial indicators/pathogens (Table 4); and (iii) between physicochemical parameters and indicators/pathogens (Table 5) in both seasons. This study found significant positive correlation among the physicochemical parameters; pH, EC, BOD, nitrate and temperature in both seasons. Wherein, with increase in EC and temperature, there is significant reduction in BOD and nitrate, respectively. Among the indicator organisms,

Table 3: Pearson correlation between physicochemical parameters

Winter season										
	pH	Temp	EC	BOD	DO	Nitrate	Ca+Mg	Na^+	K^+	Chloride
pH	1									
Temp	0.068	1								
EC	0.503*	0.549**	1							
BOD	0.694**	0.584**	0.773**	1						
DO	0.164	0.169	-0.632**	-0.270	1					
Nitrate	0.185	-0.439*	0.710**	0.601**	-0.680**	1				
Ca+Mg	-0.625**	-0.224	-0.259	-0.341	-0.264	0.417	1			
Na^+	0.432	0.476*	0.440*	0.413	-0.302	0.343	-0.062	1		
K^+	-0.214	-0.012	0.174	0.237	-0.410	-0.056	-0.254	0.060	1	
Chloride	0.302	0.191	0.098	0.320	0.209	-0.473*	0.841**	0.002	0.638**	1
Summer season										
pH	1									
Temp	-0.016	1								
EC	0.523*	0.567**	1							
BOD	0.513*	0.555**	0.852**	1						
DO	-0.252	0.183	-0.479*	-0.236	1					
Nitrate	-0.342	-0.553**	0.555*	0.490*	-0.304	1				
Ca+Mg	-0.340	-0.315	-0.037	0.023	0.017	0.672**	1			
Na^+	0.329	0.029	0.568**	0.533*	0.036	0.103	0.277	1		
K^+	0.328	0.380	-0.467*	-0.847**	-0.042	-0.663**	-0.192	-0.426	1	
Chloride	-0.530*	-0.111	0.306	0.493*	-0.019	0.146	-0.421	0.173	0.576**	1

Temp: water temperature; EC:electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K: potassium
***significant at the p<0.05**
****significant at the p<0.01**

Table 4: Pearson correlation between indicator and emerging pathogens

Winter season											
	THPC	TC	FC	*E.coli*	UTI bacteria	*V. cholera*	*S. enterica*	*S. flexneri*	*L. monocytogenes*	*A. Hydrophila*	*S. aureus*
THPC	1										
TC	0.504*	1									
FC	0.664**	0.833**	1								
E.coli	0.549**	0.563**	0.528*	1							
UTI bacteria	0.151	-0.790**	-0.740**	0.179	1						
V. cholera	0.468*	0.502*	0.654**	-0.292	0.059	1					
S. enteric	0.774**	-0.011	0.061	0.092	-0.046	0.481*	1				
S. flexneri	0.684**	0.077	0.233	0.363	-0.209	0.463*	0.463*	1			
L.monocytogenes	-0.031	0.028	-0.004	0.401	-0.121	-0.319	-0.298	0.377	1		
A. hydrophila	0.704**	0.059	-0.112	-0.495*	-0.059	0.761**	0.560**	0.436*	-0.334	1	
S. aureus	-0.303	0.812**	0.907**	-0.065	-0.820**	-0.025	0.093	0.038	-0.163	-0.166	1
Summer season											
THPC	1										
TC	0.584**	1									
FC	0.742**	0.898**	1								
E.coli	0.606**	0.543*	0.643**	1							
UTI bacteria	-0.577**	-0.777**	-0.756**	-0.109	1						
V. cholera	0.594**	0.568**	0.602**	-0.194	-0.235	1					
S. enteric	0.092	0.309	0.083	0.158	-0.239	0.280	1				
S. flexneri	-	-	-	-	-	-	-	1			
L.monocytogenes	-0.079	-0.659**	-0.445*	-0.042	0.263	-0.602**	-0.122	-	1		
A. hydrophila	0.258	0.097	0.051	-0.776**	-0.292	0.319	-0.261	-	-0.332	1	
S. aureus	0.069	0.481*	0.278	-0.380	-0.782**	-0.032	0.282	-	-0.276	0.332	1

THPC: Total Heterotrophic Plate Count; TC: Total Coliform; FC: Fecal Coliform; UTI bacteria: Urinary Tract Infection bacteria
***significant at the p<0.05**
****significant at the p<0.01**

Table 5: Pearson correlation between physicochemical parameters and microbial indicator/pathogens

Winter season										
	pH	**Temp.**	**EC**	**BOD**	**DO**	**Nitrate**	**Ca+Mg**	**Na^+**	**K^+**	**Chloride**
THPC	0.634**		-0.738**		-0.750**	-0.651**		0.436*		
TC	0.581**	-0.448*		0.556*	-0.567**	0.529*		0.576**		
FC	0.655**				-0.536**					
E.coli	0.660**			0.785**			-0.608**	0.478*	0.453*	-0.692**
UTI bacteria	0.109*	0.543*						0.809**		
V. cholera	0.574**			0.608**			-0.744**			0.661**
S. enteric		-0.610*	-0.596**	0657**	-0.718**	0.730**				
S. flexneri	0.594**									
L.monocytogenes									-0.195**	-0.602**
A. hydrophila			0.586**	0.517*	-0.441*		-0.640**		0.452*	0.525*
S. aureus		-0.454*	-0.458*					-0.558**		
Summer season										
THPC	0.811**		-0.594**	0.484*	-0.525*	-0.536*				
TC	0.531*	-0.575*		0.514*	-0.587**	0.509*		-0.459*		
FC	0.779**				-0.560**					0.491*
E.coli	0.655**			0.780**			-0.748**	0.497*		-0.759**
UTI bacteria	0.720**	0.512*						0.560**		
V. cholera	0.514*			0.576**	-0.421*	0.552**				
S. enteric		-0.693**	-0.515*	0.680**	-0.411*	-0.535*				
S. flexneri										
L.monocytogenes					-0.160*	-0.546*		0.455*		-0.179*
A. hydrophila			0.621**	0.584**			-0.817**			0.610**
S. aureus		-0.454*						-0.523**		

Temp: water temperature; EC: electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K: potassium; THPC: Total Heterotrophic Plate Count; TC: Total Coliform; FC: Fecal Coliform; UTI bacteria: Urinary Tract Infection bacteria

***significant at the $p<0.05$**

****significant at the $p<0.01$**

Table 6: Water quality index (WQI) based on physicochemical and biological parameters of water samples.

		Percentage of water samples	
Levels	**Water quality index score**	**Winter**	**Summer**
Level 1	0-25: Extremely polluted	99%	100%
Level 2	26-50: Suitable for transportation purposes	1%	0
Level 3	51-75: Suitable for irrigation purposes	0	0
Level 4	76-90: Suitable for domestic usage	0	0
Level 5	91-100: Suitable for domestic water supply	0	0

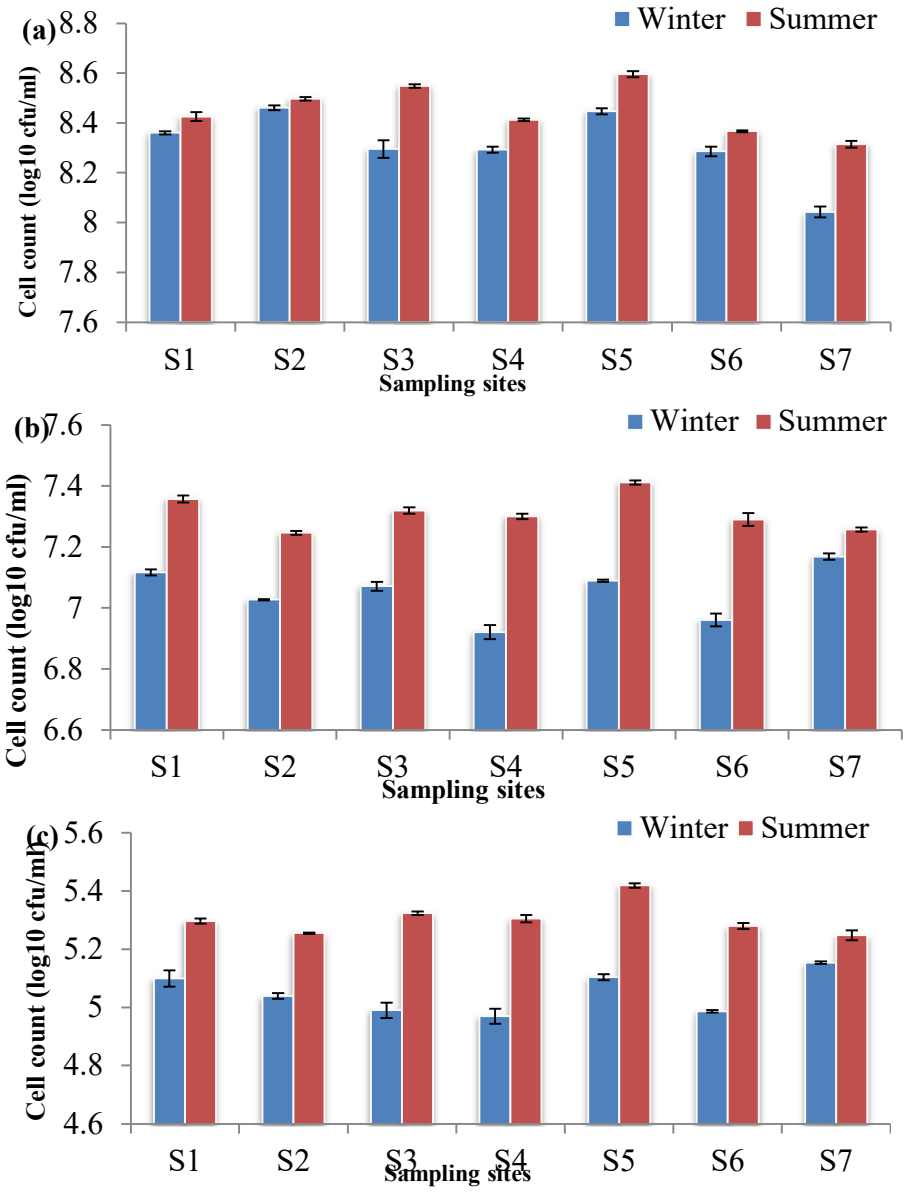

Figure 2: Bacterial cell count (log10 cfu/ml) of (a) Total Heterotrophic plate count (THPC); (b) Total Coliform (TC) and (c) Fecal Coliform in winter and summer season

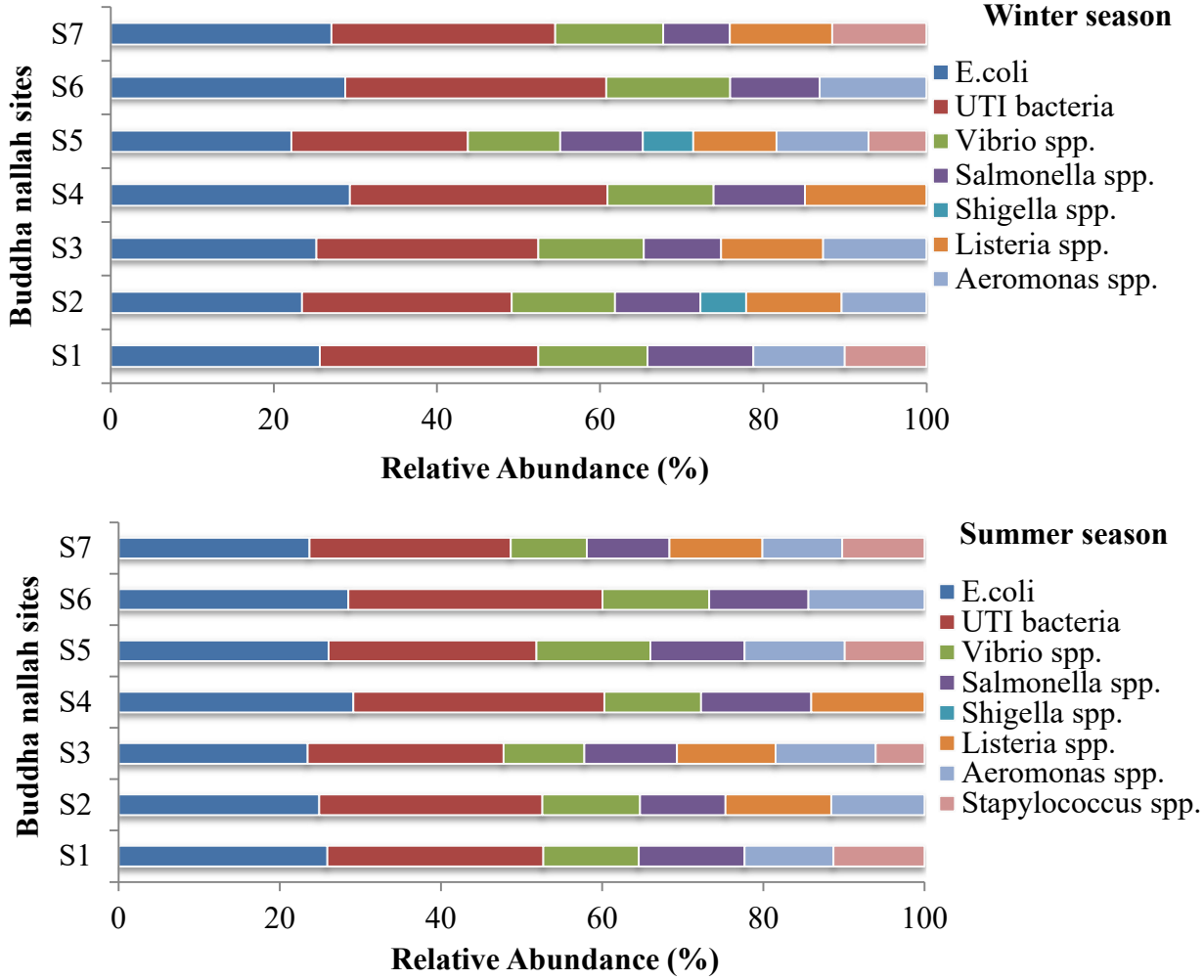

Figure 3: Relative abundance of water pathogens at different sites of Buddha nallah, Ludhiana, Punjab.

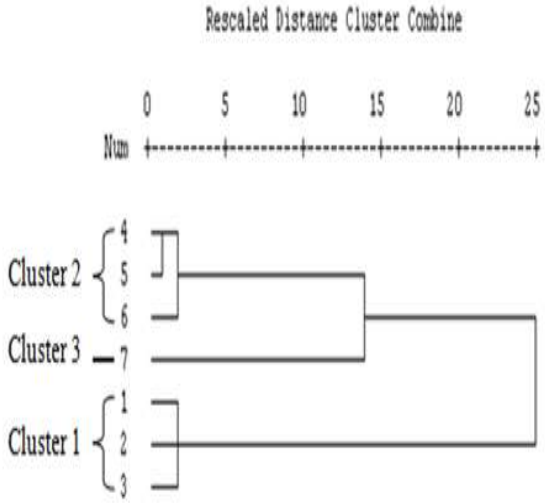

Figure 4:Clustral analyses of Buddha nallah sites (S1-S2) for winter season.

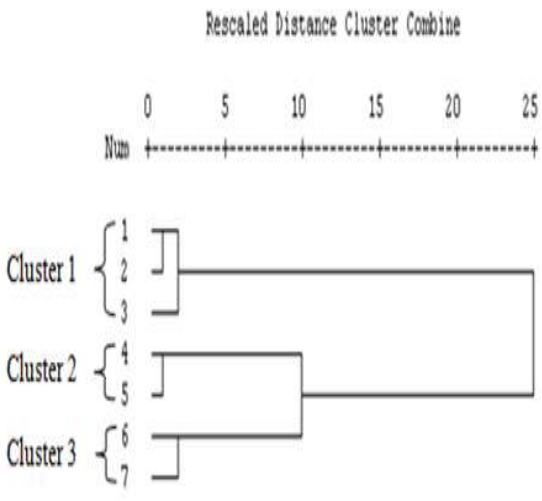

Figure 5: Clustral analyses of Buddha nallah sites (S1-S2) for summer season.

significant correlation was found between the Total Coliform (TC), Fecal Coliform (FC) and *E.coli.* In this study, the correlation between indicator organisms and emerging pathogens were found very weak and doesn't support the concept of using coliforms as a signal of presence of pathogens in the water quality assessment practices. Among all the pathogens, *Vibrio cholera* and *Staphylococcus aureus* were only emerging pathogens that were positively correlated with coliform (r =0.502-0.901).The results from this study revealed the overall weak correlations among the pathogens. In terms of the physicochemical parameters, an increase in pH and biological oxygen demand (BOD) significantly increased the prevalence of indicator/pathogens in water. While temperature, electrical conductivity (EC), dissolved oxygen (DO), nitrates, chlorides and nutrients showed the opposite trend. The correlation analysis also showed that with the seasonal variation there is no significant relation between the physicochemical parameters and microbial indicators/pathogens.

Clustral analysis

Clustral analysis (CA) is a multivariate statistical tool to classify different places or objects on the basis of distance and proximity. They classified different clusters on the basis of similarities and differences in their components. The dendrogram generated by the group linkages cluster analysis divided 7 Buddha Nallah sites into three cluster groups with significant differences between the groups. In summer as shown in Fig. 5, cluster 1 included sites S1 to S3; cluster 2 included S4-S6 sites and cluster 3 comprised only S7 site. Whereas in winter, cluster 2 comprised only two sites S4 and S5; cluster 3 includes S6 with S7 site as shown in Fig. 4. Therefore, the spatial and temporal change in Buddha Nallah water quality depends largely on local climate conditions (winter and summer) and pollutant sources (domestic, industrial and agricultural runoffs). Based on clustral analyses, the cluster 1 group included the sites that present the highly polluted areas, the cluster 2 group sites are moderately polluted areas and cluster 3 group sites in less polluted areas. In cluster 1, the main source of pollutants was discharges from textile, dairy and domestic wastes. The cluster 2 sites were mainly polluted with paper industry discharges. However due to strict actions for industrial discharges in Ludhiana, the wastewater discharged from paper mills should be reduced and cause moderate pollution. As compared to other areas, the cluster 3 is somehow less populated as it was away from industrialized and urbanized areas.

Water Quality Index (WQI)

Results in Table 6 represent the water quality index based on physicochemical and biological parameters of water samples during the winter and summer season. The classification of water quality into different levels was adapted from VEA 2011. Based on the WQI assessment, 99% and 100% of the Buddha Nallah water samples was found to be extremely polluted during winter and summer season, respectively. These results revealed that the water of Buddha Nallah is not suitable for domestic or irrigation purposes because it poses high public health risks.

Conclusion

High electrical conductivity (EC) in upstream as compared to the downstream sites clearly indicate that water contain high amount of dissolved solids and is highly polluted with industrial and domestic discharges. There was no significant difference in the pathogen abundance for the two seasons except for the *S. flexneri* which had significantly higher abundance in winter season. The correlation analysis showed that with the seasonal variation there is no significant relation between the physicochemical parameters and microbial indicators/pathogens. Based on clustral analyses, the cluster 1 group included the sites that present the highly polluted areas, the cluster 2 group sites are moderately polluted areas and cluster 3 group sites in less polluted areas. Only 1% of water samples during the winter were categorized under level 2. The quality of water evaluated from WQI score indicates that water samples contain highlevel contamination and suggests the prevalence of water-borne pathogens in Buddha Nallah.

Conflict of interest

The authors declare that they have no conflict of interest.

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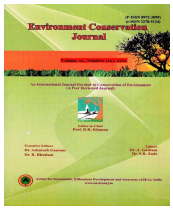

Assessment of groundwater quality from Sahibabad to Modinagar Meerut Uttar Pradesh, India using water quality index

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ABSTRACT

Groundwater quality and quantity both are important for the survival of human beings on this planet. In the present study an attempt has been made to assess the groundwater quality at mass using points. To fulfil the objectives of the present study, four sites (Sahibabad, Ghaziabad, Muradnagar, and Modinagar) were selected along the metro line construction from Delhi to Meerut. At all these sites, workers of metro line projects are living and working and using the groundwater for drinking purpose. Sampling was carried out from July 2021 to June 2022 using grab method of sampling. The samples were analysed for pH, total dissolved solids (TDS), total hardness (TH), calcium, magnesium, chloride, sulphate, nitrate, and fluoride. The data was processed using water quality index (WQI) and Pearson correlation metrix. TDS at all the study sites ranged from 514mg/l to 549.3mg/l and the values are above the standard limit of BIS (500mg/l). Values of TH, calcium and magnesium were found above the limits prescribed. Concentration of Chloride, nitrate, sulphate, and fluoride were found below the limits prescribed by BIS. However, nitrate is approaching to the standard limit (45mg/l). Correlation metrix shows that calcium is responsible for increasing values of TDS. As per the values of WQI, water quality of site 2 (46.7762), 3 (48.3523) and 4 (48.6281) falls in good category while at site 1 (50.9363) in poor category. There is an urgent need of strict actions to stop the increasing water pollution in the area to prevent the huge population of this area from various water related implications.

Introduction

Water is one of the vital elements necessary for the sustainable development of life on earth. In India, 85% of drinking water and 60% of irrigational water requirements are fulfilled by groundwater (Sajil Kumar, 2017; Agarwal *et al.,* 2019). In the present scenarios, many countries are facing the problem of water scarcity; even the good quality of drinking water is not available for the human society (Gleick, 2000). This situation is wide spreading day by day specially in most of the developing countries such as India, where majority of population depends on the availability of ground and surface water (Srivastava *et al.,* 2012). Physical, chemical, bacteriological and radiological characteristics of water make precious and healthful resource for all biotic and abiotic component of the ecosystem. It is reported that worldwide more than 1.5 billion people directly or indirectly depend on groundwater for drinking purpose (Shen *et al.,* 2008). The efficient use of freshwater resources and their transfer with high quality to the next generation are of great importance in terms of both human health and the ecosystem (Sener *et al.,* 2022). In various regions of the world different

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potable water sources and the associated ecosystems have undergone major modifications; therefore the availability, vitality, and quality of the water assets have been facing the human terrorization (Singh *et al.,* 2015a; Nemcic-Jurec *et al.,* 2019). Both the natural and manmade activities such as population blast, climate change, rapid urbanization and industrialisation, land conversion, extensive agricultural activities, and over abstraction (Singh *et al.,* 2015b; Nemcic-Jurec and Jazbec, 2017; Rawat *et al.,* 2018) contaminate the ground as well as surface water. Groundwater quality also depends on the nature of percolating water and geochemical reactions running in aquifers (Pandey *et al.,* 2020; Dutt and Sharma, 2022). Approximately 28% (1123 BCM-billion cubic meters) of the total water received on the geographical area of India (4000 BCM) is utilizable water resource annually (Central Water Commission, 2020). Groundwater fulfils about 85% of rural water requirement, 50% urban water requirement and more than 60% of countries irrigation water requirement (Sishodia *et al.,* 2016; Adimalla and Venkatayogi, 2017; SubbaRao *et al.,* 2017; Adimalla *et al.,* 2020). In India, the yearly abstraction and consumption of groundwater is highest. The exploitation of groundwater in India (244.92BCM in 2020) is higher than the consumption of both USA and China together (Singh, 2018). Furthermore, a report by the Central Ground Water Board, India reveals that the annual groundwater draft in India is approximately 245 × 109 m^3 (CGWB 2014; Adimalla and Venkatayogi, 2018; Li *et al.,* 2018; Adimalla and Li, 2019). Water quality index (WQI) is a most efficient process to convey the information of water quality concern to citizens and policy makers. WQI is used to by several authors to appraise the water quality of the concerned areas (Bhutiani *et al.,* 2018; Mukate *et al.,* 2019; Rezaie-Balf *et al.,* 2020; Uddin *et al.,* 2021; Ram *et al.,* 2021; Ruhela *et al.,* 2022; Mishra *et al.,* 2022).

Metro line construction activities are going on from Delhi to Meerut {project is named as Delhi–Meerut Regional Rapid Transit System (Delhi-Meerut RRTS)}. A lot of workers are working continuously at all the sites and utilizing the groundwater as the main source for drinking and bathing purpose. There is a need to evaluate the quality along this construction project. Therefore, in the present paper an attempt has been made to evaluate the groundwater quality at the selected sites of Delhi-Meerut Rapid Rail Corridor.

Material and Methods

The samples were collected from the selected sites (Figure 1) once in two months starting from July 2021 to June 2022 in the plastic can of capacity 2 Litre. After collection, the samples were transferred to the laboratory for the analysis of remaining parameters. The samples were analysed using the standard methodologies prescribed in APHA (2012) and CPCB manual (2010).

Figure 1: Showing the map of the study area (Source: Website of RRTS)

Water quality index (WQI)

WQI is an extremely valuable and efficient method which can provide a simple indicator of water quality and it is based on some very important parameters. In this study, WQI was calculated by using the Weighted Arithmetic Index method as described by Cude (2001) and Brown *et al.* (1970). In this method unit weight (Wi) and quality rating (Qi) was calculated first and then sub index of each parameter was calculated by multiplying the unit weight (Wi) and quality rating (Qi). The overall WQI was calculated by aggregating the sub index of each parameter by using the following equation:

$$WQI = \frac{\Sigma Qi Wi}{\Sigma Wi}$$

Where,

- Qi = Quality rating
- Wi = Relative weight

Results and Discussion

The average and comparative values of all the parameters are given table 1. pH is the negative log of hydrogen ion values. It measures the acidic and basic strength of the particular solution. Usually, pH values have no direct impact on human health but it alters the other characteristics (it promotes corrosivity inside the pipes which has a direct impact on human health) which affect the human health (Wu *et al.,* 2020). pH of groundwater depend on the certain factors like geology, atmospheric precipitation and anthropogenic activities in that area. During the study period, highest pH was observed at site 4 (7.7±0.04) and at the remaining site, same pH (7.6) was observed with different standard deviation. pH was observed within the limit of BIS (6.5 to 8.5). A strong negative correlation was observed between pH and chloride (-0.726) while a very week positive correlation with fluoride (0.050). Agarwal *et al.* (2019) observed the pH in the range of 7.31 to 8.97. The author also observed the anthropogenic activities in the area which are continuously altering the pH of the groundwater. Singh and Tripathi, (2016) also reported the pH between 7.1 and 7.9 in the same study area. Total dissolved solids (TDS) are the total of dissolved ions in the groundwater primarily calcium, magnesium, potassium, sodium, carbonates, sulphates, bicarbonates and chloride. As the TDS is directly correlated with electrical conductivity (EC) therefore an increase in TDS results in an increase in the EC of water. During the study period, minimum TDS (514.0 mg/l±3.94) was observed at SS-4 and maximum TDS (549.3mg/l ±9.89) was observed at SS-2. At all the studied sites, TDS was found above the standard limit of BIS (500mg/l). The obtained results are lesser than the obtained range (514.0–549.3 mg/l) from the report of Singh and Tripathi (2016) and Agarwal *et al.* (2019) for the NCR region. A strong negative correlation was observed between TDS and calcium (+0.742) indicating that calcium is the major ions responsible for increased TDS level in the area. Suitability of groundwater samples for domestic, industrial and irrigation purposes depends on the values of total hardness; therefore total hardness is considered as an important parameter (Farid *et al.,* 2022). The hardness in water is because of the presence of the carbonates and bicarbonates of calcium, magnesium, chloride and sulphate (Bhutiani *et al.,* 2021a&b). During the study period, minimum total hardness (358.4mg/l±3.37) was observed at SS-4 and maximum total hardness (380.7mg/l ±12.64) was observed at SS-1. Hardness was found above the standard limit of BIS (200 mg/l) at all the studied sites. All the samples fall in hard water category. Increased level of hardness is the causes of many stomach problems and reduced amount of minerals in human body (Rawat *et al.,* 2018). Therefore there is a need of water treatment before consumption in the study area. Prolonged use of hard water can cause urolithiasis (Agarwal *et al.,* 2019). Ahmad and Khurshid, (2019) observed the average values of hardness as 301.53mg/l in Hindon River basin area of Ghaziabad. Hardness was found moderately positively correlated with chloride (+0.629) and strongly correlated with sulphate (+0.954). TH was found moderately negatively correlated with calcium (-0.610) and weekly positively correlated with magnesium (+0.388). The results of correlation show that TH was highly influenced with chloride and sulphate in spite of their low quantity. However, concentration of calcium and magnesium were high but their impact was low. During the study period, minimum calcium (116.7mg/l±1.62) was observed at SS-4 and maximum calcium (124.6mg/l ±3.05) was observed at SS-2. Calcium was found above the

Table 1: showing the average values of physicochemical characteristics of the all the selected four sites (All the values are in mg/l except pH)

Parameters /Month	SS-1	SS-2	SS-3	SS-4	Standard (BIS, 2012)
pH	*(7.43-7.76) 7.6±0.11	*(7.43-7.62) 7.6±0.07	*(7.53-7.68) 7.6±0.05	*(7.69-7.79) 7.7±0.04	6.5-8.5
TDS	*(510.7-567.9) 527.6±21.02	*(539.8-567.9) 549.3±9.89	*(538.1-549.1) 542.8±4.80	*(511.5-521.9) 514.0±3.94	500
Total Hardness	*(360.3-394.3) 380.7±12.64	*(362.5-374.2) 367.9±4.13	*(345.9-376.2) 363.9±10.36	*(353.8-362.1) 358.4±3.37	300
Chloride	*(66.2-79.7) 74.3±4.94	*(75.2-83.9) 78.9±2.93	*(55.2-65.2) 59.9±3.53	*(55.8-67.3) 62.9±4.11	250
Magnesium	*(75.2-79.3) 77.8±1.57	*(72.8-77.8) 75.0±1.82	*(75.2-79.3) 77.8±1.57	*(70.4-74.1) 72.1±1.49	30
Calcium	*(113.7-123.5) 117.7±3.65	*(121.2-129.5) 124.6±3.05	*(115.8-128.2) 121.4±4.39	*(114.4-119.3) 116.7±1.62	75
Sulphate	*(27.2-34.5) 30.3±2.70	*(34.5-39.8) 37.9±2.08	*(28.5-31.5) 29.9±1.12	*(27.6-29.5) 28.9±0.67	200
Nitrate	*(29.3-33.5) 31.1±1.65	*(31.7-39.2) 34.6±2.75	*(31.3-34.9) 33.8±1.33	*(29.8-30.7) 30.1±0.33	45
Fluoride	*(0.24-0.32) 0.28±0.03	*(0.21-0.28) 0.24±0.03	*(0.23-0.28) 0.26±0.02	*(0.24-0.32) 0.21±0.27	1

***=range (n=06)**

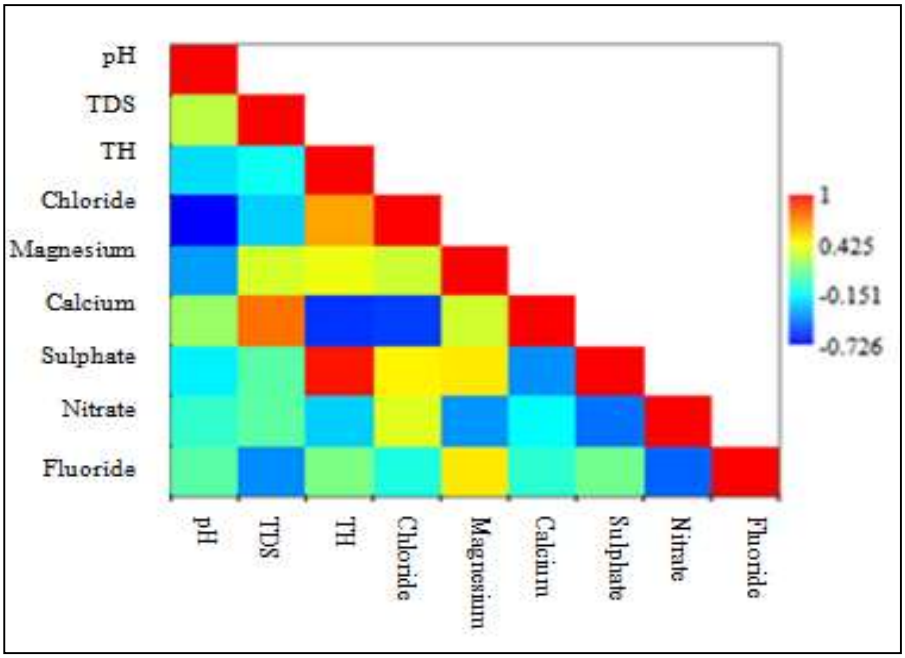

Figure 2: Showing the correlation between average values of physicochemical characteristics.

standard limit of BIS (75 mg/l) at all the studied sites. During the study period, minimum magnesium (72.1mg/l±1.49) was observed at SS-4 and maximum magnesium (77.8mg/l ±1.53) was observed at SS-1 and SS-3. Magnesium was found above the standard limit of BIS (30 mg/l) at all the studied sites. Chloride is considered as an indicator of sewage contamination in water. Higher quantity of chloride is responsible for salty taste and bleaching property of water (Sadat-Noori *et al.,* 2014). The higher concentration of chloride ion is responsible for salinity problem in ground water.

During the study period, minimum chloride (59.9mg/l±3.53) was observed at SS-3 and maximum chloride (78.9mg/l ±2.53) was observed at SS-2. Chloride was found within the limit of BIS (250 mg/l) at all the studied sites. Occurrence of sulphate in groundwater is due to nature of rocks present there, nature of fertilizers used and solid and liquid industrial waste dumped in the area. Sulphate beyond the permissible limit is harmful to plumbing structures. During the study period, minimum sulphate (28.9mg/l±0.67) was observed at SS-4 and maximum sulphate (34.6mg/l ±2.75) was observed at SS-2. Sulphate was found within the limit of BIS (200 mg/l) at all the studied sites. Sulphate was found strongly positively correlated with TH (+0.954) and moderately positively correlated with chloride (+0.448) and magnesium (+0.472). Sources of presence of nitrate in groundwater are nitrogen-based fertilizers, atmospheric precipitation, residues of crops (Shakerkhatibi *et al.,* 2019), and septic tanks (Nakagawa *et al.,* 2017). Increased quantity of nitrate in water (beyond the permissible limit) causes blue baby syndrome (Logeshkumaran *et al.,* 2015). During the study period, minimum nitrate (30.1mg/l±0.33) was observed at SS-4 and maximum nitrate (34.6mg/l ±2.75) was observed at SS-2. Nitrate was found below the standard limit of BIS (45mg/l) at all the studied sites. Similar lower concentrations of nitrate ion have also been found by Lone *et al.* (2021). A week negative correlation of nitrate was found with TH (-0.258), magnesium (-0.152), and moderately negative with sulphate (-0.468). Both the natural and anthropogenic factors are responsible for fluoride occurrence in groundwater. A minimum quantity of fluoride is a dietary requirement for strong bones (Aravinthasamy *et al.,* 2020) while in excess quantity it causes bones fluorosis (whether teeth or skeletal) and different other implications. During the study period, minimum fluoride (0.23 mg/l±0.02) was observed at SS-4 while maximum fluoride (0.28mg/l ±0.03) was observed at SS-1. Fluoride was found within the limit of BIS (1.0 mg/l) at all the studied sites.

Water quality index (WQI)

Standard values, ideal values and unit weight used in the calculation WQI are given in table 2 while the values of WQI at all the sites are given in table 3. The WQI is a widely acknowledged method for determining the fitness of groundwater for human use. Twelve water quality parameters (Cl^-, pH, Ca^{2+}, Mg^{2+}, TDS, SO_4^{2-}, NO_3^-, and F^-) were involved in estimating the integrated groundwater quality by the WQI method. Standard values recommended by Bureau of Indian Standard (BIS) for drinking water were used as reference for WQI calculation. Water quality was categorized based on Chaterjee and Raziuddin (2002) classification, as (I) excellent, (WQI is 0-25); (II) good (26–50); (III) poor water (51–75); (IV) very poor water (76–100); (V) unsuitable for drinking, when WQI is >100. At all the studied sites magnesium was considered as criteria pollutant due to highest value quality rating (Qi). The value of WQI at site 2, 3 and 4 ranged from 46.7762 to 48.6281. Therefore, groundwater at these sites falls in second category i.e. good. At site 1, the value of WQI was found as 50.9363, therefore, groundwater quality at this site falls in third category i.e. poor. Values of WQI at all the sites indicate that water quality is continuously degrading in the area. Therefore there is a need of awareness among the society and stake holders regarding the water quality and its impacts on human health.

Table 2: Showing the standards value, ideal value and unit weight of each parameter used for the calculation of WQI.

Parameters	Standard value	Ideal Value	Unit weight
pH	7.5	7	0.0844
TDS	500	0	0.0013
TH	300	0	0.0021
Chloride	250	0	0.0025
Ca	75	0	0.0084
Mg	30	0	0.0211
Sulphate	200	0	0.0032
Nitrate	45	0	0.0141
Fluoride	1	0	0.6330

Conclusion

The present study was conducted at the selected sites along the metro line construction from Delhi to Meerut. The objective of the present study was to evaluate the water quality in terms of physicochemical parameters. The groundwater in study area was found slightly acidic in nature. Values of dissolved solids were found beyond the

Table 3: Showing the values of sub-index of each parameter and WQI at all the sites

Parameters/Site	SS-1		SS-2		SS-3		SS-4	
	OV	WiQi	OV	WiQi	OV	WiQi	OV	WiQi
pH	7.6	10.7469	7.6	9.3965	7.6	10.0155	7.7	12.4349
TDS	527.6	0.1336	549.3	0.1391	542.8	0.1374	514.0	0.1302
TH	380.7	0.2677	367.9	0.2587	363.9	0.2560	358.4	0.2521
Chloride	74.3	0.0752	78.9	0.0799	59.9	0.0607	62.9	0.0637
Ca	77.8	0.8749	75.0	0.8436	77.8	0.8749	72.1	0.8108
Mg	117.7	8.2759	124.6	8.7600	121.4	8.5408	116.7	8.2102
Sulphate	30.3	0.0479	37.9	0.0600	29.9	0.0473	28.9	0.0457
Nitrate	31.1	0.9732	34.6	1.0805	33.8	1.0555	30.1	0.9409
Fluoride	0.28	17.8295	0.24	15.4030	0.26	16.2470	0.23	14.5590
∑WiQi	39.2250		36.0214		37.2351		37.4475	
WQI	50.9363		46.7762		48.3523		48.6281	

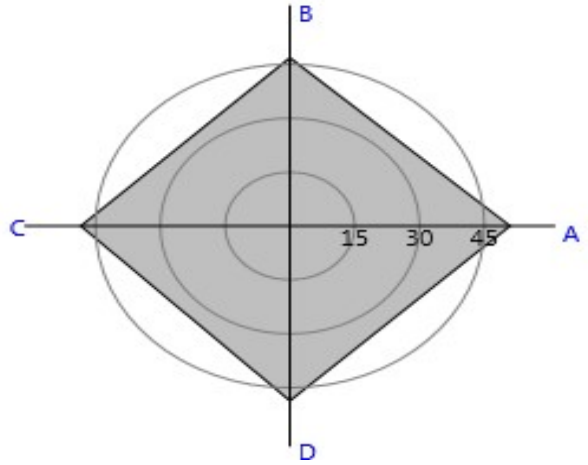

Figure 3: Radar chart showing the values of WQI at all the sites.

permissible limits at all the sites showing the problem of salinity in the study area. Values of hardness, calcium and magnesium were found beyond the limits but the values of chloride and sulphate was found below the permissible limits showing that the temporary hardness is present in the groundwater. Therefore, there is a necessity to spread the awareness among the workers to use the water after proper boiling otherwise various abdominal implications will happen in long term use of this water. Fluoride was also observed below the standard limit. Similarly, the values of nitrate was also observed below the standard limit but the values are approaching towards limit, therefore there is a necessity to spread the awareness among the farmers regarding the use of nitrogen based fertilizers. Values of WQI indicate that water quality in the area falls from good to poor category. Therefore, there is an urgent necessity to take the appropriate steps to protect the water quality as well as quantity in the study area.

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Conflict of interest

The authors declare that they have no conflict of interest.

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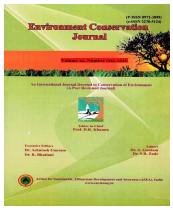

Urbanization's environmental imprint: A review

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ABSTRACT

The urban population interact with their environment and change it through the consumption of resources. The changed condition may impact the wellbeing and life nature of the urban population. The uncontrollable growth of urbanization has been reported to be dangerous for mental health and sanity of many citizens. Urban areas whether small or megacities each generate an ecological foot print.The present day urbanization, consequently requires a sustainable development pattern and changes in present day styles of boom to cause them to be more equitable and more resource and energy efficientAs of now, urban administration is to a great extent concentrated on single issues, for example, water, transportation, or waste. A multi scale administration framework that expressly inscribes interconnected asset chains and interconnected spots is essential as a way to transition closer to sustainable urbanizationwhich require, prevention of urban pollution, decreasing production capacity and supporting recycling, while discouraging non-profit development and fading poor and rich differences. . Therefore, the present study was conducted to clarify the effect of urbanization on the environmental quality, resource use and sustainability. The study conducted throughout the globe indicated sustainable urbanization can be achieved by following concept of development wherein natural resources are restored and not replaced by technology.

Introduction

One of the greatest social changes of current time is urbanization. It is the segment procedure whereby most extreme portion of the national populace lives in urban settlements (Arouri*et al.,* 2014).Historically, it has been viewed as a crucial device for social turn of events. It is exceptionally viable for financial development, as it produces economies of scale for business, fosters an innovative environment and delivers higher wages and profits (Cohen, 2006). A 2.5 billion increment in urban populace is anticipated by 2050, with 90% of the expansion moved in Asia and Africa (Aguilar *et al*., 2022 ; UN, 2015).Quick and unregulated development of the world's urban areas is joined by a few externalities. As per reports by UNCHS (1987) 600 million urban tenants resides in dangerous situations because of poor sanitation and housing (Gelbard*et al.,* 1999). The detonatingpopulaces of the evolving world compound everlasting issues, for example, starvation, destitution and ecological debasement. The ecological ramifications of quick urbanization are extraordinary and wide extending, with the effects regularly outflanking the populace development.Quick endless suburbia is a significant variable influencing different natural issues since it straightforwardly influences the spatial grouping of individuals, vitality utilization, water use, industry, business, squander age, and other ecological stresses (Bartone*et al.,* 1992).In China, India and Mexico City ubiquitous degrees of air contamination has been seen as of late. Municipal solid waste is a noteworthy issue for urban communities. around, 3 billion urban inhabitants

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create 1.2 kg of waste a day - 1.3 billion tons for each year (World Bank, 2012). This brings different difficulties, for example, GHG from moving waste, deficiency of land for landfill destinations marine contamination from beach front urban areas, and wellbeing risks from casual dumps and untreated waste. The natural effect of city size is commonly viewed as negative. It is expected that, bigger the city, more prominent is the per capita ecological expenses or harms. PrudhommeI(1994)expressed that what at last tallies isn't the manner by which contamination released, yet rather contamination released less contamination disposed of. The connections among urbanization and ecological crumbling are mind boggling, including interlinkages with the common and the constructed condition, just as different social, political and financial components.Hence, to maintain environmental sustainability, it is obligatory to understand the linkage between them and also how natural environment is being affected by urbanization. This article reviews first handknowledge linking environment and urbanization, emphasizing on variousaspects such as biodiversity, land use, air quality, water quality, solid waste generation and way forward for sustainable urbanization.

Urban land use and environmental impacts

In spite of the fact that lion's share of the total populace is controlled by urban communities, urban zones comprise under 1% of the World's property cover (Schneider *et al*., 2010).The effect of urban land use change is excessively huge when contrasted with the zone it possesses and these effects happen at a scope of scales, from nearby and territorial to worldwide, as an outcome of physical progressions of living and nonliving materials, and teleconnections of practical assets (Grimm *et al.,* 2010). It shapes neighbour-hood and worldwide atmosphere by adding to altered rainfall an altered rainfall and heat island effects (Wang *et al*., 2004), drives international trade in agriculture and forestry (Meyfroidt*et al.,* 2013) and alters local biodiversity and the environment (Aronson*et al.,* 2014). Urban focuses tend to agglomerate, framing urban groups or urban halls, along which transportation and different types of improvement regularly occur (Wang *et al*., 2004).Such spatial bunches can go about as hubs that impact land-use mosaics of whole regions (Seto *et al.,* 2012).Older urban settlements tend to be compact with with slower growth rates, moderately unsurprising mosaic of focused development, while new urban settlements show significant and spatially complex development patterns (Ramalho and Hobbs, 2012). There are exchange offs that should be deliberately viewed as, for example, urban smallness may lessen non-renewable energy source use for transportation and energy consumption, however it reduces groundwater infiltration and enhances UHI (Jabareen, 2013).Huge harm to the environment is brought about by uncontrolled development, frequently named as "concrete jungles". For example, energy needs causes resource depletion, expanding number of vehicles adds to extreme air contamination and untreated sewage causes water contamination.

The process of urbanization, and the related increase in impervious surfaces, affects other types of land use and land cover, triggering a series of effects on the environment, which results in it being the main cause of land degradation. The main effects consist of the loss of fertile soils, the adverse impact on water balance, the increase in surface water runoff and flood risk, the negative influence on local microclimates due to urban heat islands, landscape fragmentation,and the loss of biodiversity (Assennato *et al.,* 2022).

In order to oblige theever-developing populace spontaneous development has been completed, which generates dangers, aggravates hitherto problematic circumstances in urban communities, and makes them untenantable.The methodology of keen urban communities is picking up prominence with governments over the world, have fabulous designs to update existing urban communities by means of the combination of frameworks advances to expand efficiencies of utilization. Be that as it may, they have been studied, for the absence of law based interest of individuals in the arranging procedure, for the predominant pretended by outside private ventures, and for escalating urban ecological treacheries, as saw in various brilliant city ventures in India and across Africa (Watson, 2014).

Urbanization and biodiversity

As the global urban population is poised to grow by 2.5 billion over the next 30 years, urban land

conversions are expected to be an increasingly prominent driver of habitat and biodiversity loss (Simkin *et al.,* 2022). The interlinkage among urbanization and biodiversity is manifold and compound (McKinney, 2002). Urban development may prompt habitat fracture, bringing about segment or hereditary detachment of local species (Ricketts, 2001).It have both immediate and aberrant effects on biodiversity. Direct effects basically comprise of altered disturbance regimes, habitat degradation, changed soils and other physical changes brought about by the development of urban regions. Circuitous effects includes increases in abiotic stressors, competition rivalry from non-local species, changes in water and supplement accessibility and changes in herbivory and predation rates (Pickett and Cadenasso, 2009).Biodiversity is influenced by both the size and spatial arrangement of urban areas (Tratalos, 2007).The composition of the landscape is changed by physical expansion and may modify or take out the conditions inside a living space that is required by species to endure. Urban extension influences local species through changes in living space arrangement and connectivity (Bierwagen, 2007). It additionally presents significant danger to endemic species because of expanded occurrence of colonization by presented species (McKinney, 2008). It decreases, fragments and confinecommon patches by modifying the shape estimate and inter connectivity of the regular terrain (Alberti, 2005).IAnthropogenic activities exercises in urban communities can have a heap of falling impacts that impacts biodiversity, remembering changes for biogeochemistry (Grimm *et al.,* 2008)neighborhood temperature (Arnfield, 2003), atmosphere change (Wilby and Perry, 2006) and hydrographic systems (Booth *et al.,* 2004).Urban development delivers the absolute most noteworthy nearby eradication rates and as often as possible disposes of the vast larger part of local species (Marzluff, 2001).It replaces the local species with across the board "weedy" nonnative species bringing about diminished organic uniqueness of neighborhood ecosystems (Blair, 2001).Different urban slope contemplates uncovered that, for some taxa, for instance, plants (Kowarik, 1995)and flying creatures and butterflies (Blair and Launer, 1997) the number of local species diminishes toward focuses of urbanization, while non local species increments.The most minimal species decent varieties along the urban rustic inclination happen in the seriously "fabricated" situations of the urban center as revealed by numerous investigations.

Urbanization and air quality

Air pollution has become significant worry all through the world in both, advanced and advancingnations. urban populace have brought about the predominance of the segment of private vehicles in urban transportation framework prompting serious air contamination influencing the encompassing environment (Guli*aet al.,* 2015). Modern transformation presented the creation of tremendous amounts of toxins discharged into the air that are hurtful to condition, despite the fact that it was an extraordinary accomplishment regarding society, innovation and the arrangement of different administrations.Urbanization and industrialization are arriving at extraordinary and upsetting extents worldwide in our period. Anthropogenic air contamination represents around 9 million passings for each year and is one of the greatest general wellbeing perils around the world.The significant wellsprings of air contamination is the road transport division. It has been accounted for that more than 70–80% of air contamination in creating countries is attributed to vehicular emanations brought about by an enormous number of more seasoned vehicles combined with poor vehicle support, low fuel quality and insufficient street infrastructure (Badami, 2005).Re-suspension of street dust because of development of traffic and tire and brake wear likewise add to surrounding PM concentrations in urban areas (Amato *et al.,* 2014).The non uniform appropriation of encompassing air contamination focuses in urban territories, make problem areas generally in focal business region, traffic crossing points and signalized roadways (Kandlikar, 2007).Air contamination predominantly influences those living in enormous urban regions, where street outflows contribute the most to the corruption of air quality (Manisalidis*et al.,* 2020). Due to rapid urbanization cities are growing in terms of population and physical size, resulting in increased demand for travel and change in travel behavior (Chowdhury, 2013). Huge urban communities in Europe, Latin America and North America have better air quality, though those in India and China have the most exceedingly terrible air quality (Han

et al., 2016). India's urban communities are under extensive hazard because of air contamination. Urban contamination as estimated by PM2.5 level is as of now about 40% over as far as possible across significant Indian urban communities, (for example, Delhi, Mumbai, Kolkata, Pune and so on.) In India, there exists spatial heterogeneity as territories with various climatological conditions populace and training levels create distinctive indoor air characteristics, with higher PM2.5observed in North Indian states (557–601 μg/m^3) contrasted with the Southern States (183–214 μg/m^3) (Saud *et al*., 2012).

Urbanization and water quality

With the emergence of urbanization the scope of necessities for water has expanded along with more noteworthy requests for better quality water.With development of an area, various water quality issues are created. According toUN WWAP (2003)each day, 2 million tons of sewage and business and agricultural waste are discharged into the world's water. As per United nation's estimation measure of wastewater created every year is around 1,500 km^3, multiple times more water than exists in all the streams of the world. The ecosystem structure is altered because of the absence of adequate interest in foundation contrasted with the quick pace of urbanization, frequently prompting a diminished limit of biological system administrations, for example, water cleansing.Mallin*et al.* (2016)reported that urban territories have the most steady and universal impacts on water quality due noteworthy heap of pollutants from point and non-point sources and the expanded impenetrable surface spread. Direct overflow from urbanized surfaces has risen as a genuine danger to the biological estimations of water environments and arrangement of good quality water required for all financial functions (Brion*et al.,* 2015). Serious release of supplements and contaminants from urban regions prompts a predictable decrease in the wellbeing of urban amphibian biological systems, a condition for the most part alluded to as the urban stream disorder. When rain water comes in contact with urban surfaces, it becomes contaminated with pollutants resulting in contamination of water bodies.Production industries boom as a result of urbanization, resulting into production of even more wastes than before. There is increase in the proportion of impervious surface with the growth of urban area resulting in reduced infiltration of water and lower water tables (Simwela, 2018).Populace development and increments in untreated sewage were the fundamental driver of water quality weakening in waterways in the Territory of Sao Paulo, Brazil (Groppo*et al.,* 2008).Ma *et al.* (2009)statedthat sharp increments in modern contamination and household release were the significant reasons for water quality weakening in the Shiyang Waterway, Northwest China. Zhang *et al*. (2015) revealed that ammonium fixations in urban streams were three to multiple times higher than in nonurban waterways. Extending masses, urbanization and developing interest from farming just as industry have brought India's water assets under pressure (Kurunthachalam, 2013). As per WHO half of India's grimness is water related (Murty and Kumar, 2011). Improper sewage disposal, unchecked industrial effluents entering the water sources, may be sometimes due to runoff phenomenon and the unprotected nature of natural water sources itself, is leading to poor water quality of surface water sources (Pandit and Bhardwaj, 2020).

Urbanization and waste generation

Solid waste has emerged as one of the most serious problem being faced by urban centres all over the world. Changing ways of life, insufficient strategies, and absence of mindfulness in creating nations may expand it exponentially throughout the following decade. Large economies like China, India, Japan, USA and France are seeing the unabated development in squander age and its effect on condition is developing quick which may exacerbate in future (Ray, 2008). As the reaction of urban lifestyle, the measure of urban solid waste is building up significantly speedier than the pace of urbanization.World waste creation is depended upon to associate with 27 billion tons for every year by 2050, 33% of which will start from Asia, with critical commitments from China and India (Modak*et al.,* 2010). In urban locales waste generation of India will be 0.7 kg per individual consistently in 2025, roughly four to multiple times higher than in 1999 (Kumar *et al*., 2017). Urban populace generates 0.045 Kg methane per kg waste by solid waste disposal (IPCC, 1996). A great part

of the squanders produced around the world (57 to 85%) are every now and again arranged in landfills, including open and built landfills (Wilson, 2015). Leachate control varies throughout the landfills of the growing world and it poses a hazard to local surface and ground water systems (Alam and Ahmade, 2013).The IPCC evaluated that solid waste management represented roughly 3% of worldwide Green House Gases outflows in 2010 with the vast majority of that as an outcome of methane emanations from landfill sites (IPCC, 2013). However, current endeavors to "decouple the loss from the riches" has caused the lower period of waste per unit of Gross domestic product in certain nations, which shows the lucky opening for urban communities to discover higher responses for this basic open bearer of present day cities (UNEP, 2013).

II Resource use pattern of urban and rural population

In 1800 just around 2 percent of the total populace lived in urban zones. The onlystrategy urban zones kept up their reality till as of late was by the nonstop movement of provincial folks (Keyfitz, 1989).In precisely two hundred years, the world's urban populace has developed from 2 percent to about 50. The most putting instances of the urbanization of the arena are the megacities of 10 million or more people (UNDESAPD, 2004).A great deal of urban movement is pushed by method of country populaces' decision for the advantages that city territories offer (National Research Council, 2003).A lot of urban relocation is driven by rustic populaces' craving for the preferences that urban regions offer. Urban points of interest includes more possibilities to get hold of schooling, fitness care, and services inclusive of amusement. Urbanization of the world is probably going to slow populace development andconcentrate a few environmental effects geographically. Individuals who remain in city territories have altogether different asset use designs than residents in rustic areas (Parikh, 1991).Urban populacesdevour far more food, energy, and goods that are durable as compared to rural populations (Taylor and Hardee, 1986). This expanded utilization is an element of urban work markets, wages, and family unit structure. The utilization of energy for power, transportation, cooking, and warming is far higher in urban zones than in rustic towns. Increased energy consumption is likely to have deleterious effects that are environmental. It makes urban warmth islands that can change nearby weather patterns and weather downwind from the warmth islands. The blend of the expanded energy distinction and utilization in albedo implies that urban communities are hotter than provincial areas (Goudie, 1997).These urban heat islands become traps for atmospheric pollutants. Urbanization also affects the broader environments that are regional. Locales downwind from huge mechanical buildings additionally observe increments in the measure of precipitation, air contamination, and thunderstorms. Urban areas affect weather patterns and the spillover designs for water. They for the most part create more downpour, however the water invasion is reduced thus lowering the water tables resulting in greater run off with greater peak flows. A large number of the effects of urban regions on the earth are not so much immediate.Bigger urban locales don't for the most part make progressively common issues and small urban territories can cause issues that are huge.The degree of the ecological effects is dictated by how the urban populaces carry on, their utilization and living examples, not exactly how enormous they are and little urban regions can cause gives that are colossal.

III Urban sustainability

Urban sustainability is defined as the process by which measurable long-term social development can be achieved through actions in the environment, economic and social magnitude. The use of sustainability indicators is increasingly needed to achieve sustainability (Pandit *et al*., 2021). It truly is a multiscale and multidimensional issue that centersaround as well as rises above urban wards and which must be tended to by tough initiative, resident association, and territorial organizations alongside vertical collaborations among various legislative levels.There are four main principles to advance urban sustainability viz:Human and characteristic frameworks are solidly interlaced and met up in urban zones; Urban imbalance undermines supportability attempts; The planet has biophysical limits; Urban communities are astoundingly interconnected. A city or district can't be supportable if its standards and activities toward its own, neighborhood level supportability don't scale up to manageability all around. As the world quickly urbanizes, accomplishing sustainable

urbanization in urban networks is quickly transforming into an overall concern (Newman and Jennings, 2008).Since the late 19th century, the problems resulted from modern urban development has appeared as different urban crisis in three dimensions of environment, social and economic, which has made these communities unsustainable and made experts think about other methods of urban development patterns. The issue of achieving urban sustainable development is in this manner a significant test (Moussiopoulos, 2010). With sustainability since the objective, the use of pointers for urban checking and guideline has gotten progressively more in demand (Repetti and Desthieux, 2006).Sustainability indicators are a strong base with regards to normal and long haul track of the advancement enlisted in the accomplishment of key goals of economical turn of events and furthermore the assessment of different regions of sustainability (Hernándezmoreno and Dehoyosmartínez, 2010).They provided the past and present trends of any particular issue and acts as a supporting tool for future decisions. There are three sustainability indicators namely economic, social and environment. Economic indicators emphasize on local financial resilience, Social indicators focus on participation in democratic process and Environment indicators highlight use of resources at sustainable rate (Kotharkar *et al*., 2011). Later and progressing research infers that human demands on our planet's systems are expanding, possibly beyond sustainable operating limits (Rockström*et al.,* 2009).This suggests the need for systemic, crosscutting appraisals, which could address and look at the contending requests from the planet's limited biosphere.Ecological Footprint Accounting(Wackernagel*et al.,* 2002) distinguishes a specific ecological budget – biocapacity- and the degree to which human demands for biocapacitysurpass this budget – the Ecological Footprint.Ecological footprint is an example of a sustainability indicator with an environmental core interest. It evaluates the amount of space that an individual or a city utilizes in order to survive on an international level respectively worldwide (WackernagelandRees, 1996).It mirrors the urban lifestyle (Weiland, 2006). While urban areas can be focal points of advancement and multifaceted collaboration, the ecological footprint of the world's urban communities reaches out a long ways past these urban focuses' physical limits, and glaring financial inconsistencies exist inside and between cities (Keivani, 2010).From 1961 to 2010, accounts show that human interest for boundless resources and natural administrations extended by about 140% (from 7.6 to 18.1 billion worldwide hectares), showing up at a point where the planet's bioproductive region (extended from 9.9 to 12 billion worldwide hectares) isn't any more extended adequate to help the contending demands (Galli*et al.,* 2014).Today, the World's human people is about 7.62 billion and the environmental impression of the planet is high so much, that it will take for all intents and purposes 1.7 Earths to satisfy the requests of this populace. India has an ecological footprint of 1.12 global hectares (gha) per individual and a biocapacity of 0.45 gha per individual which implies it is a 'biocapacity debtor' or an 'ecologically deficit country' with there being a 148 per cent more interest than flexibly on its normal resources (NFA, 2014).

Conclusion

Urbanization has been, and will keep on being, one of the greatest cultural changes. While urban zones can be habitats for social and financial portability they are beset by growing problems of environmental deterioration. Numerous types of development disintegrate deteriorate the natural assets whereupon they ought to be based, and ecological debasement can sabotage financial turn of events. As opposed to fear "the unavoidable," we have to figure out how to relieve the environmental imprints of urbanization. Unquestionably, urban sustainability is the need of great importance. Urban areas carry out their activities With the advent of energy efficient innovation, an agreeable connection between environment and development is feasible. It is time that every single one of us receive a 'energy proficient and green' mentality and utilize the regular assets accessible fairly, reasonably and spare them for our people in the future, as the most ideal approach to anticipate future is to make it. Each and every one of us should grasp a 'energy effective and green' outlook and use common assets accessible impartially, wisely and spare them for our people in the future, as the most ideal approach to anticipate future is to make it.

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Conflict of interest

The authors declare that they have no conflict of interest.

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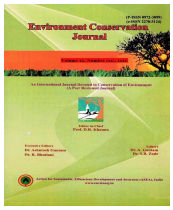

Bio-management of rice root-knot nematode, *M. graminicola* by using various organic amendments and bio-control agents on rice nursery

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ABSTRACT

A screen house experiment was conducted during kharif season, 2020-21 to know the effect of organic amendments and bio-agents (alone and in combination) in comparison with chemical on the population of rice root-knot nematode (*Meloidogyne graminicola*) in rice. Nursery was grown in 5 kg soil capacity earthen pan filled with infested soil having initial nematode population 285 J2/200 cc soil. Seeds of the rice (Variety- Pusa 1121) were soaked in tap water for 24 h and the sprouted seeds were sown in pots. Hundred seeds of rice were sown in each pot and each treatment was replicated four times. Organic amendments (neem cake and FYM) and bio-agents (*Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride*) were added 10 days before sowing. Weighted amount of bio-agents was mixed in neem cake and FYM for enrichment with 7-10 days waiting period. Carbofuran (Furadan 3G) was added at time of sowing. The earthen pots without organic amendments, bio-agents and chemical were treated as control. Observations on plant growth parameters (seedling length in cm fresh and dry seedling weight in g) and nematode multiplication (number of galls/20 seedling, number of eggs/20 seedling and final nematode population in the soil/200 cc soil) were made at transplanting time. The plant parameters were maximum and significantly highest in treated nursery (neem cake @ 50 g/pot + *Pseudomonas fluorescens* @ 50 g/pot) under screen house conditions. Nematode reproduction and multiplication parameters such as number of galls/seedling, number of eggs/seedling and final nematode population were significantly reduced in neem cake @ 50 g/pot + *Pseudomonas fluorescens* @ 50 g/pot.

Introduction

Rice (*Oryza sativa* L.) belongs to the Poaceae family and classified as semi-aquatic crop plants that thrive in a variety of soil and water conditions. Approximately, 90 per cent of the world's rice is grown and consumed in Asia. In India, it was cultivated from ancient time and ranked first in area and second in production after China. Annually, the country produces approx. 175.58 million tonnes of rice (FAOSTAT, 2018). Rice is cultivated in almost all the states of country and among which West Bengal is the highest in rice production and Tamil Nadu has first place in productivity. As rice is grown under various conditions so it is affected by a number of abiotic and biotic factors. Among biotic factors, plant parasitic nematodes (PPNs) proved most damaging pathogens (Jain *et al.,* 2012)

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which causes 16-32 per cent yield loss under irrigated and 11-73 percent under flooded conditions in India (Tian *et al.,* 2018). Due to these tiny worms on rice, annually estimated globally yield loss ranges from 10-25% (Bridge *et al.,* 2005). Among PPNs, four major nematode species of rice crop are taken into account i.e. *Meloidogyne graminicola*, *Aphelenchoides besseyi*, *Ditylenchus angustus* and *Heterodera oryzicola* that caused combined yield loss to be estimated 10.50 per cent (Jain *et al.,* 2007). However, rice root-knot nematode, *M. graminicola* has become the most destructive pest and serious problem in major rice producing countries of the world including in India (Jain *et al.,* 2012). The diagnostic symptom of *M. graminicola* affected rice plants are less vigor, stunting growth, yellowing (nurseries and main field), production of poorly filled kernels and late maturity of crop. The main problem of this pest is seen particularly in nursery where flooding is intermitted. Since, the initial infection starts from the nursery, the management of this nematode in rice becomes most important. Various effective strategies should be applied in preventing the spread of *M. graminicola* from infected areas to uninfected areas by infected seedlings. Among these preventive or management methods, chemical control should be avoided due to their indiscriminate use which enhance the problem of resistance and risk to the environment.

So, there is current need of the adoption of eco-friendly management approaches like organic amendments particularly deoiled cakes and use of effective bio-control agents which can mostly used in rice nursery application. Therefore in present study, we tried to define the effects of integrated management practices on rice root-knot nematode, *M. graminicola* using organic amendments and bio-agents under rice nursery conditions.

Material and Methods

The present study was conducted under screen house conditions to know the effect of organic amendments, bio-agents (alone and in combination) by comparing with chemical on the population of rice root-knot nematode in rice. Nursery were grown in 5 kg soil capacity earthen pan filled with infested soil having initial nematode population 285 J2/200 cc soil. Seeds of the rice (Variety - Pusa 1121) were soaked in tap water for 24 h and the sprouted seeds were sown in pots. Hundred seeds were sown in each pan and every treatment was replicated four times. Organic amendments (neem cake and FYM) and bio-agents (*Pseudomonas fluorescens, Purpureocillium lilacinum* and *Trichoderma viride*) were added ten days before sowing. Weighted amount of bio-agents was mixed in neem cake and FYM for enrichment and kept for 7-10 days as waiting period for proper multiplication of bio-agents in organic amendments. Carbofuran (Furadan 3G) was added at time of sowing. The earthen pans without organic amendments, bio-agents and chemical were treated as control. After thirty-five days of sowing, all the plants were uprooted carefully. The roots of plants were retrieved carefully and kept under running tap water to clear it from adhering soil particles and recorded the observations such as plant growth parameters (seedling length in cm, fresh and dry seedling weight in g) and nematode multiplication factors (number of galls/20 seedling, number of eggs/20 seedling and final nematode population in the soil/200 cc soil). For recording final soil population, soil from each pan after depotting was analyzed by Cobb's Sieving and Decanting technique (Cobb, 1918 and Schnidler, 1961) and nematode were extracted by Modified Baermann's Funnel technique (MBFT).

Treatment details:

T1: Farm yard manure @ 50 g/pot
T2: Neem cake @ 50 g/pot
T3: T1 enriched with *Pseudomonas fluorescens* @ 50 g/pot
T4: T1 enriched with *Purpureocillium lilacinum* @ 50 g/pot
T5: T1 enriched with *Trichoderma viride* @ 50 g/pot
T6: T2 enriched with *Pseudomonas fluorescens* @ 50 g/pot
T7: T2 enriched with *Purpureocillium lilacinum* @ 50 g/pot
T8: T2 enriched with *Trichoderma viride* @ 50 g/pot
T9: Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check)
T10: Untreated control

Statistical analysis:

The data obtained in the experiment was analyzed by complete randomized design (CRD).

Results and Discussion

Generally, the infestation of *M. graminicola* is suppressed by various management components *viz.,* organic amendments, bio-control agents, chemical control etc. The present investigation was on integrated management of *M. graminicola* using organic amendments and bio-agents on rice nursery. After thirty-five days of sowing, observations were recorded on plant growth parameters and nematode reproduction and multiplication factors.

Maximum and significantly higher seedling length (66.1 cm) was recorded in neem cake enriched with *Pseudomonas fluorescens* @ 50 g/pot over untreated check followed by FYM enriched with *Trichoderma viride* @ 50 g/pot (63.9 cm). This was statistically at par with that of Furadan 3G (carbofuran) @ 200 mg/pot (Figure 1). The minimum seedling height (41.1 cm) was observed in untreated check. However, all the treatments significantly increased the seedling height as compared to untreated check except FYM @ 50g/pot. Between organic amendments higher seedling height was obtained in neem cake followed FYM. Maximum and significantly higher fresh (4.45 g) and dry seedling weight (1.29 g) was observed in plants treated with neem cake enriched with *P. fluorescens* @ 50 g/pot. This was statistically at par with that of FYM enriched with *T. viride* @ 50 g/pot, neem cake enriched with *P. lilacinum* @ 50 g/pot, neem cake enriched with *T. viride* @ 50 g/pot and carbofuran @ 200 mg/pot. However, all the treatments significantly increased the fresh and dry seedling weight as compared to untreated check except individual application of FYM @ 50g /pot. The findings present study confirms the results of Bansal *et al.* (2005). Similarly, Kumar (2019) who reported that soil application of neem cake @ 50 g/pot + *P. fluorescens* @ 50 g/pot had significantly higher seedling growth of rice under nursery conditions as compared to untreated check (Table 1). These results are in conformity with those of Anitha and Rajendran (2005) who revealed that the integration of *P. fluorescens* at 2.5 kg/ha, neem cake at 1 t/ha and carbofuran at 1 kg a.i./ha was highly effective in improving rice plant growth parameters in the nursery as well as in main field. Shukla and Chand (2018) also reported that among the various botanicals neem cake @ 15 g/kg soil proved more effective against *M. graminicola* under pot conditions and significantly increase the plant growth parameters and reduce the nematode parameters as compared to botanicals.

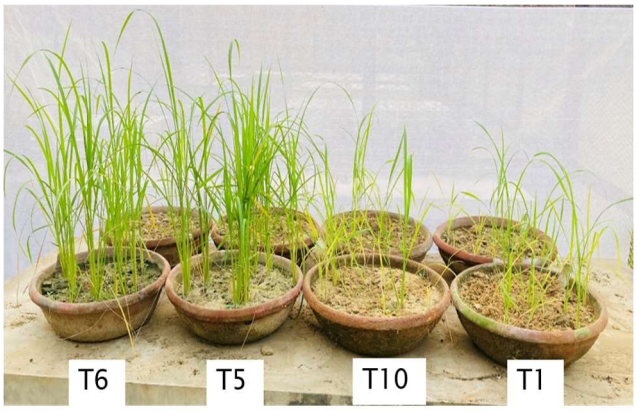

Figure 1. Difference among effective and less-effective treatments T6, T5, T10 and T1 (from left to right) on the plant growth parameter of rice infected with *Meloidogyne graminicola.*

In the present investigation, combined soil application of different organic amendments and bio-agents on rice nursery were used, among which neem cake with *P. fluorescens* was superior as compared to other treatments. Significantly higher seedling growth was also recorded in pre sowing application of with neem cake enriched with *P. fluorescens* @ 50 g/pot followed by FYM enriched with *T. viride* @ 50 g/pot. In individual application organic amendments, maximum seedling growth was found in neem cake @ 50 g/pot than FYM @ 50 g/pot. According to Pankaj *et al.* (2010), use of carbofuran as soil application considerably decreased galling of rice root-knot nematode at 1 kg/ha or above, at 2 kg dose causing the largest reduction in galling (82%). In the year 2013, Mukesh and Sobita also reported that the neem leaf extract significantly increased the plants growth parameters and reduced number of galls in different concentration.

Minimum and significantly lowest number of galls/seedling (29.00) was observed in neem cake enriched with *P. fluorescens* @ 50 g/pot over untreated check. This was statistically at par with that of neem cake enriched with *P. lilacinum* @ 50 g/pot (33.50). The maximum number of galls/seedling (155.75) was however observed in untreated check. Between organic amendments

Table 1. Effect of various treatments on the plant growth parameter of rice infested with *Meloidogyne graminicola*

Sr. No.	Treatments	Seedling height (cm)	Fresh seedling weight (g)	Dry seedling weight (g)
T1	Farm yard manure @ 50 g/pot	47.6	2.83	0.82
T2	Neem cake @ 50g/pot	51.6	3.25	0.95
T3	FYM enriched with *Pseudomonas fluorescens* @ 50 g/pot	56.4	3.69	1.07
T4	FYM enriched with *Purpureocillium lilacinum* @ 50 g/pot	54.7	3.44	1.00
T5	FYM enriched with *Trichoderma viride* @ 50 g/pot	63.9	4.27	1.24
T6	Neem cake enriched with *P. fluorescens* @ 50 g/pot	66.1	4.45	1.29
T7	Neem cake enriched with *P. lilacinum* @ 50 g/pot	62.2	4.09	1.19
T8	Neem cake enriched with *T. viride* @ 50 g/pot	61.3	4.15	1.21
T9	Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check)	60.7	3.96	1.15
T10	Untreated control	41.1	2.48	0.77
C.D. at 5%		8.3	0.53	0.16

Date of sowing: 17/07/2020; Date of termination: 23/08/2020

Table 2. Effect of various treatments on reproduction and multiplication of *Meloidogyne graminicola* on rice

Sr. No.	Treatments	Number of galls/ seedling	Number of eggs/ seedling	Final nematode population in the soil (200 cc soil)
T1	Farm yard manure @ 50 g/pot	137.75 (11.78)	15525.00 (124.56)	246.25 (15.71)
T2	Neem cake @ 50g/pot	131.00 (11.48)	11162.50 (105.64)	217.50 (14.74)
T3	FYM enriched with *Pseudomonas fluorescens* @ 50 g/pot	95.50 (9.81)	6800.00 (81.80)	192.50 (13.86)
T4	FYM enriched with *Purpureocillium lilacinum* @ 50 g/pot	126.50 (11.29)	8792.50 (93.71)	198.75 (14.10)
T5	FYM enriched with *Trichoderma viride* @ 50 g/pot	65.25 (8.11)	6570.00 (80.92)	187.50 (13.71)
T6	Neem cake enriched with *P. fluorescens* @ 50 g/pot	29.00 (5.32)	1397.50 (36.30)	125.00 (11.13)
T7	Neem cake enriched with *P. lilacinum* @ 50 g/pot	33.50 (5.69)	1830.00 (42.33)	165.00 (12.38)
T8	Neem cake enriched with *T. viride* @ 50 g/pot	34.50 (5.79)	1877.50 (41.12)	171.25 (13.10)
T9	Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check)	35.00 (5.86)	1907.50 (42.90)	172.50 (13.16)
T10	Untreated control	155.75 (12.52)	20327.50 (142.41)	311.25 (17.13)
C.D. at 5%		(1.58)	(12.43)	(3.37)

Figures in parentheses are √ n transformed value
Date of sowing: 17/07/2020; Date of termination: 23/08/2020

lowest number of galls/seedling was obtained in neem cake than FYM.

Minimum and significantly lowest number of eggs/seedling (1397.50) and final nematode population (125.00) was observed in neem cake enriched with *P. fluorescens* @ 50 g/pot over untreated check and this was statistically at par with that of neem cake enriched with *P. lilacinum* @ 50 g/pot. The maximum number of eggs/seedling (20327.50) was however observed in untreated check. Ammonia released during decomposition of the different organic amendments, which are poison to PPNs including root knot nematodes (Mian *et al.,* 1982; Mian and Rodriguez-Kabana, 1982) due to which it may be partially involved in suppression *M. graminicola* and reduced root galling. Similar findings were also observed by Kumar (2019) who concluded that integration of neem cake with *P. fluorescens* under nursery conditions significantly reduced the nematode reproduction and multiplication parameters (Table 2).

Similar results were recorded by Seenivasan *et al.* (2012) who observed that talc based formulation of *P. fluorescens* and *P. lilacinum* significantly reduced the root invasion and soil population of nematode but *P. fluorescens* was most effective when applied as seed cum soil application and seed treatment alone. Panigrahi and Mishra (1995) also reported that carbofuran significantly reduced galling and also buildup low population of *M. graminicola* when with seedlings in fields planted treated with carbofuran @ 1 kg a.i. per ha. The results of this study is not confirmatory with Devi *et al.* (2019) who revealed that castor cake @ 10 g/kg soil was found best in improving plant growth parameters and reducing nematode parameters as compared to other organic amendments (neem cake and mustard cake) @5 and 10g/kg against this nematode in rice nursery. This may be due to some ecological and environmental factors. Dangal *et al.* (2008) also observed number of J2 and plant growth parameters were non-significant in different organic amendments including neem leaves (*Azadirachta indica*) when applied @ 1, 2 and 3 t/ha against this nematode in direct seeded rice.

Conclusion

The current study is more realistic because nematode invasion caused significant damage to rice nurseries. Under screen house conditions, rice growth metrics were maximum and significantly higher in treated nursery (neem cake @ 50 g/pot + *Pseudomonas fluorescens* @ 50 g/pot). Nematode reproduction and multiplication parameters such as the number of galls per seedling, the number of eggs per seedling, and the final nematode population were all reduced dramatically.

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Conflict of interest

The authors declare that they have no conflict of interest.

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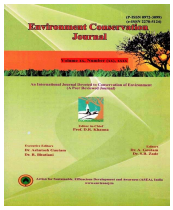

Economic characters of muga silkworm cocoons influenced by regions and commercial seasons

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ABSTRACT

Muga cocoons were procured from Jorhat, Kamrup and Lakhimpur districts in spring and autumn season. The objective was to determine the impact of three regions and commercial seasons on cocoon characters in terms of cocoon size, cocoon weight, pupal weight, shell weight and shell ratio. The experiment was laid out in completely randomised design with three replications. The impact of three regions and two seasons was found to be significant on cocoon parameters. Large sized cocoons were obtained from Kamrup district followed by Lakhimpur and Jorhat in autumn season compared to those obtained in spring season. Cocoon weight, shell weight, pupal weight and shell ratio percentage (5.73g, 0.51g, 5.19g and 8.74% respectively) were found to be significantly higher in Kamrup as compared to Lakhimpur (5.4g, 0.46g, 4.94g and 8.43% respectively) while lowest was recorded in Jorhat district (5.27g, 0.43g, 4.83g and 8.20% respectively) irrespective of the seasons. Considering the seasons, cocoons obtained from autumn were superior in terms of cocoon characters (5.66g cocoon weight, 0.49g shell weight, 5.16g pupal weight and 8.63% shell ratio) than those of spring season. Kamrup district and autumn season have turned out to be the best region and best season clearly indicating that region and season influences the cocoon characters of muga silkworm.

Introduction

The silkworm, *Antheraea assamensis* Helfer is found only in north-east India from which muga silk is obtained. Muga culture is especially practiced in Kamrup, Goalpara, Udalguri, Kokrajhar, Tinsukia, Dibrugarh, Sibsagar, Jorhat, Golaghat, Lakhimpur and Dhemaji districts of Assam (Bayan Borah and Borgohain, 2018). Muga silkworm is polyphagous, semi-domesticated and producing six broods in one year. *Katia* (autumn, September-October) and *Jethua* (spring, April-May) are commercial crops and *Aherua*, *Bhodia*, *Jarua, Chotua* are seed crops (Goswami *et al.,* 2013). The climatic conditions prevailing during the commercial seasons in Assam are favourable for muga culture in comparison to seed crops. The availability of the primary and secondary food plants (*som, soalu* etc.) in the north-eastern India both in the Brahmaputra valley and in the Eastern Himalayan ranges made this region an ecological niche. In India, the total production of muga raw silk during the year 2019-20 was 240.50 MT out of which Assam produced 197.90 MT of muga raw silk (Anonymous, 2020a). The districts selected for the study i.e. Jorhat, Kamrup and Lakhimpur contributed about 27.06% of the total muga production of the state and Lakhimpur scored the

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highest production (41.11MT) with the muga silkworm food plant area of 2238.17 ha engaging 4297 number of families (Anon., 2020b). Therefore, every possibility must be explored to enhance the production in these potential muga growing areas based on the quality.

Cocoon and its standard are the crucial factor which leads to better silk output and sustainability of silk industry. The quality of cocoon is determined through its characters (size and weight of cocoon and shell, shell ratio percentage) which vary as it is reared in outdoor condition. According to Rahmathulla (2012), variation in the environmental elements affect the genotypic constitution in the form of phenotypic output such as cocoon weight, shell weight, shell ratio percentage. Hence, it can be inferred that the cocoon quality and ultimately the quality of raw silk is influenced by wide range of factors starting from climatic conditions that changes with different crops, seasons and regions. The present study was undertaken with a view to study the regional variations in cocoon characteristics of muga silkworm reared in two commercial seasons from three randomly selected regions (Jorhat, Kamrup and Lakhimpur). The cocoon parameters considered during the study were cocoon size, weight, shell weight, pupal weight and shell ratio percentage.

Material and Methods

The experiment was carried out in two commercial seasons *viz*., Spring (*Jethua*) and Autumn (*Katia*) during the year 2018-2020. Freshly stifled cocoons were procured from private rearers of Jorhat, Kamrup and Lakhimpur districts of Assam in spring and autumn season. The cocoons were stored in a well-ventilated wire mesh cage in a properly disinfected insect and rat proof room by following the standard method of Mishra *et al.,* 2016. Inside the cage the cocoons were kept open and spread thinly in a single layer. The cocoons were exposed to sunlight periodically to avoid fungal attack. Muga cocoon shell and pupa are presented in figure 1(A-F).

Cocoon parameters assessment

Cocoons were subjected for assessment of quality.

a) Size of the cocoons: Size of the cocoon is regarded as one of the most important characteristics which indicate the productivity in silk reeling and quality of raw silk (Gowda *et al.,* 2014). Size of the cocoon was calculated by following the standard method of Anonymous, 2021a. The number of cocoons that can be enclosed in a container of one litre indicates the size. The more the number of cocoons the smaller will be the size and vice versa.

b) Cocoon weight: The weight of the cocoons along with the pupa was recorded in grams procured from different locations in different seasons.

c) Cocoon shell weight: The weight of the cocoon shell was measured in grams by cutting the shell and removing the pupa from the cocoon.

d) Pupal weight: Weight of pupa was recorded in gram after removing it from cocoon.

e) Shell ratio: Shell ratio is the ratio of the weight of cocoon shell to the weight of cocoon with pupa. It is the amount of silk present in the cocoon and expressed in percentage. It was calculated by following the standard method of Bashir *et al*. (2014).

$$\text{Shell ratio} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

Statistical analysis of data was performed in Completely Randomised Design (CRD) to study the effect of different regions and commercial seasons on cocoon parameters as described by Panse and Sukhatme (1989). Data taken were mean of three replications. The number of cocoons in each replication was ten.

Results and Discussion

It is apparently manifested in Table 1 that the cocoon size of Jorhat, Kamrup and Lakhimpur regions was significantly different. The number of cocoons per litre ranges between 38.00-44.00 numbers in different regions. Considering the regions, Kamrup (38.50 nos.) registered significantly less cocoon numbers per litre while Lakhimpur (approx. 40 nos.) and Jorhat (42.00 nos.) was observed as more. Between the two commercial seasons, the numbers of cocoon per litre was significantly less in autumn season (39.22 nos.) than the spring season (41.56 nos.). The interaction effect due to region and season was non-significant. The lowest number of cocoons per litre (38.00 nos.) was recorded in Kamrup district in

Table 1: Effect of different muga growing regions and commercial seasons on cocoon size (nos./lt.) of muga silkworm.

Region	Season		Mean
	Spring	Autumn	
Jorhat	44.00	40.00	42.00
Kamrup	39.00	38.00	38.50
Lakhimpur	41.67	39.67	40.67
Mean	41.56	39.22	
	SED (±)	CD (5%)	
Region	0.61	1.34	
Season	0.50	1.10	
Region x Season	0.86	NS	

Data are mean of 3 replications, NS = Non significant, SED = Standard error of difference

Jorhat district (spring season)

Jorhat district (autumn season)

(C) Kamrup district (spring season)

(D) Kamrup district (autumn season)

(E) Lakhimpur district (spring season)

(F) Lakhimpur district (autumn season)

Figure 1(A-F): Shell and pupa of muga cocoon in spring and autumn season.

autumn season whereas the highest (44.00 nos.) was found in Jorhat district in spring season. Less number of cocoons indicated bigger size while small size is denoted by a more number of cocoons. Thus, it can be concluded that the cocoons procured from Kamrup in autumn season were comparatively bigger in size than Lakhimpur and Jorhat district.

Cocoons from different regions showed significant variation in weight (Table 2). The highest cocoon weight was registered in Kamrup district (5.73g) while the lowest was observed in Jorhat district (5.27g). Irrespective of the regions, significantly the highest cocoon weight was observed in autumn season (5.66g) and in the spring season (5.27g) recorded the lowest. But notably, the interaction effect of region and season was found to be non-significant. Table 3 evidently indicate that shell weight of muga cocoons varied considerably in different regions and seasons. Highest shell weight was recorded in cocoons procured from Kamrup while the lowest was observed in cocoons obtained from Jorhat (0.43g). Cocoons harvested in autumn (0.49g) showed significantly higher shell weight in comparison of those harvested in spring season (0.44g). The interrelation between region and season was found to be non-significant. Results pertaining to pupal weight are presented in Table 4. Pupal weight was significantly highest in cocoons obtained from Kamrup district (5.19g) followed by pupal weight in cocoons obtained from Lakhimpur (4.94g) which was found *at par* with the pupal weight (4.83g) of Jorhat district. Irrespective of regions, pupa obtained from the cocoons of autumn season (5.16g) was significantly higher in weight than spring season (4.81g). The study showed that the interaction effect of region and season was not significant. Similar trend was noticed in case of shell ratio percentage (Table 5). The results displayed that shell ratio percentage differed significantly majorly across the regions. Irrespective of the season, shell ratio percentage was maximum in Kamrup district (8.74%) and minimum was observed in Jorhat (8.20%) region. Cocoons obtained in autumn season (8.63%) exhibited significantly higher shell ratio percentage compared to spring season (8.28%). The interaction effect of region and season was trivial to notice. However, the maximum shell ratio percentage was recorded in Kamrup district (8.86%) in autumn season whereas the lowest was observed in Jorhat district (8.01%) in spring season. Cocoon acts as defensive covering for the pupa inside to overcome unfavourable environmental conditions and its natural enemies. It is spun by mature silkworm with the help of protein secretion from silk gland at the end of its larval period. Cocoon is the raw material of silk industry. Size and weight of cocoon and shell, shell ratio percentage are key parameters which help to access the quality of cocoon. Seasonal variations in different silkworm species have been reported by many researchers (Bashir *et al.,* 2014 and Sarkar, 2018 in mulberry; Bhatia and Yousuf, 2014, Chattopadhyay *et al.,* 2017 in tasar; Naik *et al.,* 2010, Chattopadhyay *et al.,* 2017 in eri; Barman and Rana, 2011, Padaki *et al.,* 2014 in muga, respectively) which directly or indirectly influence the silkworm in the form of commercial characters. Literature is also available on effect of host plants on economic traits of silkworm (Bahar *et al.,* 2011, Singh and Goswami, 2012, Deka and Kumari, 2013, Subharani *et al.,* 2017). Rahman *et al.* (2015) observed that environmental conditions play a significant role which influence the quantitative and qualitative characters of silkworm such as cocoon size, weight, length and shell ratio. The contribution of silkworm race is about 4.2% for successful cocoon production (Neog *et al.,* 2016) along with other factors like rearing conditions, environmental conditions during rearing, quantity and quality of leaves, harvesting conditions, all these factors influence the healthy growth of larva which ultimately affecting the size of cocoon (Aruga, 1994 and Lee, 1999). In case of uni/bivoltine mulberry silkworm species, the number of cocoons per litre ranges from 90-120 (Anon., 2021a). Saikia (2008) reported that the cocoon volume of eri silkworm ranged from 52-55 nos., 50-51 nos. and 46-51 nos. reared on borkesseru, borpat and castor, respectively.

The present results are in accordance with Borpuzari *et al*. (2020) who observed that the weight of muga cocoons obtained from autumn (*Katia*) was significantly higher than cocoons produced in other seasons. Bordoloi (1999) while studying the effect of various seasons and host plants on cocoon characteristics of muga silkworm revealed that there is significant variation in regards of cocoon weight (5.53g, 4.96g, 4.89g and 4.81g in

Table 2: Effect of different muga growing regions and commercial seasons on cocoon weight (g) of muga silkworm.

Region	Season		Mean
	Spring	Autumn	
Jorhat	5.15	5.39	5.27
Kamrup	5.49	5.97	5.73
Lakhimpur	5.18	5.61	5.40
Mean	5.27	5.66	
	SED (±)	CD (5%)	
Region	0.06	0.13	
Season	0.05	0.11	
Region x Season	0.08	NS	

Table 3: Effect of different muga growing regions and commercial seasons on shell weight (g) of muga silkworm.

Region	Season		Mean
	Spring	Autumn	
Jorhat	0.41	0.45	0.43
Kamrup	0.48	0.53	0.51
Lakhimpur	0.43	0.48	0.46
Mean	0.44	0.49	
	SED (±)	CD (5%)	
Region	0.01	0.01	
Season	0.01	0.01	
Region x Season	0.01	NS	

Table 4: Effect of different muga growing regions and commercial seasons on pupal weight (g) of muga silkworm.

Region	Season		Mean
	Spring	Autumn	
Jorhat	4.71	4.94	4.83
Kamrup	4.97	5.41	5.19
Lakhimpur	4.75	5.12	4.94
Mean	4.81	5.16	
	SED (±)	CD (5%)	
Region	0.08	0.17	
Season	0.06	0.14	
Region x Season	0.11	NS	

Table 5: Effect of different muga growing regions and commercial seasons on shell ratio percentage of muga silkworm.

Region	Season		Mean
	Spring	Autumn	
Jorhat	8.01	8.38	8.20
Kamrup	8.61	8.86	8.74
Lakhimpur	8.22	8.64	8.43
Mean	8.28	8.63	
	SED (±)	CD (5%)	
Region	0.05	0.11	
Season	0.04	0.09	
Region x Season	0.07	NS	

Katia, Bhodia, Aherua, Jethua respectively), pupal weight (5.12g, 4.50g,4.49g and 4.46g in *Katia, Bhodia, Aherua, Jethua* respectively), shell ratio percentage (7.40%, 9.00%, 8.27% and 7.37 % in *Katia, Bhodia, Aherua,Jethua* respectively) but non-significant effect on shell weight. Kemprai

(2012) also observed that season and host plant significantly influenced the cocoon characters like cocoon weight, shell weight, pupal weight and autumn season were the best season. However, season had non-significant effect on shell ratio percentage. According to Choudhury *et al*. (1998), the plants containing more sugar and less fibre in the leaf were suitable for muga worm and there exist considerable variation in the chemical constituents of som leaf collected from various locations of geo-meteorological areas of Assam and a positive relation has been found between the sugar content of leaf and cocoon weight. Padaki *et al*. (2014) concluded that significant variation was observed among three prominent muga growing regions (Tura, Goalpara and Sonapur) and cocoons of Tura region performed better in terms of cocoon quality in both *Katia* and *Jethua* as compared to other two regions. According to Tikader (2012), the cocoon weight and shell weight of semi-domesticated muga silkworm lies in the range 2.9-7.7 g and 0.18-0.65 g, respectively. Neog *et al*. (2016) found the male cocoon weight and shell weight was higher from larvae fed with *som* leaves while female cocoon weight was higher from *soalu*. The weight of pupa (4.16-6.10g in male and 5.50-8.47g in female) were found to vary among various wild and semi domesticated stock and more in wild accessions (Kalita and Dutta, 2015). Chattopadhyay *et al*. (2018) also reported that the average weight of muga cocoons was 7.10g, shell weight 0.60g and shell ratio 8.50%. As per findings of Kalita and Dutta (2020), significant differences were observed in *Antheraea assamensis* population collected from eleven different regions of north-east India in terms of cocoon colour, weight, shell weight and shell ratio. Sarmah *et al*. (2013) reported that wide range of variation exist in nutrient status of soil under *Persea bombycina* plantation in Jorhat and Lakhimpur district which influenced the nutrient content of *som* leaf and in turn affect overall cocoon production. Borpuzari *et al*. (2020) revealed that the rearing performance as well as biochemical constituents was better in *katia* crop followed by *Jethua* when rearing was conducted on *soalu* plant. The changes in various cocoon parameters may be due to the variations in environmental factors and quality of food plants. Kumar *et al*. (2012) studied the effect of different host plants *viz*., *Terminalia arjuna, T bellirica, T chebula, T tomentosa, L. parviflora and L. speciosa* on cocoon weight, shell weight, silk ratio of *Antheraea mylitta* during July-August under agro-climatic condition of Ranchi and found that the parameters varied with the host plants. Pallavi and Sannappa (2018) concluded that commercial characters of eri silkworm varied significantly among six eco-races (Borduar, Dhanubanga, Khanapara, Kokrajhar, Mendipathar and Titabar) of eri silkworm when reared on castor leaves.

Conclusion

Cocoon has immense commercial importance as it is the raw material of silk industry and is processed after harvesting from the mountage. Size and weight of cocoon and shell, shell ratio percentage are key parameters which help to access the quality of cocoon. These parameters or properties vary from region to region and season to season. Different regions of Assam experience different climatic factors in terms of temperature, humidity, rainfall etc. which has its impact on existing fauna and flora of that particular region as a consequence variation can be noticed on product quality of crops. Soil nutrient quality and quantity also varies in different agro-climatic zones of Assam which may also affect quality of the food plant and ultimately growth and development of muga silkworm and their product i.e. cocoon. From the results, it can be inferred that all the parameters were better in Kamrup as compared to Lakhimpur and Jorhat district. Considering the seasons autumn season was found better in respect of cocoon characteristics. In order to improve the quality of muga cocoons in Lakhimpur and Jorhat district as well as in spring season necessary measures should be taken into consideration. Rearers should also follow proper package of practices regarding host plants and silkworm rearing to enhance productivity as well as quality of cocoons.

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Conflict of interest

The authors declare that they have no conflict of interest.

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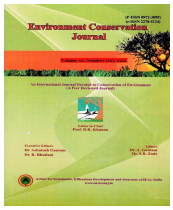

Fertility capability classification (FCC) of soils of a lower Brahmaputra valley area of Assam, India

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ABSTRACT

Fertility capability classification (FCC) is a system of classification which uses pedological data of soils and coverts it into capability classes based on major fertility constraints portrayed by the soils. The present study was aimed to classify the soils major landforms of a lower Brahmaputra valley region of Assam, India in to FCC classes, to suggest specific management practices in order to overcome the fertility constraints and improve the crop productivity.The major strata types used were found to be loamy top soil, 'L' and clayey top soil 'C'. The sub-strata type found were loamy sub soil, 'L' and clayey sub soils, 'C'. The major condition modifiers or the major fertility constraints were found to be Al toxicity 'a' and 'a$^-$', high leaching potential, 'e', low nutrient reserves 'k' and 'g' waterlogging. The paddy soils of alluvial plains were classified into La$^-$eg and Lg$^+$a$^-$e. The tea growing soils of younger alluvial plains were classified into Car$^+$e. The non-paddy soils of alluvial plains were categorized as Ca$^-$gke. The soils of uplands and inselberg were categorized into LCae class. The study revealed that FCC classification can successfully bring out the soil fertility constraints and can be very much helpful in soil fertility management for sustainable crop productions.

Introduction

Soil properties are the outcome of several soil-forming processes as influenced by basic soil-forming factors as outlined by Jenny (1942). Climate and geology are the two important factors affecting the soil development process through the type and quantity of material deposited at a place and these are reflected in the soil horizons and soil properties (Glina*et al*., 2013; Waroszewski*et al*., 2015). Geological history and climate affect the soils at a regional or continental scale whereas land use plays an important role at small catchment scale (Wang *et al*., 2001). The soil properties of a landscape are continuously influenced by land use, soil management practices, soil erosion, mineralization, and leaching of organic carbon and other nutrient elements (Xiao *et al*., 2010; Vasu *et al*., 2016). For sustainable agricultural production and environmental health, it is important to measure specific soil properties to monitor soil quality (Karlen*et al*., 1997; Mukherjee and Lal, 2014; Choudhury and Mandal, 2021). Soil fertility is the inherent capacity of soil to supply plant nutrients in balanced proportion and adequate quantity for optimum plant growth (Bharti *et al*., 2017). Soil fertility is affected by natural as well as anthropogenic factors (Kavitha and Sujatha, 2015). The detailed knowledge about soil fertility is a prerequisite for the adoption of improved soil and

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crop management practices to get an optimum level of crop productivity (Delsouz*et al*., 2017). Soil taxonomic information generated at different scales has limited direct applicability in assessing soil fertility as they focus to quantify subsurface soil properties that are permanent (Sanchez *et al*., 2003) and they do not include the dynamic properties of topsoil which are more important for fertility assessment (Chandrakala *et al.,* 2020; Vasu *et al.,* 2016). To overcome this limitation fertility capability classification (FCC) was first introduced by Buol *et al.*, (1975) with an intent to bring together soil classification and soil fertility into a single frame. Soil fertility capability classification (FCC) is a technical system to group the soils according to the kind of physical and chemical constraints they present under agronomic management (Sanchez *et al*., 1982; Chandrakala *et al.*, 2020). The system comprises of three gradations viz., type (texture of surface soil), sub strata type (texture of sub-surface soil) and modifiers with respect to their characteristics in the 50 cm depth of soil. The term "topsoil" refers to the plough layer or the top 20 cm of the soil, whichever is shallower. The term "subsoil" includes the depth interval between the topsoil and 50 cm depth. The modifiers are the various physical and chemical constraints portrayed by the soils (Sanchez *et al.,* 1982). After several revisions (Sanchez *et al.,* 1982; Sanchez *et al.,* 2003), the FCC system is now widely accepted and has been used by many researchers in the different parts of the world (Babalola *et al.,* 2019; Prasad, 2000; Das, 2015; Vasu *et al.,* 2016; Kalaiselvi*et al.,* 2019; Hota *et al.,* 2021). Scanchez*et al.,* (2003) in their fourth approximation also introduced modifiers related to biological properties of soil to relate importance of biological processes and soil organic matter in maintaining crop productivity (Bhutiani and Ahamad, 2019). Prasad (2000) used the FCC system to convert the taxonomic units into fertility classes for soils of Konheri watershed in semi-arid tropics of India and concluded that FCC can give a basic idea of soil fertility without analysing routine fertility parameters for N, P and K fertilizer recommendation. Kalaiselvi*et al.,* (2019) have used FCC to classify soils of Palani block in a semi-arid tropical region of Tamil Nadu. They concluded that the FCC was beneficial in identifying the potential constraints of fertility and for suggesting better management options. Hota *et al.,* (2021) successfully identified the major fertility constraints of soils under major land uses of north-eastern India to as low K reserves, highly acidic soil and low cation exchange capacity (CEC), and suggested management practices. Even though FCC does not include routine soil tests of fertility measurement it can be a useful approach to assess the capability of soils to sustain crop production and soil quality in the rainfed systems with low fertilizer input (Smithson and Sanchez, 2001; Vasu *et al.,* 2016).

Assam is a state with 90% of area under rainfed agriculture which contributes around 34% of food production of the state(Indiastat, 2017). Agriculture is the dominant land use practiced in the state of Assam and it accounts for about 54.11 per cent of the total geographical area of the state (Environmental Information System network, Assam, 2015). Though 80 per cent of the total population in Assam is involved in agriculture, the state is quite backward in the national list of agricultural productivity i.e., the total food grain productivity of Assam is 2.08 MT/ha which is less than that of the national average of 2.29 MT/ha. Similarly, in case of horticulture, the total productivity is 9.92 MT/ha, which is quite low compared to the national average of 12.26 MT/ha (Indiastat, 2018). However, the agriculture productivity of the state of Assam is quite below the national average due to many constraints including low agricultural inputs (Saud, 2018). The poor soil fertility management is common in the Brahmaputra valley in general and in the lower Brahmaputra valley in particular, due to smaller sizes of land holding and subsistent agricultural practices (Sharma, 2011). Hence, the present study was undertaken with the aim to classify the soils of the study area into fertility capability classes to identify specific fertility constraints for adoption of best soil and crop management practices for sustaining higher level of productivity.

Material and Methods

The study was conducted in the Rangjuli block of Goalpara district in the lower Brahmaputra Valley region of Assam. Goalpara district is one of the aspirational districts selected by NITI ayog Government of India for faster socio-economic

development of the district. The block extends its boundary between 25° 53.3 ' 3.637" to 26° 6' 4.73 " N latitude and 90°53.3' 8.231 " to 90° 5'59.075 " E Longitude and comes under Hot humid eco subregion (15.2) of agroecological regions of India (Velayutham*et al.,*1999), having udic moisture regime and hyperthermic temperature regime (Soil Survey Staff, 2014) with a mean annual precipitation of 2000 to 2500 mm. The block is dominantly of agrarian economy and more than 90% of population is dependent on agriculture. The major crops grown in the block are paddy (as kharif crop followed by fallow), arecanut and banana. Other than that, rubber and vegetables crops are also grown by the farmers.A detailed soil survey of the study area was conducted in the year 2020. Different landform classes were delineated based on slope, aspect, and drainage etc using SRTM DEM and Sentinel-2 satellite data using ArcGIS 10.2 software. From each of the landform class soil profiles were studied for important morphological features in the field and layer wise soil samples were collected from entire profile depth (up to 150 cm) and brought to laboratory for analysis of various physical and chemical properties of soils. Soils were classified into taxonomic classes following USDA Soil Taxonomy (Soil Survey Staff, 2014). To classify the profiles in to FCC, representative profiles from each landform were selected. The collected soil samples were air-dried and grounded to pass through 2 mm sieve for analysis of soil physical and chemical parameters and part of the 2mm sieved soil samples were finely grounded and passed through 0.5 mm sieve and used for soil organic carbon (SOC) analysis. The soil samples were analysed for particle size distribution using international pipette method (Robinson, 1922). Soil organic carbon (SOC) was determined by Walkley and Black (1934) method. Cation exchange capacity (CEC) and exchangeable cations were determined following standard procedures (Schollenberger and Simon, 1945; Sumner and Miller, 1996). FCC was carried out using fourth approximation by Scanchez*et al.,* (2003) and FCC designations were allotted accordingly. FCC designations were allotted as per the three gradations, first, type (surface texture) and substrata type (subsurface texture). Second, the condition modifiers, which includes fertility constraints or limitations (physical and chemical properties) and third, superscripts + or – which indicates the magnitude of modifiers. FCC considers the properties of 0-50 cm soil depth only, where 0-20 cm is considered surface soil, and 20-50 cm is considered as subsurface soil. Hence, the values of chemical properties were weighed to calculate the values for 0-20 cm and 20-50 cm for each profile. Descriptive statistics of soil parameters were worked out using data analysis tool of Microsoft excel 2019.

Results and Discussion

The major landforms of Rangjuli blocks were delineated in to active flood plain, younger alluvial plain, older alluvial plain, upland and inselbergs. Form each landform 1 or 2 representative profiles have been selected and named as P1 to P8. The detailed taxonomic classifications of the soils are given in Table 1. The descriptive statistics of the soil parameters that were used to carryout FCC classification are presented in the Table 2 and 3 for surface and subsurface soils respectively.The weighted mean values of considered soil properties for each pedons from each landform is given in Table 4. The strata type used were (i) loamy top soil, 'L'; (ii) clayey top soil 'C' and sub-strata type used (i) loamy sub soil, 'L'; (ii) clayey sub soils, 'C'. The condition modifiers appropriate for the soils under study were (i) Al toxicity for most common crops in terms of base saturation < 33% of sum of cations at pH 7 'a'; (ii) Al toxicity for very sensitive crops like cotton in terms of Al saturation of 10-60% 'a^{-}'; (iii) high leaching potential, 'e' with low activity clay minerals; (iv) low nutrient reserves 'k'; (v) gravel 10-35 % 'r^{+}'; (vi) 'g' waterlogging. P1 of active flood plain was categorized into fine loamy, mixed, hyperthermic Fluvaquentic Endoaquepts. These soils are mostly cultivated with paddy double cropping in *kharif* and *rabi*. They had loamy topsoil and subsoil. The loamy texture of these soils is due to the close proximity to river channel. For the very same reason, these soils remain saturated with water for more than 60 days in normal years. Due to continuous submergence in most of the time in a year, paddy cultivated soils show glei condition leading to a chroma of <2 (Gangopadhyay*et al.,* 2015) in the top 50 cm soils indicating aquic moisture regime. The soils also showed high leaching potential and low activity clays and these soils were categorized in to La^{-}eg FCC class (Table 5)

Table 1: Pedons and their respective landforms

Pedon number	Taxonomic classification	Landform
P1	Fine loamy, mixed, hyperthermic Fluvaquentic Endoaquepts	Active flood plain
P2	Fine,mixed,hyperthermicIncepticHapludalfs	Mounds of Younger alluvial plain
P3	Fine, mixed, hyperthermicTypicEndoaqualfs	Younger alluvial plain
P4	Fine, mixed, hyperthermicFluvaquenticEndoaquepts	Older alluvial plain
P5	Fine loamy, mixed, hyperthermicFluventicEutrudepts	Older alluvial plain
P6	Fine loamy, mixed, hyperthermicTypicKanhapludults	Upland
P7	Fine loamy, mixed, hyperthermicTypicKandihumults	Upland
P8	Fine, mixed, hyperthermicIncepticHapludalfs	Inselberg

Table 2: Descriptive statistics of soil parameters at 0-20 cm depth

Parameters	Mean	Minimum	Maximum	Std. Dev.	SE(m)±
Sand (%)	20.40	3.62	61.15	18.24	6.45
Silt (%)	42.93	9.74	63.64	17.65	6.24
Clay (%)	36.68	29.11	47.49	6.98	2.47
pH	4.70	4.24	5.01	0.25	0.09
SOC (%)	1.09	0.87	1.40	0.17	0.06
CEC [c mol (p^+) kg^{-1}]	8.00	6.47	8.96	0.80	0.28
Ca^{2+}[c mol (p^+) kg^{-1}]	1.53	0.80	3.03	0.79	0.28
Mg^{2+}[c mol (p^+) kg^{-1}]	1.87	0.98	3.88	0.95	0.34
Na^+[c mol (p^+) kg^{-1}]	0.32	0.23	0.43	0.06	0.02
K^+[c mol (p^+) kg^{-1}]	0.15	0.11	0.19	0.02	0.01
Sum of Bases [c mol (p^+) kg^{-1}]	3.88	2.20	6.28	1.51	0.53
BS (%)	49.38	25.72	79.91	21.60	7.64
Al^{3+}[c mol (p^+) kg^{-1}]	2.19	0.93	4.49	1.33	0.47
H^+[c mol (p^+) kg^{-1}]	1.43	0.32	3.45	1.07	0.38
ECEC[c mol (p^+) kg^{-1}]	7.50	5.37	10.14	1.56	0.55
Al saturation (%) of CEC	19.36	7.04	52.41	14.82	5.24
K saturation (%) of sum of bases	4.62	2.10	8.16	2.26	0.80

Table 3: Descriptive statistics of soil parameters at 20-50 cm depth

Parameters	Mean	Minimum	Maximum	Std. Dev.	SE(m)±
Sand (%)	18.53	2.95	45.23	14.60	5.16
Silt (%)	39.79	24.01	65.56	14.01	4.95
Clay (%)	41.68	25.58	54.70	10.05	3.55
pH	4.94	4.55	5.33	0.27	0.09
SOC (%)	0.90	0.58	1.27	0.26	0.09
CEC [c mol (p^+) kg^{-1}]	8.15	6.63	9.35	1.05	0.37
Ca^{2+}[c mol (p^+) kg^{-1}]	1.69	0.63	3.63	1.19	0.42
Mg^{2+}[c mol (p^+) kg^{-1}]	2.19	0.99	4.63	1.33	0.47
Na^+[c mol (p^+) kg^{-1}]	0.28	0.14	0.35	0.07	0.02
K^+[c mol (p^+) kg^{-1}]	0.14	0.07	0.35	0.09	0.03
Sum of Bases [c mol (p^+) kg^{-1}]	4.30	2.04	8.66	2.50	0.89
BS (%)	52.95	22.56	92.66	29.24	10.34
Al^{3+}[c mol (p^+) kg^{-1}]	1.52	0.37	4.08	1.50	0.53
H^+[c mol (p^+) kg^{-1}]	0.88	0.01	2.90	1.27	0.45
ECEC[c mol (p^+) kg^{-1}]	6.70	3.00	9.16	2.36	0.83
Al saturation (%) of CEC	14.03	0.01	39.63	13.46	4.76
K saturation (%) of sum of bases	4.88	0.84	14.30	4.43	1.57

The soil reaction was found to be highly acidic (4.65 to 5.02). The management of soil acidity is crucial in these soils. Similar results have been reported by Tabi *et al*., (2013), where the paddy growing soils showed modifiers ofAl-toxicities (a), low nutrient capital reserves (k) and high leaching potential (e). Usually, paddy tolerates acidic soil conditions but if a second crop is to be recommended, like rabi pulses, the management of soil acidity with regular liming has to be undertaken. The soils with 'g' modifiers or glei conditions are usually prone to denitrification (Sanchez *et al*., 2003). Hence, the split doses of N fertilizer and nitrification inhibitors are recommended.

Similarly, the paddy growing soils of the younger alluvial plain (P3) were loamy in texture. The major constraints are prolonged waterlogging, high leaching potential and Al toxicity for sensitive crops like cotton. These soils were categorized into $Lg^{+}a^{-}e$ (Table 5). The glei condition of these soils are due to high-water table. However, these soils are dominantly cultivated with paddy and water saturation in most part of the year is advantageous for growing paddy. But it also makes these soils unsuitable for taking a non-paddy second crop, unless artificially drained. Hence, instead of monocropping of paddy, a second paddy crop can be taken in these soils. Only, the denitrification and leaching losses have to be addressed using nitrification inhibitors and slow- release fertilizers.

P2 is the representative Pedon of younger alluvial plains, which are found in the mounds within the alluvial plains, and was categorized into fine, mixed,hyperthermicInceptic Hapludalfs. Due to shifting course of braided rivers like Brahmaputra, small mounds are left in places between two river channels, and in the long-run these lands become isolated uplands between two narrow valleys of alluvial plains (Hazarika *et al*., 2015).These lands are called as char land.These mounds of younger alluvial plain of our study area, are cultivated with tea. These soils have clayey topsoil and subsoils. The major constraints found in these soils are, Al toxicity for most of the crops and low buffering capacity with gravelly subsoil.

The Al toxicity of these soils arise due to prolonged leaching and washing away of bases down the profile (Reza *et al*., 2018) due to prevalent heavy rainfall of the area. The low buffering capacity of the soils arise due to the presence of low activity clays (Bhattacharyya *et al*., 2010). Similar results have been reported by Bandyopadhyay *et al*., (2014) for tea cultivated soils of upper Brahmaputra valley.These soils also showed highly acidic soil reaction (4.7 to 4.9), which falls within the ideal pH range for tea(Kacar, 1984). P2 was hence, categorized into $Car^{+}e$ FCC class (Table 5). These soils are suitable for tea and rubber (Naidu *et al*., 2006). However, integrated nutrient management and liming is crucial for realizing optimum yields from these soils. Because inappropriate nutrient application may aggravate soil acidity, which will be detrimental for tea growth and productivity (Ozyazici *et al*., 2010). The land use of older alluvial plains ispaddy in the level slopes (0-1 %) whereas at 1-3 % slopes vegetables like potato and bananaorchards are common. Two representative profiles one from paddy cultivation (P4) and another from homestead plantation (P5) were selected from older alluvial plains for categorising soils into FCC. Soils of the pedon P4 were categorized into fine, mixed, hyperthermicFluvaqueticEndoaquepts. These soils were found to be extremely acidic in reaction (pH 4.61-4.78), clayey in texture, with low permeability and highwater holding capacity. Low nutrient reserves, heavy leaching potential, and Al toxicity for very sensitive crops like cotton are the major constraints of these soils. Hence, these soils were designated as $Ca^{-}gke$. The high leaching potential is indicated by low CEC (7.96- 9.35 c mol (p^{+}) kg^{-1} soil). The reasons of low CEC are usually low clay content, low organic matter content in the surface soils, or clay minerals with low activity (Minh, 2011).

The first two reasons are not applicable in this case because the soils showed high SOC (0.87 to 1.02 %) and high clay content (47-48 %). Hence, the reason must be presence of low activity clays similar to the other pedons P1, P2 and P3. These soils are suitable for paddy cultivation and presence of high residual moisture after paddy harvesting provide an opportunity to grow short duration second crop such as mustard, pea, gram etc in the Rabi season. However, application of lime and residue management is important for neutralizing extreme acidity of soils for optimum productivity. It is desirable to apply nitrogen fertilizers in split

Table 4: Soil properties relevant for FCC of each pedon

Pedon	Soil depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	SOC (%)	CEC[c mol (p^+) kg $^{-1}$]	Exchangeable bases [c mol (p^+) kg $^{-1}$]				Sum of bases [c mol (p^+) kg $^{-1}$]	BS (%)	Exchangeable Acid cations [c mol (p^+) kg $^{-1}$]		ECEC [c mol (p^+) kg $^{-1}$]	Al saturation (%)	K saturation (%)
								Ca^{2+}	Mg^{2+}	Na^+	K^+			Al^{3+}	H^+			
P1	0-20	7.0	29.3	29.3	4.65	1.00	7.81	1.80	2.01	0.36	0.15	4.32	55.1	1.38	1.10	6.80	17.8	17.8
	20-50	2.9	31.5	31.5	5.01	1.23	6.63	1.66	1.96	0.32	0.15	4.09	60.9	1.40	1.07	6.55	22.1	22.1
P2	0-20	3.6	44.9	44.9	4.7	1.18	7.42	0.97	1.14	0.30	0.18	2.58	34.7	1.15	1.64	5.37	7.0	7.0
	20-50	4.2	54.7	54.7	4.9	0.59	7.75	0.79	1.11	0.28	0.35	2.53	32.6	0.47	0.00	3.00	3.3	3.3
P3	0-20	11.1	32.8	32.8	5.01	1.09	8.66	2.06	2.27	0.36	0.15	4.83	55.7	1.64	0.70	7.17	8.2	8.2
	20-50	15.1	34.1	34.1	5.06	0.58	9.14	2.69	3.20	0.28	0.13	6.29	68.6	0.37	0.00	6.66	0.01	0.0
P4	0-20	24.1	47.5	47.5	4.61	0.87	7.96	1.84	3.88	0.43	0.13	6.28	79.0	1.63	0.70	8.60	9.6	9.6
	20-50	26.1	48.3	48.3	4.78	1.02	9.35	3.63	4.63	0.33	0.07	8.66	92.7	0.49	0.00	9.16	2.6	2.6
P5	0-20	61.1	29.1	29.1	4.98	0.91	6.47	3.03	1.72	0.30	0.16	5.21	79.9	0.93	0.32	6.46	14.3	14.3
	20-50	45.2	25.6	25.6	5.23	0.79	6.91	2.77	3.12	0.35	0.08	6.33	91.2	0.68	0.10	7.11	9.8	9.8
P6	0-20	18.2	32.5	32.5	4.86	1.05	8.96	0.90	2.01	0.23	0.11	3.25	36.3	2.34	0.99	6.6	26.1	26.1
	20-50	14.2	48.1	48.1	5.33	0.83	8.19	0.68	1.51	0.14	0.07	2.40	29.2	0.96	0.17	3.5	11.7	11.7
P7	0-20	12.0	37.0	37.0	4.24	1.40	8.57	0.80	0.98	0.28	0.15	2.20	25.7	4.49	3.45	10.1	52.4	52.4
	20-50	9.4	46.4	46.4	4.70	1.27	9.28	0.67	0.99	0.28	0.15	2.09	22.6	3.68	2.81	8.6	39.6	39.6
P8	0-20	26.0	40.3	40.3	4.57	1.12	8.17	0.84	0.98	0.32	0.19	2.33	28.5	3.95	2.59	8.86	19.4	19.4
	20-50	31.1	44.9	44.9	4.55	0.91	7.92	0.63	0.99	0.29	0.13	2.04	25.9	4.08	2.90	9.02	23.1	23.1

Table 5: FCC of pedons and interpretations

Pedon	FCC designation	Description	Interpretations
P1	La^{-}eg	Loamy top soil and subsoil, aquic condition and saturated with water for >60 days in a year. High leaching potential with Al toxicity for sensitive crops like cotton.	Glei condition indicates low water infiltration. These soils are suitable for paddy cultivation. However, anaerobic conditions in the subsoils lead to denitrification. Hence, nitrification inhibitors are recommended. Split application of nitrogen fertilizers should be practiced to prevent leaching losses. Prone to the deficiency of Zn micronutrient due to heavy leaching. Hence, micronutrient management is crucial. To take a second crop (rabi pulses) the acidic soil conditions are to be managed by regular liming.
P2	Car^{+}e	Clayey topsoil and subsoil. Low base saturation and Al toxicity for most of the crops and low buffering capacity. Gravelly subsoil.	High water holding capacity. Highly acidic soils. Suitable for tea and rubber cultivation. Integrated nutrient management is necessary for optimum yield and maintain fertility levels.
P3	Lg^{+}a^{-}e	Loamy topsoil and subsoil, prolonged waterlogging, High leaching potential with Al toxicity for sensitive crops like cotton.	Similar to P1. But due to prolonged waterlogging in most of the time in the year and high-water table, paddy can be taken in rotation with another crop of paddy only.
P4	Ca^{-}gke	Clayey topsoil and subsoil, aquic condition and saturated with water for >60 days in a year. High leaching potential with Al toxicity for sensitive crops like cotton, low nutrient reserves.	Glei condition and clayey texture indicate low water infiltration. These soils are suitable for paddy cultivation. However, anaerobic conditions in the subsoils lead to denitrification. Hence, nitrification inhibitors are recommended. Split application of nitrogen fertilizers should be practiced to prevent leaching losses. Prone to the deficiency of Zn micronutrient due to heavy leaching. Hence, micronutrient management is crucial. To take a second crop (rabi pulses) the acidic soil conditions are to be managed by regular liming. Heavy K fertilizers and band application of K fertilizers is highly recommended.
P5	La^{-}ek	Loamy top soil and subsoil, High leaching potential with Al toxicity for sensitive crops like cotton, low nutrient reserves.	Moderate water holding capacity. Soils are suitable for wide range of vegetable and pulse crops. Well drained. But the leaching loss of bases are to be addressed by band placement of K and P nutrients. N, P and K should be applied in split. Foliar spray of Zn and other micronutrients are recommended. Soil acidity should be addressed with regular liming.
P6	LCae	Loamy top soil and clayey subsoil, high erosion risk, Al toxicity and low base saturation for all crops, low buffering capacity.	Top soil management is crucial. These soils are suitable for cultivation of rubber, tea and arecanut, which are among the major crops of Assam. Major management practices should involve covering the top soil with cover crops and application of organic matter over the soil surface.

doses, application of slow-release fertilizers, use of nitrification inhibitors to increase the use efficiency of nitrogen fertilizers by minimizing the leaching and volatilization losses (Minh, 2011). Application of K fertilizers as band application in split doses is highly recommended to minimize nutrient losses. Due to prolonged submergence, these soils are prone to the deficiency of Zn micronutrient(Minh, 2011). Hence, integrated nutrient management including micronutrients is highly recommended.

The non-paddy soils of older alluvial plains (P5) were classified in to Fine loamy, mixed, hyperthermicFluventicEutrudeptscategorized into FCC class La^{-}ek (Table 5). They have loamy top soil with major constraints of high leaching potential, Al toxicity for sensitive crops like cotton and low nutrient reserves. The soils are lighter in texture compared to the paddy cultivated soils of older alluvial plains, because these soils were confined to the river banks of Deosila river, a main tributary of Brahmaputra in Goalpara district. Due to the lighter texture of these soils, and higher sand content (45 - 60 %), the leaching of K might have occurred leading to the low nutrient reserve status. Similar results have been reported by Orimoloye, (2016) for flood plain soils of Nigeria. Also, because of the very same reasons of low CEC due to low activity clays, the nutrient holding capacity reduce and nutrients leach down the profile.These soils are suitable for wide range of vegetable and pulse crops. But nutrient leaching has to be controlled by band placement of K and P nutrients. N, P and K should be applied in split to minimize these losses. These soils are also highly acidic in reaction (4.98 to 5.23) which might be due to the same reason of heavy rainfall in the area. Hence, regular liming is necessary for obtaining higher productivity.

The soils of upland (P6 and P7) were categorized into LCae FCC class (Table 5). The lighter textured soils (loamy) in the surface and heavier textured soils (clayey) in the subsurface indicate susceptibility to top soil erosion (Vasu *et al*., 2016). Low base saturation and low buffering potential are the major constraints of these soils. Due to slope gradient of 3-8 % in combination with the prevalent heavy rainfall, the water erosion of top soil is a major risk of these soils. Also, low buffering potential and low base saturation can potentially cause nutrient deficiencies to all the crops. These soils are suitable for tea and rubber. Tea cultivation can provide sufficient cover to prevent erosion but cultivation of rubber and arecanut should be taken along with covers crops as intercrops. Low buffering capacity of soils can be managed with regular incorporation of organic matters in the top soil to increase the CEC. If rubber cultivation is to be taken, organic matter incorporation in the first 4-5 years in necessary. Afterwards, the leaf litters from rubber can be incorporated or left on the soil surface. Integrated nutrient management including micronutrients is very much essential.

The soils of inselberg (P8) were also categorized into LCae FCC class (Table5). Hence, the risks and limitations remain same as that of P6 and P7. However, the inselbergs are mostly covered by mixed forest and not cultivated. A very few inselbergs are cultivated with arecanut and rubber. For these cultivated soils, management practices recommended for P6 and P7 can be followed.

All the soils under study showed highly acidic soil reaction which might be due to the leaching of bases down the profile due to heavy rainfall. Similar results have been reported by Reza *et al*., (2018) for soils of upper Brahmaputra valley. The common constraints in each soil under study were Al toxicity and high leaching potential or low buffering capacity. The Al toxicity could be attributed to the low base saturation. The low base saturation and low buffering capacity of soils might be due to the low CEC of the soils, which is due to presenceof low activity clays in the Brahmaputra valley (Bandyopadhyay *et al.,* 2017). The low nutrient reserves in the soils of older alluvial plains might have aroused due tothe soluble nature of K nutrient which is easily leached by the excess soil moisture in the lowlands (Ojanuga, 2006). Other reasons might be the geology of the alluvial plains of the study area, which is derived by quaternary alluvial sediments (Dasgupta and Biswas, 2000) which are inherently low in Kreserves.

Conclusion

The soils of the lower Brahmaputra valley were grouped by similar set of characteristics that put them into single class in terms of fertility management. The major FCC classes found were La^{-}eg, Lg^{+}a^{-}e, Car^{+}e, Ca^{-}gke and LCae. High soil

acidity, Al toxicity, high leaching potential and low CEC are the major problems of the soils of the block. Apart from these constraints, moisture saturation is the common problem in active flood plain, younger alluvial plains and paddy growing soils of older alluvial plains. This moisture saturation condition can be made beneficial by taking proper soil management practices of regular liming and proper nutrient management strategies. The problem of top soil erosion risk can be managed by cover crops and surface residue management with organic matter application. Our study revealed that converting the pedological parameter data into FCC for the lower Brahmaputra valley could successfully bring out the fertility constraints of soils and has provided insight into proper management of these soils for sustainable crop productions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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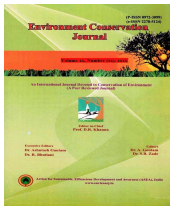

Assessing the genetic diversity for yield traits in rice (*Oryza sativa* L.) genotypes using multivariate analysis under controlled and water stress conditions

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ABSTRACT

The genetic diversity of yield and yield attributing characteristics was explored in this research. In the topical study, fifty-two rice genotypes including four checks were used under three environmental conditions i.e. irrigated (IR), rainfed (RF) and terminal stage drought (TSD) conditions. The prevalence of genetic divergence was evaluated using clustering and Principal component analysis (PCA) was used to determine the relative contribution of various traits. To fulfill the aim of the study, fifty-two genotypes were grouped into three distinct and non-overlapping clusters among these 3 clusters, cluster-I was the largest with the highest number of genotypes i.e. 47, 49 and 49 under IR, RF and TSD conditions, respectively. The highest average intra-cluster distance was observed in cluster-I, also the genotypes showed high variability under all three conditions. The highest inter-cluster distance between the cluster-II and cluster-III (IR and TSD) and cluster-I and cluster-II (RF) was observed, indicated that genotypes from the group should be considered for direct use as parents in hybridization programme to produce high yield. Only five of the 13 principal components (PCs) have been considered in the study based on the Eigen values and variability criteria. From the complex matrix it was revealed that the first-PC accounted for the highest variability. Genotypes which fall under a common PC were observed to be the most important factor for grain yield.

Introduction

Rice (*Oryza sativa* L.) is a major staple crop that feeds more than half of the world's population (Ricepedia, 2020; USDA, 2020). Rice genotypes from Chhattisgarh are critical for preserving and maintaining rice biodiversity. Rice germplasm is a valuable resource that must be protected. In order to produce superior hybrid and desirable transgressive segregants, genetic diversity plays a critical role in selecting suitable parents for the hybridization programme (Burman *et al.,* 2019). Cluster analysis is a numerical approach used for measuring genetic divergence in the germplasm lines. Although yield is a complex trait that is influenced by a variety of factors and the environment, principal component analysis was used to discover and minimize the number of traits for effective selection (Gaur *et al.,* 2019). Because it is a simple, non-parametric method for extracting crucial data from confusing data sets, PCA has become a standard tool in modern data analysis. It is a mathematical process that converts a set of (potentially) correlated variables into a set of

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(smaller) uncorrelated variables known as principal components. It decreases the data's dimensionality while preserving the majority of data set variation. The first principal component accounts for as much variability as possible. Multivariate analysis (PCA) has been widely used in the selection of diverse parents in any hybridization programme. The main advantage of PCA is that it quantifies the value of each dimension in characterizing the variability of a data set (Raj *et al.,* 2020). The current study was conducted to assess genetic divergence and PCA in 52 rice germplasm lines to discover yield related characteristics whose selection would result in an increase in rice grain yield.

Material and Methods

The current research was conducted at the Research Farm, Department of Genetics and Plant Breeding, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during *kharif* 2018 and *kharif* 2019 using Randomized Block Design (RBD) with two replications. The experimental material consists of 52 germplasm lines which were grown in two rows with row to row and plant to plant spacing of 20 × 20 cm maintained under three environmental conditions (IR, RF and TSD). In IR and TSD conditions, seeds were sown in nurseries and seedlings were transplanted as a single plant after twenty-one days and under RF condition, seeds were sown directly in both the seasons. The collected data were pooled over the season and the data recorded for thirteen yield traits were days to 50% flowering, plant height in cm, flag leaf length in cm, flag leaf width in cm, number of tillers per m^2, panicle length in cm, biological yield per plot in g, grain yield per plot in g, harvest index in per cent, thousand grain weight in g, number of filled grains per panicle, number of unfilled grains per panicle and total number of grains per panicle. Cluster analyses for the above characters were done by following Agglomerative hierarchical clustering (AHC) using XLSTAT. The Hierarchical clustering method's structure is represented by a dendrogram. For the traits, intra and inter cluster distances, as well as mean cluster performance were calculated (Sudeepthi *et al.,* 2020). Similarly, Multivariate Analysis (PCA) was performed by following Pearson correlation type (Kumari *et al.,* 2019) using XLSTAT. For the traits, Eigen values, factor loading and principal component scores were calculated.

Results and Discussion

Cluster analysis

Cluster analysis divides the fifty-two rice genotypes into 3 clusters under three conditions (Table 1) and dendrogram showed in Figure 1. Cluster-I with 47 genotypes was the biggest cluster followed by cluster-II with 2 genotypes, while cluster-III was mono-genotypic under irrigated condition. Cluster-I with 49 genotypes had the most genotypes under rainfed and TSD conditions, followed by cluster-II, which had 2 genotypes and cluster-III was mono-genotypic. As per the topical study the intra and inter cluster under irrigated, rainfed and TSD conditions are shown in Table 2. The highest intra-cluster distance under all three conditions were found in cluster-I (IR-14.41), (RF-8.23), (TSD-8.18), and crossing between the genotypes of cluster-I produces better segregants with greater genetic diversity and genetic advance. The highest inter-cluster distance between the clusters-II and cluster-III under irrigated (30.56) and TSD (12.46) conditions and between the cluster-I and cluster-II under rainfed (13.38) condition, followed by cluster-I and cluster-II (26.96), cluster-I and cluster-III (15.87) under irrigated condition; cluster-II and cluster-III (10.87), cluster-I and cluster-III (9.91) under rainfed condition; cluster-I and cluster-III (10.53), cluster-I and cluster-II (8.21) under TSD condition, revealed greater diversity among these clusters and may be used in hybridization for the development of germplasm lines. Based on mean performance of three clusters (Table 3), the traits which showed high mean values were biological yield per plot, grain yield per plot, number of tillers per m^2 and total number of grains per panicle in all conditions. The highest percent contribution (Table 4) were showed by the traits, harvest index under irrigated (16.104) and rainfed (13.598) conditions and thousand grain weight under TSD (11.800) condition. Kali Mai was the only genotype commonly observed in all three clusters. The genotypes falling in the same cluster (intra-cluster) are more closely related and less divergent than those which are placed in different clusters (inter-cluster).

Table 1: Pooled clustering pattern of fifty two rice genotypes in different water regimes during *Kharif* 2018 and 2019.

Cluster No.	No. of genotypes	Name of genotypes	No. of genotypes	Name of genotypes	No. of genotypes	Name of genotypes
Irrigated (Pooled)			**Rainfed (Pooled)**		**Terminal Stage Drought (Pooled)**	
I	47	Bega hudi, Aajan, Banko, Barangi, Khurabal, Peelee Luchai, Nagbel, Bangoli-5, Byalo, Duggi, Saja chhilau, Surmatia, Basa bhog, Dhusari, Gandhak, Cross 116, IR 62266, Laloo-14, Aganni, Safri 17, Tarunbhog, Chepti Gurmatia (3011), Basmati 370, Kalanamak, Moroberekan, Pakshi Raj, Dokra Dokri, Parmal, Tedesi, Bisni, Dhaniya Phool, Tulsi Manjar, Sarai Phool, Bharma Tripal, Dudh Malai, Shonth, Chhind Guchchhi, Naykain Jhaba, Ramali Chonch, Roti, Hathi Panjara, Nangodar, Soth, Bajarang Bali, Kurso bhog, Swarna, Maheshwari	49	Bega hudi, Aajan, Banko, Barangi, Khurabal, Peelee Luchai, Nagbel, Bangoli-5, Byalo, Duggi, Saja chhilau, Surmatia, Basa bhog, Dhusari, Gandhak, Cross 116, IR 62266, Laloo-14, Aganni, Safri 17, Tarunbhog, Chepti Gurmatia (3011), Kalanamak, Moroberekan, Nagina-22, Pakshi Raj, Dokra Dokri, Parmal, Tedesi, Bisni, Dhaniya Phool, Tulsi Manjar, Sarai Phool, Kharani, Bharma Tripal, Dudh Malai, Shonth, Chhind Guchchhi, Naykain Jhaba, Ramali Chonch, Roti, Hathi Panjara, Nangodar, Shoth, Bajarang Bali, Kurso bhog, Maheshwari, Mahamaya, MTU 1010	49	Bega hudi, Aajan, Banko, Barangi, Khurabal, Peelee Luchai, Nagbel, Bangoli-5, Byalo, Duggi, Saja chhilau,Surmatia, Basa bhog, Dhusari, Gandhak, Cross 116, IR 62266, Laloo-14, Aganni, Safri 17, Tarunbhog, Chepti Gurmatia (3011), Basmati 370, Kalanamak, Moroberekan, Nagina-22, Pakshi Raj, Dokra Dokri, Parmal, Tedesi, Tulsi Manjar, Sarai Phool, Kharani, Bharma Tripal, Dudh Malai, Shonth, Chhind Guchchhi, Naykain Jhaba, Ramali Chonch, Roti, Hathi Panjara, Nangodar, Soth, Bajarang Bali, Kurso bhog, Swarna, Maheshwari, Mahamaya, MTU 1010
II	4	Nagina-22, Kharani, Mahamaya, MTU 1010	2	Basmati 370, Swarna	2	Bisni, Dhaniya Phool
III	1	Kali Mai	1	Kali Mai	1	Kali Mai

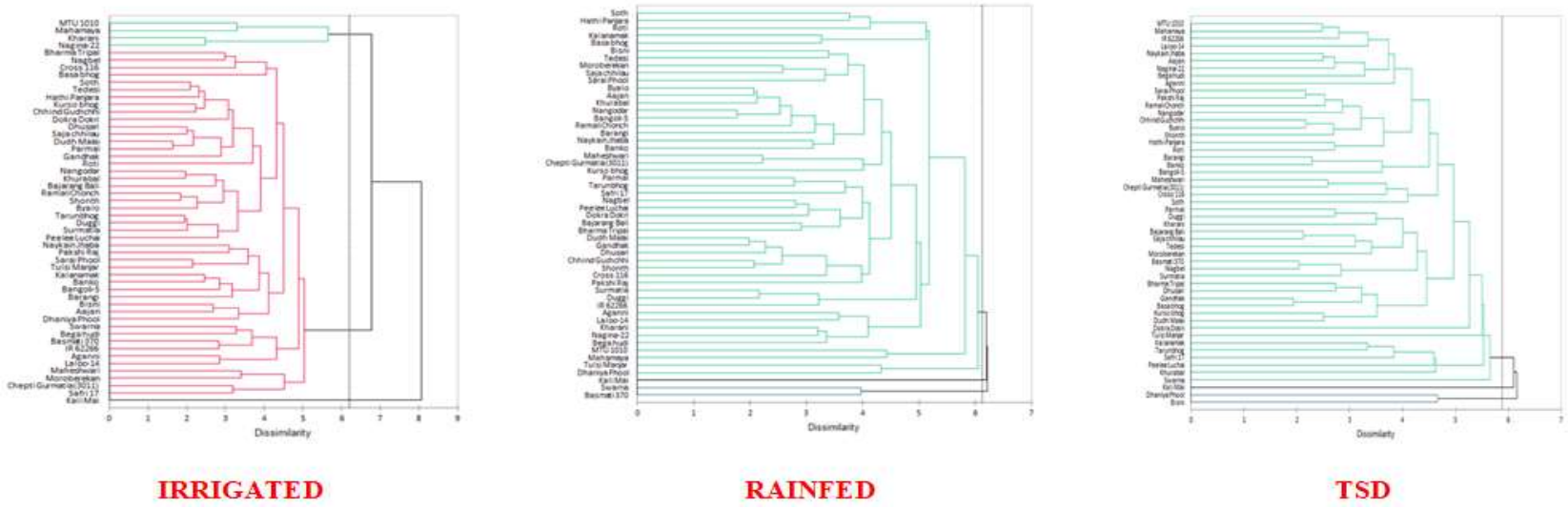


Figure 1: Dendrogram of fifty-two rice genotypes in different conditions.

Table 2: Average intra (diagonal and bold) and inter cluster distance for irrigated, rainfed and terminal stage drought

Cluster	Irrigated			Rainfed			TSD		
	I	II	III	I	II	III	I	II	III
I	**14.41**	26.96	15.87	**8.23**	13.38	9.91	**8.18**	8.21	10.53
II		**13.25**	30.56		**5.35**	10.87		**7.62**	12.46
III			**0.00**			**0.00**			**0.00**

Table 3: Cluster mean value for different traits under different conditions.

	Class	DTF	PH	FLL	FLW	NT	PL	BY	GY	HI	TGW	NFG	NUFG	TNG
Irrigated	**I**	108.388	133.421	32.352	1.528	185.213	25.808	1691.862	279.351	17.071	28.502	98.156	29.537	127.693
	II	91.938	101.738	28.986	1.375	217.188	20.929	876.870	313.800	39.121	27.878	86.705	30.963	117.668
	III	118.000	128.450	36.885	1.450	217.500	27.668	1890.800	361.600	19.238	38.971	143.810	72.710	216.520
Rainfed	**I**	97.776	103.423	30.222	1.340	122.742	24.435	373.192	69.648	19.221	22.434	82.444	36.851	119.275
	II	104.125	80.550	27.995	1.285	146.250	23.048	206.100	74.264	37.205	19.576	75.020	41.803	116.823
	III	109.000	91.150	29.800	1.175	103.750	25.550	322.900	75.300	23.347	37.250	110.380	45.050	155.430
TSD	**I**	92.939	109.473	32.428	1.412	174.834	23.741	542.895	110.371	20.828	27.727	85.212	24.757	109.969
	II	80.625	130.100	29.768	1.178	181.875	26.148	434.325	124.215	30.013	19.926	79.962	32.645	112.606
	III	103.000	110.850	33.555	1.345	108.750	27.000	617.400	102.000	18.016	27.813	91.647	11.680	103.327

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m^2, PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW=thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TNF= total number of grains per panicle

Table 4: Percent contribution of each character under various conditions

Traits	% contribution of each character		
	IRRIGATED	RAINFED	TSD
DTF	3.771	3.530	4.192
PH	6.353	5.837	6.292
FLL	5.715	6.231	7.054
FLW	5.945	3.126	6.519
NT	5.118	8.958	6.128
PL	5.087	4.440	3.554
BY	8.364	8.789	6.356
GY	5.620	9.902	9.803
HI	16.104	13.598	11.733
TGW	6.730	9.298	11.800
NFG	9.749	10.531	9.238
NUFG	12.187	8.145	10.107
TNG	9.256	7.615	7.225

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m^2, PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW=thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TNF= total number of grains per panicle

Table 5: Eigen values of yield and yield related traits of 52 rice germplasm accessions under different conditions

		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
IR	Eigenvalue	3.877	2.924	1.171	1.088	0.911	0.714	0.651	0.546	0.412	0.380	0.271	0.057
	Variability (%)	29.820	22.489	9.008	8.366	7.007	5.489	5.009	4.197	3.171	2.920	2.087	0.438
	Cumulative %	29.820	52.309	61.317	69.683	76.690	82.178	87.187	91.384	94.555	97.475	99.562	100.000
RF	Eigenvalue	2.506	2.244	1.588	1.386	1.145	0.986	0.878	0.814	0.601	0.558	0.264	0.031
	Variability (%)	19.281	17.258	12.214	10.662	8.806	7.583	6.751	6.261	4.622	4.295	2.031	0.235
	Cumulative %	19.281	36.539	48.753	59.415	68.221	75.804	82.555	88.816	93.439	97.734	99.765	100.000
TSD	Eigenvalue	2.607	2.101	1.709	1.595	1.158	1.004	0.840	0.633	0.491	0.432	0.417	0.014
	Variability (%)	20.056	16.165	13.144	12.268	8.905	7.720	6.459	4.866	3.780	3.323	3.204	0.109
	Cumulative %	20.056	36.221	49.365	61.633	70.539	78.259	84.717	89.583	93.364	96.687	99.891	100.000

Table 6: Factor loading (Eigen vectors) of 52 rice germplasm accessions for yield traits under different conditions

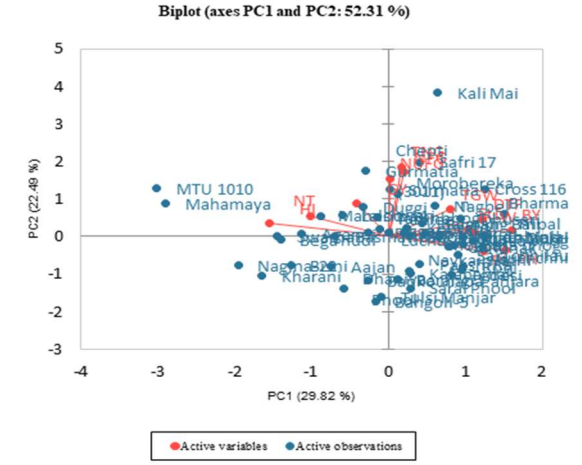
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
	Irrigated					**Rainfed**					**TSD**				
DTF	**0.634**	0.233	-0.078	-0.002	-0.468	**0.653**	0.158	-0.150	0.243	0.282	**0.501**	-0.189	-0.469	0.336	0.177
PH	**0.788**	-0.186	-0.077	0.071	0.102	-0.271	**0.549**	-0.361	-0.128	0.404	-0.125	-0.002	**0.638**	0.314	0.323
FLL	0.532	0.110	**0.595**	0.092	-0.180	0.205	**0.475**	-0.188	0.371	-0.385	0.202	-0.394	0.292	0.063	**0.530**
FLW	**0.575**	0.056	-0.153	0.368	0.482	-0.027	0.396	-0.219	**0.642**	-0.127	0.028	-0.500	0.271	**0.593**	-0.090
NT	**-0.526**	0.278	-0.351	0.078	-0.452	0.157	-0.068	-0.089	**-0.442**	-0.033	-0.087	0.227	**0.523**	-0.429	-0.301
PL	**0.636**	-0.198	-0.112	0.401	-0.367	0.249	0.587	-0.280	-0.119	**0.590**	-0.225	-0.117	-0.127	-0.183	**0.678**
BY	**0.825**	0.090	0.183	-0.286	-0.039	-0.508	0.011	**-0.629**	-0.316	-0.174	0.396	**-0.644**	-0.075	0.012	-0.209
GY	-0.212	0.449	**0.681**	0.345	-0.005	0.264	**0.627**	0.188	-0.449	-0.178	**0.664**	0.491	-0.092	0.445	-0.061
HI	**-0.800**	0.176	0.106	0.477	0.022	0.570	0.401	**0.671**	-0.168	-0.042	0.346	**0.818**	-0.023	0.374	0.107
TGW	0.417	0.369	-0.341	**0.491**	0.129	0.085	-0.175	0.124	**0.453**	0.241	-0.286	-0.287	0.340	**0.536**	-0.318
NFG	0.108	**0.881**	-0.035	-0.254	0.173	**0.791**	-0.246	-0.450	-0.086	-0.206	**0.853**	-0.209	0.182	-0.308	-0.042
NUFG	0.010	**0.796**	-0.123	0.060	-0.144	0.039	**-0.593**	0.134	-0.021	0.471	-0.002	0.369	**0.581**	0.021	0.147
TNG	0.090	**0.957**	-0.067	-0.185	0.094	**0.768**	-0.433	-0.385	-0.090	-0.044	**0.844**	-0.090	0.363	-0.298	0.005

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m^2, PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW=thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TNF= total number of grains per panicle

Table 7: Principal component score of rice genotypes under irrigated, rainfed and TSD conditions

Accessions	Score														
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
	IRRGATED					**RAINFED**					**TSD**				
Bega hudi	-2.766	-0.138	0.021	-1.135	-1.501	-1.242	0.448	**1.119**	0.398	-1.603	-0.031	-2.095	-0.309	-1.262	-1.695
Aajan	-1.475	-1.374	**1.305**	-1.770	0.734	-1.383	-0.625	0.431	-0.125	0.303	-0.930	-1.021	-0.140	-0.588	0.108
Banko	-0.526	-2.000	0.272	**1.221**	-1.274	-1.674	0.241	-0.256	**0.922**	0.562	-2.134	-1.669	**1.318**	-1.122	**1.117**
Barangi	0.233	-1.948	**1.292**	**2.254**	0.233	-1.216	-0.396	-1.055	**1.240**	**1.613**	-2.073	-2.352	**1.690**	-2.071	**2.484**
Khurabal	0.478	0.376	-0.364	-1.077	0.556	-0.086	-0.447	0.116	0.820	0.370	**3.598**	-1.654	**2.183**	-0.345	-0.778
Peelee Luchai	-0.217	0.333	0.774	-0.672	-0.354	0.706	**1.837**	-1.062	-0.159	-1.139	**2.085**	-0.963	1.165	-0.639	0.405
Nagbel	1.190	**1.397**	**1.176**	-0.190	0.535	**2.179**	1.174	-0.217	0.484	-1.742	0.865	**1.207**	-0.398	0.107	0.408
Bangoli-5	-0.314	-2.955	0.771	0.713	0.490	-1.687	0.077	-0.707	-0.103	0.068	-1.118	-2.025	0.459	0.220	0.530

Byalo	0.004	0.162	-0.226	-0.633	0.614	-1.417	-0.590	-0.440	0.348	0.165	-2.737	0.560	0.155	0.462	0.812
Duggi	-0.638	**1.343**	0.524	-0.929	-0.414	1.017	-2.027	-0.411	0.210	-0.290	0.896	0.515	-0.916	**1.348**	-1.576
Saja chhilau	1.792	-0.848	0.757	0.814	-0.392	0.021	**1.949**	-1.553	-0.360	-0.610	**1.625**	0.961	**1.419**	1.173	-0.048
Surmatia	0.033	**2.131**	0.509	-0.888	0.144	1.484	-2.445	0.985	0.640	-0.951	0.906	**3.225**	0.104	0.265	-0.053
Basa bhog	**1.917**	-0.346	**2.824**	0.031	-1.235	-0.870	**2.753**	0.329	-0.220	-1.547	**2.257**	-0.967	-1.274	0.411	1.023
Dhusari	**1.856**	0.821	0.440	0.350	-0.160	**1.567**	-1.209	-1.819	-0.635	-0.679	1.138	0.148	-2.765	0.643	**1.328**
Gandhak	1.557	-0.460	**1.756**	0.244	-0.301	1.546	0.049	-1.179	0.485	-0.405	**2.802**	-1.419	-1.692	0.417	0.086
Cross 116	**2.456**	**2.158**	**1.898**	0.101	**1.495**	1.046	-1.512	-2.679	0.095	0.133	0.536	-2.565	**1.636**	-0.611	0.543
IR 62266	-0.525	0.182	0.981	0.087	-0.874	0.070	-3.237	**1.264**	0.549	-0.231	-0.904	-1.120	-1.597	-1.257	-0.710
Laloo-14	-1.187	0.956	-0.770	-1.215	**1.579**	-0.744	-1.500	**1.141**	**1.056**	0.126	-1.787	0.437	-1.864	-1.114	-0.922
Aganni	-2.217	0.124	-0.032	-1.255	0.128	-0.824	-2.363	-0.184	-1.384	-1.158	-1.598	**1.092**	**1.299**	-2.369	-0.728
Safri 17	0.799	**3.387**	-0.344	-0.502	-0.917	**2.128**	-1.286	-0.198	-1.682	**0.639**	**2.448**	0.718	0.118	-1.035	-0.366
Tarunbhog	-0.305	0.845	**1.202**	-1.836	-0.506	**3.029**	0.764	0.292	-1.674	-0.012	**2.819**	0.503	**1.322**	-1.748	-0.184
Chepti Gurmatia (3011)	-0.584	**2.981**	-1.161	-0.649	**1.274**	0.006	-0.234	0.606	-1.154	-0.369	0.795	-1.554	0.258	-0.366	-1.690
Basmati 370	-1.371	0.005	0.114	-1.240	-1.373	**2.374**	-0.125	**3.519**	-1.280	-0.237	**1.536**	**1.714**	-1.627	-0.580	0.370
Kalanamak	0.564	-1.692	**1.081**	0.579	-0.680	-1.787	1.325	0.647	0.579	-1.318	**2.513**	0.808	1.020	-1.925	**1.403**
Moroberekan	0.241	**1.956**	0.450	**1.422**	**2.053**	0.009	0.896	-0.816	-0.560	0.385	**2.136**	0.650	0.796	**2.497**	-1.025
Nagina-22	-3.853	-1.331	0.353	**1.598**	**1.585**	-3.063	-0.268	**1.314**	-0.012	-2.660	-0.737	0.172	1.120	-0.975	-1.036
Pakshi Raj	0.794	-1.255	-0.691	-0.232	0.522	-0.767	-0.015	-2.264	-1.049	-1.922	-1.141	-0.561	0.914	0.283	-0.189
Dokra Dokri	**2.480**	-0.029	-1.553	**1.322**	-0.506	**2.013**	0.405	0.517	0.845	-0.802	-1.478	0.814	0.814	**1.999**	**1.828**
Parmal	1.279	-0.034	-0.752	0.378	-0.842	1.543	**2.090**	0.049	-1.707	0.119	-0.260	**1.098**	-0.105	**1.559**	0.145
Tedesi	1.610	-1.743	-1.953	0.681	0.559	-0.424	**3.120**	0.218	-1.021	**1.273**	1.061	0.689	1.061	**1.940**	-1.594
Bisni	-2.480	-1.333	**1.421**	-0.302	-0.051	-0.771	1.003	-0.661	-1.290	**1.195**	0.014	**4.482**	0.960	-1.082	**1.264**
Dhaniya Phool	-1.132	-2.382	-0.242	-0.591	-0.404	-1.945	**1.755**	0.517	-2.997	**2.558**	-1.088	**2.474**	**1.780**	-0.337	**2.400**
Tulsi Manjar	-0.183	-2.768	-0.730	-1.445	-1.440	-0.746	1.121	**1.607**	-2.769	0.496	-0.117	0.519	-2.168	-1.467	0.054
Sarai Phool	0.573	-2.362	-0.576	-1.177	-0.775	-2.048	**1.914**	-1.636	0.354	-0.254	-1.655	-0.487	0.376	0.268	-0.523
Kharani	-3.243	-1.785	0.771	0.608	0.703	-3.489	-0.697	**1.134**	0.137	-0.403	-1.058	**1.925**	0.812	0.556	-1.512
Bharma Tripal	**2.976**	1.046	**1.080**	0.565	-0.720	**1.875**	0.867	-0.396	**1.193**	0.408	1.183	0.530	-1.401	**1.620**	**1.600**
Dudh Malai	**1.912**	-0.006	0.175	0.054	-0.838	1.505	0.127	-1.522	0.259	0.225	1.374	-0.747	-0.810	-0.649	**1.321**
Shonth	0.526	0.027	0.614	-0.097	**0.947**	0.993	-0.505	-2.261	0.076	0.462	-2.488	**1.896**	-0.162	**1.225**	-0.448
Chhind Guchchhi	**2.394**	-0.453	-1.100	-0.058	0.736	1.462	-0.402	-1.076	0.207	0.565	-2.261	0.435	0.162	0.584	0.673
Naykain Jhaba	0.547	-1.558	0.011	-1.043	**1.457**	-1.797	-0.968	0.460	**2.083**	-0.073	0.007	-0.129	0.808	0.026	-0.816
Ramali Chonch	0.931	0.101	-0.172	-0.723	**1.091**	0.120	0.047	-0.795	**1.008**	0.268	-2.088	-1.491	**1.503**	0.608	-0.339
Roti	**1.903**	-1.379	-1.714	**1.600**	-0.446	-0.760	1.146	0.215	**2.056**	**3.435**	-1.812	-0.793	-0.507	**3.186**	-0.012
Hathi Panjara	**1.878**	-1.538	-0.746	**0.977**	-0.128	0.057	**1.467**	**1.133**	**3.260**	**0.638**	-1.668	-0.923	-0.848	**1.346**	0.045
Nangodar	0.388	0.167	-0.622	-1.184	**1.380**	-1.380	-0.088	-0.222	**1.042**	0.048	-1.580	-0.157	-0.709	0.954	-1.235
Soth	1.674	-0.412	-0.942	**1.014**	0.629	-0.237	**2.214**	**1.638**	**1.067**	0.401	0.802	-2.000	**2.102**	**2.232**	-0.623
Bajarang Bali	0.852	0.608	-1.815	-0.813	-0.723	**2.111**	**2.102**	0.384	0.621	-1.364	1.060	0.104	0.744	0.038	-0.539
Kurso bhog	**2.189**	-0.178	-1.958	0.289	0.202	0.792	-1.201	0.158	0.643	**0.913**	1.108	0.314	-0.697	0.524	1.066
Kali Mai	1.268	**6.564**	0.004	**1.157**	-1.616	**3.131**	-1.802	0.394	0.595	**1.550**	0.334	-2.601	-3.262	0.703	**1.836**
Swarna	-2.848	0.001	-2.308	-0.381	-1.867	**1.968**	-0.534	**3.872**	0.116	0.092	-0.272	0.674	-2.756	-1.561	-1.365
Maheshwari	-1.749	0.941	-1.199	0.281	**1.346**	-0.674	-0.414	-0.429	-0.067	-0.439	-0.019	-0.752	-0.228	-0.883	-0.470
Mahamaya	-5.725	**1.486**	0.069	**3.118**	-0.655	-1.650	-2.639	0.901	-1.955	**1.071**	-0.781	**1.280**	-0.739	-1.629	-1.366
MTU 1010	-5.956	**2.207**	-0.678	0.576	0.002	-2.071	-3.361	-1.122	-1.182	0.127	-2.083	0.100	-1.124	-1.579	-1.007



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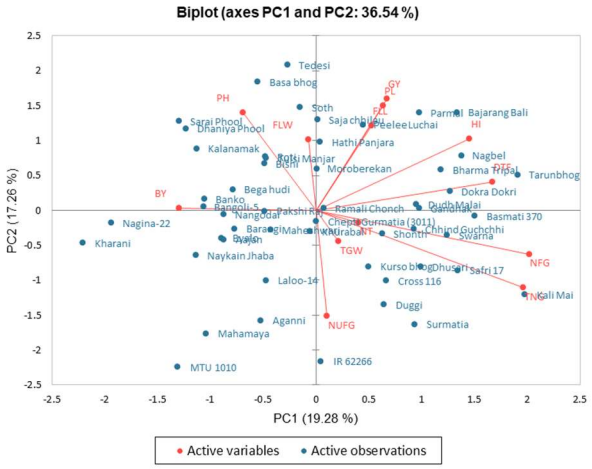
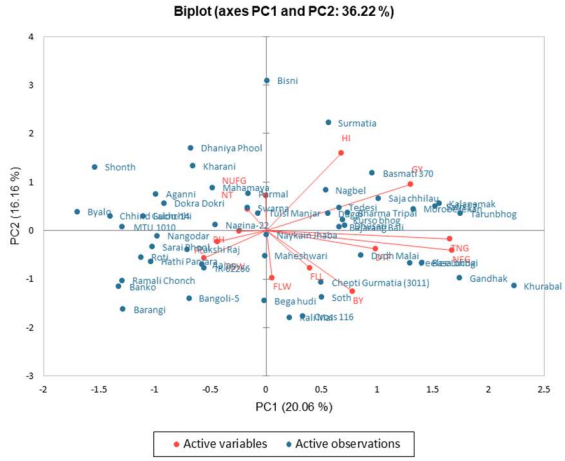


RAINFED



TSD

Figure 2: Biplot graph representing the active variables and observations taking PC_1 and PC_2 under different conditions

The greater the distance between two clusters, greater is the divergence (Singh and Narayanan, 2013). If crossing takes place among genotypes between clusters, they produce more diverse and better progenies when compared to the crossing of genotypes within the same cluster. Those genotypes can be used as parents in future crossing programmes. The results were found in agreement with Amegan *et al.,* 2020; according to Bekis *et al.,* 2021 the highest inter-cluster distance was recorded between cluster II & III. The results depicted that cross-genotype from cluster II & III, cluster I & III to get genotypes of rice with high grain yield and early maturing genotypes; Burman *et al.,* 2019; Iqbal *et al.,* 2018 and Shrestha *et al.,* 2021 revealed that cluster II & cluster IV showed the highest distance between cluster centroids. The genotypes in cluster II would be grown for higher grain yield. Genotypes in clusters of different conditions with high cluster mean value may be directly used for adaptation, or intercrossing may be recommended to produce the wide spectrum of variability, followed by effective selection for those traits according to the results.

Principal Component Analysis (PCA)

PCA was used in the topical study to analyze thirteen yield and yield-related parameters in 52 rice germplasm accessions (Table 5). Biplot graph representing the active variables and observations taking PC_1 and PC_2 under different conditions presented in Figure 2. The PC with Eigen value >1 that described at least 5% of the fluctuations in the data was evaluated in the current investigation, according to the criteria provided by Brejda *et al*. (2000) and Dhakal *et al*. (2020). The PC with the highest Eigen values and variables with the highest factor loading was deemed to be the most representational of system characteristics. Only five of the 13 principal components (PCs) had an Eigen value greater or nearer to 1. As a result, these five PCs were given due consideration for additional explanation. For the variables under research, the PC-1, PC-2, PC-3, PC-4, and PC-5 genotypes showed 29.82%, 22.489%, 9.008%, 8.366%, and 7.007% variability, in irrigated; variability 19.281%, 17.258%, 12.214%, 10.662% and 8.806% in rainfed; variability 20.056%,

16.165%, 13.144%, 12.268% and 8.905% in TSD conditions respectively. Each subsequent component accounts for as much of the remaining variability in the data as possible, with the first and second PCs accounting for as much as possible in all three conditions.

The factor loading for thirteen yield-related traits showed in Table 6. Only the most highly loaded factors were retained for further analysis within each PC. From the complex matrix it was revealed that the PC-1 (first PC) accounted for the highest variability (29.82%) was mainly related to traits like biological yield per plot and plant height in irrigated condition; variability in rainfed (19.281%) and TSD (20.056%) was mainly related to traits like total number of grains per panicle and number of filled grains per panicle in both rainfed and TSD conditions. Table 7 showed the top ten (bold values).

Principal Component scores for all genotypes, split down into five principal components. These scores can be used to develop exact selection indices, the intensity of which is determined by the variability described by each principal component. A high PC score for a given accession in a certain component indicates that the variables in that genotype have high values. It was revealed in the results that Bharma tripal (2.976), Kali Mai (3.131) and Khurabal (3.598) had the best PC score in PC-1; Kali Mai (6.564), Tedesi (3.120) and Bisni (4.482) in PC-2; Basa bhog (2.824), Swarna (13.872) and Khurabal (2.183) in PC-3; Mahamaya (3.118), Hathi panjara (3.260) and Roti (3.186) in PC-4, and Moroberekan (2.053), Roti (3.435) and Barangi (2.484) in PC-5 under irrigated, rainfed and TSD conditions respectively.

The results were found in agreement with Raj *et al.,* 2020 and Burman *et al.,* 2021 revealed that first PC showed the most variability among the five principal components, all of the principal components contributed positively to yield and its contributing traits. As a result, Tarunbhog, Safri 17 and Basmati 370 are the common genotypes with high PC1 scores and highly correlated with yield component traits under RF and TSD conditions. Hence, selecting these genotypes would result in higher yield and yield related traits under drought condition.

The PCA emphasizes the features with the maximum variability. As a result, intensive selection processes can be developed to improve yield and yield-related traits rapidly. PCA can also be used to rank genotypes based on PC scores in the corresponding component. The results showed that the selected accessions might be utilized as donors in a varietal development programme to improve yield attributes.

Conclusion

Both multivariate statistical analysis tools showed the existence of the wide genetic diversity among the germplasm lines in the study. In accordance with the current findings, the cluster-I have more genetic variability in specific conditions. Hence, the genotypes present in this cluster could be selected as parents in future breeding programmes. The traits biological yield per plot, grain yield per plot, number of tillers per m^2 and total number of grains per panicle revealed that, they play a crucial role in genetic divergence among fifty-two rice genotypes and we would select these traits of rice lines for the diversity purpose. PCA revealed that, PC1 was dominated by the yield and yield contributing traits such as biological yield per plot and plant height under IR condition and the traits total number of grains per panicle and number of filled grains per panicle under both RF and TSD conditions. So, selecting the germplasm lines with a high score in PC1 could result in greater yield and yield related characters.

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Conflict of interest

The authors declare that they have no conflict of interest.

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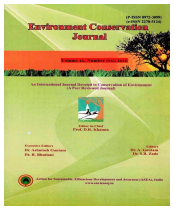

Effect of preceding rice herbicide residue towards control of weeds and urdbean productivity in rice -Bhendi-urdbean sequence under high rainfall area

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ABSTRACT

Field experiment was conducted at Field Crops Research unit, ICAR- Central Island Research Institute, Bloomsdale, Port Blair during summer season, 2018. To study the preceding rice herbicide residual effect in control of weeds, growth and yield of urdbean in rice-bhendi-urdbean sequence under high rainfall area. At 20 DAS, grassy weeds (48.00 weeds/m^2) were predominant species of weeds followed by sedges and broad leaf weeds of 29.67 and 22.67 nos./m^2 respectively. Among the irrespective of weed control treatments, at 20 DAS, residual effect of 1.25 kg/ha butachlor at 3 DAP + manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in both bhendi and urdbean recorded significantly lower grass, sedges, broad leaf weeds population and weed biomass of 11.67, 8.67, 6.67 weeds/m^2 and 2.93, 2.02, 1.84 g/m^2 respectively. Residual effect of 1.25 kg/ha butachlor applied at 3 DAP along with manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in both bhendi and urdbean obtained 299 kg/ha higher seed yield as compared to weedy control.

Introduction

Urdbean is an essential short duration food leguminous crop which is more suitable and extensively grown under intensive cropping system. Urdbean contains higher content of protein, vitamins, minerals and fibre for direct human being consumption. Integration of urdbean, in different cropping system such as rice fallow, mixed cropping, catch crops and sequential cropping in India, because of it fixes atmospheric nitrogen and also enriches soil nutrient status for succeeding crops. In India, urdbean is grown in an area 4.48 m.ha with the average productivity of 641 kg/ha (Indiastat, 2018). In Andaman and Nicobar Islands, productivity of urdbean was 49.7% lower than national average productivity. There are many reasons for reduction of urdbean productivity. Among them, weeds compete with crops for natural resources and reduced productivity of urdbean. Productivity of urdbean is reduction was noticed upto 43.2 to 90.0% owing to weed infestation during critical stages of crop growth. Hence, weeding should be done at appropriate time by using suitable weed control method is more essential to obtain higher yield of urdbean (Singh *et al.,* 2010). Manual weeding is laborious, tedious; time consuming as well as costly, even availability of manpower is also scarce during peak stage of crop growth. Hence, farmers are not shown much interest to spend more cost because of its grown in residual crop or fallow situation. Now days, scarcity of agricultural labor increasing day by day which leads to farmers for adoption of chemical weed control methods that is more effective in minimizing weed infestations for prolong period and also reduced soil weed seed bank. For this reason, farmers should be given more emphasis during selection of herbicide because, it causes more persist in soil for prolong period which affect succeeding crops in the cropping sequence. In the above facts, present investigation was conducted to

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find out the preceding rice herbicide residue in control of weeds and urdbean productivity under rice-bhendi-urdbean cropping sequence.

Material and Methods

Experimental details

Field experiment was conducted at Field crops Research unit of ICAR – Central Island Agricultural Research Institute, Bloomsdale, Port Blair during Summer season 2018. To study the preceding rice herbicide residual effect in control of weeds and yield of urdbean in rice-bhendi-urdbean sequence under high rainfall area. The experimental soil type was "Clay loam" soil with 0.38% organic carbon and pH of 6.9, EC (0.3 dS/m) besides, soil available nitrogen (92.0 kg/ha), phosphorus (18.3 kg/ha) and potassium (55.4 kg/ha) content.

The experiment was laid out in previous rice crop experiment without any disturbance in layout for subsequent okra and urdbean. The details of eight weed control treatments imposed to previous rice were "T_1- Oxadiargyl loading with biochar applied on 3 days after planting (DAP), T_2- Oxadiargyl loading with zeolites applied on 3 DAP, T_3- Oxadiargyl entrapped in starch applied on 3 DAP, T_4- Oxadiargyl entrapped in water-soluble polymers applied on 3 DAP, T_5- Application of 100g ha^{-1} of oxadiargyl on 3 DAP, whereas, slight modification was done in Treatment T_6 to T_8 viz., T_6- Application of 1.25 kg/ha of butachlor on 3 DAP along with manual weeding at 40 DAP+ two manual weeding on 20 and 40 days after sowing (DAS) in bhendi and urdbean, T_7- Weed free control and T_8- Weedy Control (entire crop sequence). After harvest of rice crop, bhendi seeds sown in rice stubbles. After harvest of bhendi residues were removed from the field and applied glyphosate at 1.0 kg/ha.

After 7 days of glyphosate application, urdbean variety of VBN (Bg)-8 dibbled with recommended spacing of 30cm and 10cm apart rows and lines. The crop was nourished with 25 kg of Nitrogen, 50 kg of Phosphorus and 25 kg of Potassium/ha was applied as basal though urea, Diammonium, Phosphate (DAP) and muriate of potash respectively. Agronomic practices were adopted as per standard packages. Crop growth and yield attributes registered as per standard procedure.

Calculating relative density of weeds

Relative density (RD) of predominant species of weeds registered separately in category wise recorded and calculated with standard protocol suggested by Kim and Moody (1983).

$$\text{RD}(\%) = \frac{\text{No. of weeds of individual species}}{\text{Total no. of weeds}} \times 100$$

Statistical analysis

Data on weeds showed high variation; hence they were subjected to transformation of $\sqrt{x+2}$ and analyzed statistically as described by Gomaz and Gomez (2010). Wherever, statistical significant was observed, critical difference at 0.05 level of probability was worked out for comparison. Non-significant effects are indicated as NS.

Results and Discussion

Absolute density and relative density of weed

The predominant weeds were noticed in experimental field *viz.,* four spices of grassy weeds such as *Echinochloa colonum, Leptochloa chinensis, Acrachne racemose, Setaria glauca* and sedges such as *Cyperus haspan, C. iria, C. eragrostis, F.aestivalis* and six weed spices of broad leaved weeds such as *Wedelia chinensis, Ammannia baccifera, Phyllanthus maderaspatensis, P. niruri, Boerhavia diffusa, Cleome viscosa* were observed in urdbean. The absolute density and relative density of individual weeds presented in Table 1.

At 20 DAS, grassy weeds of 48.00 weed/m^2 were predominant species of weeds followed by sedges and broad leaf weeds of 29.67 and 22.67 weeds/m^2 respectively. Among the grassy weeds *Echinochloa colonum* was predominant weed species with higher absolute density of 15.67 weeds/m^2 with relative density of 15.62%. However, at 60 DAS sedges were predominant weeds species followed by broad leaf weeds and grasses respectively. *Cyperus haspan* was dominant weeds species among sedges with 10.67 weeds/m^2 and 10.63% of absolute density and relative density respectively which was followed by *F. aestivalis*. It might be due to weeds were adapted to wide range of environmental condition and also deplete natural resources for their growth. Similar species of weeds were also reported by Bommayasamy *et al.* (2018).

Table 1: Preceding rice herbicide residue on absolute and relative density of weeds at 20 and 60 DAS in urdbean under high rainfall area

Weed species	20 DAS		60 DAS	
	AD (weeds/m^2)	RD (%)	AD (weeds/m^2)	RD (%)
Grasses				
Echinochloa colonum	15.67	15.62	41.67	14.25
Leptochloa chinensis	10.67	10.63	19.33	6.61
Acrachne racemosa	13.33	13.28	16.67	5.70
Setaria glauca	8.33	8.30	16.00	5.47
Total grasses	**48.00**	**47.84**	**93.67**	**32.04**
Sedge				
Cyperus haspan	10.67	10.63	46.67	15.96
Cyperus iria	5.33	5.31	19.33	6.61
Cyperus eragrostis	4.67	4.65	15.00	5.13
F. aestivalis	9.00	8.97	23.33	7.98
Total sedges	**29.67**	**29.57**	**104.33**	**35.69**
Broad leaved weeds (BLW)				
Wedelia chinensis	8.67	8.64	36.67	12.54
Ammannia baccifera	6.67	6.65	22.67	7.75
Phyllanthu .maderaspatensis	5.33	5.31	12.00	4.10
Phyllanthus niruri	2.00	1.99	9.33	3.19
Boerhavia diffusa	0.00	0.00	7.33	2.51
Cleome viscosa	0.00	0.00	6.33	2.17
Total BLW	**22.67**	**22.59**	**94.33**	**32.27**
Total weed density	**100.34**	**100.00**	**292.33**	**100.00**

AD- Absolute density RD- Relative density Data statistically not analysised

Table 2: Preceding rice herbicide residue on grass, sedges, BLW weed population (weeds/ im^2) and dry biomass production (g/m^2) at 20 DAS in urdbean under high rainfall area

Treatments	Weed population (weeds/m^{-2})			Weed dry biomass (g/m^2)		
	Grass	Sedges	BLW	Grass	Sedges	BLW
T_1- Oxadiargyl loading with biochar applied on 3 DAP	4.65 (19.67)	3.36 (9.33)	2.94 (6.67)	2.66 (5.06)	2.12 (2.49)	1.97 (1.87)
T_2- Oxadiargyl loading with zeolites applied on 3 DAP	5.20 (25.00)	4.20 (15.67)	3.56 (10.67)	2.81 (5.90)	2.40 (3.75)	2.28 (3.22)
T_3-Oxadiargyl entrapped in starch applied on 3 DAP	4.72 (20.33)	3.56 (10.67)	3.11 (7.67)	2.73 (5.45)	2.13 (2.52)	2.07 (2.28)
T_4-Oxadiargyl entrapped in water-soluble polymers applied on 3 DAP	5.37 (27.00)	4.27 (16.33)	4.04 (14.33)	2.86 (6.17)	2.43 (3.94)	2.55 (4.49)
T_5—Application of 100g/ha of oxadiargyl on 3 DAP	5.83 (32.00)	4.43 (17.67)	4.51 (18.33)	3.20 (8.22)	2.51 (4.31)	2.60 (4.77)
T_6-Application of 1.25 kg/ha of butachlor on 3 DAP along with manual weeding at 40 DAP+ two manual weeding on 20 and 40 DAS in bhendi and urdbean	3.69 (11.67)	3.26 (8.67)	2.94 (6.67)	2.22 (2.93)	2.00 (2.02)	1.96 (1.84)
T_7- Weed free control	1.41 (0.00)	1.41 (0.00)	1.41 (0.00)	1.41 (0.00)	1.41 (0.00)	1.41 (0.00)
T_8- Weedy control	7.05 (48.00)	5.63 (29.67)	4.96 (22.67)	4.38 (17.25)	3.77 (12.27)	3.39 (9.54)
SE.d	0.26	0.16	0.12	0.12	0.10	0.08
CD (P=0.05)	0.56	0.35	0.25	0.26	0.21	0.18

Values in parentheses are original and transformed to √x+2

Table 3: Preceding rice crop herbicide residue on leaf area index, DMP, root characters of urdbean under high rainfall area

Treatments	Growth attributes at 25 DAS			
	LAI	DMP (kg/ha)	Root length (cm)	Root dry weight (kg/ha)
T_1- Oxadiargyl loading with biochar applied on 3 DAP	1.09	288	12.43	36.0
T_2- Oxadiargyl loading with zeolites applied on 3 DAP	1.00	219	12.10	32.9
T_3- Oxadiargyl entrapped in starch applied on 3 DAP	1.05	230	12.30	33.2
T_4- Oxadiargyl entrapped in water-soluble polymers applied on 3 DAP	1.03	192	10.70	29.5
T_5—Application of 100g/ha of oxadiargyl on 3 DAP	0.94	187	9.77	27.0
T_6- Application of 1.25 kg/ha of butachlor on 3 DAP along with manual weeding at 40 DAP + two manual weeding on 20 and 40 DAS in bhendi and urdbean	1.25	334	15.10	37.6
T_7- Weed free control	1.88	425	16.83	43.0
T_8- Weedy control	0.78	184	8.43	20.0
SE.d	0.04	21.54	0.55	1.33
CD (P=0.05)	0.10	46.09	1.18	2.84

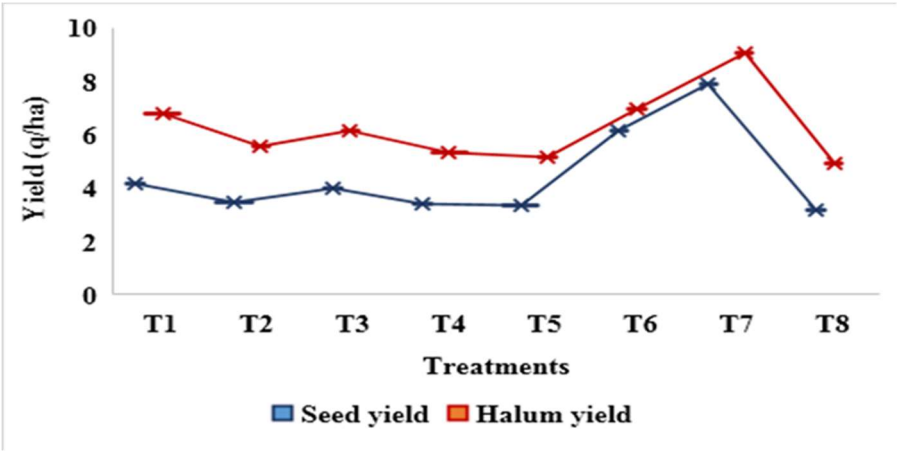

Figure 1: Preceding rice herbicide residue on seed yield and halum yield (q/ha) of urdbean.

Herbicide residual effect of weeds

Herbicide residual effect of preceding rice crops weed control treatments exerted significant influence on grasses, sedges and broad leaf weed population and dry biomass production on 20 DAS (Table 2). Lowest value of grassy, sedge and broad leaf weed population under weed free control. At 20 DAS, significantly lower grass weed density registered in residual effect of 1.2 5kg/ha butachlor applied at 3 DAP supplement with manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in bhendi and urdbean. The next best treatment was residual effects of oxadiargyl loading with biochar applied at 3 DAP which was at par with oxadiargyl entrapped with starch applied at 3 DAP. Similar trend had noticed in sedges and broad leaved weed density. Higher grass weed density was recorded in weedy control.Weed free control recorded superiority in diminution of grassy, sedge and broad leaf weeds dry biomass production at 20 DAS of observation. Irrespective of weed control measures, at 20 DAS, grass weed dry weight showed significant influence among the treatments. Residual effect of 1.25 kg/ha butachlor application

at 3 DAP supplemental with manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in bhendi and urdbean recorded lesser dry weight of grasses (2.93 g/m^2). It was followed by residual effect oxadiargyl loading biochar applied at 3 DAP, oxadiargyl entrapped starch applied at 3 DAP and residue of oxadiargyl entrapped water-soluble polymers applied at 3 DAP. Grassy, sedges and broad leaved weed had registered highest biomass weight of 17.25, 12.27, 9.54 g/m^2 respectively under weedy control treatment. It might be owing to greater weed count and dry biomass production in preceding rice and bhendi crop which depleted more resource. Similar line of findings was reported by Bommayasamy and Chinnamuthu (2019; 2022).

Herbicide residual influence on crop growth attributes

Herbicide residue of preceding crops weed management treatments exerted marked influence on leaf area index, plant biomass production, length of roots and its dry weight at 25 DAS (Table 3). The impact of weed control treatments on dry matter production was well exhibited at 25 days after of sampling. Weed free check exhibited its superiority by registering higher LAI, dry matter production, root length and root dry weight of 1.88, 546 kg/ha, 16.83 cm, 43.0 kg/ha respectively on 25 DAS. Among the preceding crop residual treatments, preceding rice herbicide of 1.25 kg/ha of butachlor applied at 3 DAP along with manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in bhendi and urdbean was recorded higher DMP of 334 kg/ha which was accumulated 81.5% higher dry matter as against to weedy control. It's mainly owing to weed free condition during critical stage and effective utilization of resources like moisture, nutrient, light and space resulted in better plant growth, crop canopy coverage which leads to enhanced crop biomass production. Yadav *et al.* (2004) found that the herbicide oxadiargyl had no negative impact on the plant stand or yield of succeeding harvest of pearl millet or moth bean. The similar line of findings was reported by Chandolia (2009). Herbicide applied to unpuddled transplanted rice showed no effect on subsequent wheat, lentil, and sunflower germination, leaf chlorophyll content, shoot length, or dry matter (Zahan *et al.*, 2016). Residual effect of 1.25 kg/ha butachlor application at 3 DAP along with manual weeding at 40 DAP + two manual weeding on 20 and 40 DAS in okra and urdbean was recorded 79.1 and 88.0% higher root length and root dry weight respectively as compared weedy control. This may be owing to higher competition of weed as well as heavy drain in soil nutrient by weed; main crop suffered and resulted in reduced root length and dry weight.

Effect on seed and haulm yield

Significant difference among herbicide residual effect of preceding rice crop weed control treatments were evidenced with seed yield of urdbean (Fig.1). Among the weed control treatments, residual effect of 1.25 kg/ha butachlor application at 3 DAP along with manual weeding at 40 DAP in rice + two manual weeding on 20 and 40 DAS in okra and urdbean registered 299 kg/ha higher seed yield as compared to weedy check. Similar trend was observed in halum yield. Results showed that positive effect on seed and halum yield in these treatments was perhaps leads to accumulation of higher crop biomass due to effective weed control. The results of experiment were in agreement and conformity in the earlier findings of Naidu *et al.* (2012). Significantly least urdbean seed yield was obtained under weedy control which was comparable with residual effect of 100 g/ha oxadiargyl at 3 DAP. It might be due to lesser number of cluster, pods and seeds/pod recorded under weedy check than other weed control treatments. Reduction in urdbean yield attributing characters observed with the heavy weed infestation in rice fallow urdbean (Rao *et al.,* 2010; Aggarwal *et al.,* 2014; Bommayasamy and Chinnamuthu, 2021).

Conclusion

It was concluded that the residual effect of butachlor @ 1.25 kg/ha after hand weeding on 40 DAT in rice and manual weeding twice at 20 and 40 DAS (both bhendi and urdbean) efficiently controlled weeds and significantly reduced soil weed seed bank in future crops, resulting in improved urdbean growth and seed yield.

Conflict of interest

The authors declare that they have no conflict of interest.

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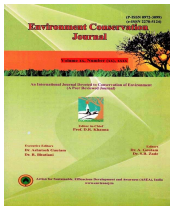

Biodegradation of harmful industrial dyes by an extra-cellular bacterial peroxidase

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ABSTRACT

Nowadays the treatment of environmental pollutants such as synthetic dyes (used in multiple industries such as paper, textile, food, plastic and pharmaceutical) has received much attention, especially for biotechnological treatments using both native and artificial enzymes. In this context, many enzymes have been reported to efficiently perform dye degradation. Peroxidase is one such enzyme, which causes dye degradation either by precipitation of chemical structure of aromatic dyes or by opening up their aromatic ring structure. In the present study an extra-cellular peroxidase extracted from a bacterial strain *Bacillus* sp. F31 JX984444.1 was tested for its capability to decolorize 16 different dyes used in various industries. Out of 16 different textile dyes the *Bacillus* sp. peroxidase efficiently decolorized 5 dyes out of which 4 triphenyl methane dyes (Basic Fuchsin (BF), Rhodamine B (RB), Coomassie Brilliant Blue (CBBG) and Malachite Green (MG) showed decolorization up to 95.5%, 70.8%, 70% and 40%, respectively, while a polymeric heterocyclic dye Methylene Blue (MB) showed 66.2% decolorization. These 5 dyes were studied to further enhance their decolorization by peroxidase after purification by optimizing different reaction conditions (temperature, time, enzyme concentration, buffer pH, dye concentration and effect of various salt ions, H_2O_2 concentration). This study indicates that the extracellular peroxidase (purified) from *Bacillus* sp. can be used as a useful tool for the treatment (degradation/decolorization) of industrial effluents contaminated with harmful industrial dyes.

Introduction

Industrial pollution refers especially to any contamination caused by industrial activities or industrial waste. Different industrial activities, whether it is the extraction of raw material, processing, manufacturing, or waste disposal, leads to environmental degradation. Industrial pollution is a big issue because, most harmful types of pollutants are produced by industries, making it a significant form of pollution on the planet. The effects of industrial pollution are vast, causing contamination, by release of toxic wastes into water, soil, air, and it is the cause of some of the most significant environmental disasters of all time. Water constitutes more than half of our planet, and it is getting polluted day by day due to different causes including human activities, one of the most limelight is industrial chemicals and wastes. The colored wastewater from various industries is the main cause of water pollution. Effects of industrial water pollution are very harmful to animals, plants, human beings as well as entire environment (Ong *et al*., 2011; Hynes *et al.*, 2020; Arif, *et. al*., 2020). The ecological balance is continuously disturbing in whole world every year because, the production of industrial effluents is more than the production of industrial products. The food web/ food chain of organism also severely affected with the toxic pollutants released from industrial effluents in the environment (Sugano *et al*., 2009; Pal and Vimala, 2012; Ramachandran *et al*., 2013; Telke *et al*.,

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2015; Pandey *et al*., 2016; Lellis *et al*., 2019; Verma *et al*., 2021). Therefore, their proper discharge and economical treatment of industrial waste is a matter of great concern.

The various dyes used by several industries are the main cause of colored wastewater and the most problematic groups of pollutants. Harmful synthetic dyes are mostly used in textile, refining, oil, plastic, tannery, paper/pulp, electroplating and cosmetics production units, in the food and pharmaceutical industries (Huber, P., & Carré, 2012; Chanwun *et al*., 2013; Guerra *et al*., 2018; Dhankhar *et al*., 2020). Synthetic dyes used in the textile industry represent a class of highly durable environmental pollutants and non-biodegradable, that are released in large amounts in the rivers and other nearby water reservoirs. These dyes are highly resistant to chemical and physical decomposition methods because colour durability is the most important goal of dying process (Zucca *et al*., 2012; Celebi *et al*., 2013; Blánquez *et al*., 2019). Industrial dyes or their degraded products drown in various sources of water cause many human health disorders such as skin diseases, ulcer, kidney damage, reproductive system diseases, disorders of liver, brain and nervous system (Husain, 2010). Dyes are characterized in specific groups on the basis of chemical structure of the chromophoric groups (Mathur and Kumar *et al*., 2013; Tian *et al*., 2016). Wastewater from various industries is estimated to contain approximately 15-25% of dyestuff that is used in the dyeing process, many of these dyes are recalcitrant compounds that is highly stable to heat cold, light and microbial attack, making them serious pollutants to damage environment (Singh *et al*., 2010). Most experiments on degradation of dyestuffs by microbial peroxidase have been carried out using either whole culture, crude or purified preparations of the peroxidase. Many kids of peroxidases decolorize or degrade the dyes by asymmetric cleavage of bonds present in chromatophoric groups in the dyes (Dawkar *et al*., 2009; Fujihara *et al.,* 2010; Du *et al*., 2011; Renugadevi *et al*., 2011; Saladino *et al*., 2013; Chen & Li, 2016; Ledakowicz & Paździor, 2021). All the currently available techniques for dye removal, such as chemical oxidation, incineration, photo-catalysis or ozonation, reverse osmosis, adsorption are highly efficient but with some disadvantages or limitations (Telke *et al*. 2010). All these treatments may result in the production of toxic by-products, these are highly costly, limited applicability, high energy input etc. Due to the inherent drawbacks of physical, chemical and photo-chemical approaches (Singh *et al*., 2010) the use of biocatalytic or biological methods for the degradation/ decolorization and detoxification of wastewaters dyes has proved as a low cast alternative, nature friendly as well as the property of producing a less quantity of by-products (Franciscon *et al*., 2012; Qin *et al*., 2018).

A number of extracellular oxidative enzymes extracted from different sources such as bacteria, fungi, yeast as well as plants like laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase, cellobiose dehydrogenase and other oxidoreductases enzymes have been reported on the degradation of industrial dyes (Alam *et al*, 2010; Joshi *et al.,* 2010; Kurade *et al*., 2011; Marco-Urrea and Reddy, 2012; Qin *et al*., 2018; Chang *et al*., 2021). The oxidoreductase enzymes like peroxidase and laccase have an advantage over other enzymes or chemical processes because of their ability to completely mineralize various dyes to carbon-dioxide and water during degradation/decolorization process (Faraco *et al*., 2007; Krishnaveni *et al*., 2011, Ilić Đurđić *et al*., 2021).

Peroxidase is an oxidoreductase enzyme, the bound cofactor essential for its activity is haem. Peroxidase catalyzes the transfer of oxygen from the H_2O_2 to an appropriate substrate (like dyes) and consequently brings about oxidation of the substrate. Peroxidases can degrade/decolorize a wide range of industrial dyes because of their less-specific nature, as well as are able to transform or mineralize many other organo-pollutants (Lauber *et al*., 2017; Morsy *et al*., 2020). Hence, the aim of the present research work was to study the biodegradation/bio-decolorization of various industrial dyes using an extra-cellular peroxidase purified from a bacterial isolate *Bacillus* sp. F31 JX984444.1 which was isolated from onion waste and crude oil-polluted soil samples. Further, the purified bacterial peroxidase showed different specificities and efficiencies toward different industrial dyes.

Material and Methods

Organism

The bacterial isolate *Bacillus sp.* F31 JX984444.1 was originally isolated by serial dilution plate technique (Dhingra and Sinclair, 1993) using agar medium, from onion waste soil samples from District Mandi (Himachal Pradesh, India).

Preparation of crude enzyme

The seed culture of *Bacillus sp. F31 JX984444.1* was raised at 37°C (120 rpm) for 24 h. The 50 ml of production broth [containing beef extract (0.1%), glucose (1.4 %), yeast extract (0.2%), peptone (0.5%), NaCl (0.5), H_2O_2 (0.06%; v/v) with final pH of 7.5] was used to culture seed (10%, v/v). Then the culture seed was incubated for 2 days (48 h) under shaking conditions at 120 rpm at 37°C. Then the cell-free broth termed as crude peroxidase was extracted from harvesting incubated broth by centrifugation (10,000 X g for 20 min at 4°C; SIGMA 3K30, Germany).

Protein and peroxidase assay

Protein concentration was measured by a standard protein estimation method (Bradford, 1976) using Bovine serum albumin (BSA) as a standard. The assay of peroxidase in the broth or purified enzyme fraction was done by a colorimetric method using *o*-phenylenediamine (OPD) as a chromogen and H_2O_2 as a substrate (Bao *et. al*., 2020). The A_{492} values were recorded and activity of the peroxidase was determined.

Enzyme unit of peroxidase

The amount of enzyme needed to convert 1.0 µM of chromogenic substrate (OPD) to its product (2, 3 diamino-phenazine) per min at pH 5.2 and temperature 37ºC is known as one unit (U) of peroxidase enzyme.

Purification

The crude bacterial peroxidase (extracellular) produced from bacterial strain *Bacillus* sp. F31 JX984444.1 was purified by ammonium sulphate precipitation, dialysis and DEAE-cellulose column chromatography (anion-exchange chromatography). SDS-PAGE was used to determine molecular mass of purified peroxidase.

Application of an extracellular peroxidase (purified) from *Bacillus* sp. F31 JX984444.1 in degradation of dyes

Screening of industrial dyes for decolorization by purified peroxidase

To test the ability of purified peroxidase to decolorize selected industrial dyes, a total of 16 different dyes namely, Bromophenol Blue (BPB), Reactive Yellow FN2R (RY), Congo Red (CR), Xylidine (XY), Methyl Orange (MO), Rhodamine B (RB), Erichrome Black Y (EB), Bismark Brown R (BBR), Basic Fuchsin (BF), Bismark brown Y (BBY), Direct Violet 21 (DV), Methylene-Blue (MB), Black RL (BRL), Coomassie Brilliant Blue G-250 (CBBG), Direct Black-154 (DB) and Malachite Green (MG) were studied using dye decolorization assay mixture containing 50 µl of different dyes (20 µM), 700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2 and 0.75 U purified peroxidase to make the final volume 1 ml in different cuvettes. Decolorization of the test dye was assayed after incubation at 37ºC for 30 min, by observing the absorbance (A) at the respective wavelength (λmax) of the dye and decolorization (%) was determined as follows:

$$\textbf{\textit{Decolourization/Degradation}}\ \% = \frac{\textbf{\textit{Initial absorbance}} - \textbf{\textit{observed absorbance}}}{\textbf{\textit{Initial absorbance}}} \times \mathbf{100}$$

Reaction conditions optimization for dye degradation by purified peroxidase from *Bacillus sp. F31 JX984444.1*

Out of 16 dyes tested for decolorization, only 5 dyes (BF, RB, MB, CBBG and MG) that were efficiently decolorized by bacterial peroxidase were selected for further studies. The purified peroxidase was used to evaluate the effect of various reaction conditions (or reaction parameters) such as temperature, reaction time, enzyme quantity, buffer system pH, effect of concertation of dye, effect of salt-ions on degradation of selected dyes and optimized conditions for dye degradation were ascertained.

Optimization of temperature (°C) for degradation of dye with purified peroxidase

To determine optimum temperature for degradation or decolorization of selected dyes (BF, RB, MB, CBBG and MG), the degradation reaction [1 ml reaction mixture containing 15 µl H_2O_2, 0.75 U purified peroxidase, 50 µl (20 µM) of different dyes and 700 µl (0.1 M) phosphate citrate buffer (pH 5.2)] was carried out at selected temperatures (25, 30, 35, 37, 40 and 45°C) in Eppendorf tubes in a

dry heating block for each dye, separately for 30 min. The decolorization was assayed after incubation of 30 min by measuring the absorbance at the respective wavelength of the dyes, and decolorization % was determined and analyzed.

Optimization of decolorization reaction time for degradation of dye with purified peroxidase

In order to determine the optimum reaction time for degradation or decolorization of few selected dyes (BF, RB and MB, CBBG, MG) the time of dye degradation assay [700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2, 0.75 U purified peroxidase and 50 µl (20 µM) of different dyes in 1 ml reaction mixture] was varied from 0 to 40 min for each dye separately. After incubation decolorization (%) of each dye was calculated after incubation at 30°C for RB, 35°C for MB; 40°C for BF, CBBG and MG.

Optimization of peroxidase (biocatalyst) concentration for degradation of dye with purified peroxidase

Purified peroxidase concentration was varied from 0.70 U to 1.1 U in 1 ml final reaction volume to perform the dye degradation assay [700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2 and 50 µl (20 µM) of selected dye in 1 ml reaction mixture]. The decolorization (%) of different dyes was calculated after 35 min of incubation in dry bath at 35°C for BF; 45 min at 35°C for MB, 40 min at 40°C for RB; 40 min at 30°C for CBBG and 40 min at 40°C for MG.

Optimization of (buffer system or reaction buffer) pH for degradation of dye with purified peroxidase

Effect of reaction buffer (phosphate citrate buffer) pH on the dye decolouration of selected dyes by purified bacterial peroxidase (extracellular) was studied at different buffer pH values (3-7) of 0.1 M phosphate citrate buffer in (1 ml; containing 15 µl H_2O_2, 50 µl (20 µM) of selected dye). The decolorization (%) of the dyes was calculated after incubation at 35°C for BF after 35 min, at 40°C for RB after 40 min, at 35°C for MB after 45 min, at 30°C for CBBG after 40 min and for MG after 40 min at 40°C.

Optimization of concentration (mg/ml) of selected dye for degradation of dye with purified peroxidase

The dye concentration of each selected dye was varied from 100 to 1000 mg/l [in dye decolouration assay mixture containing, 15 µl H_2O_2, 50 µl of different dyes, 700 µl (0.1 M) phosphate citrate buffer of optimised pH and optimized concentration purified peroxidase for each dye]. Optimized temperature, pH and time were used in decolorization assay for respective dyes and decolorization (%) was determined thereof.

Optimization of hydrogen peroxide concentration (mM) for degradation of dye with purified peroxidase

The concentration of H_2O_2 was varied from 0.25 mM to 3.50 mM in the decolorization reaction mixture [containing 700 µl (0.1 M) phosphate citrate buffer, 50 µl (20 µM) of selected dyes, and optimized concentration of purified peroxidase]. The decolorization (%) was determined in each case by using respective optimized parameters like temperature, pH and time for each of the dye in decolorization assay.

Effect of salt-ions and inhibitors for dye decolorization by purified peroxidase

The decolorization (%) of BF, RB, MB, CBBG and MG with purified peroxidase was determined in the presence of 1 mM (w/v) of selected salt ions and inhibitors (Li^{+3}, Mn^{+2}, Mg^{+2}, K^{+2}, Na^{+}, Hg^{+2}, Cu^{+2}, Fe^{+2}, Zn^{+2}, Ca^{+2}, EDTA, SDS, sodium azide and DTT) at optimized conditions. The mixture of enzyme and metal ion/ inhibitors in ratio 1: 1 was pre-incubated at 37°C in a dry heating bath for 30 minutes. Thereafter, the pre-incubated peroxidase was separately incubated with each of the selected dye in decolorization assay. Respective optimized temperature, pH and time for each of the dye were used in decolorization assay and decolorization (%) was determined in each case.

Results and Discussion

Purification

The purification of peroxidase from *Bacillus sp. F31 JX984444.1* was done by ammonium sulphate precipitation, dialysis and anion exchange chromatography (DEAE-cellulose). The protein analysed by the SDS-PAGE and the native-PAGE resulted in a single band of approximately 37 kDa and 95 kDa, respectively. The extracellular bacterial peroxidase was purified with a yield of 12.6 % up to 14.6-fold. The purified peroxidase was further used for dye decolorization experiments.

Application of an extracellular peroxidase (purified) from *Bacillus* sp. F31 JX984444.1 in degradation of industrial dyes

Screening of industrial dyes for decolorization by purified peroxidase

To test the ability of bacterial peroxidase to decolorize a few selected industrial dyes namely BPB, RY, CR, XY, MO, RB, EB, BBR, BF, BBY, DV, DB, MB, BRL, CBBG and MG, each of these dyes was subjected to treatment with purified peroxidase (0.75 U) at 37ºC for 30 min in phosphate citrate buffer (pH 5.2). Out of these 16 textile dyes, the purified peroxidase efficiently decolorized 5 dyes, out of which 4 belonging to triphenyl methane group (BF, RB, CBBG and MG) showed decolorization up to (95.5, 70.8, 70.0 and 40.0%, respectively) while a polymeric heterocyclic dye (MB) showed 66.2% decolorization (Table 1 and Table 2). These 5 dyes were selected to further enhance their decolorization by purified peroxidase by optimizing reaction conditions (temperature, time, enzyme concentration, buffer pH, dye concentration and effect of various salt ions). Therefore, it could be stated that the peroxidase of *Bacillus sp. F31 JX984444.1* has higher affinity for triphenyl methane dyes as substrates. It has been reported by the present studies that different types of peroxidase(s) decolorize specific type of dyes more efficiently as compared to others. The Triphenyl methane dyes were efficiently decolorized as compared to other groups of synthetic dyes by peroxidase from *Hevea brasiliensis* in a previous study (Chanwun *et al*., 2013; Blánquez *et al*., 2019). Many factors influence the decolourization of dyes by an enzyme such as their chemical structure, molecular weight (*M*r), redox potential, molecular weight, complexity of side chains and most importantly the binding site of enzyme (Pereira *et al*., 2018; Ardila-leal *et al*., 2021). The dye BF having a simple structure with small functional groups (NH_2; side chains) and a relatively lower *M*r (337.8 g/mol) was efficiently decolourized as compare to other dyes bearing more complex side chains. The side chains often account for stearic hindrance in binding to the enzyme. This can be possibly the reason for greater decolourization of the BF dye by purified peroxidase of *Bacillus* sp. F31.Many types of peroxidases purified from various microbial sources such as bacterial, fungi, yeast and plants etc. were found efficient to decolorize different industrial dyes (Fetyan *et al*., 2013; Telke *et al*., 2015, Pandey *et al*., 2016; Ilić Đurđić *et al*., 2021; Guo *et al*., 2021). The MnP (manganese-independent peroxidase) purified from *Dichomitus squalens* was also able to degrade selected azo dyes and anthraquinone dyes (Šušla *et al*., 2008). The MnP sourced from *Auricularia uricular-judae* was found to be highly stable and effective in degradation of very complex dyes (Liers *et al*., 2010). Peroxidase isolated from *Ganoderma cupreum* AG-1 (grown on decaying wood) was evaluated for its strong ability to degrade many azo dyes (Gahlout *et al*., 2013). A newly isolated and purified peroxidase from *Sterigmatomyces halophilus* (yeast strain), under the optimized conditions, showed a complete degradation efficiency on many azo dyes within 2 days (Al-Tohamy *et al*., 2021).

Reaction conditions optimization for dye degradation by purified peroxidase from *Bacillus sp. F31 JX984444.1*

Optimization of temperature (°C) for degradation of dye with purified peroxidase

Temperature of decolorization reaction system was varied from 30 to 45°C for decolorization of BF, RB, MB, CBBG and MG separately in the reaction mixture (1 ml) containing 0.75 U of purified peroxidase. The optimum temperature for each of these dye with purified peroxidase was 30°C for RB (72.1%), 35°C for MB (82.0%), 40°C for BF (92.1%), CBBG (90.2%) and MG (65.2%), respectively (Fig. 1). The recorded data showed that purified peroxidase performed efficient decolourization of chosen common textile dyes at 30-40°C. The previous studies for dye degradation have proved that peroxidase works efficiently at a range of 25-45ºC for different dyes, though in some cases free peroxidase retained its activity at a temperature as low as 5°C (Carmen *et al*., 2012). The decolourization of MG, a constant temperature of 25± 0.5°C (Satapathy *et al*., 2011) and 30°C for the peroxidase of a fungal strain *Cunninghamella elegans* was reported (Roushdy *et al*., 2011). The decolorization of different dyes by peroxidase from *Trametes versicolor* was found to be effective at 30°C (Celebi *et al*., 2013). The peroxidase from *Pleurotus ostreatus* (Shin *et al*., 1998) efficiently decolorized triphenyl methane dyes as BB and MB

Table 1: Screening of dyes for decolourization by purified peroxidase.

SN	Name of dye (1 mM)	Type of dye	Dye decolourization (%)
1.	BPB	Triphenyl methane	3.0
2.	RY		5.0
3.	CR	Azo	-
4.	XY	*Dimethylanilin e*	-
5.	MO	Azo	3.0
6.	**RB**	**Triphenyl methane**	**70.8**
7.	EB	Azo	-
8.	BBR	Diazo	5.0
9.	**BF**	**Basic dye**	**95.5**
10.	BBY	Diazo	7.0
11.	DV	Azo	-
12.	DB	Azo	8.0
13.	**MB**	**Polymeric heterocyclic**	**66.2**
14.	BRL	Azo	4.0
15.	**CBBG**	**Triphenyl methane**	**70.0**
16.	**MG**	**Triphenyl methane**	**40.0**

at 25ºC, whereas BF was 93% decolorized at 30ºC by peroxidase from *Aeromonas hydrophila* (Ogubue *et al*., 2012). The activity of different oxidative enzymes from different microbial sources such as manganese peroxidase, tyrosinase and laccase were evaluated for decolorization of Indanthrene Blue-RS and other industrial dyes, then the optimized temperature for decolorization reaction was found 37°C (Mohanty and Kumar, 2021). In general, peroxidase enzyme tends to lose its dye degradation capability beyond 45ºC and retained only about half of its activity at 65ºC (Yao *et. al*., 2013). This is possibly due to the reason that the higher temperature results in thermal inactivation of proteins (enzymes), which also affects cell structures and activity of enzymes (Shah *et al*., 2013).

Optimization of decolorization reaction time for degradation of dye with purified peroxidase

Reaction time of decolorization for each of the selected dyes (BF, RB, MB, CBBG and MG) was varied from 0 to 45 min. The maximum decolorization by purified peroxidase was observed between 30-45 min for MB (82.1%, 30 min) at 35°C, BF (96.1%, 35 min) at 40°C, RB (76.2%, 40 min) at 30°C, CBBG (90.0%, 40 min) and MG (78.3%, 40 min) at 40°C (Fig. 2). The purified biocatalyst efficiently performed decolourization of selected dyes between 35-45 min at the chosen optimized temperature. The decolourization of the dye enhanced with the increase of reaction time but little increase in decolourization was noticed after 40 min for each dye. In another study, the optimum time for MB decolourization was also found 40 min (Satapathy *et al*., 2011). The effective and eco-friendly biodegradation of Reactive Black dye by peroxidase from *Sterigmatomyces halophilus* within effective rection time of 1-2 hours has been reported recently. It was also proved effective for textile azo dye wastewater processing and detoxifcation (Al-Tohamy *et al*., 2020). The chemical structure of dye was reported to have a relation with degradation time. Generally, dyes with low molecular weights and simple structures exhibited higher rates of dye removal than high molecular weight dyes (Chen *et al*., 2003).

Optimization of enzyme (biocatalyst) concentration for degradation of dye with purified peroxidase

The concentration of enzyme (purified peroxidase) used in dye decolorization assay of 5 selected dyes (BF, RB, MB, CBBG and MG) was varied from 0.77 to 1.05 U for purified peroxidase in the 1 ml final volume of reaction mixture. The maximum decolorization was observed with 0.94 U of purified peroxidase for BF (95.1%) at 40°C in 35 min; 1.05 U for RB (82.2%) at 35°C in 40 min, MB (85.1%) at 35°C in 30 min and MG (78.2%) at 35°C in 40 min, and 1.01 U for CBBG (92.1%) at 40°C in 40 min (Fig. 3). The removal of an organic pollutant (that act as a substrate for enzyme catalysis) is dependent on the amount of biocatalyst added and the contact time between enzyme and substrate. Increasing enzyme concentration will speed up the enzymatic reaction, as long as there is substrate available to bind. When all the substrate is bound, there will be no increase in the speed of reaction, since there will be nothing for additional enzymes to bind to. In a previous study, an increase in concentration of the Soyabean peroxidase in reaction mixture from 15 U/ml to 85 U/ml resulted in slow increase in the dye degradation (16-64%) that appeared to be constant at optimized dye concentration 80 U/ml (Kalsoom *et al*., 2013). Published studies showed that degradation of an

Table 2: Efficient degradation of selected dyes with purified peroxidase.

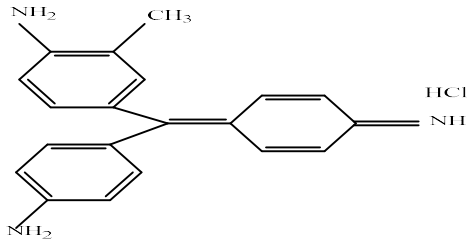
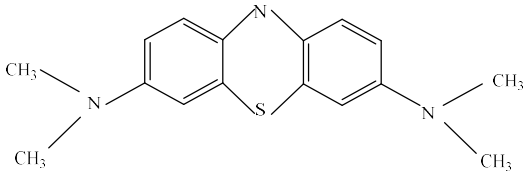
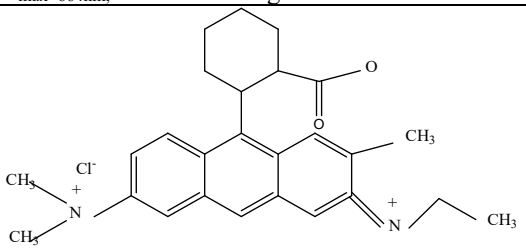
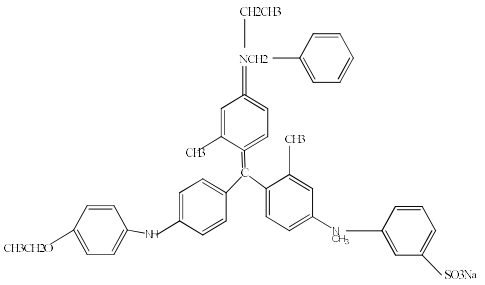
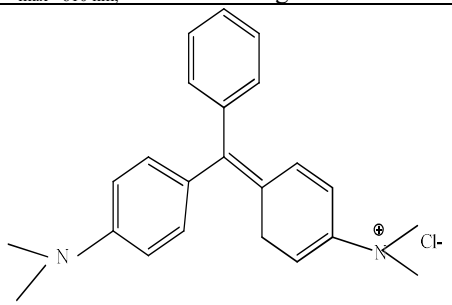
Dye	Structure of dye/ λ_{max}/ Molecular weight (g/mol)	Type of dye	Decolorization (%)
BF	 $\lambda_{max=545nm,}$ molecular weight= 337.85	Triphenyl methane dye	95.5
MB	 $\lambda_{max=664nm,}$ molecular weight= 319.85	Triphenyl methane dye	66.2
RB	 $\lambda_{max=\ 555nm,}$ molecular weight= 479.01	Polymeric/heterocyclic dye	70.8
CBBG	 $\lambda_{max=\ 610\ nm,}$ molecular weight =854.02	Triphenyl methane dye	70.0
MG	 $\lambda_{max=550\ nm,}$ molecular weight= 364	Triphenyl methane dye	40.0

Table 3: Effect of salt-ions and inhibitors on degradation of dyes with purified peroxidase

Metal ion/ Inhibitor (1 mM)	Relative decolourization (%) at stated λ_{max}				
	BF (A_{545})	RB (A_{555})	MB (A_{664})	CBBG (A_{610})	MG (A_{550})
None	100.0	100.0	100.0	100.0	100.0
Li^{+3}	97.8	88.4	97.6	98.5	95.0
Zn^{+2}	**100.5**	**101.2**	**101.1**	**101.1**	**101.2**
Mg^{+2}	**101.6**	**101.2**	**100.5**	**101.6**	**102.5**
K^{+2}	98.9	98.7	98.8	95.5	92.5
Na^{+}	97.8	80.7	84.1	93.3	87.5
Hg^{+2}	63.8	62.8	47.0	38.8	31.2
Ca^{+2}	94.6	98.7	96.4	94.4	88.7
Cu^{+2}	96.8	96.1	97.6	97.7	93.7
Fe^{+2}	95.7	93.5	98.8	96.6	96.2
Mn^{+2}	**100.0**	**100.6**	99.4	99.3	**100.2**
EDTA	88.9	80.9	78.9	74.5	72.0
SDS	62.5	56.5	60.7	58.4	54.2
Sodiun azide	45.8	42.3	35.7	50.4	41.5
DTT	58.9	64.5	62.1	60.4	67.0

azo dye required a much higher amount (15-times higher concentration) of enzyme than in the case of the anthraquinonic dyes (Mohan *et al*., 2005). Researchers found out that 3.3 µg/ml of peroxidase enzyme helped in the efficient removal of an anthraquinonic dye, the Reactive Blue 19 (Celebi *et al*., 2013).

Optimization of reaction buffer (phosphate citrate buffer) pH for degradation of dye with purified peroxiadse

The optimum pH of the assay buffer system for efficient decolorization of each selected dye (BF, RB, MB, CBBG and MG) with purified peroxidase was determined by varying buffer pH (phosphate citrate buffer) from 3-7. The optimum pH of reaction buffer for each of the selected dyes was 5.0 for RB (81.2% at 35°C for 40 min with 1.05 U of purified peroxidase); pH 5.5 for BF (96.1% at 40°C for 35 min with 0.94 U peroxidase) and also for MB (83.5% at 35°C for 30 min with 1.05 U of peroxidase) and MG (78.3% at 40°C for 40 min with 1.05 U of peroxidase); pH 6.0 for CBBG (92.2% at 40°C for 40 min with 1.01 U of peroxidase) (Fig. 4). All the selected dyes were effectively decolorized in pH range 5.0-6.0 and at the higher values, the dyes showed less degradation. These results seem similar to what other researchers have reported (Marchis *et al*., 2011; Zhang *et al*., 2012; Tian *et al*., 2016) and suggested the usefulness of this enzyme to degrade industrial effluents or decolorize various industrial dyes which might be very acidic in nature. In another study, it was observed that strong to moderate acidic pH (pH 2-6) of buffer system is suitable for decolorization of most of the dyes including MG (Zucca *et al*., 2012). In the catalytic cycle of peroxidase all the reactions steps are pH dependent so enzyme was appeared to do decolorization best under acidic pH range (Kalsoom *et al*., 2013; Blánquez *et al*., 2019). In addition, HRP was reported to catalyze degradation of Remazol Blue at a pH of 2.5 (Šekuljica *et al*., 2020). The reaction steps of the catalytic cycle of peroxidase were pH-dependent and appeared to work best at an acidic pH. On checking pH, optimum researchers have obtained a bell-i shaped curve with an optimum at pH 5-5.5 (Kinnunen *et al*., 2017). Another study showed the lignin-peroxidase to work at an optimum pH of 3, where it was used to degrade azure B dye (Silva *et al*., 2013).

Each enzyme has an optimum pH range. Changing the pH beyond of this range will slow enzyme activity. Extreme pH values can cause denaturation of enzymes. At native state an enzyme is a combination of both cationic and anionic groups at any given pH. These groups are part of the active site of an enzyme (Gomare *et al*. 2008). The rate of enzymatic reaction varies with the changes in the pH of the reaction medium because the ionic state of active site in enzyme changes hence, activity of enzyme is also changed (Hossain and

Anantharaman. 2006). Moreover, these organic dyes have different redox potentials, which may also affect the decolorization activity of the designed heme enzyme (Jenkins *et al*., 2021).

Optimization of selected dye concentration (mg/ml) for degradation of dye with purified peroxidase

In the present work, the maximum decolorization was found in case of BF (97.1%) at concentration 800 mg/l at 40°C in 35 min at pH 5.5, for RB (86.2%) at 600 mg/l at 30°C in 40 min at pH 5.0, MB (84.2%) at 35°C in 30 min at pH 5.5 and MG (78.1%) at 400 mg/l at 40°C in 40 min at pH 5.5 and CBBG (92.1%) at 40°C in 40 min with 200 mg/l at pH 6.0 (Fig. 5). In the present work, the maximum decolorization was found in case of dye BF (97.1%) The results were in accordance with a previous study, which showed that dye-degradation efficiency of enzyme peroxidase decreased with increasing concentration of dye, then eventually resulted in an inhibition effect, thus presenting less degradation (Boucherit *et al.*, 2013). Other microbial peroxidase showed similar results where the degradation rate decreased with an increase in dye concentration (Silva *et al*., 2012). Enzyme-catalysed reactions were affected by the concentration of the substrate present in the aqueous phase (Robinson *et al*., 2015). It is appeared quite possible due to the reason that if the substrate concentration is slowly increased and the concentration of enzyme is kept constant the reaction rate would continue to increase until it reaches equilibrium state. At equilibrium, there will be maximum rate of reaction after that any further addition of the substrate would not increase the rate of reaction. The effect of dye concentration in reaction mixture is an important consideration for its application in industrial dye removal or decolorization. In effluent from textile printing house, the dye concentration is approximately 100-800 mg/l (Zhao and Hardin, 2007). According to a study, with increase in dye concentration, the decolourization efficiency of dye decreased and a marked inhibition was exhibited when the dye (Remazol Brilliant Blue R) concentrations were above 100 mg/l (Silva *et al*., 2013). The FTIR spectroscopy, NMR and GC-MS of several dye degradation products using purified peroxidase by *Bacillus cereus*, the results confirmed that decolorization of various industrial dyes was due to breakdown of dyes into unknown colourless products (Fetyan *et al*., 2013).

Optimization of hydrogen peroxide concentration (mM) for degradation of dye with purified peroxidase

H_2O_2 reacts with the native enzyme (purified peroxidase from *Bacillus* sp. F31 JX984444.1) to form an enzyme intermediate, which easily combine with an aromatic compound such as dyes to carry out its oxidation and produce a free radical form. To figure out an appropriate concentration of H_2O_2 is definitely a key factor in obtaining a higher rate of dye degradation (Yao *et al*., 2013). In this regard, in present study experiments were done to decolouration/ degradation of the selected textile dyes (BF, RB, MB, CBBG and MG) the H_2O_2 concentration varied while keeping the other reaction conditions of degradation reaction constant. The concentration of H_2O_2 was varied from 0.25 mM to 3.5 mM in dye decolorization assay, the optimized concentration of H_2O_2 for decolorization of all selected dyes (BF, RB, MB, CBBG and MG) using purified peroxidase was found between 1 to 1.5 mM with a decolorization of 97.1% for BF at 40°C in 35 min, 86.2% for RB at 30°C in 40 min, 84.1% for MB at 35°C in 30 min, 92.3% for CBBG at 40°C in 40 min and 78.1% for MG at 40°C in 40 min (Fig. 6). Activity of Peroxidase is known to change considerably with change in the concentration of the presence of H_2O_2 (Gholami-Borujeni *et al*., 2011). Many harmful organic compounds including industrial dyes can be oxidised and degrade/ decolourize by using H_2O_2 alone or in conjunction with other materials. The addition of a small amount H_2O_2 in the reaction mixture may lead to the generation of free radicals like •OH that facilitates faster degradation of many organic compounds. On the other hand, higher concentration of H_2O_2 was detrimental to the reaction process, most likely due to the damage to the enzyme being a protein itself. So, it becomes necessary to optimize the H_2O_2 concentrations in the enzyme-based dye degradation approaches (Zhang *et al*., 2012). For the decolourization of MB by HRP, 0.15 mM H_2O_2 concentration in the reaction mixture was found to be the optimum (Satapathy *et al*., 2012). In case of Soya bean peroxidase, increase in H_2O_2 concentration in

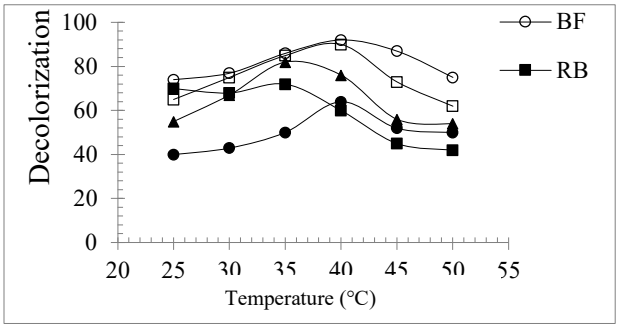


Figure 1: Optimization of temperature (°C) for dgradtion of dye with purified peroxidase.

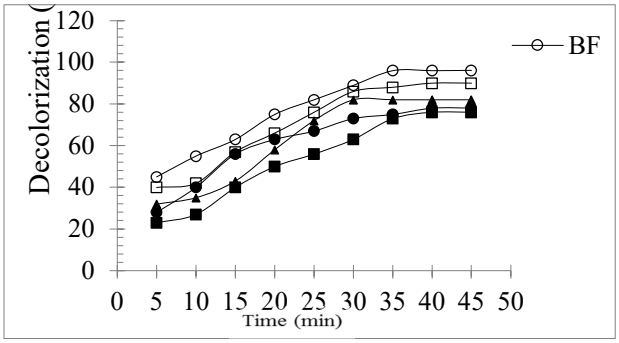


Figure 2: Optimization of reaction time for degradation of dye with purified peroxidase.

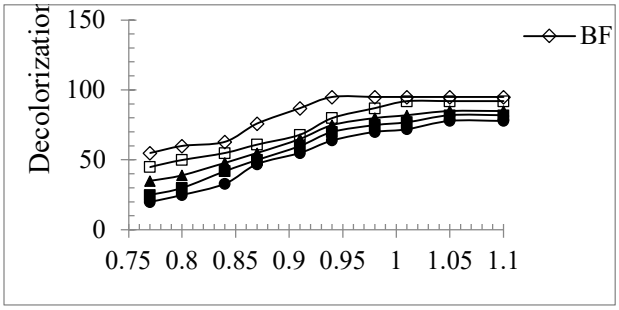


Figure 3: Optimization of biocatalyst (Enzyme) concentration for degradation of dye with purified peroxidase.

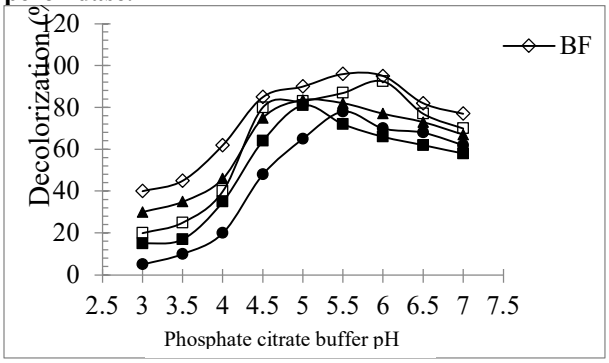


Figure 4: Optimization of reaction buffer (phosphate citrate buffer) pH for degradation of dye with purified peroxidase.

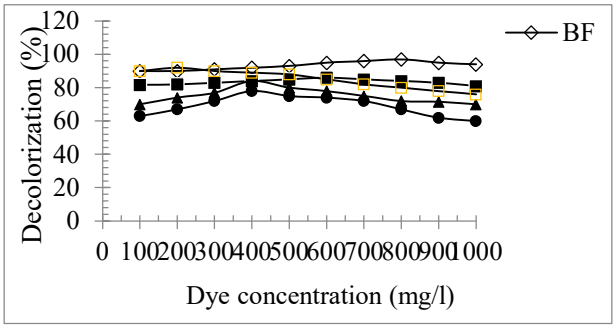


Figure 5: Optimization of dye concentration (mg/ml) for degradation of dye with purified peroxidase.

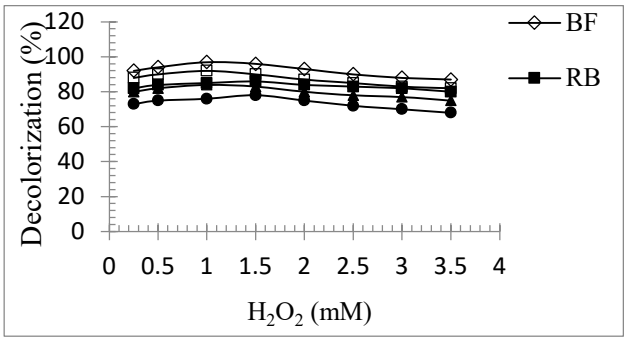


Figure 6: Optimization of hydrogen peroxide concentration (mM) for dye degradation with purified peroxidase.

reaction mixture led to an increased dye decolourization. At 64µM concentration of H_2O_2 maximum dye decolourization was observed, any further increase in H_2O_2 concentration did not cause any additional effect (increase /decrease) on dye decolorization (Kalsoom *et al*., 2012).

Effect of salt-ions and inhibitors on dye decolorization by purified peroxidase

The decolorization (%) of BF, RB, MB, CBBG and MG with purified peroxidase was also determined in the presence of selected salt ions and inhibitors under optimized conditions. The decolorization of all the five dyes with purified peroxidase was inhibited by the presence of Hg^{+2}, EDTA, sodiun azide, DTT and SDS. However, the decolorization was found to be slightly stimulated by purified peroxidase in the presence of Zn^{+2} (BF 100.5%, RB 101.2%, MB 101.1%, CBBG 101.1% and MG 101.2%), Mg^{+2} (BF 101.6%, RB 101.2%, MB 100.5%, CBBG 101.6% and MG 102.5%) and Mn^{+2} (RB 100.6% and MG 100.2%), (Table 3). As in previous study, the decolourization was slightly stimulated for extracellular purified peroxidase in the presence of some bivalent metal ions such as

Mg^{+2}, Zn^{+2} and Co^{+2}, so the use of such ions could be considered in dye decolourization experiments as additives for efficient dye decolourization (Dawkar *et al*., 2009; Irshad and Asgher, 2011; Si and Cui, 2013). In another study, decolorization of MG by peroxidase from *Pseudomonas* sp. was observed to significantly enhanced in the presence of Mg^{+2} and Mn^{+2} (1-2 mM) ions (Du *et al*., 2011).

Different industries like textile, food, pharmaceuticals, cosmetics etc., release a huge amount of the effluents daily in the form of wastewater into rivers and other water reservoirs causing serious health issues. That leads to enhanced biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of water and affect the ecosystem drastically. Therefore, it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and the presence of metal ions (Anjali *et al*., 2007). Treatment of various industrial effluents discharged with harmful compounds like synthetic dyes becomes necessary before their final discharge into the environment and different water sources. There are many conventional methods for the effective removal of dyes such as degradation of dye by anaerobic reaction, adsorption of dye by activated carbon, silica, functional polymer granules, biomaterials, nanofiltration, oxidation and precipitation by Fenton's reagent, bleaching with chloride, ozone photo degradation and membrane filtration (Robinson *et al*., 2001). These methods have been outdated because of many drawbacks such as low efficiency, high cost and in-applicability to a wide variety of complex hazardous dyes. In recent years a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes (Shah *et al*., 2013; Šekuljica *et al*., 2020: Chauhan & Kanwar 2020; Barathi *et al*., 2020). Many types of peroxidases purified from various sources were reported to decolorize different industrial dyes (Shin *et al*., 1998; Hong *et al*., 2012; Celebi *et al.,* 2013, Salvachúa *et al*., 2013; Chen & Li, 2016). The MnP purified from *Dichomitus squalens* was also able to degrade selected azo and anthraquinone dyes (Šušla *et al*., 2008). A thermostable peroxidase from *Bacillus stearothermophilus* and peroxidase sourced from *Auricularia auricula-judae* has been found to be stable in decolourization of the many complex structured industrial dyes such as Reactive Black-5 and Reactive Blue-5 (Loprasert *et al*., 1988; Liers *et al*., 2010). Peroxidase isolated from *Ganoderma cupreum* AG-1 was evaluated for its ability to decolorize many industrial azo dyes Gahlout *et al*., (2013). In some another previous studies, bacterial and fungal peroxidase(s) have been found to be very efficient biological decolourization tools (Zucca *et al*., 2012; Saladino *et al*., 2013, Lauber *et al*., 2017, Morsy *et al*., 2020). The decolorization or degradation of industrial dyes by using biocatalysts or enzymes from various sources like bacteria, fungi, yeast and even plants is an eco-friendly and low-cost process. Biocatalytic methods of decolorization also produce a low quantity of by-products as compare to physical, chemical and photochemical approaches.

Present results suggested that the extracellular peroxidase (purified) from *Bacillus* sp. F31 JX984444.1 was effective in discolouration of many common textile dyes. The purified bacterial peroxidase assisted dye-degradation parameter(s) optimization resulted in increased dye degradation. The optimized dye-degradation process was most successful for BF, where 97.1% of dye decolorization was achieved and minimum decolorization 78.1% was found in case of MG. The selected dyes for decolorization experiments i.e., BF, RB and MG are basic triphenyl methane dyes mostly used in textile, pharmaceutical and chemical industries; while RB is a cationic triphenylmethane dye used in textile industries for dying, wool, silk, nylon and cotton. Sometimes it is also used in medicine, paper, leather, food, cosmetics industries. On other hand, MB is a polymeric/ heterocyclic dye used in textile dyeing, pharmaceuticals, paper industry and also as a biological strain. The great potential of peroxidase produced by *Bacillus* sp. F31 could offer a cheaper and efficient alternative treatment of wastewaters contaminated heavily with industrial dyes. Further extension of work shall comprise the study of the combination of the extracted enzyme(s) with other established methods for wastewater management to see if enzyme alone or in combination with other methods m ay be helpful as an industrially efficient treatment procedure for efficient dye-degradation.

Conclusion

In present study the purified peroxidase from Bacillus sp. F31 was found to be highly effective in decolourisation/degradation of 5 different industrial dyes (BF, RB, CBBG, MG and MB). This approach will provide an effective application of peroxidase in managing industrial effluents containing dyes. Proper management of industrial effluents by using peroxidase can give environmental and economic benefits.

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Conflict of interest

The authors declare that they have no conflict of interest.

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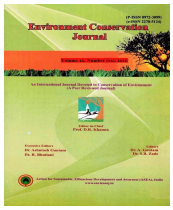

Effect of planting geometry and fertilizer levels on growth and yield of finger millet (*Eleusine coracana* L)

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ABSTRACT

The experiment was done on finger millet during the *zaid* season of 2021-22 at crop research farm, Department of Agronomy, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj (Uttar Pradesh). The treatments consisted of three planting geometry *viz.*, 20 cm × 20 cm, 25 cm × 25 cm and 30 cm × 30 cm and three NPK levels *viz.*, 75%, 100% and 125%. The experiment was conducted in randomized block design with nine numbers of treatments and replicated thrice. The results showed that treatment with 30 cm × 30 cm spacing at 125 % NPK /ha growth parameters *viz.*, maximum plant height (69.73 cm), number of tillers per plant (17.36 g/plant), dry weight per plant (7.36 g) while yield were recorded highest with treatment 20 cm × 20 cm spacing at 125 % NPK /ha. *viz.*, Grain yield (45.76 t/ha), Straw yield (4.33 t/ha) and harvest index (44.91) of finger millet at harvest. This may due to the highest plant population with close spacing treatment and higher number of heads/ m^2 as compare with wide spacing.

Introduction

Fingermillet is commonly known as *ragi* or *mandua* or bird's foot millet [*Eleusine coracana* (L) *Gaertn*]. India has third rank in area and production. Finger millet having highest productivity among all small millets (Seetharamand Krishnegowda, 2007). It is primary food crop for majority of hilly regions of country. The crop is cultivated up to elevations of 3000 meters above mean sea level and utilized for both grain and fodder purposes. The crop is well adapted to very poor and marginal uplands where other crops cannot be grown successfully (AICSMIP, 2014). It is an annual herbaceous plant and contains a lot of protein, calcium, fiber, and energy. It is also rich in iron, essential amino acids (riboflavin, thiamine, leucine, isoleucine and trypsin inhibitory factors) (Chethan and Malleshi, 2008). The average area, production and productivity of India in 2018-19 were 891, 1239 and 1390 respectively. In India, more than 50% area occupied by Karnataka, Maharashtra and Uttrakhand. If we look at the area and production of these three major growing states from 2007-08 to 2018-19, we will find that there has been a lot of volatility. The area and production of finger millet of Karnataka, Maharashtra in 2007-08 were (833 thousand hectare, 1497 MT) and (128 thousand hectare, 124 MT) respectively but in 2018-19 area and production of Karnataka and Maharashtra dropped to (527 thousand hectare, 678 MT) and (80 thousand hectare, 93 MT) (Anonymous, 2020). The area and production of finger millet have decreased over the last three

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decades due to low market prices and lack of better cultivation practices *viz.,* fertilizer application, planting geometry etc due to which, the majority of farmers shifted to cash crops. Major constraint of low productivity and profitability in finger millet due to lower fertilizer dose and less fertilizer use efficiency. (Kalaraju *et al.,* 2011).

A better crop geometry will result in a better harvest of moisture and nutrients from the soil (root spread) and from the plant canopy as well as better photosynthesize formation (Uphoff *et al*., 2011). In Karnataka, the average yield of finger millet is reported more under square planting of young seedlings with single seedling hill (Kalaraju *et al*., 2011). Nitrogen, phosphorous and potassium are the essential elements required for plant growth in relatively large amounts (Dhhwayo and Whhgwin, 1984). Nitrogen fertilizer is one of the most yield limiting nutrients for crop production and it is applied in large quantity for most annual crops (Huber and Thompson, 2007). Phosphorus plays an integral role in maintaining membrane structure, bimolecular synthesis, and high-energy molecule synthesis. As well as cell division, enzyme activation and inactivation, and carbohydrate metabolism (Razaq *et al*., 2017). Potassium increases water use efficiency and transforms sugar into starch in the grain filling process (Srinivasarao *et al.,* 2013).

Material and Methods

The present experiment was conducted at crop research farm, Sam Higginbottom University of Agriculture, Technology and Science, Prayagraj in *Zaid* season of 2021-22. The crop research farm is situated at 25º24‘41.27” N latitude, 81°50‘56” E longitude and 98 m altitude above the mean sea level. The experimental field located approximately 7 kilometers from Prayagraj city, near the River of Yamuna, on the left side of the Prayagraj-Rewa Road.

There is a subtropical and semiarid climate in Prayagraj, with hot summers and pleasant winters. The area's average temperature is 46°C to 48°C, with temperatures seldom dropping below 3°C or 4°C. The relative humidity levels range from 45% to 92 %. In this location, is requires about 600-650 mm annual rainfall during crop period for optimum production. The soil chemistry analysis reveals sandy loam texture with a pH of 7.4 [Glass electrode pH meter (Jackson, 1973)], low amounts (0.32 percent) of organic carbon (Walkley and Black’s rapid titration method (Piper,1966), nitrogen [(188.3 kg/ha) Alkaline permanganate method (Subbiah and Asija, 1956)], phosphorus [(35.4 kg/ha) Olsen’s colorimetric method (Olsen *et al.,* 1954)] and potassium [(87 kg/ha) Flame Photometer method (Jackson, 1958)]. The soil was electrically conductive and had an electrical conductivity of [(0.270 dS/m) Method No.4 USDA Hand Book No.16 (Richads, 1954)].

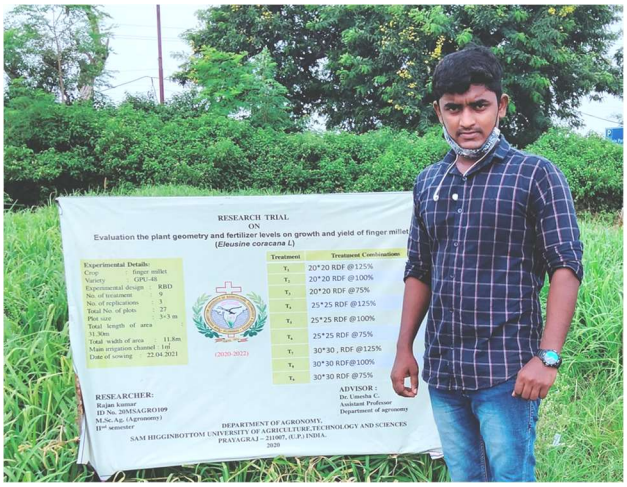

Figure: 1 General view of experimental field

Three replications of the experiment were done in an experimental design with randomized block design and nine treatments *viz.,*T_1- 20 cm × 20cm spacing at 75 % NPK /ha, T_2- 20 cm × 20cm spacing at 100 % NPK /ha, T_3- 20 cm × 20cm spacing at 125 % NPK /ha T_4- 25 cm × 25cm spacing at 75 % NPK /ha, T_5- 25 cm × 25cm spacing at 100 % NPK /ha, T_6- 25 cm × 25cm spacing at 125 % NPK /ha, T_7- 30 cm × 30cm spacing at 75 % NPK /ha, T_8- 30 cm × 30cm spacing at 100 % NPK /ha, T_9- 30 cm × 30cm spacing at 125 % NPK /ha. In order to meet the nitrogen, phosphorus, and potassium requirements, urea, DAP, and MOP were used as nutrient sources. The recommended dose of fertilizer *viz.,* N, P_2O_5 and K_2O (50:40:25) was applied respectively. Nitrogen was given in two split, half of nitrogen and entire quantity of phosphorus and potassium applied as basal dose and remaining half quantity of nitrogen applied as top dressing. Observation recorded as plant height, number of tillers, total dry weight, crop growth rate, relative growth rate, grain yield, straw yield and harvest index. The F test was

Table I- Effect of planting geometry and fertilizer levels on growth of finger millet at harvest

Treatments	Plant height (cm)	Total tillers/ hill	Plant dry weight (g/plant)
1. 20 cm × 20 cm at 75% NPK/ha	89.25	7.07	18.73
2. 20 cm × 20 cm at 100% NPK /ha	92.34	7.74	20.88
3. 20 cm × 20 cm at 125% NPK/ha	94.98	8.87	21.12
4. 25 cm × 25 cm at 75% NPK/ha	90.44	8.07	19.77
5. 25 cm × 25 cm at 100% NPK/ha	93.13	8.80	21.58
6. 25 cm × 25 cm at 125% NPK/ha	98.44	9.39	22.17
7. 30 cm × 30 cm at 75% NPK/ha	91.69	8.48	20.47
8. 30 cm × 30 cm at 100% NPK/ha	96.57	9.40	22.72
9. 30 cm × 30 cm at 125% NPK/ha	101.23	10.27	23.11
F test	S	S	S
S. EM ±	2.28	0.49	0.82
CD (P = 0.05)	6.85	1.47	2.47

Table II- Effect of planting geometry and fertilizer levels on yield attributes and yield of finger millet

Treatment	Productive Tillers/ hill	No. of fingers /earhead	No. of grains /earhead	Length of Finger (cm)	Test Weight (g)
1. 20 cm × 20 cm at 75% NPK/ha	5.56	4.57	1633	7.50	2.27
2. 20 cm × 20 cm at 100% NPK /ha	6.37	4.93	1692	8.01	2.19
3. 20 cm × 20 cm at 125% NPK/ha	7.21	5.67	1839	9.09	2.11
4. 25 cm × 25 cm at 75% NPK/ha	6.00	4.87	1638	7.80	2.68
5. 25 cm × 25 cm at 100% NPK/ha	7.78	5.07	1713	8.40	2.57
6. 25 cm × 25 cm at 125% NPK/ha	8.25	6.10	1878	9.50	2.19
7. 30 cm × 30 cm at 75% NPK/ha	7.03	4.90	1687	7.93	2.92
8. 30 cm × 30 cm at 100% NPK/ha	8.79	5.33	1821	8.62	2.84
9. 30 cm × 30 cm at 125% NPK/ha	9.10	6.51	1908	9.73	2.71
F test	S	S	S	S	NS
SEm±	0.30	0.33	64.89	0.45	0.35
CD(p=0.05)	0.92	1.00	194.5	1.35	-

Table III- Effect of planting geometry and fertilizer levels on growth of finger millet

Treatments	Grain Yield (t/ha)	Straw Yield (t/ha)	Harvest Index (%)
1. 20 cm × 20 cm at 75% NPK/ha	2.45	4.93	33.19
2. 20 cm × 20 cm at 100% NPK /ha	2.74	5.49	33.29
3. 20 cm × 20 cm at 125% NPK/ha	3.32	5.58	37.30
4. 25 cm × 25 cm at 75% NPK/ha	2.31	4.87	32.17
5. 25 cm × 25 cm at 100% NPK/ha	2.62	5.30	33.08
6. 25 cm × 25 cm at 125% NPK/ha	3.10	5.50	36.04
7. 30 cm × 30 cm at 75% NPK/ha	2.19	4.67	31.92
8. 30 cm × 30 cm at 100% NPK/ha	2.54	5.06	33.42
9. 30 cm × 30 cm at 125% NPK/ha	3.03	5.28	36.46
F test	S	S	NS
S. EM (±)	0.11	0.24	4.62
CD (P = 0.05)	0.35	0.74	-

used to test for the significance of the overall difference among treatments using the experimental data analyzed statistically by analysis of variance (ANOVA) prescribed for the design, and the conclusion was drawn at a 5% probability level. Economics of treatments was also worked out (Gomez and Gomez, 1984).

Results and Discussion

I. Growth parameters at harvest

A. Plant height (cm)

In the present observation plant height is shown in (table 1). It is a most important growth attributing character which increases with crop age. In all treatment combinations, significant differences were observed at harvest. The highest plant height (101.23 cm) was found in treatment with the application of 125% NPK + 30 cm x 30 cm (T_9), which was significantly superior among all treatments, except treatment with application 125% NPK + 25 cm x 25 cm (T_6) was found (98.44 cm) to be statically at par with (T_9). It was at par for all the spacing with 125%NPK, please elaborate and add more review to justify. The shortest plants height (89.25 cm) was associated with 75% NPK at 20 cm x 20 cm (T_1). With increased fertilizer levels, a wider spacing resulted in reduced plant competition, increased solar radiation absorption, photosynthesis, and nutrient supply, which leads to the robust growth of transplanted finger millet. This was evidenced by Prakasha *et al., (*2018).

B. Number of tillers/ hill

Data related to total number tillers is shown in (table 1). It was recorded at harvest. The maximum number of tillers (10.27) was found in treatment with the application of 125% NPK + 30 cm x 30 cm (T_9), which was significantly superior among all treatments, except treatment with application 100% NPK + 30 cm x 30 cm (T_8) and 125%NPK+ 25cm x 25cm . The lowest number of tillers (7.07) was associated with 75% NPK at 20 cm x 20 cm (T_1). Higher availability of nutrients to the plant at higher NPK levels and wider spacing resulted in good growth and development of auxiliary buds leading to higher number of tillers. Similar results were reported by Prakasha *et al*., (2018). As a result of planting in a square format at a wider spacing, there is less competition between plants in a hill and in the field, resulting in more efficient tailoring. The results found by Kewat *et al.,*(2002) and Nayak *et al.,*(2003).

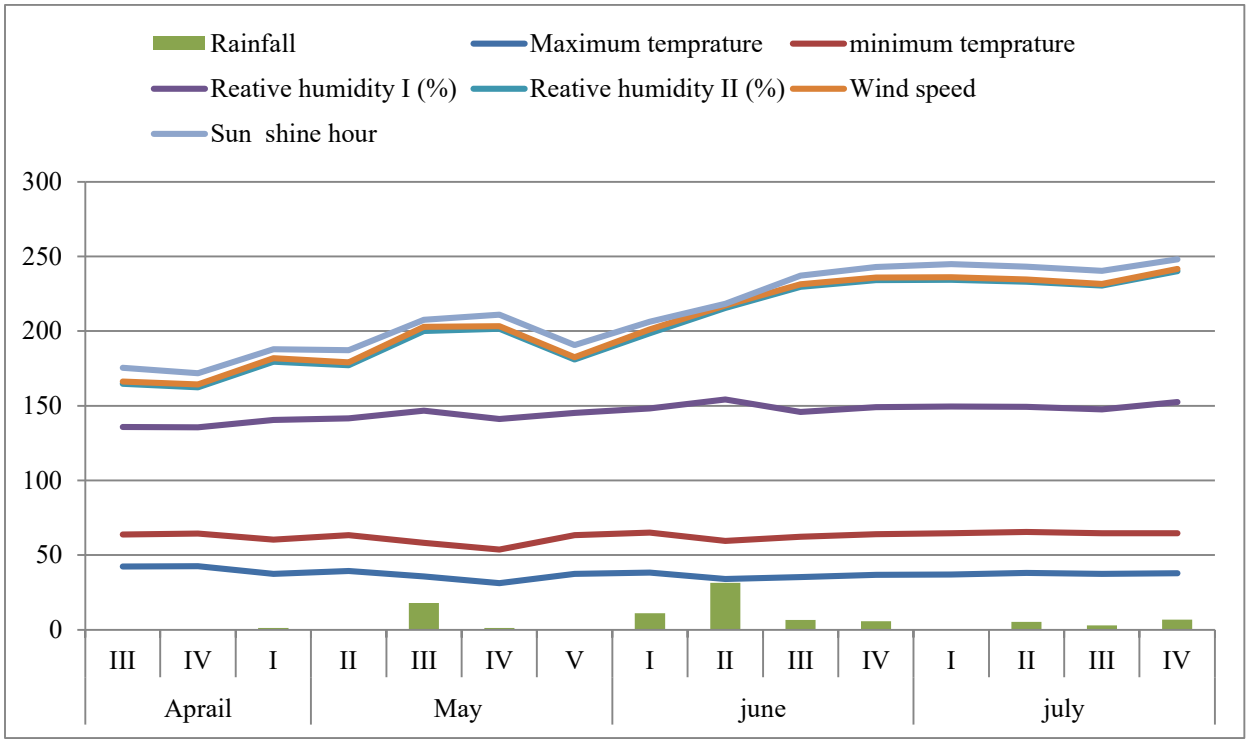

Figure: 2 Mean weekly weather parameters and total rainfall during the cropping season *Zaid* 2021

C. Dry matter Production (g)/ plant

The production of dry matter consistently increased with age of plant from seedling to vegetative stage, after this stage it starts decreasing in vegetative part of plant and accumulating in grains. It shown in (Table 1). Finger millet has the highest (23.11 g/plant) estimated dry matter production at harvest was found in treatment with the application of 125% NPK + 30 cm x 30 cm (T_9), which was significantly superior among all treatments except all spacing combinations of 100% and 125%NPK, respectively. Primary nutrients like nitrogen, phosphorus and potassium showed significant effect on dry matter producing characteristics like-number of tillers, leaves, length of leaves etc. wide spacing helps in better solar radiation penetrations and interception to plant. So, it results better dry matter accumulation in plant parts Prakesha *et al.,* (2018).

II. Yield Attribute

A. Productive tillers/hill

In tillering crops, productive tillers play important role to deciding crop yield. The number of productive tillers/ hill found during research is given in (Table 2). Planting geometry and different fertilizer levels statistically influenced the number of productive tillers/ hill in finger millet. Data recorded at harvest, maximum number of productive tillers/ hills (9.10) was recorded with application 125% NPK + 30 cm x 30 cm (T_9), which was significantly superior among all treatments, except treatment with application 100% NPK + 30 cm x 30 cm (T_8) and 125%NPK + 25 cm x 25cm (T6). The minimum productive tillers/ hill (5.56) were produced with 75% NPK + 20 cm x 20 cm (T_1). Vijay *et al.,* (2019) reported that yield attributing factor like productive tillers (7.3) found maximum in wide plant spacing and highest fertilizer levels.

B. Number of fingers/ earhead

Number of finger/ earhead differed significantly due to planting geometry and different fertilizer levels (Table 2). Among the different planting geometry and fertilizer levels, the highest number of fingers/ earhead (6.51) was recorded with 125% NPK + 30 cm x 30 cm (T_9), which was at par with all spacings of 125% NPK. The lowest number of fingers/ earhead (4.57) was produced with 75 % NPK + 20 cm x 20 cm (T_1). Kumar *et al*., (2019) reported that yield attributing traits were significantly influenced by the crop geometry and fertilizer levels, where yield attributing characters *viz*., number of fingers/ ear head was recorded the maximum with transplanting of seedlings at 50 cm ×50 cm + 100% RDF.

C. Number of grains/ earhead

Number of grains/ finger differed significantly due to planting geometry and different fertilizer levels (Table 2). The maximum number of grains/ finger (1908) was recorded with 125% NPK + 30 cm x 30 cm (T_9), which was statistically superior over other treatments, however, at par with all spacing of 125% NPK and 100% NPK+ 30 cm x 30 cm. The minimum number of grains/ finger (1633) was produced with 75% NPK + 20 cm x 20 cm (T_1). A planted area of 30 cm x 30 cm + 125% of NPK provides a favorable microclimate for crops to effectively utilize available nutrients and moisture, and early adoption of this practice results in the partitioning of photosynthesis to reproductive parts, resulting in greater productivity. Similarly, Prakasha *et al.,* (2018) reported that different nutrient levels and spacing i.e., 60 cm x 60 cm + 100% RDF found maximum number of grains/ earhead.

D. Length of finger (cm)

Length of finger differed significantly due to planting geometry and different fertilizer levels (Table 2). The maximum finger length (9.73 cm) was recorded with 30 cm x 30 cm + 125 % (T_9) which was statistically superior to all other treatments except all other spacings of 125% NPK and 25 cm x 25 cm and 30 cm x 30 cm of 100% NPK. The lowest finger length (7.50 cm) was recorded with 20 cm x 20 cm + 75 % NPK (T_1). This may be to increase in plant spacing and level of fertilizer increases the length of finger millet.

E. Test weight (g)

The results from the data revealed that no significant difference exists between the treatments on test weight (Table 2). However, the maximum test weight (2.92 g) was recorded with 30 cm x 30 cm + 75 % (T_7) and the lowest test weight (2.11 g) was recorded with 20 cm x 20 cm + 125 % (T_3). It's not affected by planting geometry and fertilizer levels because weight of seed highly influenced by genetic characters of variety.

III. Yield

A. Grain yield(t/ha)

Planting geometry and fertilizer levels played significant role increasing the grain yield of finger millet shown in (Table 3). The maximum grain yield (3.32 t/ha) was obtained with 20 cm x 20 cm + 125 % (T_3). This was statistically superior over the all other treatments. Another best treatment was 25 cm x 25 cm + 125 % (T_6) was recorded (3.10 t/ha) followed by 30 cm x 30 cm + 125 % (T_9) was recorded (3.03 t/ha). The lowest grain yield (2.19 t/ha) was recorded with 30 cm x 30 cm + 75 % (T_7). The closer spacing was most likely to have resulted in more heads and grains, as there were more plants, as opposed to a wider spacing. According to Shinggu *et al.,* (2012) narrow spacing suppresses weeds and eventually leads to increased yields. Similar results were also reported by Shinggu and Gani (2016) reported that closer inter-row spacing produced a higher number of panicles and higher grain yield at 15 cm inter-row space compared to over 20 cm; this was attributed to higher panicle numbers according to the researchers. Wider plant spacing yielded lower grain yields because total plant number per unit area was much lower than closer planting. To exploit the potential productivity of any crop, the optimal planting pattern is critical for maximizing growth resources.

B.

C. Straw yield (t/ha)

Straw yield directly influenced by planting geometry and fertilizer level shown in (Table 3). The maximum straw yield (5.58 t/ha) was obtained with 20 cm x 20 cm + 125 % (T_3). This was statistically superior over the all other treatments. Another best treatment was found with 25 cm x 25 cm + 125 % (T_6) recorded (5.50 t/ha) followed by 20 cm x 20 cm + 100 % (T_2) was recorded (5.49 t/ha). The lowest straw yield (4.93 t/ha) was recorded with 20 cm x 20 cm + 75 % (T_1). In finger millet, higher NPK level and higher plant population were likely to lead to maximum dry matter production in stems, leaves, and roots. Furthermore, positive effects are shown on leaf area index, which contributed to increased straw yield. Similar findings were reported by (Rajesh, 2011) and Kalaraju *et al.,* (2011).

D. Harvest index (%)

The results from the data revealed that significant difference did not exist between the treatments on test weight (Table 3). However, the maximum harvest index (37.30) was recorded with 30 cm x 30 cm + 75 % (T_7) and the minimum harvest index (27.42) was recorded with 30 cm x 30 cm + 125 % (T_9).

Conclusion

Optimum planting geometry and fertilizer levels shows great effect on growth and yield of finger millet. In view of the obtained results from the experiment, application of 125% NPK at 20 cm × 20 cm (T_3) produces maximum grain yield and straw yield. So, application of 125% NPK at 20 cm × 20 cm (T_3) is economically viable to the farmer. Only one season of experimentation has been conducted, so recommendations require further confirmation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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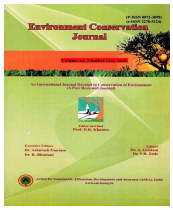

Nutritional quality evaluation of oil and fatty acid profile in various genotypes/varieties of Indian mustard [*Brassica juncea* Czern & Coss (L.)]

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ABSTRACT

Brassicas are one of the most agronomically eminent oilseeds that are employed as a variety of oilseed, vegetable, and fodder crops. The experiment was conducted with thirty-four genotypes/varieties seeds of Indian mustard [*Brassica juncea* Czern & Coss (L.)] for oil content, Iodine value, oil stability index and fatty acid composition during 2018-2019. The experiment was laid out in completely randomized design with three replications. The range of variability of contents of oil, palmitate, stearate, oleate, linoleate, linolenate, ecosenate, doecosenate, iodine value and oil stability index varied from 33.52 to 42.15%, 1.53 to 4.98%, 0.16 to 2.71%, 5.06 to 17.78%, 17.88 to 32.15%, 11.82 to 19.85%, 5.44 to 11.89%, 28.82 to 47.66%, 114.43 to 131.71 and 1.08 to 1.99, respectively. The *Brassica juncea* genotype-KMR-15-6 followed by genotype-KMR-17-6 had the higher oil content, oleic acid content and low value of erucic acid which indicates that seed oil this *B. juncea* species genotype is possibly suitable for both human consumption and industrial purposes.

Introduction

Oilseed crops have long held a prominent position in human nutrition, as they continue to be the major source of calories and proteins for a substantial section of the world's population. Groundnut, soybean, palm kernel, cotton seed, olive seed, sunflower seed, rapeseed mustard, sesame seed, linseed, and safflower seed are some of the most well-known oilseeds or conventional oilseeds. (Ajala *et al.,* 2014; Aremu *et al.,* 2006). *Brassica juncea*, a member of the crucifereae family of oilseeds, is the world's third largest source of edible vegetable oils, after soyabean and palm oils. Its seeds have a high energy level, with oil content ranging from 28 to 32 percent and protein content ranging from 28 to 36 percent. It contributes over 27 percent of the country's edible oil and accounts for over 30 percent of total production (Sutariya *et al.,* 2011). The fatty acid profile of mustard oil, which includes palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, ecosenoic acid, and erucic acid, determines its nutritional properties. Essential fatty acids with a content of less than 3 percent linolenic acid generally preferred for oil stability. Linolenic acid is necessary for growth and development, but it also shortens the shelf life of oil due to auto-oxidation, which causes an off flavour (Priyamedha *et al.,* 2014). Edible oil containing more than 2% erucic acid is not recommended for human consumption since it causes myocardial infarction and high blood cholesterol, although mustard oil contains a larger percentage of erucic acid, making it industrially

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essential.Saturated fatty acids (SFAs) include palmitic acid and stearic acid, unsaturated fatty acids (MUFAs) include erucic acid, eicosenoic acid, and oleic acid, as well as polyunsaturated fatty acids (PUFAs) like omega-3 alpha-linolenic acid and omega-6 linoleic acid, that are nutritionally important. Conversion interfering factors for Alpha linolenic Acid (ALA) to Eicosapentaenoic Acid (EPA) and Docasahexaenoic Acid (DHA) provide a therapeutic approach in major depressive disorder. Omega-3 fatty acids play a key role in the development and function of the central nervous system. Cardioprotective properties, regulation of the inflammatory response, and a favourable impact on both central nervous system function and behaviour are among potential benefits of ALA (Reifen *et al.,* 2008). Linoleic acid is the most commonly ingested PUFA in the human diet, and while it is required for normal metabolic activities, it has been linked to inflammation and cancer when produced in excess (Fritsche *et al.,* 2013). Erucic acid in high concentrations is not recommended for human ingestion because it has been associated to cardiac lipidosis (Wendlinger*et al.,* 2014).

Material and Methods

The experiment consisting of thirty-four genotypes/varieties was carried out in Completely Randomized Design (CRD) with three replications of mustard at Oilseed Research Farm, Kalyanpur from C.S. Azad University and Technology, Kanpur under uniform agronomic conditions (80 kg N+60 kg P_2O_5+60 kg K_2O/ha) during Rabi season. The crop was irrigated two times. After harvesting seeds were sun-dried followed by oven dried before chemical analysis. The Oil content in seed was determined by soxhlet extraction apparatus by using petroleum ether of boiling point ranged between 40-60°C as described by Anonymous (2020).The oil was cooled and weighed and oil percentage was then calculated. The methyl esters of their fatty acids were prepared by the procedure of Luddy *et al.* (1968). The methyl ester was analyzed on CIC-Model Gas chromatograph using FID. The column used for fatty acid analysis was 2m x 2mm stainless steel packed with 15% DEGS on chromosorb-W (80-100 Mesh). The injector and oven temperature were maintained at 248°C and 190°C, respectively. The fatty acid composition was calculated by measuring the area under each peak by triangulation method. The iodine value was determined by method described by Jamieson (1943). Oil Stability Value was measured by procedure given by Carpenter *et al*. (1976). The statistical analysis was done by method suggested by Rangaswamy (2016).

Results and Discussion

Triglycerides content -Significant variation in oil content was noted among genotypes/varieties of Indian mustard (Table 1) that the range of variation of oil content recorded from 33.52-42.15%. The lowest and highest values of oil content were observed in genotype-KMR-17-5 and variety-Varuna, respectively. The genotypes-TM-108, TM-179 and varieties-Rohini, Urvashi exhibited 40.06%, 40.16%, 40.18% and 40.39% of oil content which was statistically at par result with Varuna. The mean oil content was recorded as 37.765% which was similar in the result of Dar *et al*. (2011), Abul-Fadl *et al*. (2011), Gupta *et al*. (2011), Singh *et al*. (2011), and Ahmad *et al*. (2012), who revealed a significant difference in oil quality traits of Indian mustard, including oil content.

Saturated fatty acid content- It is obvious from the data as shown (Table 1) that there was a significant variation in stearic acid in the Indian mustard. The highest stearic acid was statistically observed in genotype- KMR-16-6 (2.71%) and lowest was recorded in genotype-KMR-16-302 (0.16%). The similar finding exhibited closed conformity with the results of Singh *et al*., 2007 reported the range of variation of stearic acid content in Indian mustard was found to be 0.22-2.40%. Dietary stearic acid is critical for regulating mitochondrial shape and function in humans *In vivo* via a specialized signaling pathway (Senyilmaz *et al.,* 2015). In healthy guys, it aids in the improvement of thrombogenic and atherogenic risk factor profiles (Kelly *et al.,* 2001).

The data shown on palmitic acid (Table 1) of Indian mustard indicated that the variability of palmitic acid content ranged from 1.53-4.98%. The maximum and minimum values of palmitic acid were shown by genotype-KMR-16-304 and genotype-KMR-18-402, respectively. Statistically, almost similar values of palmitic acid was noted in genotypes-KMR-18-403 and TM-179.The similar

Table 1: Oil content and fatty acid profile in Indian mustard (*B. juncea*) of certain promising genotypes/varieties.

SN	Genotypes/ Varieties	Oil (%)	Fatty acid profile (%)							I.V.	O.S.I.
			16:0	18:0	18:1	18:2	18:3	20:1	22:1		
1.	TM-117	39.22	3.79	0.59	11.13	31.37	14.43	5.91	32.60	129.35	1.08
2.	TM-108	40.06	3.23	0.33	9.84	19.0	13.78	5.89	47.66	115.77	1.93
3.	TM-106	39.85	2.95	0.49	10.10	32.15	14.62	5.66	33.93	131.71	1.06
4.	TM-108-1	38.98	2.32	0.55	9.74	26.73	14.94	5.44	40.13	126.38	1.32
5.	TM-179	40.16	4.20	0.21	11.51	22.89	15.69	10.43	34.77	122.91	1.46
6.	Varuna	42.15	2.79	0.61	10.80	20.87	13.95	7.21	43.73	118.40	1.77
7.	Rohini	40.18	1.92	0.36	10.51	21.32	19.64	5.90	40.15	130.30	1.46
8.	Urvashi	40.39	3.69	0.29	8.84	18.43	17.31	7.80	43.52	121.56	1.68
9.	KMR-17-5	33.52	2.40	0.69	14.05	26.02	13.83	6.97	33.94	122.60	1.37
10.	KMR-17-6	35.11	3.23	0.38	17.78	23.99	15.92	9.83	28.82	126.12	1.41
11.	KMR-16-5	36.19	2.42	0.59	15.05	23.80	16.82	6.96	34.20	127.3	1.38
12.	KMR-16-6	35.12	1.66	2.71	11.32	20.18	13.62	9.90	40.49	121.56	1.82
13.	KMR-15-5	33.78	2.61	0.64	14.05	26.02	15.83	6.97	33.74	127.70	1.30
14.	KMR-15-6	35.79	3.23	0.38	17.78	23.99	15.92	9.83	28.82	126.12	1.41
15.	KMR-18-401	38.88	2.00	0.64	10.59	18.65	15.90	9.96	42.96	118.75	1.88
16.	KMR-18-402	36.51	4.98	0.59	5.06	26.46	16.71	7.68	38.51	126.95	1.17
17.	KMR-18-403	38.12	4.45	0.48	14.04	25.04	14.57	6.75	34.53	123.10	1.39
18.	KMR-18-404	38.25	2.75	0.43	12.34	20.60	11.82	10.54	41.50	114.43	1.98
19.	KMR-18-405	39.13	3.00	0.82	12.64	20.28	15.55	11.64	35.95	131.37	1.68
20.	KMR-18-406	36.56	3.87	0.40	9.02	17.88	14.42	8.48	45.82	115.06	1.96
21.	KMR-18-407	37.15	3.09	0.27	10.86	21.04	16.50	11.89	36.31	123.41	1.57
22.	KMR-18-408	39.69	4.01	0.46	13.08	25.36	14.64	5.30	37.11	123.84	1.38
23.	KMR-18-409	38.11	2.84	0.68	13.49	22.03	13.61	11.15	36.13	119.17	1.70
24.	KMR-18-410	36.12	2.94	0.38	9.11	19.65	14.23	8.70	44.98	117.51	1.83
25.	KMR-16-301	38.16	2.97	0.68	12.23	20.02	13.77	9.41	40.85	117.18	1.84
26.	KMR-16-302	38.16	3.97	0.16	12.97	19.38	13.89	10.94	38.41	116.35	1.87
27.	KMR-16-303	35.23	3.60	0.49	12.20	22.43	15.04	5.77	40.16	121.58	1.55
28.	KMR-16-304	33.72	1.53	0.31	5.24	18.55	19.85	9.64	44.34	127.21	1.54
29.	KMR-16-305	35.23	2.27	0.51	12.68	20.55	13.80	10.71	39.31	118.37	1.82
30.	KMR-16-306	38.11	2.37	0.75	11.91	25.39	15.21	8.96	35.71	125.96	1.39
31.	KMR-16-307	36.25	3.34	0.56	15.81	26.27	17.29	5.70	30.47	130.22	1.19
32.	KMR-16-308	39.10	3.70	0.33	13.28	18.84	13.18	9.90	40.69	114.72	1.99
33.	Ashirwad	39.68	3.30	0.87	13.98	24.57	15.20	8.00	34.30	124.69	1.42
34.	Vardan	38.19	2.85	0.30	10.28	22.08	15.83	9.23	35.89	120.80	1.47
	Mean	37.76	3.06	0.55	11.86	22.70	15.21	8.38	37.95	122.89	1.56
	C.D. at 5%	2.22	0.18	0.04	0.71	1.35	0.90	0.50	2.25	7.24	0.09
	S.E.(±)	0.78	0.06	0.01	0.25	0.47	0.31	0.17	0.79	2.56	0.03

Note: 16:0 = Palmitic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, 18:3 = Linolenic acid, 20:1 = Eicosenoic acid, 22:1 = Erucic acid, I.V. = Iodine value, O.S.I. = Oil stability index

findings were also reported by some scientists such as Singh *et al*., 2007 and Rai *et al*., 2018 reported the variability of palmitic acid was found between range of 1.5-7.35 % and 3.08-3.85%, respectively. Palmitate has a broad range of biological activities at the cellular and tissue levels, and its steady content is ensured by its endogenous manufacture via de novo lipogenesis (Crta *et al.,* 2017). Monounsaturated fatty acid content-The result obtained on oleic acid content in genotypes/varieties of Indian mustard (Table 1) expressed that the oleic acid varied 5.06-17.78%. Genotypes-KMR-18-402 showed lowest content of oleic acid while genotypes-KMR-15-6 and KMR-

17-6 gave highest oleic acid content. Oleic acid content exhibited significant variability, the similar findings was also reported Chauhan And Kumar (2011) and Singh *et al*. (2007) who were reported the concentration of oleic acid in Indian mustard oil, a beneficial fatty acid ranged from 3.6-32.02% and 12.88-19.04%, respectively. Oleic acid, a major component of the Mediterranean diet, is thought to have a wide range of physiological effects, including a protective effect against cancer, autoimmune and inflammatory disease, and the ability to aid wound healing, metabolic disturbances, and skin injury and prominent role in drug absorption (Sales-Campos *et al.* 2013).

The data displayed on eicosenoic acid (Table 1) in genotypes/varieties of Indian mustard recorded that the variability of ecosenoic acid content ranged from 5.44-11.89% .The highest percentage of ecosenoic acid was recorded in genotype-KMR-18-407 and the lowest percentage of ecosenoic acid was recorded in genotype-TM-108-1.The similar findings were also reported by Chowdhury *et al*. (2010)reported that eicosanoic acid were present in small amount in mustard oil as compared to oleic and linoleic acid. Singh *et al.* (2007) showed the percentage variation of eicosenoic acid in mustard oil ranged from 4.89-12.99%. Ecosenoic acid, a precursor to prostaglandin, inhibits lipolysis and the conversion of ATP to cyclic-AMP in adipose tissues, *Brassica juncea* contains a higher concentration of this acid, which is hazardous to human health by Bergstrom S. (1966).

Erucic acid is a minor fatty acid, but it is vital for industrial uses such as lubricants, rubber manufacturing, additives, and oil paints. Results shown on erucic acid (Table 1) in genotypes/varieties of Indian mustard revealed that the value of range of variation of erucic acid was from 28.82-47.66%. The maximum value of erucic acid was exhibited in genotype-TM-108 and minimum value of erucic acid was found in two genotypes like KMR-17-6 and KMR-15-6. The genotype-KMR-18-406 is best for industrial purposes while genotype-KMR-16-307 is significantly best for nutrition point of view. The results achieved in the experimental findings were accordance with similar findings of Sawicka *et al*. (2020), Sharafi *et al*. (2015) who were reported the contents of erucic acid ranged from 41-46%. Similar finding were also reported by Abul-Fadl *et al.* (2011) and Singh *et al.* (2007) who were shown the contents of erucic acid was from 23.90- 37.89% and 41.36-53.50%, respectively. Some workers have reported that, by and large, due to higher intake of mustard oil having higher concentration more than 40% erucic acid may be cause of health problems like absorption, lipidosis in children and neurocardial fibrosis in adults Nolew (1981), Fatemi *et al*. (1980).

Polyunsaturated fatty acid-The data shown on linoleic acid (Table 1) indicated that the values ranged from 17.88-32.15%. The linoleic acid was highest in genotype-TM-106 obtaining 32.15% and lowest in genotype-KMR-18-406 having 17.88%. Genotype- TM-117 gave significantly 31.37% linoleic acid. The similar findings were also reported by the some workers such as Abul-Fadl *et al.* (2011)and Singh *et al.* (2007) who were reported the range of linoleic acid was from 12.37 to 21.36% and 10.51 to 18.00%.Sharafi *et al*. (2015)reported the linoleic acid content was 19% in mustard oil. It has a significant impact on human health, particularly in the prevention of cardiovascular disease (DVD), coronary heart disease, and cancer; as well as inflammatory, hypertension, diabetes type II, renal diseases, and Crohn's disease (Abedi *et al.,* 2014).

Mustard oil composition is rich in alpha-linolenic acid is the major fatty acid that has shown to exhibit anti-inflammatory properties and formation of prostaglandins which is essential for growth and development. The results of linolenic acid (Table 1) revealed that the range of variability from 11.82-19.85%. The maximum value was noted in genotype-KMR-16-304 and lowest was found in genotype-KMR-18-404. The similar findings were also reported by Singh *et al*. (2007) who revealed the percentage of linoleic acid was 4.92-15.02%. Kaushik and Agnihotri (2000)reported the range of linolenic acid was 11 to 20% in *Brassica juncea* L. The highest content of linolenic acid was 20% in *Brassica juncea* reported by Sharafi *et al*. (2015).

Iodine value- The iodine value indicates the degree of unsaturation of fat or oil. The data shown on iodine value (Table 1) indicated that the range of variation of iodine value was 114.43-131.71%. . The highest iodine value was obtained in genotype-TM-106 and lowest iodine value was found in

Table 2: Correlation studies among the various characteristic in Indian mustard of certain promising genotypes/varieties.

Characters	Oil content	16:0	18:0	18:1	18:2	18:3	20:1	22:1	I.V.
16:0	0.256711	-	-	-	-	-	-	-	-
18:0	-0.19862	-0.36992	-	-	-	-	-	-	-
18:1	-0.16531	0.028436	0.049648	-	-	-	-	-	-
18:2	-0.01371	0.191114	0.031303	0.211161	-	-	-	-	-
18:3	-0.13372	-0.17347	-0.21574	-0.23283	0.015309	-	-	-	-
20:1	-0.14053	-0.1264	0.058167	0.071125	-0.5491	-0.15107	-	-	-
22:1	0.217733	-0.16655	-0.01028	-0.6948	-0.68415	-0.12431	0.022235	-	-
I.V.	-0.09673	-0.09633	0.079784	0.091454	0.670959	0.624766	-0.35534	-0.61215	-
O.S.I.	0.108355	-0.09633	0.055743	-0.11423	-0.89598*	-0.42741	0.532041	0.703588	-0.85188*

Note: * = Significant, 16:0 = Palmitic acid,18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, 18:3 = Linolenic acid, 20:1 = Eicosenoic acid, 22:1 = Erucic acid, I.V. = Iodine value, O.S.I. = Oil stability index.

genotype-KMR-18-403.Variety-Ashirwad, Rohini and genotypes- KMR-16-307, KMR-16-306, KMR-16-304, KMR-18-402, KMR-15-6, KMR-15-5, KMR-16-5, KMR-17-6, TM-108-1, TM-106, TM-117 varied significantly. The range of variability of Iodine value of Indian mustard was almost conformity with the findings of Valentine *et al*. (2014) who reported the iodine value ranged from 102±0.07 to 113.8±0.01 in mustard oil. Sharifi *et al.* (2017) showed the iodine values were in BARI Sarisha-15 (72.50), BARI Sarisha-16 (70.60) and BARI Sarisha-17 (73.44).The oil stability index represents the storage quality of vegetable oils. The long storage quality is most beneficial for use of oils. Results represented (Table 1) on oil stability index indicated that the range of variation on oil stability index in Indian mustard oil was varied from 1.08-1.99. Genotype-KMR-16-308 gave the maximum value of OSI and genotype- TM-117 gave the minimum value of OSI. Genotypes-TM-108, KMR-18-404 showed significantly higher amount of oil stability index. The results of oil stability index was conformity with the findings of Chauhan *et al.* (2010)who were reported the oil stability index was 3.19 in variety-Parbati of Toria. Guimaraes *et al.* (2013)were also found the oil stability index was 0.79 in sesame oil and 5.25 in flaxseed oil.Correlation coefficient studies- The iodine value was inversely correlated with oil content, palmitic acid, erucic acid, and eicosenoic acid in genotypes/varieties of Indian mustard (Table 2). However, oil stability index was significantly negative correlated with linoleic acid and positively significant correlated with iodine value. Stearic acid was a negative and non-significant correlated with oil content and palmitic acid. Oleic acid and linoleic acid were non-significantly and negative correlated with oil content.

Linolenic acid was negatively and non-significantly correlated with oil content, palmitic acid, stearic acid and oleic acid, respectively. However linoleic acid was positive and non-significantly correlated with palmitate, stearate and oleate, respectively. Eicosenoic acid was non-significantly and negative correlated with oil content, palmitate, linolenate and linoleate, respectively. Erucic acid was a negative and non-significant correlation with palmitate, stearate, oleate, linolenate but it was positive or non-significant correlation with eicosenoic acid and oil content (Katavic *et al.,* 2001) and (Sasongko *et al.,* 2005) reported a significant negative correlation among oleic acid with erucic acid and linoleic acid. The non-significant correlation between oleic acid and linoleic was observed. Two separate biosynthetic pathways, which are genetically independent, are responsible for results on which converts oleic acid to linoleic acid and others which converts oleic to ecosenoic acid and ecosenoic acid to erucic acid (Krzymanski *et al.,* 1969).

Conclusion

The overall assessment of the selected genotypes/varieties of *Brassica juncea* was made on the basis of nutritional parameters and noted that, the genotype-KMR-15-6 followed by genotype- KMR-17-6 had the higher oil content, oleic acid content and low value of erucic acid alongwith the medium value of linoleic acid,

linolenic acid, palmitic acid, stearic acid and eicosenoic acid. Consequently, when compared to other genotypes/varieties, genotype-KMR-16-308 had a high OSI.

Conflict of interest

The authors declare that they have no conflict of interest.

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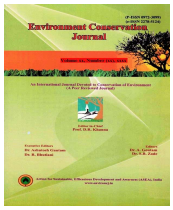

Analysis of pharmacognostical standardization, antioxidant capacity and separation of phytocompounds from five different vegetable peels using different solvents

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ABSTRACT

Vegetables are one of the most preferred food commodities and can be consumed either raw or as processed due to their health-promoting nutrients. In the present work, analysis of pharmacognostical standards, antioxidant capacity, and separation of phytocompounds through thin layer chromatography (TLC) from cabbage, cauliflower, pea, carrot, and potato peels were carried out. Microscopic analysis revealed the presence of wood fibers, trichomes, crystals, and annular xylem vessels in the vegetable peels. Physicochemical analysis showed that all the vegetable peel samples which were analysed have low (7.08%-10%) moisture content. The total ash content of vegetable peels varied in cauliflower peels (1.95±0.58) to the peels of pea (19.86±1.9). The content of acid insoluble ash varied from 1.46±0.63 to 3.09±0.59 in cauliflower and pea. Potato peel has the lowest water-soluble ash content (1.16±1.90) as compared to other peels. The highest pH value was found in the peels of pea (7), while the lowest pH was found in the peels of cabbage (4). Among all extracts, the petroleum ether extract has shown the greatest yield (5.6±0.45). The fluorescence analysis showed various colours like green, brown, pale green, and yellow under different chemical treatments. Different types of pri-secondary metabolites were detected in small, moderate, and high amounts and notified to provide numerous health benefits to humans. In case of DPPH assay, aqueous extract of cauliflower has shown the low value of IC_{50} (24.82 µg/ml) in comparison to standard, suggested the higher antioxidant activity of the extract. Among all the extracts, aqueous and methanol extracts of cauliflower have shown the better reducing and total antioxidant activity in comparison to standard. TLC profiling of methanolic extract of cabbage and cauliflower peels revealed the presence of different compounds of varying R_f values. Above results indicate that the food waste consists of valuable components and may be utilized as noticeable and cheap source in pharmaceuticals for the treatment of several life-threatening diseases.

Introduction

Due to the change in diet habits and increasing population, processing and production of horticultural crops, mainly vegetables, have been remarkably noticed as growing tool to fulfil the demands (Schreinemachers *et al*., 2018). Several studies suggested that vegetables are low in calories and rich in selected minerals, antioxidants, and fibres. It has been found in some of the researches that vegetables are rich in potassium and have relatively low sodium content. Due to all these

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amazing benefits, vegetables hold a unique contribution in a healthy diet (Chauhan *et al*., 2021). Recent studies suggest that diseases like gastric cancers and cardiac problems are protected in a better way by including vegetables in our diet. The anti-inflammatory and antiproliferative capacities of the phytochemicals and antioxidants makes them effective for the prevention of cancer and inflammation (Gazdik *et al*., 2008; Sarkar *et al*., 2022). The phytochemicals present in vegetable peels can also be utilized as food additives, colouring agents, biopesticides, fragrances, flavours, agrochemicals, and pharmaceuticals (Saha *et al*., 2012). But now a days, the scenario is changing and the agroindustrial wastes, mainly the vegetable peels, have started gaining more attention than previous days because they have potential to provide multiple benefits to the society in the field of medicine. However, the main obstacle, which has stopped the promotion of uses of vegetable peels in the developed nations, is no proper evidence of documentation. Therefore, the aim of present study was to evaluate the pharmacognostical standards, antioxidant activity, and presence of different bioactive phytoconstituents of the five different vegetable peels.

Material and Methods

Collection of plant materials

Vegetables including Cabbage (*Brassica oleracia* var. Capitata), Cauliflower (*Brassica oleracea* var. Botrytis), Pea (*Pisum sativum)*, Carrot (*Daucus carota* subsp. Sativus) and Potato (*Solanum tuberosum* L.) were obtained from the local wholesale market and their inedible part such as peels were separated with a peeler or knife. Then the vegetable peels were collected, washed, and shade dried, respectively.

Preparation of plant extracts

The shade dried samples were powdered with the help of grinder. Twenty-five grams of each sample was macerated sequentially using 100 ml of different solvent [petroleum ether (PET), chloroform (CH), methanol (ME), and water (AQ)]. Each extract of vegetable peels was air dried by the help of rotatory evaporator. After drying, the extracts were kept in the desiccator for one or two days and then were kept in the air tight containers at 5˚C for further use (Sharma and Janmeda, 2017).

Organoleptic and microscopic study

Different vegetable peel samples were examined morphologically and various microscopic characters were determined after the staining of samples as described by Janmeda and Sharma (2013).

Physicochemical analysis

Physicochemical parameters such as moisture content, total ash content, acid insoluble ash content, water soluble ash content, pH of 1% and 10% solution and extractive value were determined by the method of Mushtaq *et al*. (2014). Fluorescence was observed at different wavelengths of UV-Visible light as reported by Sharma and Janmeda (2013).

Phytochemical evaluation

Different phytochemicals from the different samples of vegetable peels were determined by using the standard methods (Saxena *et al.,* 2013; Banu and Cathrine, 2015).

Determination of *in vitro* antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl scavenging activity (DPPH)

DPPH assay was determined by using the protocol of Chaudhary and Janmeda (2022) with slight modifications. To one ml (0.2-0.5 mg/ml) of sample and ascorbic acid (standard), 4 ml of DPPH solution (25 mg/ml) which was prepared in methanol was added. The solutions were shaken and allowed to incubate in dark for 30 min. After 30 min, the absorbance of the solution was recorded at 517 nm using methanol as blank by the help of the below mentioned formulae:

$$Inhibition\ concentration\ (\%) = \left(Absorbance\ of\ control - \left(Absorbance\ of \frac{test\ sample}{Absorbance} of\ control \right) \right) \times 100$$

Ferric reducing antioxidant power (FRAP)

The reducing power of a sample was determined by using the FRAP assay (Benzie and Strain, 1999). Briefly, the FRAP reagent was prepared by mixing the acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). A potential antioxidant can reduce the ferric ion to the ferrous ion and resulted in the formation of blue coloured complex, whose absorbance was increased at 593 nm.

Total antioxidant capacity determination (TAC)

Phosphomolybdate method was applied to determine the total antioxidant capacity (TAC) of

the different samples (Prieto *et al.,* 1999). An aliquot of 0.4 ml (mg/ml) of each sample was mixed with 4 ml of reagent (4 mM ammonium molybdate, 0.6 M sulphuric acid, 28 mM sodium phosphate) solution. Then the mixture was shaken and was incubated at 95°C for 90 min in the water bath. Finally, the absorbance of the sample was recorded at 765 nm against the blank sample.

Thin layer chromatography (TLC)

TLC of the selected extracts was carried out with various solvent phase by using silica gel. For TLC analysis, Silica gel 60 F254 TLC (Merck, Germany) plates were utilized. The marking on the plate were made with the help of soft pencil. Glass capillaries were utilized to load the 1-µl of sample on TLC plate and then the plate was allowed to run in the presence of different solvent system. When the solvents reached to a certain height on the TLC plates, we removed the plate from the TLC chamber and allowed it to dry. Then the bands were observed in iodine chamber and on UV transilluminator. The movement of the analyte was expressed by its retention factor (R_f) values which was calculated by the help of below mentioned formulae (Gujjeti and Mamidala, 2013).

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent from TLC plates}}$$

Where, R_f is retention factor

Statistical analysis

All the assays and test were performed in triplicates and their outcomes were expressed as mean ± standard deviation (SD).

Results and Discussion

Organoleptic evaluation

Organoleptic evaluation and characteristic features of powdered drug of all five samples of vegetable peels are listed in Table 1 and Figure 1. The quality of vegetable peels mainly comprises of five primary attributes, 1) taste, 2) odour, 3) adulteration, 4) colour, 5) texture and the examination of these primary characteristics is generally very useful in the development of new products and in determining the product standards (Shewfelt, 1993).

Powder microscopic analysis

Powder microscopy is used to determine the specific microscopic characters after staining it with different staining solutions. The adulterants can be detected by doing a comparative study with authenticated sample (Amponsah *et al*., 2014).

Cabbage peel

The very fine powder of vegetable peels was mounted in glycerine and was stained with iodine and phloroglucinol. Microscopic analysis revealed the presence of wood fibers, trichomes, crystals, and annular xylem vessels from the cabbage peel as shown in Figure 2.

Cauliflower Peel

Powder microscopic analysis of cauliflower peel revealed the presence of different types of fibers and crystals as shown in Figure 3.

Pea Peel

The powder of pea peel shows the presence of fibers, wood fibers, different types of crystal, and xylem vessels as shown in Figure 4.

Carrot Peel

The microscopic analysis of powder revealed the presence of parenchyma, fibers, trichomes, and calcium oxalate crystals from the carrot peel as shown in Figure 5.

Potato Peel

Powder microscopic analysis revealed the presence of wood fibers, simple fibers, trichome, crystals, and spiral xylem vessel as shown in Figure 6.

Physicochemical properties of different vegetable peels

The physicochemical parameters of five different vegetable peels were determined in order to detect any type of adulteration and improper handling of plant material. Lower content of moisture represents the higher stability and less chances of microbial growth that ultimately increases the shelf life of product (Alam and us Saqib, 2015).

Results showed that all the vegetable peel samples which were analysed have low moisture content as shown in Table 2. One of the other parameters is ash content that gives information regarding the presence of organic, inorganic and any other impurities in the sample (Alam and us Saqib, 2015). The total ash content of vegetable peels varied from 1.95±0.58 in cauliflower peels to 19.86±1.9 in the peels of pea as shown in Table 2. The results of acid insoluble (Table 2) and water soluble ash content (Table 2) of different vegetable

Table 1: Organoleptic Characters of different vegetable peel powder

Samples	Taste	Odour	Adulteration	Colour	Texture
Cabbage	Bitter	Characteristic	Nil	Moss green	Rough
Cauliflower	Sweet and Sour	Characteristic	Nil	Bronze	Rough
Pea	Bitter	Characteristic	Nil	Moss green	Rough
Carrot	Sour	Characteristic	Nil	Sage green	Fibrous
Potato	Sour	Characteristic	Nil	Cider colour	Smooth
Powder drug	Cabbage	Cauliflower	Pea	Carrot	Potato
Color	Brown	Brown	Light brown	Dark brown	Brown
Odour	Characteristic				

Figure 1: Morphological features and characteristics of cabbage, cauliflower, pea, carrot, and potato vegetable peels.

peels as obtained in this study do not favor the results obtained by Parmeswaran and Murthi (2014). According to some guidelines, the adsorption, distribution, metabolism, excretion, and toxicity (ADMET) are greatly affected by the varying pH conditions. The acceptable pH value of trees, grasses, vegetables, and fruits is 4.0-7.5 (Prakash *et al*., 2019). The pH values observed in the present study were between 4 and 7 (Table 2). The pH values obtained are quite similar with those obtained by Nasreen and Qazi (2012).

Determination of extractive values

Extractive values were found to be useful in evaluating the chemical constituents and solubility of that specific constituents in particular solvent (Gupta *et al*., 2012). The percentage yields of PET, CH, ME, and AQ extracts of different vegetable peel samples are presented in Table 3 and in Figure 7.

Fluorescence analysis of different vegetable peel powder

The fluorescence analysis is utilized as a tool to determine the constituent and chemical nature of the herbal drug. The observations of fluorescence analysis of cabbage, cauliflower, pea, carrot, and potato are presented in Table 4, and 5.

Phytochemical screening

The phytochemical screening of different extracts of vegetable peels is shown in Table 6, and 7. This screening helps in determining the presence of various pharmacologically active compounds (Pandiyan and Illango, 2022). The results of present study revealed that protein, carbohydrate, cardiac glycosides, steroids, terpenoids, fats and oils are present in these vegetable peels. These secondary metabolites help in providing the defence mechanism to plant, and in turn provide numerous health benefits to humans (Sharma *et al*., 2022).

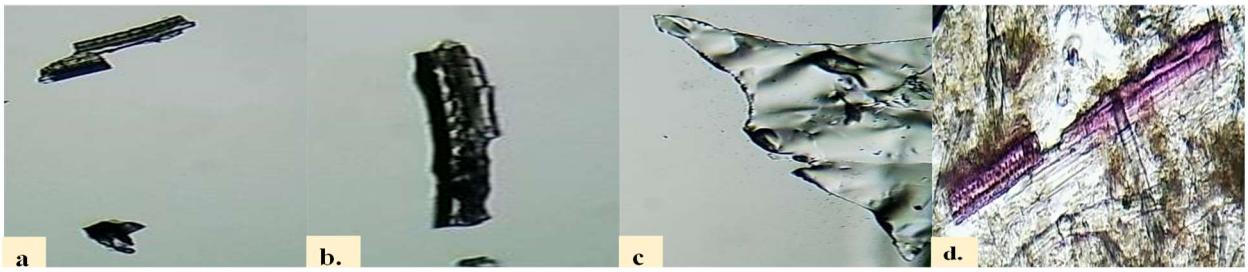

Figure 2: Powder microscopic analysis of cabbage peel. a. wood fibers, b. trichomes, c. crystals, d. annular xylem vessels.

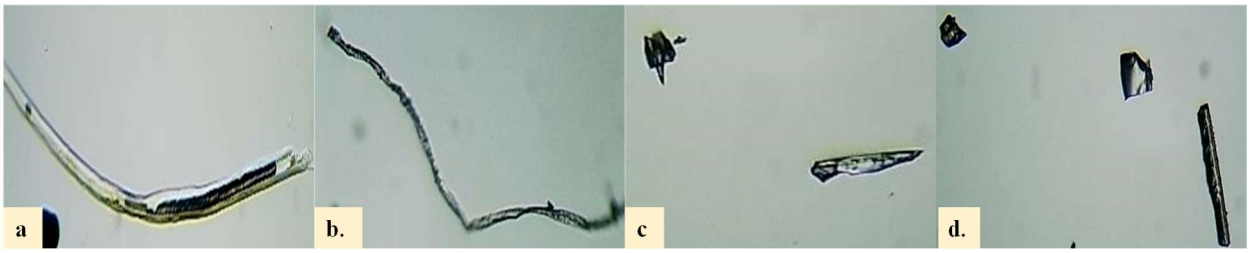

Figure 3: Powder microscopic analysis of cauliflower peel. a. fibers with lumen, b. fibers, c. & d. crystal.

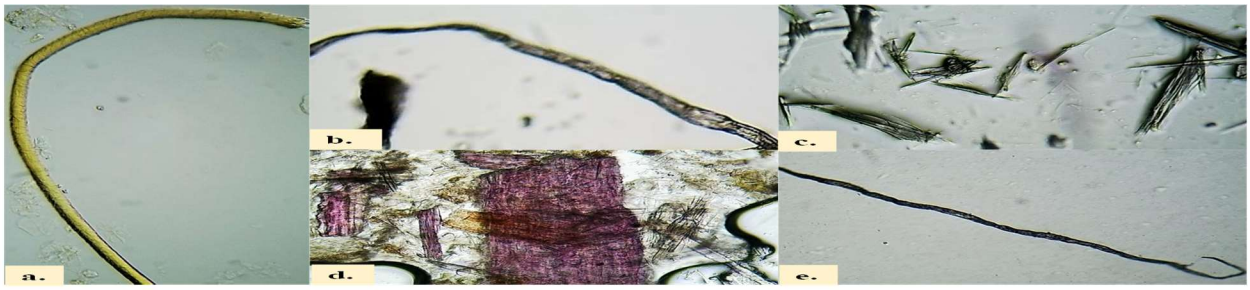

Figure 4: Powder microscopic analysis of pea peel. a. & b. fibers, c. different types of crystals, d. wood fibers, e. xylem vessels

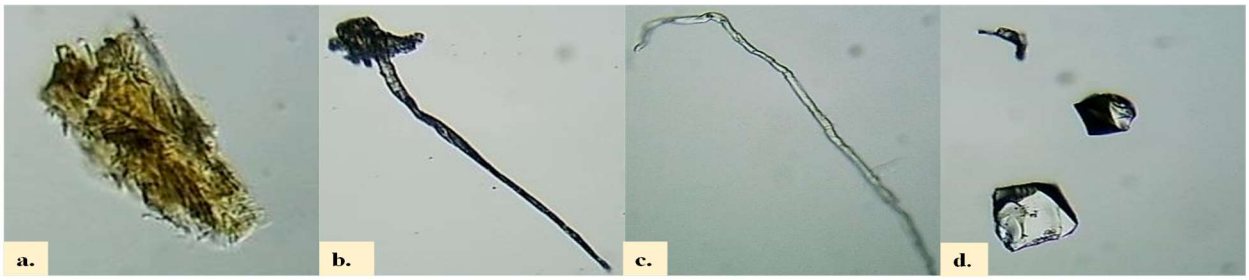

Figure 5: Powder microscopic analysis of carrot peel. a. parenchyma, b. fibers, c. trichomes, d. calcium oxalate crystal.

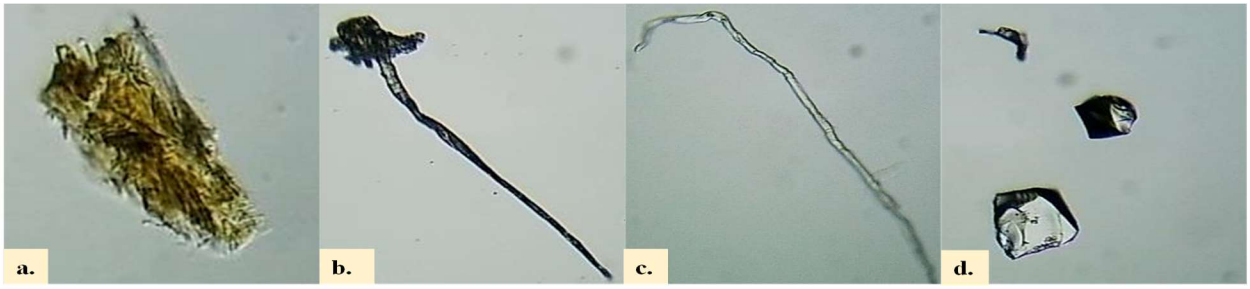

Figure 6: Powder microscopic analysis of Potato peel. a. wood fibers, b. fibers, c. trichomes, d. crystals, e. spiral xylem vessels.

Table 2: Physicochemical properties of different vegetable peels

Peel Powder	Moisture content %	Total ash content %	Acid insoluble ash content %	Water soluble ash content %	pH 1%	pH 10%
Cabbage	7.86	4.61±2.88	2.45±1.40	4.1±2.62	5.56±0.42	4.0±0.10
Cauliflower	7.52	1.95±0.58	1.46±0.63	1.81±0.51	6.36±0.20	4.9±0.10
Pea	7.08	19.86±1.90	3.09±0.59	16.31±1.88	5.91±0.10	5.7±0.10
Carrot	8.30	8.91±2.30	2.41±0.15	3.52±1.98	5.30±0.10	4.8±0.10
Potato	10.00	3.06±1.88	1.62±1.02	1.16±1.90	5.9±0.9	4.5±0.10

Note: Mean ± SD

Table 3: Preliminary phyto-profile of different vegetable peel extracts

Vegetable samples	Solvent	P.I.	B.P. of solvents (°C)	Colour	Consistency	Nature	% yield ± SD
Cabbage	PET	0.0	60-80	Olive green	Sticky	Solid	1.69±0.1
	CH	4.1	61.2	Fern green	Sticky	Solid	2.9±0.51
	ME	5.1	64.2	Olive green	Sticky	Semi- solid	3.7±0.11
	AQ	9.0	100	Greenish brown	Dry	Solid	3.9±0.21
Cauliflower	PET	0.0	60-80	Army green	Sticky	Solid	2.08±1.11
	CH	4.1	61.2	Sacramento green	Sticky	Solid	2.1±0.13
	ME	5.1	64.2	Fern green	Sticky	Semi-solid	3.12±0.12
	AQ	9.0	100	Army green	Dry	Solid	4.01±0.13
Pea	PET	0.0	0.0	Moss green	Dry	Solid	5.6±0.45
	CH	4.1	4.1	Crocodile green	Dry	Solid	2.01±1.12
	ME	5.1	5.1	Fern green	Sticky	Semi-solid	4.5±0.19
	AQ	9.0	9.0	Army green	Dry	Solid	4.9±1.1
Carrot	PET	0.0	60-80	Brick red	Sticky	Solid	1.19±0.2
	CH	4.1	61.2	Brick red	Dry	Solid	1.09±0.3
	ME	5.1	64.2	Brick red	sticky	Semi-solid	2.01±0.1
	AQ	9.0	100	Brownish red	sticky	Solid	1.1±0.22
Potato	PET	0.0	0.0	Ivory brown	Dry	Solid	0.34±0.6
	CH	4.1	4.1	Tortilla brown	Dry	Solid	0.67±0.4
	ME	5.1	5.1	Ivory brown	Sticky	Semi- solid	3.2±1.1
	AQ	9.0	9.0	Dark brown	Dry	Semi- solid	4.08±1.9

Note: PET: Petroleum ether, CH: Chloroform, ME: Methanol, AQ: Aqueous

Antioxidant potential of vegetable peels

DPPH scavenging assay

DPPH assay measured the antioxidant potential of plant extracts which reduces the DPPH free radicals to hydrazine with the change of violet colour to yellow colour and reduction in absorbance at 517 nm in a concentration dependent manner (Hossen *et al*., 2021; Chaudhary and Janmeda, 2022). The inhibitory concentration of different extracts like PET extract, CH extract, ME extract and AQ extracts of cabbage (CB), cauliflower (CA), pea (PE), carrot (CT) and potato (PT) is listed in Table 8. The IC_{50} values of DPPH assay was in the following order: CAAQ>ST>PTAQ>CBAQ> CTAQ> PEME>PEAQ> CBME>CACH>CBPET> CTME> PTME>PECH>PTCH>CAPET>PTPET>PEPET>CAC H>CBCH>CTCH. Among all extracts, CH extract of CT has shown the highest IC_{50} value whereas the AQ extract of CA has shown the low value of IC_{50} in comparison to standard, suggested the higher antioxidant activity of the extract. Kalpna *et al*. (2011) reported the IC_{50} value of 200 µg/ml and 380 µg/ml from the acetone and methanol extract of *Solanum tuberosum* whereas Biswas *et al*. (2021) reported the

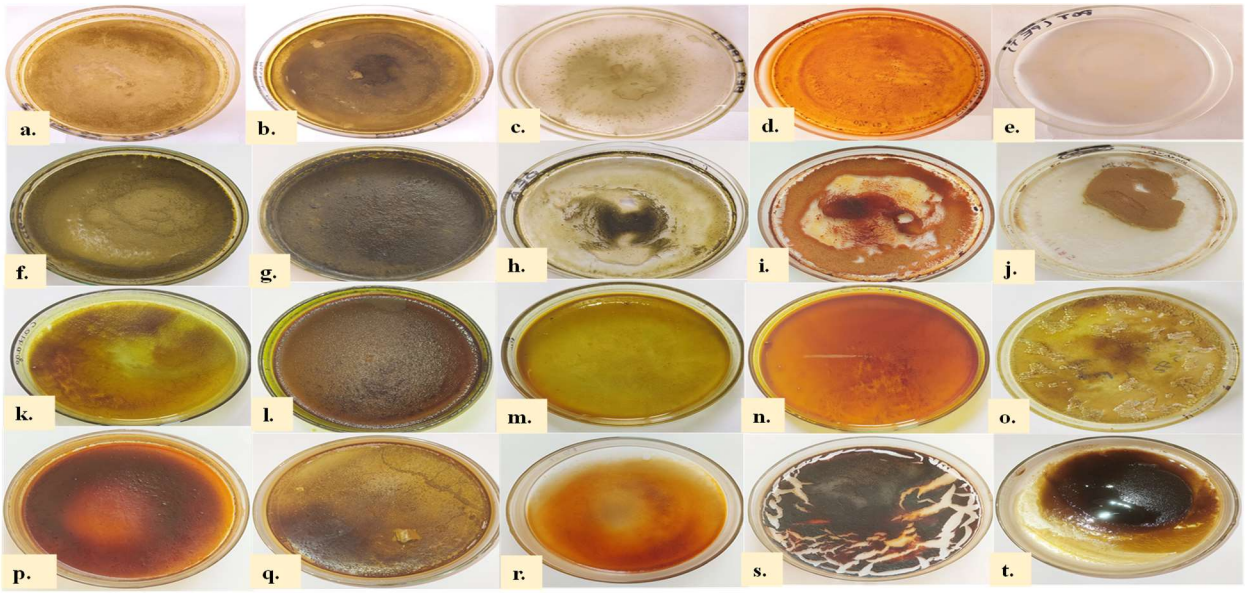

Figure 7: Sequential extracts of different vegetable peels. (a.-e.) petroleum ether extract, (f.-j.) chloroform extract, (k.-o.) methanol extract, (p.-t.). Aqueous extract of cabbage, cauliflower, pea, carrot, and potato.

Table 4: Fluorescence characteristics of cabbage, cauliflower and pea peels

Reagents used	Cabbage			Cauliflower			Pea		
	Visible	High UV (366 nm)	Low UV (254 nm)	Visible	High UV (366 nm)	Low UV (254 nm)	Visible	High UV (366 nm)	Low UV (254 nm)
HCl	Light green	Purple	Dark army green	Reddish brown	Purple	Dark army green	Reddish brown	Purple	Dark army green
H_2SO_4	Light green	Purple	Light green	Brown	Black	Light green	Brown	Black	Light green
HNO_3	Light green	Purple	Light green	Black	Violet	Light green	Black	Violet	Light green
Picric acid	Light green	Purple	Green	Light green	Light brown	Green	Light green	Light brown	Green
Ethyl acetate	Light green	Purple	Dark green	Charcoal black	Black	Dark green	Charcoal black	Black	Dark green
Glacial acetic acid	Light green	Purple	Dark brown	Light green	Brown	Dark brown	Light green	Brown	Dark brown
Methanol	Light green	Purple	Dark green	Light green	Purple	Dark green	Light green	Purple	Dark green
Chloroform	Light brown	Purple	Dark green	Light brown	Purple	Dark green	Light brown	Purple	Dark green
Water	Light brown	Purple	Army green	Reddish orange	Purple	Army green	Reddish orange	Purple	Army green
Benzene	Dark brown	Purple	Dark black	Dark brown	Black	Dark black	Dark brown	Black	Dark black
NaOH	Light green	Purple	Dark green	Reddish yellow	Brown	Dark green	Reddish yellow	Brown	Dark green
$FeCl_3$	Lighr green	Purple	Dark green	Lighr green	Purple	Dark green	Lighr green	Purple	Dark green
NH_4OH	Light green	Purple	Dark green	Light green	Purple	Dark green	Light green	Purple	Dark green
Iodine	Light green	Purple	Dark green	Light green	Purple	Dark green	Light green	Purple	Dark green
Powder	Brown	Purple	Greenish brown	Reddish brown	Purple	Greenish brown	Reddish brown	Purple	Greenish brown

Table 5: Fluorescence characteristics of peels of carrot and potato

Reagents used	Carrot			Potato		
	Visible	High UV (366 nm)	Low UV (254 nm)	Visible	High UV (366 nm)	Low UV (254 nm)
HCl	Dark brick red	Purple	Blackish brown	Dark brick red	Fluorescent purple	Blackish brown
H_2SO_4	Dark brick red	Dark purple	Blackish brown	Dark brick red	Dark purple	Blackish brown
HNO_3	Brown red	Dark purple	Blackish brown	Brown red	Dark purple	Blackish brown
Picric acid	Brown red	Dark purple	Dark brown	Brown red	Dark purple	Dark brown
Ethyl acetate	Brown red	Purple	dark green	Brown red	Purple	Fluorescent dark green
Glacial acetic acid	Brown red	Purple	Dark green	Brown red	Purple	Dark green
Methanol	Brick red	Purple	Dark brown	Brick red	Purple	Dark brown
Chloroform	Brick red	Purple	Dark brown	Brick red	Purple	Dark brown
Water	Reddish brown	Purple	Blackish brown	Reddish brown	Purple	Blackish brown
Benzene	Reddish brown	Purple	Blackish brown	Reddish brown	Fluorescent purple	Blackish brown
NaOH	Reddish brown	Purple	Blackish brown	Reddish brown	Fluorescent purple	Blackish brown
$FeCl_3$	Green	Purple	Green	Fluorescent green	Fluorescent green	Fluorescent green
NH_4OH	Light brown	Purple	Green	brown	Cream	Green
Iodine	Light brown	Purple	Green	Light brown	Fluorescent purple	Green
Powder	Light brown	Purple	Green	Light brown	Fluorescent purple	Ivory

Table 6: Phytochemical screening of cabbage, cauliflower and pea

S.No	Test	Cabbage				Cauliflower				Pea			
		PET	CH	ME	AQ	PET	CH	ME	AQ	PET	CH	ME	AQ
Proteins													
1.	**Millon's test**	-	-	-	+	-	-	-	-	-	-	-	+
2.	**Sulphur containing protein**	-	-	-	+	-	-	-	++	-	-	-	++
Carbohydrates													
3.	**Fehling's test**	-	-	+	+	-	-	+	-	-	-	-	+
4.	**Benedict's test**	-	-	+	+	-	+++	-	-	-	-	+	-
Fats and oil													
5.	**Filter paper test**	-	-	-	-	-	-	++	-	-	-	-	+
Alkaloids													
6.	**Mayer's test**	-	-	-	-	-	-	-	-	-	-	-	+
7.	**Tannic acid test**	+	-	+	+	_	-	+	+	-	-	+	+
Flavonoids													
8.	**Sulphuric acid test**	++	+	-	+	-	-	-	+	-	+	-	-
9.	**Alkaline reagent test**	+	-	++	++	-	-	++	++	-	+	++	++
Phenol and tannins													
10.	**Ferric chloride test**	-	-	-	-	-	-	+	+	-	-	+	-
11.	**Nitric acid test**	-	-	-	+	-	-	-	+	-	-	+	+
Cardiac glycosides													
12.	**Legal's test**	-	-	+	++	-	-	+	+	-	-	++	++
13.	**Keller-killiani test**	+	++	+	++	+	+	-	+	++	+	++	++
Steroids													
14.	**Salkowski test**	-	-	-	-	-	-	-	-	-	-	-	+
Saponin													
15.	**Foam test**	-	-	-	-	++	-	-	-	-	-	-	+
16.	**Olive oil test**	-	-	-	-	+	-	+	-	-	-	+	-

Terpenoids													
17.	**Salkowski test**	++	++	-	-	++	++	-	-	++	+	-	+
Anthocyanins													
18.	**Hydrochloric acid test**	+	++	-	++	++	+	+	+	++	+	+	+

PET: Petroleum ether, CH: chloroform, ME: Methanol, AQ: aqueous

Table 7: Phytochemical analysis of carrot and potato

S.No.	Test	Carrot				Potato			
		PET	CH	ME	AQ	PET	CH	ME	AQ
Proteins									
1.	**Millon's test**	++	-	+	+	-	-	-	++
2.	**Sulphur containing protein**	-	++	-	+	++	+	-	-
Carbohydrate									
3.	**Fehling's test**	-	+	-	-	-	-	-	-
4.	**Benedict's test**	-	-	+++	+	-	-	++	++
Fats and oil									
5.	**Filter paper test**	-	-	-	-	-	-	-	-
Alkaloids									
6.	**Mayer's test**	+	+	++	+	-	-	-	-
7.	**Tannic acid test**	+	+	++	++	+	+	+	+
Flavonoids									
8.	**Sulphuric acid test**	-	-	-	-	-	-	-	-
9.	**Alkaline reagent test**	-	-	+	++	++	++	++	++
Phenol and tannins									
10.	**Ferric chloride test**	-	-	+	+	-	-	-	-
11.	**Nitric acid test**	-	-	+	+	-	-	-	+
Cardiac glycosides									
12.	**Legal's test**	-	-	+	+++	-	-	++	+
13.	**Keller-killiani test**	+	+	-	-	+	_	_	_
Steroids									
14.	**Salkowski test**	-	-	+	+	-	-	-	-
Saponin									
15.	**Foam test**	-	-	+	+	+	+	-	-
16.	**Olive oil test**	+	-	-	+	++	+	-	-
Terpenoids									
17.	**Salkowski test**	+	+	+	+	+	+	-	-
Anthocyanins									
18.	**Hydrochloric acid test**	++	-	+	+	_	++	-	++

PET: Petroleum ether, CH: chloroform, ME: Methanol, AQ: aqueous

Table 8: IC_{50} values of DPPH, FRAP, and TAC assay of different vegetable peel extracts

Different vegetable peel samples	DPPH Values (µg/ml)					FRAP Values (µMFe(II)/g)					TAC Values (µg/ml)				
	PET	CH	ME	AQ	ST	PET	CH	ME	AQ	ST	PET	CH	ME	AQ	ST
Cabbage	49.28	85.76	43.31	29.28	26.7	44.2	51.2	52.2	42.2	30.8	78.9	75.8	36.2	34.3	28.3
Cauliflower	69.792	84.69	46.29	24.82	26.7	50.22	43.22	41.2	39.22	30.8	74.8	58.2	24.4	31.2	28.3
Pea	80.312	61.11	31.87	34	26.7	61.2	89.34	48.3	41.2	30.8	59.2	87.32	49.3	35.9	28.3
Carrot	62.35	92.75	51.6	31.23	26.7	58.2	62.5	49.2	48.7	30.8	68.5	81.8	55.9	35	28.3
Potato	79.1	64.2	56.11	28.3	26.7	71.22	42.33	45.2	44.22	30.8	94	91.3	36.66	35	28.3

Note: ST: standard, PET: petroleum ether, CH: chloroform, ME: methanol, AQ: aqueous

Table 9: TLC analysis of methanolic extract of cabbage and cauliflower

Vegetable peel samples	Solvent	Ratio	No. of spots	No. of spots	No. of spots	Total Spots	RF Value
			Visible	I.C	UV		
Cabbage	M:n-H:EA	1:3:1	2	3	2	3	0.38, 0.56, 0.81
Cabbage	C:M	8:2	1	2	2	2	0.12, 0.56
Cabbage	DCM:M	8:2	1	2	2	2	0.66, 0.79
Cauliflower	C:M	8:2	0	2	2	2	0.51, 0.53
Cauliflower	DCM:M	8:2	0	3	3	3	0.88, 0.34, 0.65
Cauliflower	M:n-H:EA	1:3:1	0	0	0	0	0

M: methanol, nH: n-Hexane, EA: ethyl acetate, I.C: iodine chamber, and UV: ultraviolet

DPPH radical scavenging activity of 13.34 ± 0.11 mg AAE/g DW from the peel of *Solanum tuberosum*. The IC_{50} value of methanol and acetone extract was found to be 859 µg/µL and 76 µg/µL in the case of carrot peel (John *et al*., 2017).

FRAP assay

FRAP assay is based on the reduction capability of an antioxidants to reduce ferric ion into ferrous (Chaudhary and Janmeda, 2022). Results of FRAP activity of different extracts of all five vegetable peels are shown in Table 8 and Figure 9. The reducing power of the sample was found to be in the following order: ST>CAAQ>CAME>PEAQ>CBAQ>PTCH>CACH>CBPET>PTAQ>PTME>PEME>CTAQ>CTME>CAPET>CBCH>CBME>CTPET>PEPET>CTCH>PTPET>PECH. Among all extracts, the AQ and ME extract of CA showed the better antioxidant activity than the other solvent system but it was lower than the standard BHT.

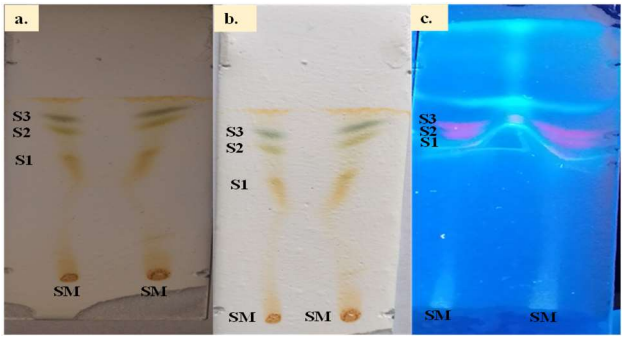

Figure 8: Thin layer chromatogram of ME extract of cabbage peel. Solvent system: methanol: n Hexane: ethyl acetate (1:3:1), a. visible, b. iodine chamber, c. ultraviolet. SM: sample.

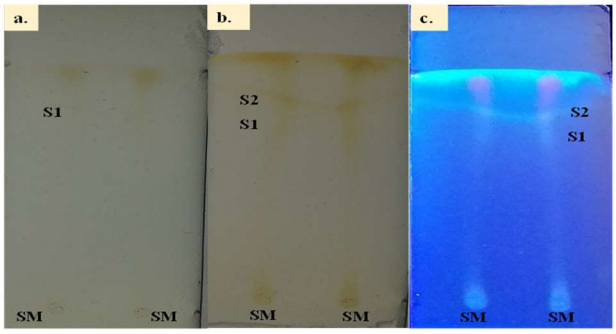

Figure 10: Thin layer chromatography of ME extract of cabbage peel. Solvent system: Dichloromethane : Methanol (8:2), a. visible, b. iodine chamber, c. ultraviolet. SM: sample.

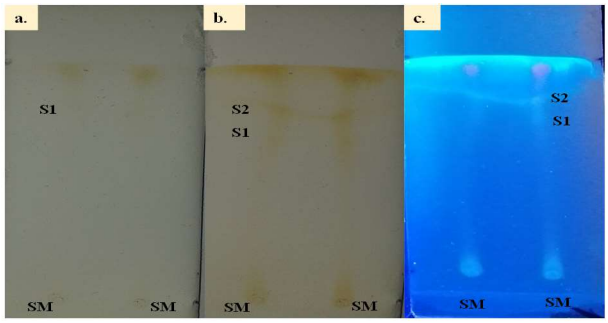

Figure 9: Thin layer chromatography of ME extract of cabbage peel. Solvent system: Chloroform: Methanol (8:2), a. visible, b. iodine chamber, c. ultraviolet. SM: sample.

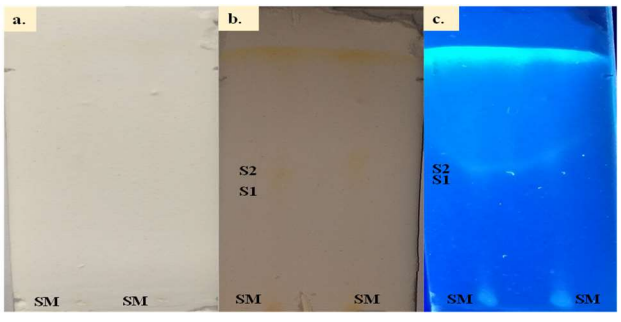

Figure 11: Thin layer chromatography of ME extract of cauliflower peel. Solvent system: Chloroform: Methanol (8:2), a. visible, b. iodine chamber, c. ultraviolet. SM: sample.

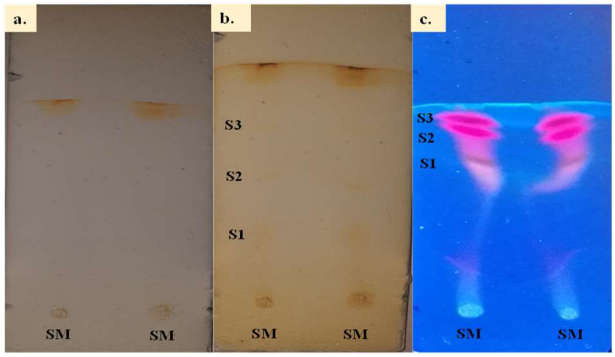

Figure 12: Thin layer chromatography of ME extract of cauliflower peel. Solvent system: Dichloromethane: Methanol (8:2), a. visible, b. iodine chamber, c. ultraviolet. SM: sample.

Nguyen *et al*. (2016) reported the reducing power of hexane, water, ethanol, and methanol extracts of a carrot peel and it was found to be 0.31, 4.82, 8.88, and 15.31 mg TE/g dry weight. FRAP assay revealed the 18.61 mmol/100g of antioxidant activity in case of potato peels extract (Rowayshed *et al.,* 2015). Though antioxidant activity of vegetables is influenced by geographical area, cultivar, harvest and storage time but variability can be seen in content between fresh vegetables and its by-products. The by-products of cabbage and cauliflower contain 20 and 15 times more reducing ability than the peels of potato and pea i.e., 20 ± 0.22 mM. Similarly, the peels of carrot have 5-30 times higher antioxidant potential and higher reducing ability than their edible parts (John *et al.,* 2017).

TAC Assay

TAC assay is based on the antioxidant activity of plant extract on the reduction of Mo(VI) to Mo(V) and subsequent generation of green coloured complexes of phosphate/Mo(V) at acidic pH. Results of TAC activity of different extracts of all five vegetable peels was found to be in the following order: CAME>ST>CAAQ>CBAQ> CTAQ>PTAQ>PEAQ>CBME>PTME>PEME>CTME> CACH>PEPET>CTPET>CAPET>CBCH>CBPET>CA CH>PECH>PTCH>PTPET (Table 8 and Figure 10). Among all extracts, the IC_{50} value of AQ extract of CA and CB was found to be low which indicated the higher antioxidant activity of this extract in comparison to standard.

Thin layer chromatography

The observations from thin layer chromatography analysis of methanolic extract of cabbage and cauliflower are listed in Table 9. TLC of methanolic extract of cabbage revealed the presence of 3 compounds with R_f values of 0.38, 0.56, and 0.81 respectively in a solvent phase of Methanol: n-Hexane: Ethyl acetate (1:3:1) as shown in Figure 8. In another solvent system i.e., Chloroform: Methanol (8:2), and Dichloromethane: Methanol (8:2), two spots were observed with R_f value of 0.12, 0.56, 0.66, and 0.79 respectively (Figure 9 and 10).TLC of methanolic extract of cauliflower revealed the presence of 2 spots of R_f value 0.51, and 0.53 (Figure 11) in solvent phase of Chloroform: Methanol (8:2). Three spots with R_f value of 0.34, 0.65, and 0.88 were observed in a solvent system of Dichloromethane: Methanol (8:2) as shown in Figure 12. and no spot was observed in the case of Methanol: n-Hexane: Methanol (1:3:1) respectively. These R_f values provide valuable information regarding the isolation of these phytochemicals in the isolation process by using an appropriate solvent system for further pharmacological applications.

Conclusion

In the present work, different pharmacognostical standardization parameters and antioxidant assays were applied to determine the quality, safety, and antioxidant potential of the five different vegetable peels. The results obtained from the present study would be useful in determining the crude extract of different peels as a potent source of antioxidants. These are economic and natural sources of antioxidants that can be utilized for the prevention of different human ailments. TLC profiling of phytochemicals showed the good separation and on the sensitivity. However, further studies are needed on isolation, identification, and characterization of specific phytocompound before it can be utilized as a novel source of antioxidant. This opens the scope for the future application of vegetable waste for different therapeutic purposes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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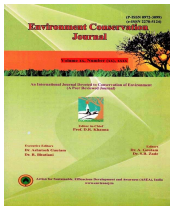

Effect of seaweed (*Kappaphycus alvarezii*) extract on rainfed aerobic rice (*Oryza sativa* L.)

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ABSTRACT

Rice is grown throughout the year in India, in a variety of agro-climatic conditions, and it is grown on 43.39 million hectares with a production of 159.20 MT with an average productivity of 3623 kg/ha. Aerobically produced rice may be an option for farmers on rainfed areas where rainfall is not sufficient or availability of water is rare and expensive too for flooded rice production but enough for cultivation of upland rice. The field experiment took place at Crop Research Farm, Department of Agronomy, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, India, during the kharif season of 2020. The experiment was done by using Randomized Block Design with three replications. The findings of the experiments revealed a considerable rise in the growth parameter viz., plant height (46.0 cm), total tillers/m^2 (564.3), plant dry matter accumulation (1938.0 g/m^2), leaf area index (19.07) and crop growth rate (49.47 g/m^2/day) and yield attributing parameters viz., effective tillers/m^2 (362.3), weight of panicle/m^2 (856.9), number of filled grain/panicle (115.7), grain yield (4.7 t/ha), test weight (28.9 g), straw yield (11.3 t/ha) with foliar application of 7.5 percent *Kappaphycus alvarezii* seaweed sap four times, plus RDF and two foliar applications of 10% K-sap yielded the greatest harvest index (32.7%).

Introduction

Rice (*Oryza sativa* L.) is a crucial staple food crop, feeding two-thirds of the world's population (Kahani *et al.,* 2015). Rice is a less expensive source of dietary calories, fibre, and proteins for the poor, especially those who are economically disadvantaged. Because there is high demand for rice, we must increase rice output and productivity. We need to implement new techniques to increase production and productivity that are economically affordable to poor farmers, as the majority of our country's farmers are marginal farmers. To achieve high demand of rice we have to increase production of rice in this limited cultivable area by using high yielding varieties to achieve the requirement of these rice varieties application of chemicals fertilizers is the only easy way out available to the farmers till now. Chemical fertilizer use not only raises production costs, but it also diminishes soil fertility and pollutes the environment. In order to relieve farmers' financial burdens, the government has increased fertilizer subsidies. However, providing subsidised cash did not fix the agricultural productivity problem. As a result, spraying extracts of natural items containing stimulants to improve nutrient absorption is a strategic approach to reduce the usage of excessive amounts of inorganic fertilizer. To prevent any of these situations, a farmer's only option is to employ organic fertilizers. Seaweed sap has been shown to promote nutrient absorption, which can help rice grow, develop, and produce more. Seaweed sap can be a good alternative as it contains growth regulators like cytokinins, auxins, gibberelins and abscisic acid, as well as micro and macro nutrients, amino acids, vitamins etc. Which stimulate growth and yield of crops. On the other hand, it is also economically cheaper than chemical fertilizers and environment friendly. Because of global warming

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monsoon delaying become a common phenomenon these days. Delayed monsoon causing a great effect on sowing of paddy and farmers have to rely on irrigation. To achieve huge demand of rice we have started cultivation of rice in low rainfall areas also where supply of water is only through irrigation of fresh water. Fresh water makes up only 3 percent of water on the surface and, the rest 97 percent in the ocean. Many parts of India facing serious problem due to over exploitation of ground water. Tuong and Bouman (2003) estimate that physical water scarcity may affect 15 million hectares of irrigated rice land in Asia, whereas economic water scarcity may affect 22 million hectares. To deal with the dilemma, researchers must find a means to lower rice's water consumption while increasing its output. Aerobic rice is going to be a great solution to this problem. Aerobic rice produces 4-6 t/ha rice with 50% saving irrigation water. This cultivation also does not need puddled field, there is no need heavy amount of water to do puddling. All these things make aerobic rice cultivation an excellent solution for water management. On the other side it also reduces irrigation cost, field preparation cost and also produces 4-6t/ha which is also beneficial in terms of return for farmers. So the combination of both aerobic rice cultivation and seaweed sap application not only saves our mother nature but also reduces production cost and increases their production.

Material and Methods

To find out the ability of seaweed sap as a productivity booster in Aerobic rice a field experiment was conducted in Crop research farm, Department of Agronomy, Naini Agriculture Institute, SHUATS, Prayagraj, Uttar Pradesh which is located at 25˚ 24' 42" N latitude, 81˚ 50' 56" E longitude and at an altitude of 98 m above mean sea level during *kharif* season of 2020 on sandy loam soil, having neutral soil reaction (pH 7.68), low in available nitrogen (226.49 kg/ha), low in available phosphorus (6.90 kg/ ha) and medium in available potassium (161.20 kg/ ha). The climate of region is sub-tropical and semi-arid.

The study used a Randomized Block Design with ten treatments replicated three times with one source of seaweed sap namely *Kappaphycus alvarezii* consisted of 3 concentrations (5%, 7.5% and 10%). Every concentration was sprayed in 3 different days combination (2 spray, 3 spray and 4 spray). Two spray was done at (20 & 40 DAS), three spray was done at (20, 40 & 60 DAS) and four spray was done at (20, 40, 60 & 80 DAS). Treatments comprise of T_1 - water spray, T_2 - 5% K-sap with 2 spray, T_3 - 5% K-sap with 3 spray, T_4 - 5% K-sap with 4 spray, T_5 - 7.5% K-sap with 2 spray, T_6 - 7.5% K-sap 3 spray, T_7 - 7.5% K-sap 4 spray, T_8 - 10% K-sap 2 spray, T_9 - 10% K-sap 3 spray, T_{10} - 10% K-sap 4 spray along with RDF, respectively in every treatment.

Sowing was done by line sowing method where seeds were planted with a row to row distance of 20 cm and plant to plant distance of 15 cm. Recommended dose of fertilizers in the form of urea, DAP and MOP were applied at the time of sowing. At the time of sowing half dose of urea was applied as a basal dose along with full dose of DAP and MOP and the other half of the urea was applied in two equal parts at 30 DAS and 60 DAS. Seaweed sap applied as per treatment as per solution as recommended. Sap application is done with the help of a sprayer.At various growth stages, observations on growth parameters such as plant height, total tillers/m2, plant dry matter accumulation, leaf area index, and crop growth rate were recorded from five randomly tagged plants from each plot, and yield attributing parameters such as effective tillers/m2, weight of panicle/m2, number of filled grain/panicle, grain yield, test weight, and straw yield were recorded at the harvesting stage. The recorded data were statistically analysed using the ANOVA approach. When the 'F' test was determined to be significant at the 5% level, the critical difference (CD) value was calculated (Mead *et al.*, 2017).

Results and Discussion

Plant height (cm)

Data of plant height was collected at 15, 30, 45, 60, 75, and 90 days after sowing, and the results were summarised in Table 1. The plant height gradually increased as the crop stages progressed in all treatment, and achieve maximum height during harvest. At 15 DAS, it showed non-significant result while at 30 DAS, RDF + 7.5 percent K-sap with 3 spray was produced significantly maximum plant height (12.7 cm). Significantly maximum

Table 1: Effect of seaweed (Kappaphycus alvarezii) extract on plant height, total tillers, and plant dry matter accumulation of aerobic rice

Treatment	Plant height(cm)						Total tillers/m2				Plant dry matter accumulation (g/m2)					
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	45 DAS	60 DAS	75 DAS	90 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
Water spray	6	11.8	15.6	23.1	24.5	35.7	72.3	380.7	423.0	467.3	8.1	30.2	106.5	201.4	907.5	1122.0
5% K-sap with 2 Spray	6.6	11.7	16.3	22.0	26.3	35.8	79	408.0	469.0	511.3	8.6	30.7	119	214.6	1060.3	1457.0
5% K-sap with 3 Spray	6.6	12.1	17.8	23.8	28.3	38.1	78.7	427.3	487.0	485.7	9.5	35.7	121.3	219.9	1076.4	1369.0
5% K-sap with 4 spray	6.4	12.1	16.9	25.4	29.9	39.5	79.7	423.7	473.7	485.0	9.2	35.7	153	247.5	1086.4	1552.7
7.5% K-sap with 2 spray	6.7	12.2	17.1	25.3	30.2	41.0	92.7	461.7	500.7	505.3	11.1	41.5	142.8	240.3	1122.8	1586.7
7.5% K-sap with 3 spray	7	12.7	18.6	25.8	33.3	43.2	95	487.7	535.0	521.3	10.8	41.8	155.3	257.7	1150.4	1802.0
7.5% K-sap with 4 spray	7.3	12.4	19.0	26.9	37.1	46	92.7	495.0	562.0	564.3	12.4	43.9	175.7	297.4	1196.0	1938.0
10% K-sap with 2 spray	5.9	11.2	14.9	20.0	23.1	32.1	74.3	378.3	391.7	419.3	5.7	27	73.7	186.3	892.6	1086.0
10% K-sap with 3 spray	6.1	11.0	16.1	22.7	26.1	35.2	74.7	381.0	446.0	451.3	6.9	27.1	113.3	214.5	995.7	1201.3
10% K-sap with 4 spray	5.7	11.2	16.7	23.2	26.9	36.9	72.3	401.0	446.7	471.3	8.1	34.2	120.1	222.8	1059.1	1212.7
SEm (±)	0.386	0.349	0.752	1.265	1.507	1.719	3.266	18.56	31.123	19.612	1.08	2.948	9.092	29.27	43.03	110.84
CD (5%)	-	1.04	2.23	3.76	4.48	5.11	9.7	55.15	92.47	58.27	3.24	8.76	27.02	-	127.86	329.34

plant height was observed at 4 foliar spray of 7.5 percent K-sap + RDF respectively at 45, 60, 75 and 90 DAS. Which was significantly superior over control treatment *i.e* RDF + water spray. While at 75 DAS treatment T_6 and at 90 DAS treatment T_5 and T_6 found to be at par with foliar application of 7.5 percent K-sap with 4 spray. Plant height increases with increase of sap concentration up to 7.5 percent. The reason behind it may be presence of plant growth regulator in seaweed sap which is resulting in enhancement of growth and development. While working with rain-fed maize, (Singh *et al.,* 2015) observed increase of plant height is proportional with the increase in K-sap concentration up to 7.5 percent. Pramanik *et al.* 2017 also reported the increase in plant height when 7.5% K-sap sprayed on the foliage of the crop along with 100% RDF on potato plants. According to Mooney and Van Staden in 1986, reported that presence of major and minor minerals, amino acids, vitamins, cytokinins, auxin, and abscisic acid like other growth boosting chemicals in seaweed extract resulting in dramatically increased plant height. The reason behind a decreased performance in high concentration may be very high salt index in higher concentration of seaweed sap (Aitken and senn 1965; Abetz 1980).

Number of tillers/ m^2

Collection of data on number of tillers/m^2 was done at 45, 60, 75 and 90 DAS. A great difference between all the treatment was observed which is depicted in Table 1. At 45 DAS 7.5 percent K-sap with 3 foliar spray along with RDF was found significantly highest total tillers (95.0/m^2). Maximum number of tillers was observed with the 4 spray of 7.5 percent K-sap along with RDF on the leaves at 60, 75 and 90 DAS. Singh *et al.,* (2016) and Shah *et al.,* (2013) observed that on wheat highest tillers/m^2 was counted with the application of 7.5 percent K-sap. Various active compound, micro and macro nutrients present in the extract of seaweeds which increases the number of tillers of rice plants (Singh *et al.,* 2018).

Dry matter accumulation (g/m^2)

Total dry matter accumulation of aerobic rice significantly influence by foliar application of seaweed sap (Table 1). As the crop progressed toward harvest dry matter accumulation also increased progressively but although the pattern of dry matter accumulation in different plant parts is different during various phases of crop growth period. In the early stages of growth leaf accumulated more dry matter than later stages of growth. During vegetative growth stage (up to 75 DAS) dry matter accumulation was at its peak in leaf and stem and in case of panicle it improved from flowering till maturity. All the treatment combinations were significantly different from each other recorded on 15, 30, 45, and 90 DAS. Significantly maximum plant dry matter accumulation was recorded with treatment of 4 foliar application of 7.5 percent K-sap + RDF (T_7) and at 60 DAS it displayed non-significant result while at 15 DAS, 30 DAS and 75 DAS treatment T_6, T_5, T_4, T_3, at 45 DAS treatment T_4, and at 90 DAS treatment T_6 was found to be similar trends with 4 foliar spray of 7.5 percent K-sap (T_7). According to Pramanik *et al.*, 2017, the seaweed sap has a considerable impact on growth and the use of 7.5% K-sap in combination with RDF produces the best results in terms of potato dry matter accumulation. Another study conducted by Singh *et al.,* 2015 showed that the dry matter production was maximised when a maize crop was sprayed with 7.5% K-sap and 100% RDF. Seaweed constitutes of various growth substances which increases number of leaf, tillers and plant height in different growth stages of paddy plant which combinedly increases dry matter.

Leaf Area Index (LAI)

In the present investigation all treatments were influenced significantly at 15, 30, 45, 60, 75 & 90 DAS were presented in Table 2. Significantly maximum leaf area index was recorded at 30, 45, 60, 75 and 90 DAS with 4 foliar application of 7.5 percent K- sap + RDF (T_7). While at 30 DAS 3 foliar spray of 7.5% K-sap, 2 foliar spray of 7.5% K-sap, 4 foliar spray of 5% K-sap, at 45 DAS 3 foliar spray of 7.5% K-sap, 2 foliar spray of 7.5% K-sap and at 75 DAS treatment 3 foliar spray of 7.5% K-sap (T_6) was found to be statistically at par with 4 foliar spray of 7.5% K-sap along with RDF. Pramanik *et al.,* 2017, revealed that foliar application of 7.5% K-sap with 100% RDF exhibit the significant results regarding the growth of leaf area index. Another study by Singh *et al.,* 2015 also showed that spraying maize crops with 7.5% K-sap with 100% RDF maximizes the leaf area index.

Table 2: Effect of seaweed (*Kappaphycus alvarezii*) extract on leaf area index and crop growth rate of aerobic rice.

Treatment	Leaf area index (LAI)					Crop growth rate (g/m^2/day)				
	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	15-30 DAS	30-45 DAS	45-60 DAS	60-75 DAS	75-90 DAS
Water spray	0.09	0.33	3.23	5.73	11.20	1.54	5.09	6.32	47.07	15.53
5% K-sap with 2 spray	0.09	0.36	3.70	6.77	12.0	1.48	5.88	7.04	56.38	26.44
5% K-sap with 3 spray	0.09	0.43	4.70	7.70	13.17	1.75	5.70	7.06	59.33	23.75
5% K-sap with 4 spray	0.10	0.43	4.93	7.63	14.17	1.77	7.82	6.90	55.93	31.08
7.5% K-sap with 2 spray	0.10	0.49	5.63	8.10	14.60	2.03	6.75	7.26	58.84	30.93
7.5% K-sap with 3 spray	0.10	0.54	6.37	10.17	16.23	2.07	7.56	7.49	59.52	43.44
7.5% K-sap with 4 spray	0.11	0.55	6.57	11.33	19.07	2.10	8.78	8.11	59.91	49.47
10% K-sap with 2 spray	0.08	0.31	2.73	4.73	9.90	1.42	3.11	6.51	47.09	15.56
10% K-sap with 3 spray	0.08	0.32	3.17	6.73	11.23	1.34	5.75	6.75	52.53	16.61
10% K-sap with 4 spray	0.09	0.32	3.93	6.40	12.33	1.74	5.73	6.85	55.75	15.99
SEm (±)	0.002	0.034	0.39	0.51	0.72	0.21	0.61	1.59	2.77	5.00
CD (5%)	0.01	0.10	1.19	1.61	2.14	-	1.82	-	8.26	14.86

Table 3: Effect of seaweed (*Kappaphycus alvarezii*) extract on yield attributes and yield of Aerobic rice.

Treatment	Effective Tillers/m^2	Weight of panicle/m^2 (g)	Number of filled grain/ panicle	Test Weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	Harvest index (%)
Water spray	249.0	365.3	81.6	21.7	3.4	7.9	30.2
5% K-sap with 2 spray	259.3	403.8	95.2	22.2	3.6	8.1	30.6
5% K-sap with 3 spray	281.0	456.9	97.1	22.3	3.8	8.4	30.9
5% K-sap with 4 spray	301.0	581.4	98.7	24.1	3.9	8.2	32.4
7.5% K-sap with 2 spray	297.7	512.3	99.6	24.4	3.9	9.0	30.3
7.5% K-sap with 3 spray	332.7	698.3	109.3	27.7	4.3	10.2	29.5
7.5% K-sap with 4 spray	362.3	856.9	115.7	28.9	4.7	11.3	29.3
10% K-sap with 2 spray	250.3	382.1	85.5	21.5	3.4	7.1	32.7
10% K-sap with 3 spray	259.3	399.4	87.8	21.1	3.5	7.8	31.1
10% K-sap with 4 spray	273.0	452.3	99.3	22.4	3.7d	8.1	31.3
SEm(±)	13.39	53.01	2.51	0.95	0.06	0.21	0.70
CD (5%)	39.81	157.49	7.48	2.81	0.18	0.62	2.09

Application of seaweed sap in plants showed that it is capable of enhancing concentration of nutrients in the leaves as growth hormone is involved in the nutrient absorption process and movements in a plant (Sunarpi *et al.,* 2010). The leaves of the rice plant increase, because there are some effective chemicals and vital nutrient present in seaweed extract, it has ability to accelerate plant growth (Abetz 1980; Finnie and Van Stadan 1985).

Crop growth rate (g/m^2/day)

Data pertaining to Crop growth rate was recorded during 15-30, 30-45, 45-60, 60-75, 75-90 days after sowing periodically and tabulated in Table 2. There was no significant difference between all treatments during 15-30 DAS and 45-60 DAS. Whereas significantly highest CGR was found with RDF + 4 foliar spray of 7.5 percent K-sap during 30-45, 60-75 and 75-90 DAS. Further these findings are permitted by Pramanick *et al.,* (2017) and Singh *et al.*, (2015) on rain-fed maize and Singh *et al.,* (2016) recorded that increase of CGR on wheat crop proportional with increasing K-sap concentration upto 7.5 percent. Because it was previously proposed that certain kinds of marine algae species found in nature contains biologically active cytokinin, gibberelin and auxin. Presence of cytokinin enhances cell division. These compounds were able to stimulate growth by increasing protein synthesis and cell division, as well as mobilising resources needed for development (Patier, 1993).

Yield attributes and yield

Observation regarding yield attributes and yield like effective tillers per m^2, weight of panicle, number of filled grain per panicle, test weight (g), grain yield (t/ha), straw yield (t/ha), harvest index (%) of aerobic rice depicted in table 3. Four foliar spray of 7.5% seaweed sap (*Kappaphycus alvarezii*) + RDF was found considerably higher effective tillers/m^2 (362.3), weight of panicle (856.9), number of filled grain/panicle (115.7), test weight (28.9 g), grain yield (4.7 t/ha), straw yield (11.3 t/ha) which was 31.2%, 57.3%, 29.47%, 24.9%, 27.6%, 30.0% more over control (Water Spray; T_1), respectively. In harvest index application of RDF along with two foliar spray of 10 percent K-sap resulted in a much higher value i.e. (32.7%) which was 10.3% more over control. On wheat, Shah *et al.,* (2012), on maize, Singh *et al.,* (2015), on maize, Singh *et al*., (2015), on rain-fed maize, Singh *et al.,* (2015), found that lower sap concentrations enhanced production while higher sap concentrations (over 7.5 percent K sap) lowered yield. May be the reason behind increased grain yield is presence of plant growth regulator in the sap and minerals elements in seaweed extract, which enhances rates of photosynthesis and delayed leaf senescence, resulting in a greater performance of photosynthetic rate, which is help for better grain filling, so grain become bigger and hence greater grain production (Beckett and Staden, 1990).

Conclusion

Seaweed liquid fertiliser, made from locally available seaweeds, is an excellent technique to boost plant growth and biochemical activity and many plant production characteristics are improved, as is soil fertility and long term output, but also hugely reduces fertilizer carbon footprint and eutrophication potential per unit of output, through which it reduces environment pollution. It is concluded that application of seaweed (*Kappaphycus alvarezii*) extract to aerobic rice (Pusa basmati-1) crop significantly influence the crop productivity and profitability. Four foliar sprays of 7.5 percent K-sap extract + RDF found to be more productive (4.7 t/ha).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Cultivation of oyster mushroom to combat pandemics: medicinal and social aspects

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ABSTRACT

Mushrooms are the spore bearings fruiting bodies that have been used by human being since ancient times for good health. The aim of the study was to utilize bulk agricultural waste that is wheat and paddy straw to cultivate oyster mushroom as its choicest food of nutrition because it provides important nutrients like proteins, selenium, potassium, riboflavin, Niacin, Vitamin D and more. Wheat straw as a substrate was inoculated with pure culture of *Pleurotus ostreatus* under aspectic and favourable conditions. High yield of mushroom was obtained with a small amount of substrate. Oyster mushroom protect the heart against cardiovascular diseases, helps in lowering the blood pressure, regulate the cholesterol level, improve immune health and have anti cancer, anti inflammatory and other medicinal properties. Thus utilization of agricultural waste appears to be an alternative for oyster mushroom cultivation. Cultivation of oyster mushroom on waste straw not only solve the pollution problem but also improves the economic conditions of farmers.

Introduction

The oyster mushroom (Pleurotus sp.), also known as "dhingri," is a member of the order Agaricales and family Agaricaceae. It naturally grows on wood logs in temperate and subtropical forests wood and may also grow on decomposing organic matter. Depending on the species, this mushroom's fruit bodies have a distinctive shell or spatula shape and come in shades of cream, white, yellow, grey, crimson, or light brown *(*Kerketta *et al.,* 2019). Typically, the term "mushroom" refers to fungi that have a stem (stipe), cap (pileus), gills (lamellae), and pores on the underside of the cap.(Masarirambi *et al.,* 2011*)*. Mushroom spawn (spores) are created on the gills and can fall off the underside of the cap as a thick powder. Most oyster mushrooms have white spore prints, and when grown, they form fruiting bodies. The oyster mushroom, or dhingri, a species of *Pleurotus,* is the second most widely grown fungus in the world. One of these mushrooms' most successfully farmed species, *Pleurotus sajor-caju,*is praised for its flavour. For the commercial production of oyster mushrooms in India, rice and wheat straw are used. Dhingri is grown extensively, which boosts the income of those who live in rural areas. In addition, compared to button mushrooms, its cultivation technique is also quite straightforward (*Agaricus bisporus*) (Sharma and Singh, 2018).The Pleurotaceae family includes the edible oyster mushroom (*Pleurotus sajor-caju*). The majority of fungi that fit the definition of a mushroom have a cap (pileus), hymenium (stipe), and spores on the underside of the cap (Masarirambi *et al.,* 2011). Temperatures between 20 and 30 degrees Celsius and relative humidity levels between 55 and 70 percent are suitable for growing oyster mushrooms. In mountainous places above 900 (masl), the optimal growing season is from March/April to September/October, and in the lower regions from September/October to March/April. It can also be grown throughout the summer months by supplying the additional humidity needed for its growth (Dubey *et al.,* 2019). The oyster mushroom, or

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Pleurotus spp., is a useful lignin-degrading fungus that may thrive on a variety of ligno-cellulosic materials, including agricultural and forestry waste (Biswas and Biswas, 2015). On a variety of agricultural waste, including wheat and paddy straw, sugarcane thrash, and vegetable waste, mushrooms can be produced. Different kinds and quantities of nutrients required for mushroom growth can be found in agricultural waste. Cellulose, hemicelluloses, nitrogen, and lignin are a few of the nutrients. A form of lingo-cellulosic substance known as a mushroom substrate is any material on which mycelium grows and aids the growth and development of mushrooms. The substrate simply needs to be pasteurised, which is less expensive, rather than sterilised. Oyster mushroom cultivation is more profitable because a significant portion of the substrate is converted to fruiting bodies. The fruiting bodies of *Pleurotus ostreatus* require little environmental restrictions, are rarely attacked by diseases and pests, and can be grown in an easy and inexpensive manner. Due to all of this, *Pleurotus ostreatus* cultivation is a superior alternative to other mushroom production (Myachikova *et al.,* 2019).

Although the white oyster mushroom is a member of the consumption mushroom family that feeds on decomposing wood, there are many other species that may be found in nature, each of which has unique traits. It can be separated into dangerous and non-toxic mushrooms based on their natural makeup. Farming white oyster mushrooms, which are regarded as a commodity in the agribusiness sector, represents a business opportunity that is still very much open (Syawal *et al.,* 2019). Mushrooms are recognised to have a wide range of purposes in both food and medicine and are a strong source of protein, vitamins, and minerals. On soil, open fields, farm lands, woods, and roadside, these are frequently discovered as saprophytes. Large enough to be seen with the naked eye are the fruiting bodies. The taste, flavour, and texture of mushrooms are varied. Fresh mushrooms have a moisture content of between 80 and 95 percent, 0.3 to 0.4 percent protein, and 1% vitamins and minerals (Biswas and Biswas, 2015). Pleurotus species have been recognized as mushroom with dual functions to humans; both as food and medicine (Adebayo and Olake, 2017).

Commonly encountered obstacles during oyster mushroom cultivation are: a lack of cultivation space, Insects make attack (flies and cockroach), Equipment deficiencies (Air conditioner, water tank, a sterilisation chamber, and a steel rack etc.), a lack of skilled and knowledgeable labour.Lack of cultivation space can be overcome by utilizing basement rooms and roof top.

Insect make attack can be overcome by pre sanitizing of growing room, and time to time monitoring, gloves should be worn during spawning, insect repeller machine should be installed in cultivation area, homemade herbal sprays (neem spray) can be sprayed in cultivation area . To overcome equipment deficiencies cultivation can be in wintersas temperature is maintained lower naturally, a pressure cooker can be used as a sterilizer machine; a wooden rack can be used in place of steel rack. A lack of skilled and knowledgeable labor problem can be overcome by taking training from KVK (Krishivigyan Kendra) which is provided free by government of India.

Cultivation Steps

- Isolation of pure culture (Tissue culture method and spore printing method)
- Preparation of mother culture & Spawn
- Preparation of substrate
- Spawning
- Spawn running
- Fruiting
- Harvesting

Isolation of pure culture

Using sterile forceps and a Petri dish containing potato dextrose agar, a pure culture of *Pleurotus ostreatus* was recovered from pileus tissues on (PDA) (Thongklang and Luangharn, 2016). The chosen fresh mushrooms were surface sterilised with 75% alcohol right away. Then, under a laminar flow cabinet, each mushroom stipe (stem) was divided into two parts with a sterilised surgical blade, and small tissues (about 4 mm2) were extracted from the pseudo-parenchymateous tissue of the stipe (Belachew and Workie, 2013). Inoculate the PDA media with tissue from a mushroom stipe that has been cultured for 4-5 days at 25°C ± 1°C. A pure culture was produced by transferring a little piece of the fungus' mycelium, which was visible around the sporophore piece in the slant, to another culture tube

and incubating it there for 3–4 days at 25°C ± 1°C (Biswas and Biswas, 2015).

Preparation of Mother Spawn

Sawdust was used to prepare the master culture substrate. Sun-dried sawdust was sieved (Miah *et al.,* 2017). The yellow-colored sorghum grain (Sorghum bicolor L), wheat bran, and calcium sulphate (gypsum), in the proportions of 88:10:2, respectively, were used to create the spawn (mushroom seed) of *Pleurotus ostreatus*. The needed amount of sorghum grain was weighed and soaked in enough water for one day. The grains had been rinsed and drained in order to remove the dead and dangling seeds from the water. Gypsum ($CaSO_4.2H_2O$) and the required quantity of wheat bran ($CaSO_4.2H_2O$) have been added to the grain after any excess water has been removed. The mixture was then transferred to 1,000 mL glass bottles (75 percent degree), with a head space above the grain, and autoclaved for 45 minutes at 121°C. After cooling, each bottle was inoculated with 20 agar blocks (1 cm x 1 cm) of a 15-day-old mushroom culture from a Petri dish and incubated at 28°C for 21 days until the substrate was fully colonised. Mycelia invasion and infection were monitored every five days, and the grain spawn was ready for use after 15 days (Getachew *et al.,* 2019).

Preparation of substrate

All of the gathered substrates were broken up into small (2-4 cm long) pieces, then soaked overnight in fresh water from the faucet. The following day, the soaked straw was cleansed with clean water three to four times until clear water was obtained. The excess water was then squeezed from the substrates or allowed to run off till the moisture level was reached and the straw was ready for sterilization (Dubey *et al.,* 2019). The bags were filled so that they only filled up to 3/4 of the way, leaving room for air for tying. The filled bags were then autoclaved for 15 minutes at 121°C and 15 PSI of pressure. Before adding the spawn, the bags were allowed to cool after sterilization (Sajid *et al.,* 2018).

Spawning

Clear monitoring of the mycelia's growth and the presence of contaminants is made possible by transparent plastic cultivation bags (65 cm long x 45 cm wide). Each substrate (500g), which had been cooled to room temperature, was combined with 10% spawn (dry weight/moist weight basis) under a laminar flow hood, and the polythene bags containing the inoculated material were then tightly sealed with cotton-based string. Once the primordial were formed, the contaminated baggage were kept in a spawn running room at room temperature (23–25°C) in the dark (Getachew *et al.,* 2019). Depending on the size, kind, and state of the bags, they are stored in the incubation chambers for 15 to 25 days. There are numerous ways to organise these bags. Typically, they are arranged in a rack with no bags touching one another (Dulal, 2019).

Spawn Running

Once the mycelium mat has fully colonised, it indicates that the mycelium mat is ready for fruiting in the poly bags. To allow the pin-heads to emerge, sterile blades were used to slit the surface of the bags at a distance of 10 cm apart. The bags were subjected to an eight-hour natural light cycle. By spraying the floor with clean borehole water, a relative humidity of 80-85 percent was maintained in the dark room. The floors could not be flooded. To avoid introducing contaminants, the room was kept closed and a footbath with chlorinated water was placed at the doorway. The temperature in the mushroom house was kept between 24 and 25°C (Musara *et al.,* 2018).

Fruiting

Fruit body produced under humid conditions (85-90 %) is bigger with less dry matter while those developed at 65 – 70 % relative humidity are small with high dry matter (Table 1).

Table 1: Environmental setup for different stages

Parameters	Spawn phase	Pinhead	Fruiting body
Temperature (°C)	24–25	10–15	15–21
Relative humidity (%)	90–95	95–100	85–90
CO2 (ppm)	5000–20,000	500-1000	≤2000
Light (lux day−1) - 500-1000	-	500-1000	500-1000

Harvesting

The best shape for picking can be determined by the shape and size of the fruit body. Before releasing the spores, the fruit bodies should be harvested by twisting so that the stubs do not remain on the beds

(straw). Harvesting can be done manually or mechanically. Mechanically with a knife and manually with clean hands by gently twisting in clockwise and anticlockwise directions. After harvesting the mushrooms, place them in a clean tray and begin post-harvest handling techniques.

Medicinal worth of Oyster Mushroom

Oyster mushrooms are white rot fungi with exceptional health benefits. They could be low in calories while being high in protein, nutrients, and minerals. Oyster mushrooms have been used as a medicinal drug in Asian continental regions since ancient times. Currently, some research studies have suggested that mushrooms play a role in a variety of health issues such as hypercholesterolemia, high blood pressure, diabetes, most cancers, infections, and so on.

Antioxidant Property

Antioxidants are substances that aid in the reduction of oxidative stress caused by reactive oxygen species within tissues or cells (ROS). Rare oxides, peroxides, and hydroxyl radicals comprise ROS. These, in general, oxidise it. As a result, antioxidants are present to react with these ROS, either by destroying them or rendering them ineffective by converting them to another residue, preventing any harm to the cell or tissue. A variety of degenerative diseases, including cancer and hepatotoxicity, have been linked to oxidative stress. As a result, the presence of antioxidants in Pleurotus sp. can be a powerful method for treating or preventing such situations.

Vitamins

Mushrooms are rich in vitamins and considered best sources of vitamins, for example vitamin B (Mattila *et al.,* 2001; Ghosh *et al.,* 2019). Mushrooms have been reported to have a low level of vitamin C, and mushrooms found in the wild have a higher level of vitamin D2 than dark cultivated *A. bisporus* (Sapers, 1999).

Immunoinflammatory

Because of its immune-modulatory properties and low cytotoxicity, oyster mushroom has the potential to be useful in the treatment of cancer patients receiving radiation and conventional chemotherapy, as it increases immune resistance while decreasing toxicity (El-Enshasy and Hatti-Kaul, 2013). *P. ostreatus* contains a large number of immunemodulatory components, including lectins, polysaccharides, polysaccharide-peptide complexes, and polysaccharide-protein complexes (Wang and TB, 2000). Deepalakshmi and Mirunalini (2014) reported that water extract from *P. ostreatus* fruit bodies and mycelia increases neutrophil production of reactive oxygen species (ROS) and has immune-modulatory properties involving all immune competent cells.

Anti microbial potential

Organic extracts of *P. ostreatus* in methanol and chloroform were found to be effective against Grampositive bacteria and were considered potential sources of antibacterial agents (Karaman *et al.,* 2010). Mirunalini *et al.* (2012) investigated the antibacterial potential of *P. ostreatus* and biosynthesized silver nanoparticles (AgNPs) against several Gram positive bacteria by measuring the diameters of the inhibition zones.

Social aspects

Waste management

According to Law *et al.* (2012), SMC of *Pleurotus pulmonarius* can reduce around 89 percent of 100mg Pentachlorophenol (PCP). Bioabsorption is another aspect of mycoremediation. *Pleurotus ostreatus* can absorb cadmium from its environment, according to Tay *et al.* (2011). *Pleurotus sajor-caju* has been found to absorb zinc.

Economy

Every year, India generates approximately 600 million tonnes of agricultural waste, the vast majority of which is left to decompose naturally or is burned in place. This can be used to produce highly nutritious foods like mushrooms, and the spent mushroom substrate can be converted into organic manure/vermicompost (Chitra *et al.,* 2018). India could produce 3 million tonnes of mushrooms and approximately 15 million tonnes of compost with a 1% conversion of agro-waste to mushroom production. Large size units were the most viable for oyster mushroom production based on the output: input ratio because the output to total cost ratio was the highest (Verma *et al.,* 2014).

Conclusion

Mushroom cultivation provides both economic opportunities and nutritional and health benefits. According to this global survey, various wastes have been shown to be beneficial for oyster mushroom growth. As a result, each oyster mushroom grower

can select the best substrate for their particular species or genera. The substrates could be used to make a valuable protein-rich food. Oyster mushroom cultivation on various agricultural residues provides economic opportunities for agribusiness to examine these residues as valuable resources and use them to produce protein-rich mushroom products.Oyster mushrooms have a prominent place in nutraceutical science because they are high in nutrients and have medicinal properties, particularly as antioxidants, antimicrobials, anti-inflammation, anticancer, immunomodulators, anti-diabetes, antineoplasics, and antihypertensives. According to the above properties, oyster mushrooms can be used to produce antibiotics, anticancer drugs, and immune boosters. Finally, mushroom cultivation can make a significant contribution to sustainable livelihoods for both rural and urban poor people because it is highly compatible with other livelihood activities and requires few physical and financial resources.

Conflict of interest

The authors declare that they have no conflict of interest.

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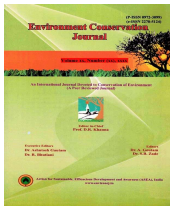

Morphometric evaluation of Ranikhola watershed in Sikkim, India using geospatial technique

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ABSTRACT

Morphological parameters are linked with the hydrological behaviour of the watershed. It helps to understand different basin characteristics. Characterization of quantitative morphology and river basin analysis is the way to implement proper river basin planning and management of soil and water conservation measures. In the present study, Cartosat-1 Digital Elevation Model (DEM) was used in Geographic Information System (GIS) environment to determine the morphometric parameters (stream length, stream order, stream frequency, bifurcation ratio, form factor, drainage density, circulatory ratio, etc.) of the Ranikhola watershed of Sikkim state, India. The slope of a major portion of the watershed area was found to be less than 30% (42 km^2) and has a drainage density of 0.585 km^{-1}. The lower value of drainage density in the watershed indicates a relatively lower streams frequency over the watershed. The elongation ratio, form factor, and circulatory ratio were estimated as 0.665, 0.347, and 0.510, respectively, which indicate that the watershed is elongated in shape, having gentle slopes and long flow paths. The relief ratio for the watershed was estimated to be 0.187, which indicates the watershed has a low elevation difference, low runoff, and high groundwater potential. This kind of morphometric analysis is required for the watershed characterization and helps to understand the hydrogeological behavior of the watershed.

Introduction

A watershed is a portion of land that drains water to a common outlet. It is a topographically delineated area that captures rainfall and contributes runoff to a single outlet in the main flow channel. The study of the watershed is essential for any developmental activities and sustainably managing the natural resources, namely, land and water, in order to minimize the negative impacts of exploitation. The watershed management practices require the qualitative and quantitative analysis of a large number of watershed features. The morphological analysis is generally performed to solve various literary problems in the basin, planning and implementing soil and water quality conservation measures, groundwater development and management, erosion control measures, etc. Morphometric measurement is the dimensional measurement and mathematical analysis of the surface composition, shape, and size of the land (Clarke, 1966). Morphometric studies of river basins were first proposed by Horton (1932), and then the ideas were developed by Schumm (1956), Coates (1958), and Strahler (1964). The watershed analysis is used as the elementary and reasonable

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approach to identify the different watershed characteristics. It offers a quantitative explanation of the drainage patterns (Strahler 1964), which helps in hydrological investigations. Drainage morphometry has a significant impact on understanding the geomorphological processes, soil physical properties, and erosion properties (Meshram *et al.,* 2020; Rai *et al.*, 2017). The analysis of the watershed morphology helps to discover the correlations between hydraulic parameters and topographical features (Abdeta *et al.,* 2020; Yadav *et al*., 2014). Morphological analysis is suitable for areas with limited access to information and the diversity of highly distributed soils (Rahmati *et al*., 2019; Sangma and Guru, 2020; Trivedi *et al*, 2021; Gautam *et al*., 2022).

The morphometric analysis includes the estimation of various basin geomorphological characteristics, which can be categorized into three groups: linear aspects (steam length, stream order, bifurcation ratio, etc.), areal aspects (shape factor, form factor, drainage density, stream frequency, elongation ratio, circulatory ratio, etc.) and relief aspects (relief ratio, relative relief, ruggedness number, geometric number, etc.). These factors are either directly or inversely belong to runoff, peak flow, soil erosion, and risk of sedimentation (Bhattacharya *et al*., 2003; Gajbhiye and Sharma, 2017). Computation of morphometric parameters using conventional methods is a tedious and time-consuming task (Sreedevi *et al*., 2005; Rao *et al.,* 2010; Ahamad *et al.,* 2022). Further, such a task is unreasonably difficult if the study area has a large areal extent. Gautam *et al.* (2021) determined the Geomorphological Characteristics of the Jakham River Basin using the GIS Technique. They analyzed that the mean bifurcation ratio ranges from 3.28 to 4.02, which indicates the effect of geological formations on the drainage pattern in the basin. Drainage density (D =1.82 km km^{-2}) of the watersheds shows the course nature of the drainage pattern and permeability for infiltration. Shape factor (circulatory and elongation ratio) indicates that sub-watersheds are less to moderate elongated in shape have less susceptibility to peak flood.

The use of satellite imagery along with remote sensing technology can be considered a practical tool for morphological analysis (Gautam *et al.,* 2022). The satellite data can offer an overview of a large area and are very useful for analyzing the morphology of the watersheds. It can be effectively used for morphological analysis and precise delineation of watersheds, sub-watersheds, mini-watersheds, and even micro-watersheds and other morphological features (Ahmed *et al.*, 2010; Samal *et al*., 2015). Cartosat-1 DEM is the product of Indian satellite IRS-P5 with a spatial resolution of 30 m, and it can be considered high-precision remote sensing data (Dabrowski *et al*., 2008; Khadri *et al*., 2013). The images obtained by this satellite can be safely used for its designed purpose (Amulya and Dhanashree, 2018; Srivastava *et al*., 2007), which is to collect high accuracy elevation data. The integration of RS and GIS for assessing the diverse topographic and morphological features of the drainage basins and watersheds provides a pliant atmosphere and an invaluable tool for the operation and analysis of spatial data (Sharma *et al*., 2014; Vincy *et al*., 2012; Singh *et al*., 2020).

In the state of Sikkim, India, few studies were carried out regarding these perspectives. Therefore, an attempt was made to estimate the morphological characteristics of the Ranikhola watershed using RS datasets in the GIS environment. Combined with the previous research, this study has the potential to provide scholars, decision-makers, and municipalities with valuable information for designing and implementing soil and water management structures along with suitable management practices.

Study Area

The Ranikhola watershed lies between the latitude 27°17’ to 27°22’ N and the longitude 88°31’ to 88°38’ E in the East district of Sikkim, India, with an area of 62.49 km^2. The watershed has hilly terrain in which Gangtok (Sikkim) is the primary urban centre with an average yearly rainfall of 3626 mm. Figure 1 represents the location map of the study area. The Ranikhola River flows into the Teesta River (a tributary of the Brahmaputra) near Singtam. The other urban settlements in the watershed include Deorali, Tadong, Sixth Mile, Rumtek, etc. (Figure 2). The major land use in the watershed is forest, followed by urban settlements and agriculture.

Material and Methods

The Cartosat-1 DEM (30-meter spatial resolution) was obtained from the Bhuvan portal. The first step was to delineate the Ranikhola watershed (Figure

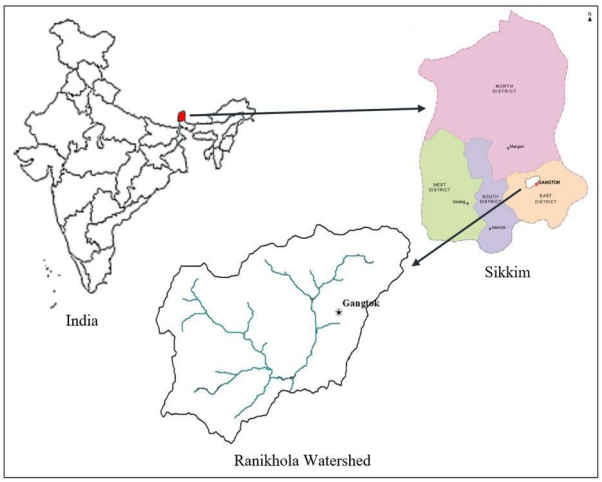

Figure 1: Location map of the Ranikhola watershed.

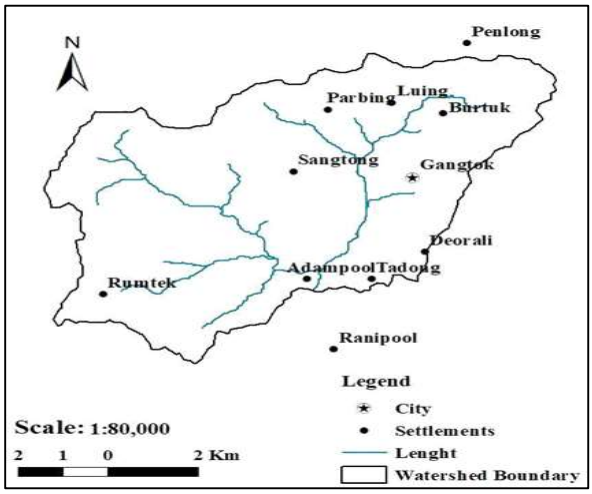

Figure 2: Urban settlements in the Ranikhola watershed.

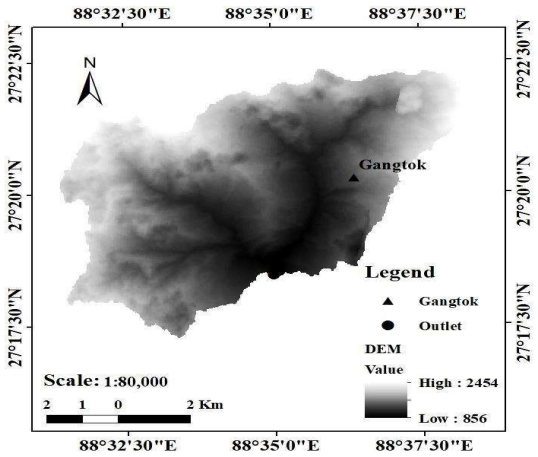

Figure 3: DEM of the Ranikhola Watershed

3). This step includes the generation of a sink, filling, flow direction, and flow accumulation maps using ArcGIS software. An outlet point was created using the generated maps, and contributing area to the outlet was delineated as the watershed. The morphometric parameters were estimated from the Cartosat-1 DEM using ArcGIS software. The formulae given by Horton (1945), Schumm (1956), Hadley and Schumm (1961) (as given in Table 1) were used to determine the morphometric parameters of the Ranikhola watershed, including linear, areal, and relief aspects. The complete methodology is presented with a flow diagram (Figure 4).

Results and Discussion

Cartosat-1 DEM data in the GIS environment is used to estimate the different geomorphological parameters. The different aspects of watershed characteristics are discussed below.

Linear Aspects

Linear aspects of a watershed deal with the rivers and their network. Generally, these are one-dimensional characteristics. They include various parameters such as stream lengths, stream order, stream frequency, bifurcation ratio, etc. The summary of linear aspects of the Ranikhola watershed is given in Table 2.

Stream order (U)

The stream order determines the size of the stream according to the hierarchical structure of the tributaries. The highest stream order in a watershed is called an order of a watershed. In the Ranikhola watershed, the highest order stream was found as fourth-order (Figure 5). The dendritic pattern of drainage of the watershed represents the homogenous soil texture and weak structural control.

Stream Number (N_u)

The Law of stream numbers asserts that the number of streams of each order is the inverse geometric function of stream order (Horton 1945; Leopld et al. 1964). The Ranikhola watershed consists of fifteen 1st order, nine 2nd order, four 3rd order, and one 4th order stream (Table 2). The results of the steam number support Horton's law. The decreasing number of streams (N_u) with the increase in stream order shows that the watershed has a hilly terrain with undulating topography.

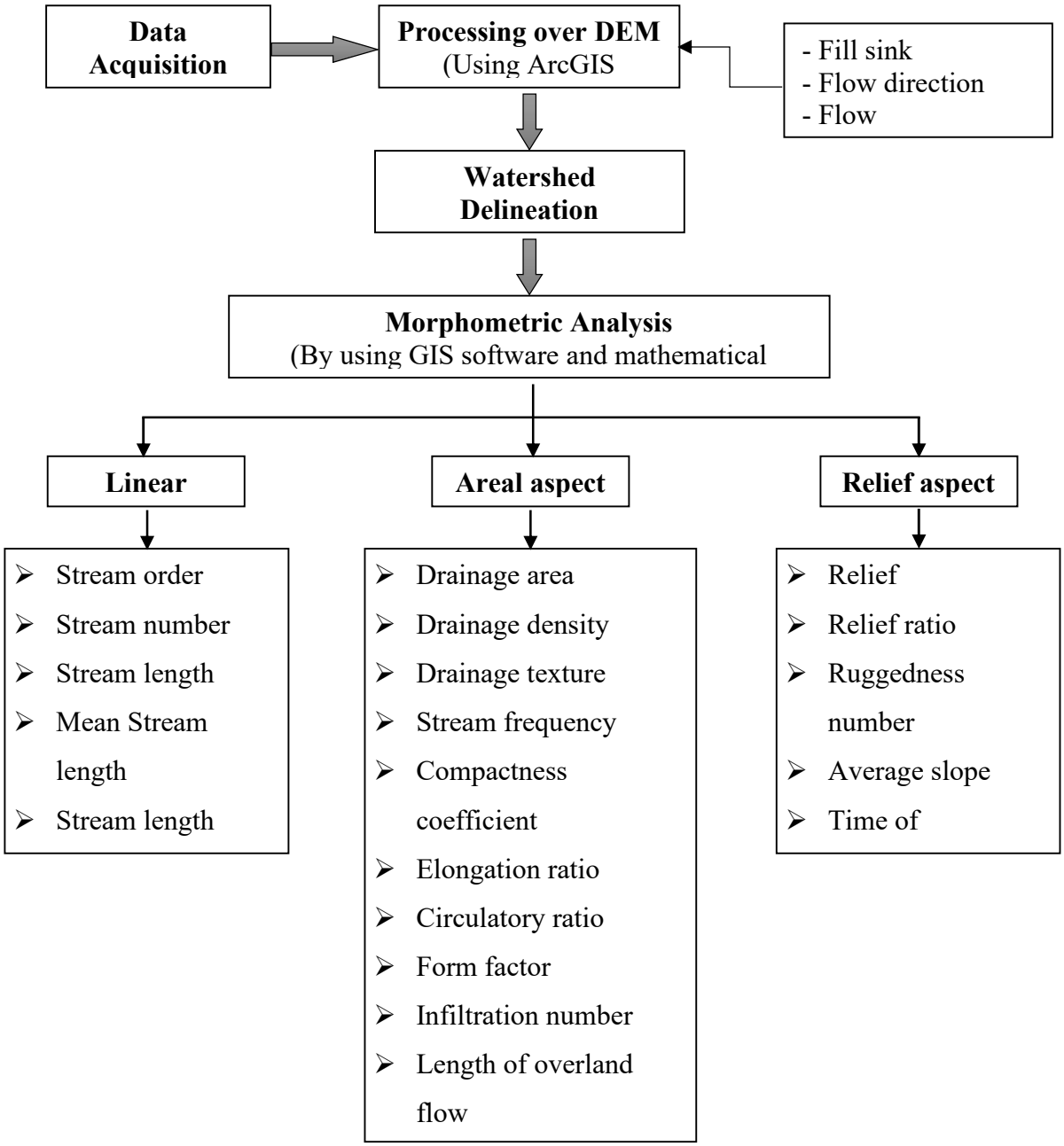

Figure 4: Methodological flow diagram for watershed morphometric analysis.

Stream Length (L_u)

Horton (1945) defined stream length as the sum of the lengths of all stream segments of each order within a watershed. It indicates the hydrological characteristics and the drainage extent of the basin. The short length of the stream represents steep slopes and finer texture, whereas longer lengths represent a relatively flatter gradient and permeable bedrock (Chitra *et al*., 2011; Withanage *et al*., 2014). For the Ranikhola watershed, the total length of all streams was estimated as 36.535 km. The longest stream length in the watershed is 10.929 km. The stream length of the 1st, 2nd, 3rd, and 4th order streams were found to be 17.785, 12.219, 5.906, and 0.624 km, respectively (Table 2).

Results showed that the stream length is an inverse function of stream order; hence Horton law is followed. This represents the variation in slope and

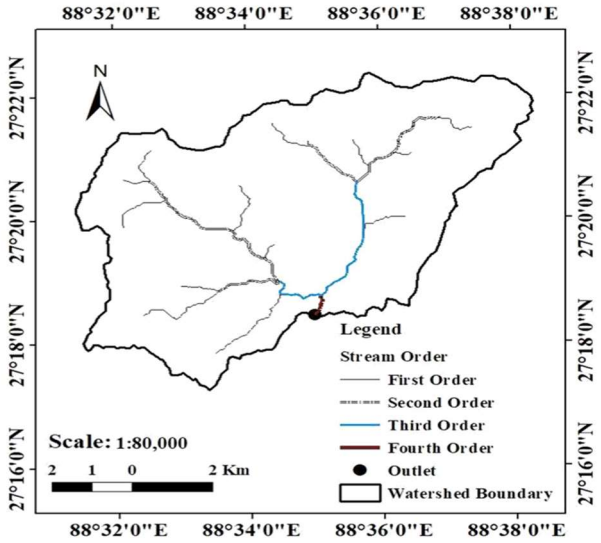

Figure 5: Stream order map of the Ranikhola watershed.

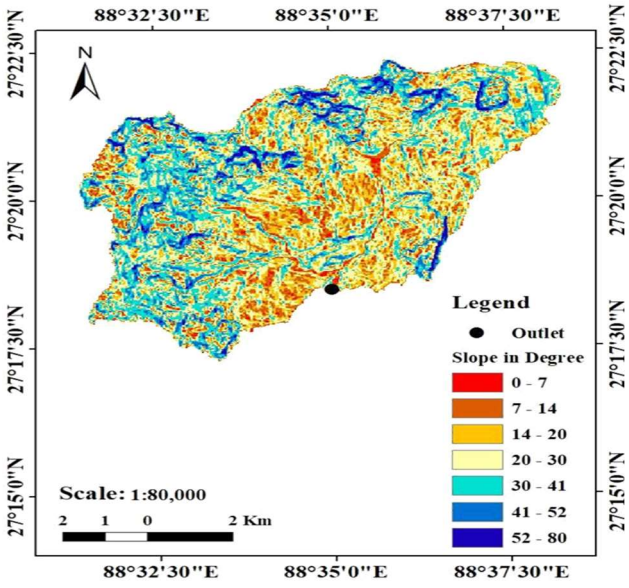

Figure 6: Slope map of the Ranikhola watershed.

the physiographic characteristics of the watershed. It further means that infiltration capacity varies with stream order across the watershed.

Mean Stream Length (L_{sm})

A watershed's mean stream length reflects the typical size of the drainage network and its contributing surfaces. It can be determined by dividing the total stream length of order 'U' by the total number of streams of the same order. The 'L_{sm}' of the Ranikhola watershed was found to be in the range of 0.624 km for the fourth-order to 1.476 km for the third-order stream, with the mean 'L_{sm}' of 1.161 km (Table 2). The smaller 'L_{sm}' was found in lower-order streams, indicating the areas with steep land slopes and finer textures, while the longer 'L_{sm}' in higher stream orders indicates the area with flatter gradients.

Stream Length Ratio (R_L)

It is the ratio of the length of stream of order 'U' to the length of stream of the next lower order, i.e., 'U-1' (Horton, 1932). The 'R_L' is the primary function of streamflow discharge and stage of erosion. 'R_L' between two successive stream order changes with the variation in slope and topography of the basin. For the Ranikhola watershed, 'R_L' was found to be ranging from 0.106 for the third-order to 0.687 for the first-order stream. These variations in 'R_L' from one order to the next show the early stage of geomorphic evolution.

Bifurcation Ratio (R_b)

It is expressed as the ratio of the total number of streams of any order 'U' to the total number of streams of the next higher-order 'U+1' (Hortan, 1932). The 'R_b' is a significant parameter in the analysis of drainage basins as it correlates the hydrological characteristics of a basin with the geological characteristics and climatic conditions (Hortan, 1945). If the 'R_b' value is between 3 and 5, it indicates the drainage pattern of the stream is independent of the geology of the watershed (Verstappen, 1983). Similarly, 'R_b' less than 3.0 indicates the flat and homogenous geological structure of the basin (Strahler, 1957). It has been found that for the Ranikhola watershed, the 'R_b' ranges from 1.67 to 4. This suggests that part of the stream network is under the influence of geological formations of the watershed and the remaining part is independent of it.

Aerial Aspects

The areal aspects of watersheds are their two-dimensional properties. These are critical to the development of watersheds. The summary of aerial aspects of the Ranikhola watershed is given in Table 3.

Table. 1 Formulae used to compute various morphometric parameters.

Sl. No.	Parameter	Formula	Originator
1.	**Stream order (U)**	Hierarchical rank	Strahler (1964)
2.	**Stream length (L_u)**	Length of stream	Horton (1945)
3.	**Mean stream length (L_{sm})**	$L_{sm} = L_u/N_u$	Strahler (1964)
4.	**Stream length ratio (R_L)**	$R_L = L_u/L_{u-1}$	Horton (1945)
5.	**Bifurcation ratio (R_b)**	$R_b = N_u/N_{u+1}$	Schumm (1956)
6.	**Mean bifurcation ratio (MR_b)**	$MR_b = (R_{b1}+ R_{b2}+\ldots+ R_{bn})/n$	Strahler (1957)
7.	**Drainage density (D_d)**	$D_d = L_{Total}/A$	Horton (1945)
8.	**Stream frequency (F_S)**	$F_S = N_{Total}/A$	Horton (1945)
9.	**Infiltration number (I_N)**	$I_N = D_d \times F_S$	Smith (1950)
10.	**Elongation ratio (R_e)**	$R_e = D_c/L_{bm}$	Schumm (1956)
11.	**Circulatory ratio (R_c)**	$R_c = 4\pi A/P^2$	Strahler (1964)
12.	**Form factor (F_f)**	$F_f = A/L_{bm}^2$	Horton (1945)
13.	**Time of concentration (t_c)**	$t_c = 0.01947\ L^{0.77}\ S^{-0.385}$	Kirpich (1940)
14.	**Relief (R)**	R = H - h	Schumm (1956)
15.	**Relief ratio (R_R)**	$R_R = R/L$	Schumm (1963)
16.	**Drainage texture (D_T)**	$D_T = L_{Total}/P$	Horton (1945)
17.	**Average slope (S)**	$S = (m \times N \times 100)/A$	Carlier and Leclerc (1964)
18.	**Compactness coefficient (C_c)**	$C_c = P/2\sqrt{(\pi A)} = P/P'$	Luchisheva (1950)
19.	**Length of overland flow (L_g)**	$L_g = 1/2D_d$	Horton (1945)
20.	**Constant of channel maintenance (C)**	$C = 1/D_d$	Schumm (1956)
21.	**Ruggedness number (HD)**	$HD = R \times D_d$	Strahler (1968)

Where,
N_u and N_{u+1} = Number of streams of order 'u' and 'u+1'
$\bar{L}_u$ and $\bar{L}_{u-1}$ = Average lengths of streams of order 'u' and 'u-1'
L_{total} = Total length of all streams of all order
N_{total} = Total number of streams of all order
D_c = Diameter of circle having area equal to as that of the watershed
L_{bm} = Maximum basin length; P = Perimeter of watershed;
A = Watershed area; H = Maximum elevation
h = Minimum elevation; N = Contour interval
L = Horizontal distance on which relief measured
m = Total length of contours within the watershed
P' = Perimeter of a circle having equal area as that of watershed

Drainage Area (A)

It is the area in which runoff from the basin's network of streams is released through a common outlet. It is one of the essential features since it directly represents the total volume of runoff generated in a watershed. The large size of a watershed intercepts greater rainfall and generates a high volume of runoff. The total drainage area of the Ranikhola watershed was estimated to be 62.49 km^2 (Table 3).

Drainage Density (D_d)

It is the ratio of the total length of all streams in the watershed to the entire area of the watershed. It reflects the infiltration capacity of the land, water and sediment discharge, and erosion susceptibility of the basin (Chorley, 1969). It is a very important measure of runoff potential and drainage texture of the basin. Smith (1950) categorized 'D_d' into five categories i.e., very fine (>8), fine (6-8), moderate (4-6), coarse (2-4), and very coarse (<2). A drainage basin with a high 'D_d' indicates the

Table. 2 Linear aspects of the Ranikhola watershed

Stream order	No. of streams	Stream length (km)	L_{sm} (km)	R_L	R_b
1st	15	17.785	1.185	0.687	-
2nd	9	12.219	1.357	0.483	1.67
3rd	4	5.906	1.476	0.106	2.25
4th	1	0.624	0.624	-	4.00
Total	29	36.535	-	Mean	2.64

Table. 3 Aerial aspects of the Ranikhola watershed

Parameter	Value
Watershed area (km^2)	62.49
Watershed perimeter (km)	39.26
Drainage density (D_d) (km^{-1})	0.585
Drainage texture (D_T)	0.931
Stream frequency (F_S) (km^{-2})	0.464
Compactness coefficient (C_c)	1.401
Elongation ratio (R_e)	0.865
Circulatory ratio (R_c)	0.510
Form Factor (F_f)	0.347
Infiltration number (I_N)	0.271
Length of overland flow (L_g) (km)	0.855
Constant of channel maintenance (C)	1.710

Table. 4 Relief parameters of the Ranikhola watershed

Parameter	Value
Relief (R)	1595 m
Relief ratio (R_R)	0.187
Ruggedness number (HD)	0.933
Average slope (S)	55.22 %
Time of concentration (tc)	52.58 minutes

Table. 5 Areas under different slope zones.

Slope (in °)	Area	
	(in km^2)	(in %)
0 – 7	1.53	2.45
7 – 14	6.85	10.97
14 – 20	11.71	18.74
20 – 30	21.18	33.90
30 – 41	14.11	22.58
41 – 52	5.35	8.56
52 – 80	1.74	2.79
Total	62.49	100

existence of a well-developed channel network, sparse vegetation, and high relief, which lead to quick runoff disposal and greater soil erosion potential of the basin (Strahler, 1964). In previous studies, it was observed that 'D_d' indirectly affects the groundwater potential of the watershed due to its surface runoff generation and permeability. The drainage density of the Ranikhola watershed was found to be 0.585 km^{-1}, which is very low. A lower value of 'D_d' indicates the low relief, weak channel network, and higher infiltration capacity, relatively good or coarser vegetative cover throughout the basin. The overland flow is the more prominent in the basin and results in low runoff disposal.

Drainage Texture (D_T)

It is the ratio of the total number of streams of all orders in a basin to the basin's perimeter (Hortan, 1945). It depends upon many factors such as climate, precipitation, soil type, vegetation, and basin relief (Darnkamp and King, 1971; Nag *et al*., 1998). Smith (1950) classified 'D_T' into five classes i.e., very fine (>8), fine (6-8), moderate (4-6), coarse (2-4), and very coarse (<2). The 'D_T'of the study area was found to be 0.931 (Table 3), which is less than 2, which indicates the Ranikhola watershed has a very coarse drainage texture; thus, the watershed is less susceptible to erosion due to its massive and resistant rocks structures.

Stream Frequency (F_S)

Horton (1932) defined 'F_S' as the total number of stream segments of all orders in a unit area. It is generally expressed in km^{-2}. The number of streams at any place depends on the structure and vegetation cover, rocks type, precipitation, and permeability of the soil. The basin having higher drainage density will lead to higher stream frequency in the basin (Rai *et al.,* 2017). Results indicated that the Ranikhola watershed has a low (< 2.5/km^2) stream frequency which was estimated to be 0.464 km^{-2} (Table 3). It indicates a watershed has low relief and high permeability of the rock. The lesser the stream frequency in the watershed, the lower will be the runoff, and therefore, flooding is less likely to occur.

Compactness Coefficient (C_c)

It refers to the ratio of the perimeter of a watershed to the circumference of a circle having an area equal to the area of the watershed (Hidore, 1965). It depends only on the slope of the basin and is independent of the watershed area. The value of 'C_c' is 1 for a circular basin and increases with the increase in basin length. Thus, it directly represents the elongated nature of the basin. Lower values of C_c indicate a more elongated basin, while higher values indicate a less elongated basin (Patel *et al.,* 2016). The 'C_c' of the Ranikhola watershed was computed to be 1.40 (Table 3), indicating the elongated shape of the watershed. Thus, having a low runoff peak for a longer time base, therefore, the flood flow of the watershed is easier to manage. From the drainage point of view, a circular basin is very hazardous because of the short time of concentration.

Elongation Ratio (R_e)

It is the ratio of the diameter of a circle having an area equal to the basin and the basin's perimeter. A circular basin has a shorter time of concentration than an elongated basin (Schumm, 1956; Chopra, 2005). Strahler (1964) categorized 'R_e' into three categories i.e. less elongated (<0.7), oval (0.9 to 0.8), circular (>0.9). Higher values of 'R_e' represent strong infiltration capacity and low runoff, whereas lower 'R_e' values indicate high sensitivity to erosion and sediment load. (Reddy *et al.,* 2004). The 'R_e'of the Ranikhola watershed was found to be 0.865 (Table 3), which indicates the watershed falls in the oval category. This facilitates watersheds with strong infiltration capacity, low runoff, and high groundwater potential.

Circulatory Ratio (R_c)

It is the ratio of the basin's area to the area of a circle having a circumference equal to the basin perimeter (Miller, 1953). The value of the 'R_c' varies with the variation in the land use and land cover, geological structure, basin length, and relief of the basin (Miller, 1953). As stated by Ahmad *et al.* (2010), for the perfect circular basin, the value of 'R_c' is equal to 1, and for an elongated watershed, its value ranges from 0.4 to 0.5. Analysis output indicates that the 'R_c' of the Ranikhola watershed was found to be 0.50 (Table 3). This depicts that the watershed is elongated in shape with fewer structural disturbances. In an elongated watershed, runoff from different parts reaches gradually to the outlet; thus, the magnitude of runoff is lower compared to that of the circular watershed.

Form Factor (F_f)

It is the ratio of the basin area to the square of the basin length. It is generally used to indicate different shapes of the basin (Horton, 1932). The 'F_f' less than 0.78 indicates an elongated basin, and greater than 0.78 indicates a circular basin (Farhan *et al*., 2016). For the Ranikhola watershed, 'F_f' was found to be 0.347 (Table 3), which shows that the watershed is elongated and has a lower peak for a longer duration. The flow of such elongated basins is easier to regulate as compared to circular basins due to the high time of concentration.

Infiltration Number (I_N)

It is the product of drainage density (D_d) and stream frequency (F_S). 'I_N' provides an idea about the infiltration capacity of the basin. The higher value of 'I_N' indicates the lower infiltration capacity of the basin, which results in more runoff generation in the basin. The value of 'I_N' of the Ranikhola watershed was estimated to be 0.271 (Table 3), which shows the strong infiltration capacity of the bedrock, lower runoff, and less susceptibility to soil erosion within the watershed.

Length of Overland Flow (L_g)

It is the distance that water flows across the ground surface before reaching stream channels. Horton (1945) expressed 'L_g' as equal to half of the inverse of a drainage density. Ratnam *et al*., (2005) classified 'L_g' in three classes i.e., high value (>0.3), moderate value (0.2-0.3), and low value (< 0.2). A low value of 'L_g' depicts a high slope, short flow paths, high runoff, and slow infiltration, which will lead to increased vulnerability to flash floods. The 'L_g' of the Ranikhola watershed, as computed from 'D_d' was found to be 0.855 km (Table 3). This means runoff has to flow for 0.855 km as a sheet flow before it reaches a stream channel, which indicates watershed slope is gentle, flow paths are long, infiltration is more, and groundwater potential is high.

Constant of Channel Maintenance (C)

Schumm (1956) defined 'C' as a reciprocal of the drainage density (D_d). This parameter signifies the area required to sustain unit channel length. Typically, a higher value of 'C' points toward the more permeable rocks in the basin. In the Ranikhola watershed, the value of 'C' was

estimated as 1.71 (Table 3), which is said to be higher (Dikpal *et al*., 2017). The greater the value of 'C' lesser the watershed is susceptible to soil erosion because of lower runoff. This also shows the dense vegetation in the watershed and higher soil infiltration rate.

Relief Aspects

The relief aspects are important terrain parameters used in assessing disaster-prone areas, erosion hazards, etc. They are of prime importance in decision-making on the needs of treatment with soil and water conservation structures in the watershed. The summary of relief aspects of the Ranikhola watershed is given in Table 4.

Relief (R)

Schumn (1956) defined relief (R) as the measure of elevation difference from the stream head to the point where it connects to the higher-order stream. It directly influences runoff velocity and sediment load transportation (Hadley and Schumm, 1961). The estimated elevations of the highest and the lowest points in the study area were 2454 m and 859 m, thus giving the maximum relief of the watershed to be 1595 m (Table 4).

Relief Ratio (R_R)

It refers to the ratio of the basin relief (R) to the maximum length of a basin parallel to the main drainage line (Schumm, 1956). It helps in comparing the relative relief of a basin regardless of the topography. 'R_R' indicates the steepness of the watershed and the erosion intensity on the slope of the basin, and morphometric characteristics (Hadley and Schumm, 1961). The high value of 'R_R' is the characteristic of hill regions whereas the low value represents plain area and valley. The longest dimension of the Ranikhola watershed measured parallel to the direction of the main channel was 8.513 km. The 'R_R' of the watershed was estimated to be 0.187 (Table 4), indicating the area has low relief, low runoff, and high groundwater potential. A lower 'R_R' indicates the characteristic features of less resistant rocks in the study location.

Time of Concentration (t_c)

It is the time required for runoff to move from the most remote point to the outlet point of a watershed. It depends on topography, geology, and land use/cover within the basin. The 't_c' of the Ranikhola watershed was found to be 52.58 minutes (Table 4). A high value of 't_c' shows a watershed with a dominant previous area, which leads to lower runoff, fewer erosion problems, and less structural disturbance in a watershed.

Ruggedness Number (HD)

It is the product of maximum basin relief (R) and drainage density (D_d) (Strahler, 1968). It indicates the degree of smoothness and roughness of the watershed topography (Dubey *et al*., 2015). Its values range from 0 to 1. The Values close to 0 show relatively smoother topography, and 'HD' close to 1 shows rough topographical characteristics. The Ruggedness value for the Ranikhola watershed was found to be 0.933 (Table 4), indicating the area has a rugged terrain. A rough topography of the watershed leads to high time of concentration and low runoff generation. This makes a watershed less susceptible to soil erosion.

Average Slope (S)

It provides information about the watershed topography. It is computed by dividing the area of the watershed by the multiplication of contour interval and the total length of all contours. The average slope of a basin has a significant impact on the time of concentration and directly affects the runoff generated into the basin. The basin slope (S) has a direct impact on the soil erodibility of the basin. Previous studies have shown that a higher land slope will lead to more erosion when other parameters remain the same. The results show the total length of all contours was estimated to be 1.38 km, and the contour interval was 250 m. It has been observed that the major portion (42 km^2) of the Ranikhola watershed has a land slope of less than 30%, as shown in Figure 6, which leads to low runoff generation in the watershed. However, the establishment of adequate soil and water conservation structures is essential in these areas to avoid soil erosion and water loss. The areas under different slope zones are given in Table 5.

Conclusion

Morphometric analysis of the Ranikhola watershed elucidates that the Ranikhola River is having a fourth-order stream, with the domination of lower-order streams. Homogeneous surface features and dendritic drainage patterns in the majority of the areas were also found, which are occupied by the streams of the first, second, and third order. The

average value of 'R_b' for the selected watershed was estimated as 2.64, which indicates the good drainage pattern of the watershed. The lower value of the 'D_d' (0.585 km^{-1}) indicates a more permeable subsoil formation under the dense vegetation cover and lower watershed relief. The 'F_f', 'R_e', and 'R_c' of the watershed indicate an elongated shape associated with mild slopes and long flow paths. Thus, a watershed has a lower peak for a longer time base on the runoff hydrograph. The lesser relief ratio would lead the watershed to be less susceptible to soil erosion on the slope, probably because of forest-dominated land use. The morphometric analysis of the Ranikhola watershed will be used in the watershed evaluation process, decision-making for prioritization of conservation structures, and micro-level management of natural resources.

Conflict of interest

The authors declare that they have no conflict of interest.

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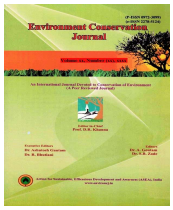

Characterization of irrigation water quality of groundnut belt of erstwhile Mahbubnagar district of Telangana

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ABSTRACT

An investigation was performed to characterize the irrigation water quality of the groundnut belt in the erstwhile Mahabubnagar district, Telangana for which 35 irrigation water samples from both canal and groundwater sources from the study area were collected through a preliminary survey in the selected farmer's fields. The samples which were analyzed for pH, EC, RSC, SAR Mg/Ca ratio and Kelly's ratio in the laboratory interpreted that the pH was slightly alkaline (pH: 7.58) with medium salinity (0.64 dS/m) and high Mg/Ca (1.15) ratio though the RSC (5.05) and SAR (2.68) fall in the safe ranges and were classified under C2S1 and C3S1 irrigation water classes. Considering the pH range in the irrigation water, proper management of the soil through incorporation of organic manures at regular intervals is suggested in all the regions of the groundnut belt (highly and marginally potential zones) having pH above 7.50 to prevent mounting up of soil pH when irrigated continuously over a period of time.

Introduction

In arid and semi-arid parts of India, groundwater is the primary source of irrigation for household, agricultural, and industrial needs. India has 2.2 percent of the world's territory, 4% of its water resources, and 16% of the world's people (Ramesh and Elango 2011; Bhutiani and Ahamad, 2019). So, development of irrigation in India has been driven by the paramount imperative of feeding a rapidly increasing population. Water quality is a major concern for humanity because it is directly linked to human welfare, particularly for drinking and agriculture (Tyagi *et al.,* 2020; Ruhela *et al.,* 2021; Bhutiani *et al.,* 2021).

The irrigation water quality can be defined based on the concentration and kind of salts and solids dissolved in it (Etteieb *et al.,* 2017). Irrigation water quality testing is necessary to ensure a safe supply of water to the crop. In recent years, there has been a growing concern over irrigation's long-term prospects and the ramifications of continuing existing water management techniques on the system's long-term viability (Chintapalli *et al.,* 2000). The information regarding irrigation water quality has critical importance in understanding the changes in the quality of the product, and the modifications that are required in the water management (Ramakrishnaiah *et al.,* 2009). The quality of irrigation water is an essential element in the assessment of salinity or alkali conditions in irrigated regions, and it is largely determined by the overall quantity of salt present, the proportion of sodium (Na) to other cations, and a number of other factors (Tiwari, 2011). The efficiency of the product and the potential for emergence of hazardous conditions of the soil should be considered during the evaluation of water quality

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for irrigation for obtaining better yields in the crop production (Bhardwaj *et al.,* 2020). Thus, evaluation of water quality is mandatory in planning, design and operation of irrigation systems (Mirabbasi *et al.,* 2008). So, the present exploration was taken up aiming for irrigation water quality characterization during rabi 2019-20 in the erstwhile Mahabubnagar district of Telangana.

Material and Methods

Study area

Mahabubnagar district of Telangana lies between 15°55' to 17°20' latitudes and 77°15' to 79°15' Northern and Eastern longitudes where the climate is generally hot. The mean monthly maximum temperature ranges between 30.5˚C in August and 38.8˚C during April-May. The average monthly minimum temperature ranged from 16.3˚C during and to 26.4˚C during May. The mean annual rainfall is 604 mm which is mostly received during South-West monsoon. The annual rainfall was hardly 64.0 per cent of the state average (940 mm). The year-to-year variation in the actual rainfall showed that there were more dry spells during the cropping season (District census handbook-Mahabubnagar, 2011). The principal soil is the chalka dubba in about 70.0 per cent of the study area and has low water holding capacity (Statistical year book-Mahabubnagar, 2017). Krishna and Tungabhadra are the two principal rivers that flowed through the district. The total ayacut area under different irrigation projects is 5.37 lakh ha. The major irrigation projects occupy an area of 3.70 lakh ha. The medium irrigation projects occupy an area of 0.20 lakh ha, and an area of 1.30 lakh ha is under minor irrigation projects. There are about 1,87,216 minor irrigation sources in the district which include shallow tube wells, dug wells, deep tube wells, surface flow and lift irrigation projects (Statistical year book-Mahabubnagar, 2017). The average irrigation intensity of the state is 1.42 (average from 2011-12 to 2015-16). Net area irrigated under different sources of irrigation in the district was 2.50 lakh ha (2010-12), out of which the area irrigated by the groundwater resources was 2.10 lakh ha, which constitutes 83.2 per cent of the net area irrigated. Area irrigated by surface water was 0.30 lakh ha, which accounts for 13.7 per cent of the total irrigated area and remaining by other sources (Madhusudhana, 2013).

Water sample collection and analysis

The groundnut crop being an important *rabi* season crop of the erstwhile Mahabubnagar district is a crop colony of groundnut. The marginally potential zones of the crop colony have high crop spread but has low productivity. So, assessment of irrigation water could reveal the reason for low crop productivity in the study area. Thirty-five (35) irrigation water samples in total were collected from both borewell (24 samples) and canal (11 samples) (Table 1 & Figure 1) sources from the study area at the time of crop harvest i.e., from 28th November, 2019 to 6th February, 2020. The samples were analysed for pH, EC (Electrical Conductivity), carbonates, bicarbonates, calcium, magnesium and sodium following the standard procedures in the laboratory Jackson, 1967; Barnes (1964); Wood (1976); Hem (1970); Diehl (1950) from which sodium absorption ratio (SAR) residual sodium carbonate (RSC), magnesium/calcium ratio and Kelly's ratio were computed and categorized them into suitable classes (Table 2). A detailed methodology followed for the assessment of quality of irrigation water samples was presented in Figure 2.

Results and Discussion

The irrigation water quality determines its suitability for the crop and its yield. So, a careful analysis was carried out for the assessment of pH, EC, RSC, SAR, Mg-Ca ratio and Kelly's ratio in the samples of the groundnut belt in the erstwhile Mahabubnagar district, Telangana and the results were detailed here under (Table 3).

pH of Irrigation water:

The study area with regards to irrigation water pH was categorized in to three classes viz., acidic (< 6.50), neutral (6.50-7.50) and alkaline (> 7.50). The pH of the water samples in the research site stretched from 6.91 to 8.10 with the mean of 7.58. The pH of borewell samples stretched from 6.91 and 7.90 with a mean of 7.56. Similarly, pH of canal water samples stretched from 7.10 and 8.10 by mean value of 7.59. The overall assessment of both the sources showed that the irrigation water in the groundnut zone falls into alkaline range. Ranjit *et al*. (2017) at Kalwakurthy mandal of Mahabubnagar also reported pH of water samples ranging from 7.78 to 8.90 with a mean value of 8.12.

Table 1: Location and coordinates of the selected groundnut crop fields.

SN	Village	Mandal	Division	Latitude	Longitude
1	Shekupally	Itikyal	Gadwal	16.11833	77.92636
2	Kothakota	Kothakota	Wanaparthy	16.36164	77.93672
3	Putanpally	Gadwal	Gadwal	16.16716	77.84795
4	Dattaipally	Wanaparthy	Wanaparthy	16.31787	78.07460
5	Maldakal	Maldakal	Gadwal	16.10839	77.68590
6	Basavapuram	Gattu	Gadwal	16.15046	77.58418
7	Mylagadda	K.T.Doddi	Gadwal	16.23944	77.60169
8	Nallahelli	Dharoor	Gadwal	16.28208	77.61575
9	Pathapalem	Dharoor	Gadwal	16.28136	77.61658
10	Mylaram	Kodair	Nagarkurnool	16.17734	78.31488
11	Buddharam	Gopalpet	Wanaparthy	16.41820	78.14000
12	Velgonda	Chinnambavi	Wanaparthy	16.10885	78.09300
13	Nallavelly	Nagarkurnool	Nagarkurnool	16.48328	78.24618
14	Ankiraopally	Kollapur	Nagarkurnool	16.10828	78.31293
15	Kottapally	Amrabad	Nagarkurnool	16.35644	78.81134
16	Pentlavelly	Pentlavelly	Nagarkurnool	16.07197	78.23838
17	Veljal	Talakondapally	Shadnagar	16.65389	78.19442
18	Gopaldinne	Veepanagandla	Wanaparthy	16.12852	78.06158
19	Uppununtala	Uppununtala	Nagarkurnool	16.52633	78.66717
20	Lingala	Lingala	Nagarkurnool	16.23610	78.08784
21	Gattunellikuduru	Telkapally	Nagarkurnool	16.40301	78.44006
22	Chennaram	Balmoor	Nagarkurnool	16.38845	78.55522
23	Kakunooru	Keshampet	Shadnagar	16.91817	78.32250
24	Kalvakolu	Peddakottapally	Nagarkurnool	16.17726	78.31484
25	Waddeman	Bijinapally	Nagarkurnool	16.48315	78.22762
26	Gummakonda	Timmajipet	Nagarkurnool	16.65451	78.19461
27	Lingotam	Achampet	Nagarkurnool	16.41383	78.62158
28	Chinna Aadirala	Jadcherla	Mahabubnagar	16.83731	78.31447
29	Chinnamylaram	Kodangal	Kodangal	17.09258	77.70106
30	Dudhyal	Kodangal	Kodangal	17.04719	77.71156
31	Pedda Aadirala	Jadcherla	Mahabubnagar	16.09869	78.29169
32	Papagal	Tadoor	Nagarkurnool	16.65180	78.30635
33	Rudrasamudram	Makhtal	Narayanpet	16.49499	77.92636
34	Rudrasamudram	Makhtal	Narayanpet	16.46476	77.93672
35	Mahabubnagar	Mahabubnagar	Mahabubnagar	16.72500	77.84795

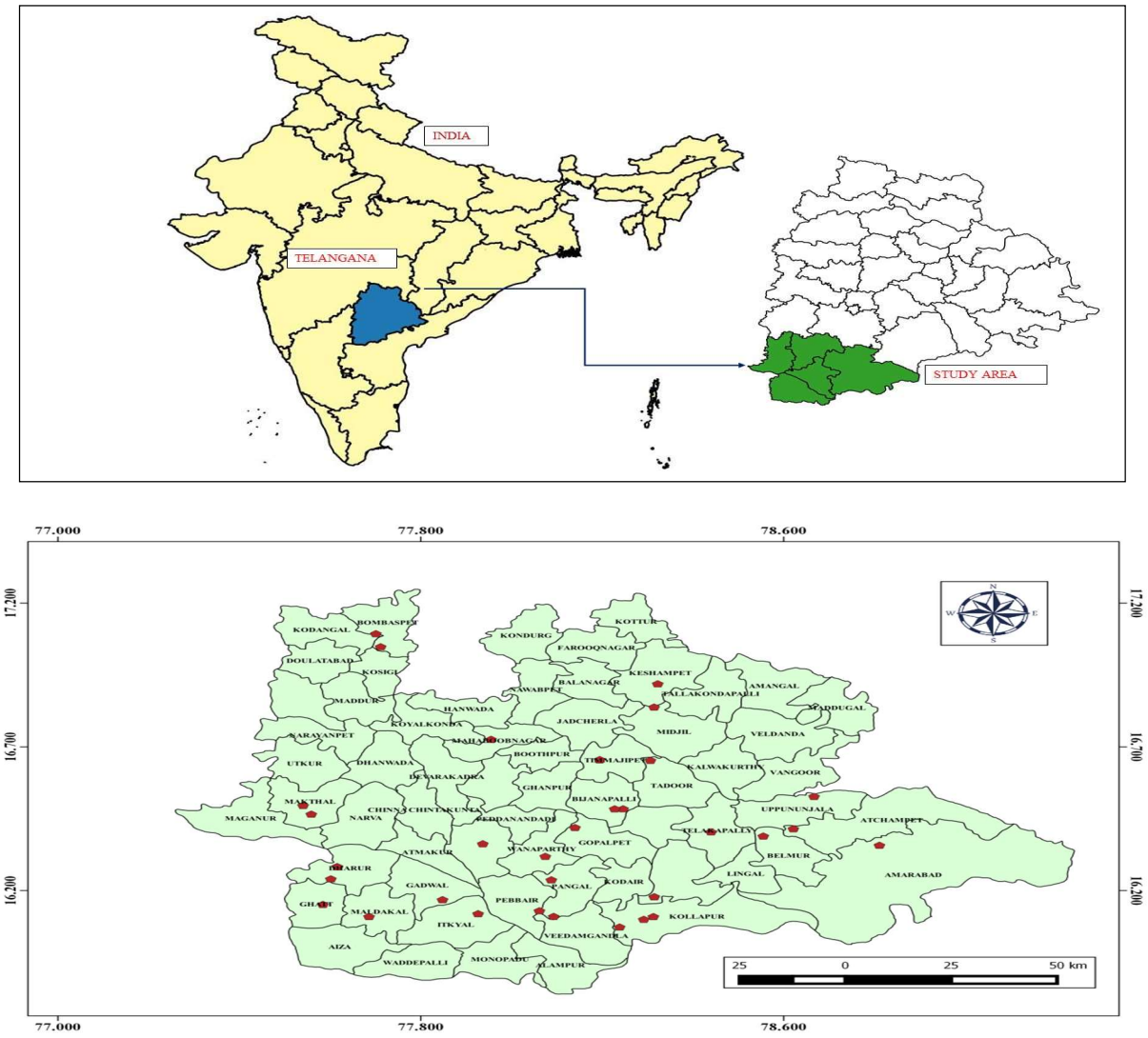

Figure 1: The study area map representing the coordinates of the groundnut fields

Table 2: Classes assigned for irrigation water parameters to assess the water quality in the study area (US Salinity Lab, 1954)

SN	Water quality parameter	No of classes assigned	Details of classes
1.	pH (1:2.5)	03	▪ < 6.00: Acidic ▪ 6.00-7.50: Neutral ▪ > 7.50: Alkaline
2.	EC (dS/m) (US Salinity Lab, 1954)	04	▪ C1: < 0.25-Low saline ▪ C2: 0.25-0.75-Medium saline ▪ C3: 0.75-2.25-Highly saline ▪ C4: > 2.25-Very highly saline
3.	SAR	04	▪ S1: 0-10.0- Low ▪ S2: 10.0-18.0-Medium ▪ S3:18.0-26.0-High ▪ S4:> 26.0-Very high
4.	RSC (me l^{-1})	03	▪ < 1.25: Safe ▪ 1.25 -2.5 0: Moderate ▪ > 2.5 0: Unsafe
5.	Mg/ Ca ratio	02	▪ < 1.00: Safe ▪ > 1.00: Unsafe
6.	Kelly's Ratio	02	▪ < 1.0: Suitable ▪ > 1.0: Unsuitable

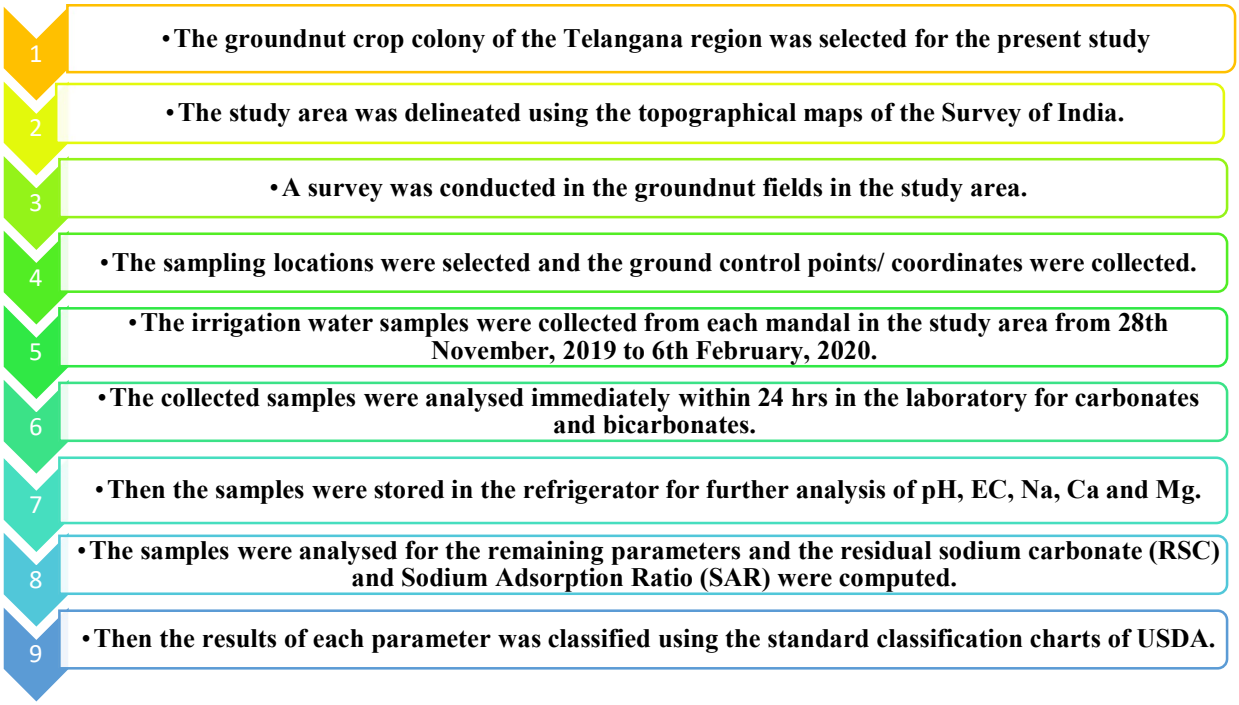

Figure 2: Detailed methodology for sampling and analysis of irrigation water samples

Assessment of groundwater quality in selected villages of Mahabubnagar by Srinivasulu *et al*. (2015) also showed that pH ranged from 7.09 to 8.19 which are slightly basic. The higher pH of the most of groundwater samples may be due to considerable Na^{+}, Ca^{2+}, Mg^{2+}, CO_3^{2-} and HCO_3^{-}. The samples from Nagarkurnool and Wanaparthy divisions of the research site in the groundnut belt showed that pH of irrigation water was mostly alkaline with exception of Timmajipet, Tadoor and parts of Bijinapally, Nagarkurnool, Telkapally, Balmoor, Uppununtala, Achampet, Kollapur, Veepangandla, Pebbair, Ghanpur mandals having neutral pH. Contrastingly, the irrigation water of entire Narayanpet and Gadwal divisions was characterized as alkaline with few exceptions in parts of Itikyal, Gadwal and Monopad mandals of Gadwal division with neutral irrigation samples. Considering the pH range, proper management of the soil through incorporation of organic manures at regular intervals is suggested in all the regions of the groundnut belt (highly and marginally potential zones) having pH above 7.50 to prevent mounting up of soil pH when irrigated continuously over a period of time.

Electrical Conductivity of irrigation water:

The irrigation water samples for electrical conductivity were classified into four classes viz., low saline (very good water: < 0.25 dS/m), medium saline (good water: 0.25-0.75 dS/m), highly saline (doubtful water: 0.75-2.25 dS/m) and very highly saline (not useful water: > 2.25 dS/m). The Electrical conductivity (EC) in the irrigation water samples (borewells and canals) in the study area ranged from 0.14 to 1.47 dS/m with a mean value of 0.64 dS/m. These values can be supported from EC values obtained at Kalwakurthy mandal which extend from 0.40 to 1.20 dS/m with a mean value of 0.71 dS/m in the irrigation water (Ranjit *et al.,* 2017). The irrigation water from borewells had reported EC ranging from 0.14 to 0.98 dS/m with a mean of 0.47 dS/m. On the other hand, the EC of the water samples of canal irrigated regions ranged from 0.15 to 1.47 dS/m with an average value of 0.72 dS/m. The EC of the water samples (both borewells and canals) indicated that the irrigation water though was doubtful (highly saline) for crop growth in some regions it was mostly good for irrigating the crop. Disintegrated regions of (doubtful) highly saline water were seen in Kodangal, Kosgi, Bomraspet, Doultabad and parts

Table 3: Results of laboratory analysis of the irrigation water samples collected from the farmer's fields in the study area.

SN	District	pH (1:2.5)	Rating	EC (dS/m)	Rating	SAR	Rating	Irrigation water class	Mg/Ca ratio	Rating	RSC	Rating	Kelly's ratio	Rating
1	**Gadwal**	6.91	Neutral	0.14	Low (C1)	1.80	Low Na (S1)	C1S1	0.79	Low	-6.70	Safe	0.32	Suitable
2	**Wanaparthy**	8.10	Slightly alkaline	0.79	Highly saline (C3)	1.99	Low Na (S1)	C3S1	1.07	Low	-6.40	Safe	0.34	Suitable
3	**Gadwal**	7.40	Neutral	0.17	Low (C1)	1.83	Low Na (S1)	C1S1	0.84	Low	-7.20	Safe	0.32	Suitable
4	**Wanaparthy**	7.70	Slightly alkaline	0.49	Medium saline (C2)	1.47	Low Na (S1)	C2S1	2.93	Medium	-7.90	Safe	0.23	Suitable
5	**Gadwal**	7.90	Slightly alkaline	0.57	Medium saline (C2)	1.30	Low Na (S1)	C2S1	2.80	Medium	-9.00	Safe	0.20	Suitable
6	**Gadwal**	8.05	Slightly alkaline	1.01	Highly saline (C3)	3.43	Low Na (S1)	C3S1	0.84	Low	-7.20	Safe	0.57	Suitable
7	**Gadwal**	7.90	Slightly alkaline	0.64	Medium saline (C2)	1.90	Low Na (S1)	C2S1	0.51	Low	-4.30	Safe	0.40	Suitable
8	**Gadwal**	8.10	Slightly alkaline	0.53	Medium saline (C2)	1.82	Low Na (S1)	C2S1	0.93	Low	-3.10	Safe	0.36	Suitable
9	**Gadwal**	8.01	Slightly alkaline	0.24	Low (C1)	1.33	Low Na (S1)	C1S1	1.95	Medium	-9.00	Safe	0.19	Suitable
10	**Nagarkurnool**	7.60	Slightly alkaline	1.43	Low (C1)	10.3	Medium Na (S2)	C1S2	1.80	Medium	-3.20	Safe	2.00	Unsuitable
11	**Wanaparthy**	7.90	Slightly alkaline	0.36	Medium saline (C2)	1.70	Low Na (S1)	C2S1	1.71	Medium	-10.5	Safe	0.24	Suitable
12	**Wanaparthy**	7.72	Slightly alkaline	0.67	Medium saline (C2)	3.26	Low Na (S1)	C2S1	1.30	Low	-6.00	Safe	0.59	Suitable
13	**Nagarkurnool**	7.30	Neutral	0.32	Medium saline (C2)	3.34	Low Na (S1)	C2S1	1.95	Medium	-4.60	Safe	0.64	Suitable
14	**Nagarkurnool**	7.80	Slightly alkaline	0.38	Medium saline (C2)	2.44	Low Na (S1)	C2S1	0.68	Low	-1.70	Safe	0.60	Suitable
15	**Nagarkurnool**	7.60	Slightly alkaline	0.15	Low (C1)	2.00	Low Na (S1)	C1S1	1.86	Medium	-7.10	Safe	0.30	Suitable
16	**Nagarkurnool**	7.16	Neutral	1.19	Highly saline (C3)	2.33	Low Na (S1)	C3S1	1.25	Low	-5.70	Safe	0.25	Suitable
17	**RangaReddy**	7.15	Neutral	0.62	Medium saline (C2)	1.33	Low Na (S1)	C2S1	0.60	Low	-3.95	Safe	0.29	Suitable
18	**Wanaparthy**	8.05	Slightly alkaline	0.96	Highly saline (C3)	3.39	Low Na (S1)	C3S1	0.10	Low	-6.95	Safe	0.22	Suitable
19	**Nagarkurnool**	7.20	Neutral	0.81	Highly saline (C3)	1.96	Low Na (S1)	C3S1	0.90	Low	-4.10	Safe	0.36	Suitable
20	**Nagarkurnool**	7.56	Slightly	0.44	Highly	2.07	Low Na	C3S1	0.60	Low	-5.55	Safe	0.08	Suitable

			alkaline		saline (C3)		(S1)							
21	**Nagarkurnool**	7.44	Neutral	0.45	Medium saline (C2)	1.92	Low Na (S1)	C2S1	1.07	Low	-4.50	Safe	0.04	Suitable
22	**Nagarkurnool**	7.20	Neutral	0.25	Low (C1)	2.92	Low Na (S1)	C1S1	0.04	Low	-3.75	Safe	0.23	Suitable
23	**RangaReddy**	7.20	Neutral	0.85	Highly saline (C3)	2.18	Low Na (S1)	C3S1	0.60	Low	-3.95	Safe	0.47	Suitable
24	**Nagarkurnool**	7.72	Slightly alkaline	1.47	Highly saline (C3)	2.89	Low Na (S1)	C3S1	0.27	Low	-1.80	Safe	0.61	Suitable
25	**Nagarkurnool**	7.54	Slightly alkaline	0.98	Highly saline (C3)	4.47	Low Na (S1)	C3S1	0.19	Low	1.00	Safe	1.18	Unsuitable
26	**Nagarkurnool**	7.52	Slightly alkaline	1.26	Highly saline (C3)	4.24	Low Na (S1)	C3S1	2.40	Medium	-5.65	Safe	0.33	Suitable
27	**Nagarkurnool**	7.79	Slightly alkaline	0.67	Medium saline (C2)	1.42	Low Na (S1)	C2S1	2.43	Medium	-7.10	Safe	0.26	Suitable
28	**Mahabubnagar**	7.10	Neutral	0.31	Medium saline (C2)	1.06	Low Na (S1)	C2S1	0.35	Low	-0.80	Safe	0.81	Suitable
29	**Vikarabad**	7.50	Neutral	0.84	Medium saline (C2)	6.57	Low Na (S1)	C2S1	0.74	Low	-2.15	Safe	0.60	Suitable
30	**Vikarabad**	7.60	Slightly alkaline	0.88	Medium saline (C2)	5.47	Low Na (S1)	C2S1	0.84	Low	-5.85	Safe	0.36	Suitable
31	**Mahabubnagar**	7.40	Neutral	0.53	Medium saline (C2)	1.16	Low Na (S1)	C2S1	1.33	Low	-2.90	Safe	0.48	Suitable
32	**Nagarkurnool**	7.26	Neutral	0.92	Medium saline (C2)	3.79	Low Na (S1)	C2S1	1.41	Low	-4.70	Safe	1.57	Unsuitable
33	**Narayanpet**	7.16	Neutral	1.19	Highly saline (C3)	1.92	Low Na (S1)	C3S1	1.08	Low	-5.34	Safe	0.66	Suitable
34	**Narayanpet**	7.15	Neutral	0.62	Medium saline (C2)	1.34	Low Na (S1)	C2S1	1.09	Low	-4.61	Safe	1.29	Unsuitable
35	**Mahabubnagar**	7.59	Slightly alkaline	0.32	Medium saline (C2)	1.42	Low Na (S1)	C2S1	1.18	Low	-4.36	Safe	1.39	Unsuitable

of Bhootpur, Timmajipet, Jadcherla, Tadoor, Midjil, Bijinapally, Ghanpur, Keshampet, Kothur, Kondurg, Farooqnagar, Uppununtala, Vangoor, Amrabad, Kothakota, Pebbair, Ghattu, Alampur, Monopadu, Veepanagandla, Pangal, Kodair, Peddakothapalli and Kollapur mandals. In these regions, while irrigating the crop precautions to be taken to manage the soil by adding organic matter once in two years to prevent build-up of soil EC due to long term irrigation with waters of high EC (0.75-2.25 dS/m). An overview of study area shows that the overall study (Nagarkurnool and Wanaparthy Narayanpet and Gadwal divisions) of groundnut cultivation receive medium saline water for irrigating the crop.

Sodium Absorption Ratio (SAR)

Corresponding to RSC contents, the SAR values were low for the irrigation water ranging from 1.06 to 10.3 with a mean value of 2.68 showing that the irrigation water was safe and sound for irrigating the crop. The sodium absorption ratio of the canal waters ranged from 1.30 to 4.48 with a mean of 2.47, while the SAR of borewell waters extended from 1.06 to 10.3 with an average value of 2.78. However, Ranjit *et al*. (2017) reported SAR values extending from 0.30 to 1.40 with a mean of 0.60 at Kalwakurthy mandal. The sodium absorption ratio was mapped with two rates viz., very low (-1.00 to -5.00) and low (-5.00 to -10.0). Entire district was portrayed as having very low SAR with few areas of low SAR in parts of Kodair, Kollapur, Kodangal, Kosgi, Bomraspet and Doulatbad mandals. A very low SAR in irrigation water was observed in the entire study area (Nagarkurnool, Wanaparthy, Narayanpet and Gadwal divisions) of groundnut except in parts of Kodair, Kollapur and Kosgi mandals with low SAR values. Ayers and Westcot (1976) reported that irrigation water having SAR of 0-10, i.e., low Na^+ water poses almost no risk of exchangeable Na^+. Since calcium is the predominantaly adsorbed cation in both seasons, soil tend to have a granular structure, which is easily worked and readily permeable (Laloo *et al.,* 2020).

Irrigation water class (EC x SAR):

In accordance with the US Salinity Lab Classification System of irrigation water class, out of 35 water samples analysed, 18 samples fell into C2S1 category, 11 into C3S1, 5 into C1S1 and 1 sample into C3S2. Similar analysis was performed in Turkey by Yilmaz and Avci (2021), where the irrigation water samples were classified into C2S1 and C3S1

Residual Sodium Carbonate (RSC) content:

The RSC content of irrigation water from both canals and borewells in the entire study area was very low ranging from -10.5 to 1.00 me l^{-1} with mean of -5.05 showing that irrigation water was safe for attaining good groundnut yields. Sundaraiah *et al*. (2014) at Kalwakurthy mandal of Mahabubnagar district indicated similar RSC values of irrigation samples varied from -6.91 to 0.19 me l^{-1}. More precisely, the RSC content ranged from -10.5 to 1.00 me l^{-1} in canal waters and -0.80 to -9.10 me l^{-1} in borewell waters with a mean of 5.05 me l^{-1} in both the cases. No large variations in either highly (Nagarkurnool and Wanaparthy divisions) and marginally (Narayanpet and Gadwal divisions) potential regions with respect to residual sodium carbonate was observed. Similar results were observed with Ranjitha *et al.* (2018) where the irrigation water was safe w.r.t SAR and RSC.

Magnesium-Calcium ratio:

The assessment of Mg/Ca ratio for irrigation water was carried out with two classes viz., safe (< 1.00) and not safe (> 1.00) for mapping. As per the classification given by U. S. Salinity Laboratory, Mg/Ca ratio < 1.50 is considered safe, 1.50 - 3.00 is moderately safe and >3.00 is unsafe (United States Salinity Laboratory, 1954). Though the irrigation water was safe in terms of sodium, the Mg/Ca ratio was higher in the study area ranging from 0.04 to 2.93 with an average of 1.15 which is not considered safe for irrigating the crops. Generally, Calcium and Magnesium maintains an equilibrium in water. But when Magnesium content increases, it promotes the increase in sodium concentration in water (Vasu *et al.,* 2015; Ayers and Wescot, 1985). Considering different sources, the Mg/Ca ratio in canal water extending from 0.04 to 2.80 with a mean of 1.06, whereas in borewell waters, it stretched from 0.10 to 2.43 with an average value of 1.20. Most of the groundnut growing regions were classified as having Mg/Ca ratio in safe limits with few unsafe areas in Bomraspet, Kodangal, Doultabad, Kosigi, Kothur, Keshampet and parts of Maddur, Kondurg, Farooqnagar, Balnagar, Talakondapally, Amangal, Midjil, Bijinapally, Uppununtala, Balmoor, Lingal, Kollapur, Pebbair,

Itikyal, Gadwal, Veepangandla, Monopadu, Alampur, Ghattu and Dharur mandals. Of the above mandals, unsafe regions were distributed in few clusters of Nagarkurnool, Wanaparthy, Narayanpet and Gadwal divisions) of groundnut belt.

Kelly's ratio (KR):

Sodium measured against calcium and magnesium was considered by Kelly (1940). The formula used in the estimation of Kelley's ratio is expressed as $KR = (Na^{+} / Ca^{2++} Mg^{2+})$. If KI value is >1, then the water is unfit for irrigation. In the present study the values of KR ranged from 0.04 and 2.00 with a mean value of 0.54 which explains the suitability of water for irrigating the crop. Maximum KR value was found in Kodair mandal of Nagarkurnool district and minimum value was found in Telkapally mandal of Nagarkurnool district. All samples showed favourable KR values except in Kodair, Bijinapally and Tadoor mandals Nagarkurnool district, Makhtal mandal of Narayanpet and Mahabubnagar mandal.

Conclusion

Irrigation water quality assessment helps to sort out the reasons for reduced crop productivity in the marginally potential zones and this helps in providing better quality of resources to the crop. The overall quality of the irrigation water samples of both canal and groundwater was good for providing irrigation to the crop. However, the salt concentration and high magnesium-calcium ratio can be reduced with good crop management practices like application of organic manures to the crop and conjunctive use of irrigation water.

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Conflict of interest

The authors declare that they have no conflict of interest.

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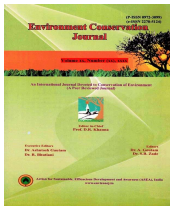

Effect of vermicompost and fertilizer on uptake and efficiency of nutrients in pot culture rice

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ABSTRACT

Rice is the major dominant crop in the asian continent and all over the world. A research was carried out at Dr. Rajendra Prasad Central Agricultural University, Pusa in *kharif, 2018* containing four different levels of vermicompost (0 t/ ha , 1.25 t/ ha, 2.5 t/ ha, 3.7 t/ ha) and three levels (0 %, 100 %, 50 % Recommended Dose of Fertilizer) of fertilizer RDF were combined with each other and analyzed for nutrient uptake and efficiencies in pot cultured rice crop variety *Rajendra Bhagawati.* Study revealed that nutrient uptake in grain (446.03 mg/ pot N, 104.95 mg/ pot P , 112.06 mg/ pot K) and straw (303.81 mg/ pot N , 49.83 mg/ pot P, 578.78 mg/ pot K) and the total nutrient uptake i.e. N (227.67 mg/ pot), P(0.083 mg/ pot), K(690.84 mg/ pot) were superior in the combined application of 3.75 t/ ha vermicompost and 100% RDF over other and showed higher stability in case of apparent nutrient use efficiency in 3.75 t/ ha vermicompost and 50% RDF except potassium for balanced growth of rice crop and declining straight 50% cost off chemical fertilizer substituted with organic sources.

Introduction

Rice is the most important food crop grown in the world with a production of nearly more than 509.8 million tons milled rice and the productivity is about 3100 kg/ha (Rice - statistics & facts-2021) as well as staple food in south East Asian region to mitigate the food crisis created by over population. To increase the crop production heavy application of chemical fertilizers are unfavourable for soil health condition along with decline in soil microbial activities. To maintain the soil fertility, soil health and enhance soil organic matter, combined applications of vermicompost with fertilizers are being recommended. Vermicompost are the ultimate fine particles secreted from casts of earthworms enriched with macro and micro nutrients i.e. N, P, K, Ca, Mg, Fe etc are readily available to plants (Piya *et al.,* 2018) along with enzymes responsible for plant growth stimulation, prevention of plant diseases and enhancement of rice crop yields by about 40% (Kamaleshwaran and Elayaraj, 2021). The combined application of vermicompost and fertilizer in integrated nutrient management helps in improving soil health and fertility status along with increasing the nutrient use efficiency (Manivannan *et al.,* 2020) which reduces the cost of cultivation and also provides a subtract for better growth of microbes in soil which will improve the soil physical, chemical and biological

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properties. Proper binding of soil particles in presence of healthy organic matter content and helps in proper establishment and growth of rice crop, ensuring higher productivity of rice.

Material and Methods

The research was carried out in department of soil science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar consisting of four levels of vermicompost (0 t/ ha, 1.25 t/ ha, 2.5 t/ ha, 3.7 t/ ha) and three levels (0 %, 100 %, 50 % RDF) of fertilizer mixed and taken in pot where rice crop (*Rajendra Bhagwati*) was grown with twelve treatment replicated thrice using Factorial Completely Randomized Design. The treatment details are as follows:

$V_0 F_{0:}$- No manure + No Fertilizer- Control
$V_0 F_{100:}$- No manure + 100% RDF
$V_0 F_{50:}$- No manure + 50% RDF
$V_{1.25} F_{0:}$- Vermicompost (1.25 t/ ha) + No Fertilizer
$V_{1.25} F_{100:}$- Vermicompost (1.25 t/ ha) +100% RDF
$V_{1.25} F_{50:}$-Vermicompost (1.25 t/ ha) + 50% RDF
$V_{2.50} F_{0:}$-Vermicompost (2.5 t/ ha) + No Fertilizar
$V_{2.50}F_{100:}$-Vermicompost (2.5 t/ ha) + 100%RDF
$V_{2.50}F_{50:}$-Vermicompost (2.5 t/ ha) + 50% RDF
$V_{3.75}F_{0:}$-Vermicompost (3.75t/ ha) + No Fertilizar
$V_{3.75}F_{100:}$-Vermicompost (3.75 t/ ha) + 100%RDF
$V_{3.75}F_{50:}$-Vermicompost (3.75 t/ ha) +50% RDF

No. of treatment	:	12
No. of replication	:	3
Crop	:	Rice
Variety	:	Rajendra Bhagwati
Recommended Dose of Fertilizer	:	N: P_2O_5:K_2O:: 120:60:40 kg/ha
Experimental Design	:	Factorial Completely Randomized Design
Pot capacity	:	10 kg
Factor	:	02
Total number of pots	:	36

A. Uptake of Nutrients (mg/ pot)

Total uptake of N, P and K by rice crop was calculated by multiplying the N, P and K content with dry matter yield. Nutrient content (%) x dry matter (mg/ pot) 100

$$\textbf{Nutrient uptake} = \frac{\textbf{Nutrient content (\%) x dry matter (mg pot}^{-1}\textbf{)}}{\textbf{100}}$$

B. Efficiency of Nutrients (%)

$$\textbf{Apparent-nutrient recovery (\%)} = \frac{\textbf{Uptake treated plot (g /pot)} - \textbf{Uptake in control plot (g /pot)}}{\textbf{Amount of nutrient applied (g /pot)}} \times \textbf{100}$$

C. Statistical analysis:

All the data obtained in the experiment will be analyzed statistically applying Factorial Completely Randomized Design by the method of "Analysis of Variance" as described by Gomez and Gomez (1984). The Significance of the treatment effect was judged with the help of variance ratio test. Critical Difference (C.D.) at 5 percent and 1 percent level of significance worked out to determine the difference between treatment means. All the statistical analysis were done by using OPSTAT (http://14.139.232.166/opstat/default.asp) analysis software.

Results and Discussion

Grain nitrogen uptake (mg/ pot):

The effect of different levels of vermicompost and fertilizer on grain nitrogen uptake is shown in the table-1. The levels of vermicompost i.e. 1.25 t/ ha, 2.5 t/ ha, 3.75 t/ ha recorded significantly higher grain nitrogen uptake over no vermicompost level. The fertilizer levels of 50 and 100 % RDF also gave significantly superior grain nitrogen uptake over no fertilizer level. The interactions among the different levels of vermicompost and fertilizer were found significant. The integrated application of vermicompost (3.75 t/ ha) + 100 % NPK showed the significantly higher amount of grain nitrogen uptake i.e. 446.03 mg/ pot at harvest stage over the control (133.47 mg/ pot).

The increase in grain nitrogen uptake might be due to the mineralization of nutrients by the favorable micro flora and availability of nutrients increased the uptake by the plant thus increased grain nitrogen uptake. The 50 % Recommended dose of nitrogen + 50 % Green manure application increased grain N uptake (Mounika *et al.* 2017) and inorganic fertilizer also enhanced nutrient uptake by plants during the crop growth (Masni and Wasli, 2019).

Grain phosphorus uptake (mg/ pot):

The table-1 showed different levels of vermicompost and fertilizer on grain phosphorus uptake. The three levels of vermicompost i.e. 1.25 t/ ha, 2.5 t/ ha, 3.75 t/ ha gave significantly higher grain phosphorus uptake over no vermicompost level. The fertilizer levels of 50 and 100 % RDF recorded significantly superior grain phosphorus uptake over no fertilizer level. The interactions among the different levels of vermicompost and

fertilizer were found significant. The integrated application of vermicompost (3.75 t/ ha) + 100 % NPK recorded the significantly higher amount of grain phosphorus uptake i.e. 104.95 mg/ pot at harvest stage over the control (24.28 mg/ pot).The increase in grain phosphorus uptake might be due to CO2 produced during mineralization of different organic sources took role in P solubilisation (Sagarika *et al.* 2012) and the comparable effect on nutrient uptake content was given by (Masni and Wasli, 2019).

Grain potassium uptake (mg/ pot):

The influence of different levels of vermicompost and fertilizer on grain potassium uptake is shown in the table-1. The levels of vermicompost i.e. 1.25 t/ ha , 2.5 t/ ha , 3.75 t/ ha recorded significantly higher grain potassium uptake over no vermicompost level. The fertilizer levels of 50 and 100 % RDF also gave significantly superior grain potassium uptake over no fertilizer level. The interactions among the different levels of vermicompost and fertilizer were found significant. The integrated application of vermicompost (3.75 t/ ha) + 100 % NPK showed the significant higher amount of grain nitrogen uptake i.e. 112.06 mg/ pot , which was statistically at par with vermicompost 2.5 t/ ha and 100 % RDF i.e. 109.17 mg/ pot .The added organic manure might have enhanced beneficial micro flora thus uptake increased (Meena *et al*. 2010) and supporting study was given by (Krishna *et al.,* 2018).

Straw nitrogen uptake (mg/ pot):

The influence of different levels of vermicompost and fertilizer on the straw nitrogen uptake is presented in the table-2. The straw nitrogen uptake varied from 153.97 to 252.00 mg/ pot with different levels of vermicompost, irrespective of fertilizer levels. The straw potassium uptake in accordance to the different fertilizer levels ranged between 143.38 to 264.10 mg/ pot , irrespective of vermicompost levels. The vermicompost levels recorded significantly higher straw nitrogen uptake over no vermicompost level. The 50 and 100 % RDF levels also gave significantly higher straw nitrogen uptake over no fertilizer level. The interactions in between vermicompost and fertilizer levels were found significant. The elevated Straw nitrogen uptake was found in the treatment receiving (vermicompost-3.75 t/ ha + 100% RDF) i.e. 303.81 mg/ pot which was significantly superior over control (no vermicompost + 0% RDF) i.e. 94.20 mg/ pot and relative result was found out by (Masni and Wasli, 2019).

Straw phosphorus uptake (mg/ pot):

The table-2 showed different levels of vermicompost and fertilizer on straw phosphorus uptake**.** The straw phosphorus uptake varied 23.67 to 40.21 mg/ pot with different levels of vermicompost, irrespective of fertilizer levels. Irrespective of vermicompost levels, the straw phosphorus uptake in accordance to the different fertilizer levels ranged between 21.71 to 42.66 mg/ pot. The vermicompost levels recorded significantly higher straw phosphorus uptake over no vermicompost level. The 50 and 100 % RDF levels also gave significantly higher straw phosphorus uptake over no fertilizer level.

The interactions in between vermicompost and fertilizer levels were found significant. The elevated straw phosphorus uptake was found in the treatment receiving (vermicompost-3.75 t/ ha + 100% RDF) i.e. 49.83 mg/ pot which was significantly superior over control (vermicompost-no manure + 0% RDF) i.e. 14.31 mg/ pot.

The increase in straw phosphorus uptake might be due to the mineralization of nutrients by the favourable micro flora and availability of nutrients increased the uptake by the plant thus increased Straw phosphorus uptake .Similar findings were observed by Chesti *et al.* (2013) and close observation was given by (Krishna *et al.,* 2018).

Straw potassium uptake (mg/ pot):

The effect of different levels of vermicompost and fertilizer on straw potassium uptake is presented in the table-2. The straw potassium uptake varied from 210.73 to 482.70 mg/ pot with different levels of vermicompost, irrespective of fertilizer levels. Irrespective of vermicompost levels, the straw potassium uptake in accordance to the different fertilizer levels ranged between 240.05 to 454.37 mg/pot. The vermicompost levels i.e. 1.25 t/ ha, 2.5 t/ ha and 3.75 t/ ha recorded significantly higher straw potassium uptake over no vermicompost level. The 50 and 100 % RDF levels also gave

Table 1: Effect of vermicompost and fertilizer on grain nutrients (N, P and K) uptakes of rice crop during growth period

Treatments	N uptake (mg/ pot)				P uptake (mg/ pot)				K uptake (mg/ pot)			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	133.47	283.28	205.19	**207.31**	24.28	63.65	42.18	**43.37**	36.33	67.68	52.90	**52.30**
$V_{1.25}$	170.78	350.33	274.46	**265.19**	31.95	83.18	60.20	**58.44**	46.17	90.76	72.76	**69.90**
$V_{2.5}$	248.13	430.88	354.78	**344.60**	56.39	100.58	86.05	**81.01**	68.52	109.17	92.00	**89.90**
$V_{3.75}$	279.05	446.03	417.34	**380.81**	65.75	104.95	98.54	**89.75**	72.54	112.06	98.99	**94.53**
Mean	**207.86**	**377.63**	**312.94**		**44.59**	**88.09**	**71.74**		**55.89**	**94.92**	**79.16**	
Factors	CD (5%)		SEm(±)		CD (5%)		SEm(±)		CD (5%)		SEm(±)	
Vermicompost(V)	11.39		3.88		2.66		0.91		2.82		0.96	
Fertilizers(F)	9.86		3.36		2.30		0.78		2.44		0.83	
V X F	19.72		6.72		4.60		1.57		4.88		1.66	

Table 2: Effect of vermicompost and fertilizer on straw nutrient (N, P and K) uptakes of rice crop during growth period

Treatments	N uptake (mg/ pot)				P uptake (mg/ pot)				K uptake (mg/ pot)			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	94.20	209.40	158.32	**153.97**	14.31	33.91	22.81	**23.67**	143.33	287.13	201.74	**210.73**
$V_{1.25}$	121.77	256.37	213.47	**197.21**	20.12	41.35	32.77	**31.41**	200.19	429.83	330.18	**320.07**
$V_{2.5}$	173.82	286.80	236.93	**232.52**	24.06	45.54	37.50	**35.70**	274.89	521.73	456.48	**417.70**
$V_{3.75}$	183.71	303.81	268.48	**252.00**	28.35	49.83	42.44	**40.21**	341.78	578.78	527.52	**482.70**
Mean	**143.38**	**264.10**	**219.30**		**21.71**	**42.66**	**33.88**		**240.05**	**454.37**	**378.98**	
Factors	CD (5%)		SEm(±)		CD (5%)		SEm(±)		CD (5%)		SEm(±)	
Vermicompost(V)	7.67		2.61		1.22		4.78		14.05		4.78	
Fertilizers(F)	6.64		2.26		1.05		4.14		12.16		4.14	
V X F	13.29		4.53		2.11		8.29		24.33		8.29	

V_0= Vermicompost (no manure), $V_{1.25}$= Vermicompost (1.25 t ha^{-1}), V2.5= Vermicompost (2.5 t ha^{-1}), $V_{3.75}$ = Vermicompost (3.75 t ha^{-1}), F_0= Fertilizer (no fertilizer), F_{100}= Fertilizer (100%RDF), F_{50}= Fertilizer (50 % RDF) and V_0F_0 = control (no vermicompost + no fertilizer).

significantly higher straw potassium uptake over no fertilizer level. The interactions between vermicompost and fertilizer levels were found significant. The integrated application of vermicompost (3.75 t/ ha) + 100 % NPK showed always the significant higher amount of straw potassium uptake i.e. 578.78 mg/ pot at post-harvest over the control (143.33 mg/ pot) and relative study was done by (Krishna *et al.,* 2018).

Total nitrogen uptake (mg/ pot):

The influence of different levels of vermicompost and fertilizer on the total nitrogen uptake is presented in the table-3. The vermicompost levels recorded significantly higher total nitrogen uptake over no vermicompost level. The 50 and 100 % RDF levels also gave significantly higher total nitrogen uptake over no fertilizer level. The interactions in between vermicompost and fertilizer levels were found significant. The elevated total nitrogen uptake was found in the treatment receiving (vermicompost-3.75 t/ ha + 100% RDF) i.e. 749.84 mg/ pot which was significantly superior over control (no vermicompost + no fertilizer) i.e. 227.67 mg/ pot .The increase in total nitrogen uptake might be due to the more availability of nitrogen to the plant in the soil thus enhanced nitrogen uptake and close finding in other crop due to vermicompost and fertilizer application was given by (Mahmud *et al.,* 2020).

Total phosphorus uptake (mg/ pot):

The (table-3) deals with influence of vermicompost and fertilizer levels on the total phosphorus content of rice crop in the pot experiment. The vermicompost of levels 1.25 t/ ha, 2.5 t/ ha and 3.75 t/ ha all were found significantly higher over no vermicompost level. The fertilizer levels of 50 % and 100 % RDF were found significantly superior over no fertilizer level. The combined application of highest level of vermicompost (3.75 t/ ha) + 100 % RDF showed total phosphorus uptake i.e. 0.083 mg/ pot , which was significantly superior over control (0.055 mg/ pot) but the interaction among the different levels of vermicompost and fertilizer was found non-significant.

Total potassium uptake (mg/ pot):

The data pertaining to total potassium uptake as influenced by vermicompost and fertilizer levels presented in table-3 is statistically significant. The levels of vermicompost of 1.25 t/ ha, 2.5 t/ ha and 3.75 t/ ha gave significantly higher total potassium uptake over no vermicompost level. The fertilizer of 50 % and 100 % RDF were significantly superior over no fertilizer level. The interactions among the levels of vermicompost and fertilizer were found significant. The integrated application of vermicompost (3.75 t/ ha) + 100 % NPK resulted in significant higher amount of total potassium uptake i.e. 690.84 mg/ pot at post-harvest over the control (179.66 mg/ pot) and supportive study was done by (Krishna *et al.,* 2018), along with close finding in other crop due to vermicompost and fertilizer application was given by (Mahmud *et al.,* 2020).

Apparent nitrogen use efficiency (%):

The influence of different levels of vermicompost and fertilizer on the apparent nitrogen use efficiency is presented in the table-4. The apparent nitrogen use efficiency varied from 28.21 to 63.16 % with application of different levels of vermicompost application, irrespective of fertilizer levels and with respect to application of different fertilizer levels ranged between 45.06 to 56.70 %, irrespective of vermicompost levels. The vermicompost level of 2.5 t/ ha recorded significantly higher apparent nitrogen use efficiency over rest of the vermicompost levels. The 50% RDF level also gave significantly higher apparent nitrogen use efficiency over 100 % RDF and no fertilizer level application. The interactions in between vermicompost and fertilizer levels were found significant. The elevated apparent nitrogen use efficiency was found in the treatment receiving (vermicompost-3.75 t/ ha + 50% RDF) i.e. 63.86 % which was significantly superior over. The increase in apparent nitrogen use efficiency might be due to the more availability of nitrogen to the plant in the soil thus enhanced nitrogen uptake (Manivannan *et al.,* 2020).

Apparent phosphorus use efficiency (%):

The data pertaining to apparent phosphorus use efficiency as influenced by vermicompost and fertilizer levels presented in table-4 are statistically significant. The apparent phosphorus use efficiency significantly increased with the application of vermicompost and varied between 14.47 to 28.61 %, irrespective of fertilizer application whereas it varied from 18.76 to 26.82 % with application of fertilizer, irrespective of vermicompost levels. The levels of vermicompost of 1.25 t/ ha, 2.5 t/ ha and

Table 3: **Effect of vermicompost and fertilizer on total nutrient uptakes of rice crop during growth period**

Treatments	Total N uptake (mg/ pot)				Total P uptake (mg/ pot)				Total K uptake (mg/ pot)			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	227.67	492.68	363.51	**361.29**	38.58	97.56	64.99	**67.04**	179.66	354.81	254.63	**263.03**
$V_{1.25}$	292.56	606.70	487.94	**462.40**	52.06	124.54	92.97	**89.86**	246.37	520.59	402.93	**389.96**
$V_{2.5}$	421.96	717.68	591.71	**577.12**	80.45	146.12	123.55	**116.71**	343.41	630.90	548.48	**507.60**
$V_{3.75}$	462.76	749.84	685.81	**632.80**	94.10	154.78	140.98	**129.95**	414.32	690.84	626.51	**577.22**
Mean	**351.24**	**641.72**	**532.24**		**66.30**	**130.75**	**105.62**		**295.94**	**549.29**	**458.14**	
Factors	CD (5%)		SEm(±)		CD (5%)		SEm(±)		CD (5%)		SEm(±)	
Vermicompost (V)	19.05		6.49		3.87		1.32		16.86		5.74	
Fertilizers (F)	16.50		5.62		3.35		1.14		14.60		4.97	
V X F	33.00		11.24		6.71		2.28		29.20		9.94	

Table 4: Effect of vermicompost and fertilizer on nutrient use efficiencies of rice crop during growth period

Treatments	Nitrogen use efficiency (%)				Phosphorus use efficiency (%)				Potassium use efficiency (%)			
	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean	F_0	F_{100}	F_{50}	Mean
V_0	0.00	41.77	42.87	**28.21**	0.00	22.51	20.89	**14.47**	0.00	98.29	84.39	**60.89**
$V_{1.25}$	48.70	49.37	57.72	**51.93**	20.48	25.54	26.84	**24.29**	66.79	122.66	118.21	**102.55**
$V_{2.5}$	72.76	54.36	62.35	**63.16**	29.08	26.17	30.57	**28.61**	81.93	119.66	127.66	**109.75**
$V_{3.75}$	58.77	50.45	63.86	**57.69**	25.47	24.02	29.00	**26.16**	78.33	107.02	114.97	**110.11**
Mean	**45.06**	**48.99**	**56.70**		**18.76**	**24.56**	**26.82**		**56.76**	**111.91**	**111.31**	
Factors	CD (5%)		SEm(±)		CD (5%)		SEm(±)		CD (5%)		SEm(±)	
Vermicompost (V)	1.77		0.60		0.82		0.28		3.35		1.14	
Fertilizers (F)	1.53		0.52		0.71		0.24		2.90		0.99	
V x F	3.06		1.04		1.42		0.48		5.80		1.98	

V_0= Vermicompost (no manure), $V_{1.25}$= Vermicompost (1.25 t ha^{-1}), V2.5= Vermicompost (2.5 t ha^{-1}), $V_{3.75}$ = Vermicompost (3.75 t ha^{-1}), F_0= Fertilizer (no fertilizer) , F_{100}= Fertilizer (100% RDF), F_{50}= Fertilizer (50 % RDF) and V_0F_0 = control (no vermicompost + no fertilizer).

Table 5: Correlation coefficients (r) among yield of rice, nutrient content and nutrient uptake during pot experiment.

a. Grain yield and nutrients (N, P, K) contents and uptakes

Parameters	Grain yield	N uptake	P uptake	K uptake
Grain yield	1.000			
N uptake	0.990**	1.000		
P uptake	0.993**	0.997**	1.000	
K uptake	0.991**	0.993**	0.993**	1.000

b. Straw yield and nutrients (N, P, K) contents and uptakes

Parameters	Straw yield	N uptake	P uptake	K uptake
Straw yield	1.000			
N uptake	0.995**	1.000		
P uptake	0.992**	0.993**	1.000	
K uptake	0.955**	0.963**	0.963**	1.000

****Significant at P = 0.01 level** ***Significant at P = 0.05 level**

3.75 t/ ha gave significantly higher apparent phosphorus use efficiency over no vermicompost level. The fertilizer of 50 % and 100 % RDF were significantly superior over no fertilizer level application.

The interactions among the levels of vermicompost and fertilizer were found significant. The integrated application of vermicompost (2.5 t/ ha) + 50 % RDF resulted in significant higher amount of apparent phosphorus use efficiency i.e. 30.57 % at post-harvest over the control (Savaliya *et al.,* 2018) and (Manivannan *et al.,* 2020).

Apparent potassium use efficiency (%):

The effect of different levels of vermicompost and fertilizer on apparent potassium use efficiency is presented in the table-4 and is statistically significant. The apparent potassium use efficiency varied from 60.89 to 109.75 % with different vermicompost levels application, irrespective of fertilizer levels. Irrespective of vermicompost levels, the apparent potassium use efficiency with respect to the different fertilizer levels ranged between 56.76 to 111.91 %. The vermicompost levels i.e. 1.25 t/ ha , 2.5 t/ ha and 3.75 t/ ha recorded significantly higher apparent potassium use efficiency over no vermicompost level. The 50 and 100 % RDF levels also gave significantly higher apparent potassium use efficiency over no fertilizer level. The interactions regarding vermicompost and fertilizer levels were significant.

However the vermicompost of 2.5 t/ ha + 50 % RDF recorded higher apparent potassium use efficiency i.e. 127.66 %, which was statistically at par with the treatment receiving 1.25 t/ ha vermicompost and 100 % RDF (122.66 %).

The increase in apparent potassium use efficiency might be due to the better utilization of nutrients as well as reduction in loss of nutrients and thus increased apparent potassium use efficiency (Manivannan *et al.,* 2020).

Conclusion

Rice crop during its growth requires a large quantity of nutrients and balanced quantity of vermicompost along with fertilizer elevates the yield during harvesting of crop. Among different treatments higher dose of vermicompost (vermicompost-3.75 t/ha + 100% RDF) and fertilizer supplied better yield and uptake of nutrients and vermicompost (2.5 t/ ha) + 50 % RDF dose provided significant results in increasing nutrient use efficiencies. Combined application of vermicompost and fertilizer enhances nutrient content and uptake in grain, straw of crop, thus maximizing the efficiencies of nutrients that check the loss of nutrients and lower the cost of cultivation. Application of vermicompost is eco friendly, cost effective and balanced application with fertilizer helps in nutrient use efficiency and recycling in soil.

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Conflict of interest

The authors declare that they have no conflict of interest.

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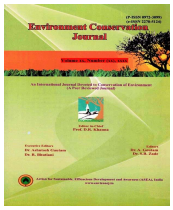

Impact of sowing dates on different growth attributes and yield of wheat in North Western Himalayas

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ABSTRACT

Sowing time is an important agronomic factor that significantly affects plant growth, development and yield. Similarly, suitable cultivar also plays an appreciable role in final productivity. Therefore, the present study was conducted to determine the Impact of sowing dates and varieties on different growth attributes and yield of wheat in North Western Himalayas. A Field experiment were conducted at Hill Agricultural Research and Extension Centre (HAREC), Bajaura (1074m altitude) and HAREC, Dhaulakuan (411m altitude) during *rabi* season of 2016-17 which comprises of three dates of sowings (25th October, 25th November, 25th December) and four varieties (HS-542, HS-490, HPW-349, VL-907) which was laid down in Factorial Randomized Block Design (FRBD) with three replications. Altitude plays a major role at both the locations and found that with decrease in temperature in delayed sowing the number of days taken to complete the physiological maturity decreased in all the dates and at all the locations. For grain and straw yield, Bajaura was found to be the most suitable location, showing significant superiority over all other locations. 25th October and 25th November sowings being at par with each other was significantly higher than 25th December sowing at all the locations. Among varieties, VL-907, HS-542 and HPW-349 being at par with each other had significantly higher grain and straw yield than HS-490. Bajaura gave significantly more number of effective tillers/m^2 and emergence count. Sowing at 25th October and 25th November being at par with each other gave significantly more number of plants per metre square than 25th December sowing at both of the locations.

Introduction

Wheat is undoubtedly one of the World's major crops and a vital component of the global food security issue. Wheat is often regarded as the king of grains, as it feeds 38 percent of the World's population and accounting for almost 22 percent of all global dietary calories (Kumar *et al.,* 2013). Being a *rabi* crop, wheat necessitates precise climatic conditions for optimal emergence, growth, and flowering, and is susceptible to high temperatures during reproductive stages (Kalra *et al*., 2008). In 2016-17, Wheat was being cultivated across an area of 340 thousand hectares with yield of 650 thousand tonnes and an average productivity of 19.21 q/ha in Himachal Pradesh (Anonymous. 2017). In a rice-wheat system, late sowing of wheat exposes the pre-anthesis stage to higher

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temperatures, which affect grain maturity and subsequently yield (Nagarajan *et al.,* 2008). The build-up of heat units above the threshold temperature is related to the phenological development of crops. To achieve a specific phenophase, a specified value of heat units is required.In cereal crops; various developmental stages are evident during which key physiological processes occur (Sikder, 2008). Before reaching phenological stages, plants require a specified temperature. At grain filling stage, the greatest loss for crop production is due to extremely high temperature i.e., temperature is the foremost determinant of development and as well as productivity of wheat. (Balla *et al.*, 2009). Several studies have discovered that optimum temperatures being 12-25 °C, affect wheat phenology, growth, development, and yield (Hakim et al., 2012).

Wheat is grown in altitudes ranging from below sea level near the Dead Sea and in California's Imperial Valley to as high as 5000 metres in Tibet. In India, it is grown upto an elevations of 4000 m above mean sea level in the Himalayas. This wider adaptability of wheat is by virtue of large variability in the genotypes/varieties available. The climate of lower or mid altitudes differ greatly from that of high altitudinal regions at the same latitude. Its altitude does not only govern climate/weather condition at a given location but other physiographic characteristics also play a significant role. Sowing time in different altitudes provide differential growth conditions such as maximum and minimum temperature, daily sunshine, precipitation, growth period and genetic potential of wheat variety (Safdar *et al.,* 2008). These periodic changes in the weather elements brings about sharp changes in the growth, development and ultimately yield of a given variety. The purpose of this research is to give an overview of the influence of elevated temperatures affect wheat phenology.

Material and Methods

A field experiment was conducted during the *Rabi* season of 2016-17, field experiments were conducted with the combination of three dates of sowing (25th October, 25th November, 25th December) and four varieties (HS-542, HS-490, HPW-349, VL-907) which was replicated three times in Factorial Randomized Block Design (FRBD) at two different location sites which were situated at Hill Agricultural Research and Extension Centre (HAREC), Bajaura about 1074m amsl and Hill Agricultural Research and Extension Centre (HAREC), Dhaulakuan about 411m amsl. The mean weekly temperature at Dhaulakuan ranged between, 12.5 ºC - 27.8 ºC, Bajaura, 5.8 ºC - 23.6 ºC, during crop growing season (October to May). The lowest minimum temperature at Bajaura has reflected in more number of days taken for completion of different phenophases at the station. The lines of mean temperature for Dhaulakuan indicating higher mean temperature. The rainfall of 425mm at Bajaura and 275mm at Dhaulakuan. The emergence count was recorded from the sampling area (1 square metre) every alternate day from the first date of seeding until emergence was constant. The number of plants per square metre were counted. To control the weeds manual weeding or hand weeding was done two times after appearing of weeds. The stage on which plants in each plot turned golden yellow and the grains did not crush with teeth was carefully judged. The date on which grains attained sufficient hardness was recorded and days taken for physiological maturity were counted from the sowing date. The number of tillers per running metre length were counted before harvest and expressed as effective tillers per metre square by multiplying with factor 4.44. After sun drying, the produce from each net plot was gathered and threshed. After threshing, the grains were cleaned and weighed. Weight of grains recorded on each plot was calculated and converted to q/ha. Weighing the sun dried harvested crop yielded the total biological yield (grain + straw) for each net plot. By deducting the grain yield from the biological yield q/ha, the straw yield was calculated.

Results and Discussion

Emergence count

Emergence count (number of plants/m^2) in different dates and varieties at different locations. With the delay in sowing from 25th October to 25th December, the emergence count decreased. All the varieties were at par with each other at both locations (Table 1). This might be due to delayed sowing of wheat tends to face high temperature which leads to reduction in emergence count.

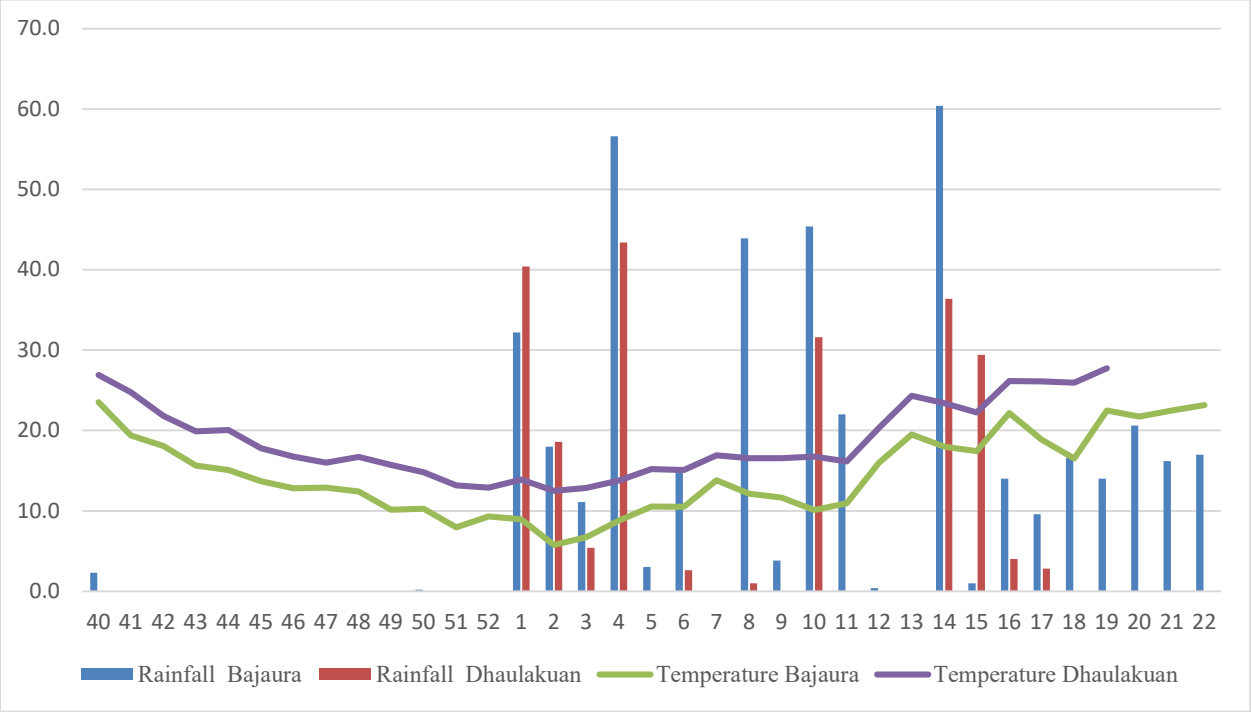

Figure 1: Mean temperature and rainfall recorded at Bajaura and Dhaulakuan locations.

Moreover, each day of sowing delay beyond the optimum time (up to mid-November) results in more days taken to emergence and also emergence count throughout the region. Similar results have been reported by Abhishek et al. (2016).

Days to physiological maturity

To complete physiological stage 25th October followed by 25th November sowing took significantly more number of days than 25th December dates at both locations. At Bajaura, it took highest number of days than Dhaulakuan (Table 1). At Bajaura, HS-542, HS-490 and HPW-349 and at Dhaulakuan, HS-542 and VL-907 were at par with each other.

As a result of the longer time between sowing to physiological maturity, the average temperature increased. The number of days taken decreased as the temperature is raised. Sial et al. (2005) discovered that delayed planting had a significant impact on plant growth and transfer of nutrients from source to sink, as well as days taken to heading, grain-filling period and days to maturity.

Number of effective tillers/m^2

Number of effective tillers per metre square in different dates and varieties at both locations. 25th October and 25th November sowing at Bajaura produced significantly more number of effective tillers per metre square than 25th December and at Dhaulakuan, first (25th October) date of sowing gave significantly more number of effective tillers per metre square than other dates of sowing (Table 1). Differences in number of effective tillers among varieties might be attributed to their genetic diversity (Shah et al. 2006).

Grain yield

Grain yield was significantly higher on 25th December sowing at both the locations and was at par with 25th October and 25th November sowing (Table 1). The four varieties evaluated at two locations exhibited significantly different response. At Bajaura, HS-490 produced significantly lower yield as compared with other varieties, which produced statistically similar yield and at Dhaulakuan, yield trend was similar, but VL-907 and HPW-349 remaining at par with each other, produced significantly higher grain yield over the other two varieties.

The partitioning of biomass from vegetative to reproductive stages were affected due to high temperature in December sowing. This leads to decrease in initials tillers, which ultimately results in small reproductive organs and consequently

Table 1: Effect of date of sowing and varieties on growth attributes and yield of wheat

Treatment	Emergence count (No. of plants/m^2)		Physiological Maturity (Days)		Number of effective tillers		Grain yield (q/ha)		Straw yield (q/ha)	
	Bajaura (1074m)	**Dhaulakuan** (411m)	**Bajaura** (1074m)	**Dhaulakuan** (411m)	**Bajaura** (1074m)	**Dhaulakuan** (411m)	**Bajaura** (1074m)	**Dhaulakuan** (411m)	**Bajaura** (1074m)	**Dhaulakuan** (411m)
Date of sowing										
D_1 (25^{th}Oct)	206.5	173.3	182.0	162.0	389.1	379.2	51.1	46.8	81.7	77.6
D_2 (25^{th} Nov)	187.9	169.3	168.8	143.7	373.7	355.1	47.8	46.3	81.4	78.6
D_3 (25^{th}Dec)	164.4	172.2	141.3	121.2	334.9	313.5	39.7	34.6	65.3	58.8
CD 5%	17.3	2.8	1.5	2.1	22.9	10.7	3.4	3.3	5.5	5.5
Variety										
V_1 (HS-542)	191.7	173.7	165.3	143.1	376.3	353.8	46.8	41.4	84.0	72.4
V_2 (HS-490)	189.2	170.0	163.7	140.2	366.7	352.7	41.9	36.9	69.4	64.6
V_3 (HPW-349)	178.3	170.8	164.8	141.9	358.2	345.0	48.0	45.4	74.2	72.3
V_4 (VL-907)	192.5	171.9	162.3	144.0	362.5	345.6	48.0	46.4	77.0	77.3
CD 5%	NS	NS	1.7	1.2	NS	NS	4.0	3.8	6.4	6.4

Table 2: Interaction effect of date of sowing and varieties yield of wheat (q/ha)

Variety	**Bajaura** (1074m)		
	25th Oct.	**25th Nov.**	**25th Dec.**
HS-542	55.8	50.1	34.4
HS-490	39.1	41.4	45.3
HPW-349	53.0	49.3	41.7
VL-907	56.4	50.3	37.4
CD 5%			
For comparison of dates*varieties		6.8	
Variety	**Dhaulakuan** (411m)		
	25th Oct.	**25th Nov.**	**25th Dec.**
HS-542	50.0	44.8	29.5
HS-490	35.1	37.5	38.1
HPW-349	50.3	52.5	33.6
VL-907	51.8	50.3	37.1
CD 5%			
For comparison of dates*varieties		6.6	

reduced yield (Mukherjee 2012). Yield variations amongst various sowing dates explains the effect of temperature rise as sowing delayed and hence, offer opportunity for yield improvement by shifting sowing time of wheat crop in last week of November.

Straw yield

Straw yield in different dates and varieties at different locations. 25th October and 25th November sowings were at par with each other and gave significantly higher straw yield than 25th December sowing across the locations. Among varieties, VL-907, HS-542 and HPW-349 were at par with each other at Dhaulakuan and VL-907 and HPW-349 at Bajaura were at par with each other. HS-490 gave significantly lower straw yield at both the locations, which were at par with HPW-349. Among locations, Bajaura in general produced higher straw yield as compared to other locations (Table 1).

Interaction Effect

Interaction among sowing dates and varieties at both locations is being presented in Table 2, which shows that all the varieties remained at par with each other for first two dates of sowing at both locations. At all, locations late sowing of VL-907, HPW-349 and HS-542 resulted in decreased grain yield. In case of HS-490, however, with delay in sowing there was increase in yield but was non-significant at Bajaura and Dhaulakuan.

Conclusion

For higher altitude like Bajaura, it was found that 25th October sowing date was recommended except others date of sowings with VL-907 and HPW-349 excepts other varieties. and low altitude areas like Daulakuan, it was recommended that 25th October sowing dates with VL-907 varieties gives better results as compare to other date of sowings and varieties.

Conflict of interest

The authors declare that they have no conflict of interest.

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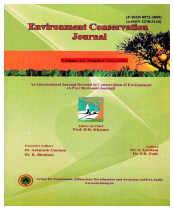

Influential role of organic sources on yield and economics of black gram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Present study for investigating the role of organic amendments on the growth and production of the black gram (*Vigna mungo*). The findings of the research illustrate that, the application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB documented a significantly greater Dry matter yield (2740 kg/ha), number of pods/plant (19.33), number of seeds/pod (6.33), the grain yield (701.33 kg/ha), the haulm yield (2038.76 kg/ha) as compared to others and higher gross returns (₹. 54540 ha^{-1}), net returns (₹. 23083 ha^{-1}) and Benefit-Cost ratio (1.74) in black gram production.

Introduction

Blackgram (*Vigna mungo* (L) Heppear), being an excellent source of high-quality protein, is considered as one of the most important pulse crops in our country where it is cultivated in an area of 35.15 lakh ha producing 21.0 lakh tonnes with a productivity of 655 kg/ha which is low compared to other pulse crops owing to its cultivation on marginal lands known to be poor in fertility (Anon, 2018). It is known that the pulses play an important role in Indian agriculture for sustainable production, improvement in soil health and environmental safety. India is the largest producer and also consumer of pulses in the world and it is a cheaper source of protein to overcome malnutrition among vegetarians. However, the pulses are more responsive to organic manures. They contain a high percentage of quality protein nearly three times more than in cereals. But the indiscriminate and continuous use of chemical fertilizers has a deleterious effect on soil physical, chemical and biological properties thereby affecting the sustainability of crop production, besides causing environmental pollution. So, there is a scope to improve the productivity of pulses by enhancing the soil fertility and its productivity through increasing soil organic carbon, soil moisture storage capacity and adopting integrated nutrient management practices. The crop productivity under the organic production system can be enhanced by optimizing the nutrient requirement of the crop at different stages. Intensive farming techniques, together with the heavy use of chemical inputs over the last four decades, have led not only to a loss of natural ecosystem balance and soil health but also many hazards such as soil salinization, soil erosion, reduction in groundwater levels and desertification, pesticide and fertilization contamination, ecological damage, genetic erosion, redness. The soil and climatic conditions in the drylands are well adapted to organic farming. The real potential of organic

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farming can be witnessed in rainfed areas and where the soil organic matter and organic carbon content are lesser. Low soil fertility is a major constraint to achieving sustainable black gram production and productivity. Continuous usage of chemical inputs will deteriorate soil physical, chemical and biological health. Due to this, the present investigation was taken up to evaluate the role of soil organic amendments on crop growth and grain yield of blackgram.

Material and Methods

A field experiment was conducted during kharif-2019 at the College of Agriculture, Vijayapur, Karnataka, India. The texture of soil at the experimental site was clayey in nature with a p*H* of about 7.82 with low organic carbon of 0.57 %. The soil is with low N (262 kg/ha), medium P_2O_5 (32.5 kg/ha) and with higher K_2O (390 kg/ha) content respectively. The experimental location contains about 12 treatments consisting of organic amendments laid out in Randomized Completely Block Design with three replications. The black gram variety TAU-1 was sown with a spacing of 45 cm x 10 cm. The recommended dose of phosphorus for black gram was enriched with various combinations of soil organic amendments with equal proportions depending on the amount of P in them. The organic manures *viz.*, Farm yard manure, Vermicompost, Ghanajeevamrutha in the required quantity were applied uniformly as per the treatments and incorporated into the soil three weeks before sowing. The quantity of organic manures was worked out equivalent to the recommended dose of fertilizer (nitrogen and phosphorus fertilizers per hectare) (20-50-0).

Treatment details includes: T_1: Application of Farm Yard Manure + vermicompost based on 100 % RDP, T_2: Application of vermicompost + ghanajeevamrutha based on 100 % RDP, T_3: Application of FYM + ghanajeevamrutha based on 100 % Recommended Dose of Phosphorus, T_4: Application of FYM + vermicompost based on 50 % RDP, T_5: Application of vermicompost + ghanajeevamrutha based on 50 % RDP, T_6: Application of FYM + ghanajeevamrutha based on 50 % RDP, T_7: Application of FYM + vermicompost based on 100 % RDP + *Rhizobium* + PSB, T_8: Application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB, T_9: Application of FYM + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB, T_{10}: Application of FYM + vermicompost based on 50 % RDP + Rhizobium + PSB, T_{11}: Application of vermicompost + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB, T_{12}: Application of FYM + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB.

Ghanajeevamrutha was prepared by using the following ingredients. Initially, 50 kg cow dung was spread on the polythene sheet. Black jaggery 1 kg was pounded to powder and added to cow dung and mixed well. Horsegram flour (1 kg) was added slowly to the mixture with hand mixing to avoid formation of lumps. One and half handful of fertile soil was added to the above mixture and mixed thoroughly until it became homogenous. Then measured quantity of cow urine (2.5 l) was added to the above mixture and this mixture was allowed to dry under the shade for 6-7 days. After a week, ghanajeevamrutha was applied to soil @ 500 kg/ha at the time of sowing as per the treatments.

Five plants from the representative plots in the field were chosen from which all yield attributes and yield were calculated *i.e*. mention now all Gross returns were calculated based on the market price of the product during the harvest and were expressed in rupees per hectare (Rs/ ha). While the net returns were calculated by deducting the cost of cultivation from gross return per hectare and were expressed in rupees per hectare (Rs/ha).

Results and Discussion

It is seen that among the organic manures application vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB resulted in higher yield attributing and grain yield characters. The application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + Phosphorus Solubilizing Bacteria recorded a higher number of pods per plant (19.33), number of seeds per pod (6.33), haulm yield (2038.67 Kg/ha) and grain yield (701.33 Kg/ha) compared to other treatments. However, the increased grain yield and yield attributing characters of black gram by application of organic manures might attribute to prolonged and unfluctuating availability of major nutrients during the entire crop growth period and inclusion of

Table 1: Effect of organics on no. of pods/ plant, no. of seeds/ pod, no. of branches and plant dry matter yield (kg/ha) in black gram variety TAU-1.

Treatments	No. of pods per plant	No. of seeds per pod	No. of branches	Dry matter (kg/ha)
T_1: Application of FYM + vermicompost based on 100 % RDP	14.67	5.33	4.67	2317.33
T_2: Application of vermicompost + ghanajeevamrutha based on 100 % RDP	15.67	5.67	5.33	2441.33
T_3: Application of FYM + ghanajeevamrutha based on 100 % RDP	15.00	5.33	5.00	2350.00
T_4: Application of FYM + vermicompost based on 50 % RDP	7.33	4.67	2.67	1792.33
T_5: Application of vermicompost + ghanajeevamrutha based on 50 % RDP	9.00	5.00	3.33	1923.67
T_6: Application of FYM + ghanajeevamrutha based on 50 % RDP	8.33	4.00	3.00	1837.00
T_7 Application of FYM + vermicompost based on 100 % RDP + *Rhizobium* + PSB	17.00	6.00	5.33	2497.33
T_8: Application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	19.33	6.67	6.33	2740.00
T_9 : Application of FYM + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	18.00	6.33	5.67	2570.00
T_{10}: Application of FYM + vermicompost based on 50 % RDP + *Rhizobium* + PSB	10.33	4.67	3.33	2017.33
T_{11}: Application of vermicompost + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	13.00	5.00	4.33	2224.33
T_{12}: Application of FYM + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	11.67	5.00	3.67	2105.33
SEm±	0.61	0.40	0.23	67.66
CD (p=0.05)	1.79	1.17	0.69	198.44

Note: FYM - Farm Yard Manure., RDP - Recommended Dose of Phosphorus (50 Kg/ha), PSB – Phosphorus Solubilizing Bacteria

Table 2: Effect of organics on grain yield (kg/ha), haulm yield (kg/ha) in black gram variety TAU-1.

Treatments	Grain yield (kg/ha)	Haulm yield (kg/ha)
T_1: Application of FYM + vermicompost based on 100 % RDP	610.00	1707.33
T_2: Application of vermicompost + ghanajeevamrutha based on 100 % RDP	634.00	1807.33
T_3: Application of FYM + ghanajeevamrutha based on 100 % RDP	622.00	1728.00
T_4: Application of FYM + vermicompost based on 50 % RDP	408.33	1390.33
T_5: Application of vermicompost + ghanajeevamrutha based on 50 % RDP	491.33	1432.33
T_6: Application of FYM + ghanajeevamrutha based on 50 % RDP	411.00	1426.00
T_7 Application of FYM + vermicompost based on 100 % RDP + *Rhizobium* + PSB	659.33	1838.00
T_8: Application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	701.33	2038.67
T_9 : Application of FYM + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	680.00	1890.00
T_{10}: Application of FYM + vermicompost based on 50 % RDP + *Rhizobium* + PSB	537.00	1480.33
T_{11}: Application of vermicompost + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	592.67	1631.67
T_{12}: Application of FYM + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	573.00	1532.33
SEm±	30.54	60.81
CD (p=0.05)	89.60	178.37

Table 3: Influence of different organic sources on cost of cultivation, gross returns, net returns and benefit cost ratio of black gram.

Treatments	Cost of cultivation (₹)	Gross returns (₹)	Net returns (₹)	B:C
T_1: Application of FYM + vermicompost based on 100 % RDP	29320	45280.70	15961	1.54
T_2: Application of vermicompost + ghanajeevamrutha based on 100 % RDP	31320	46963.75	15644	1.50
T_3: Application of FYM + ghanajeevamrutha based on 100 % RDP	29320	45635.50	16316	1.56
T_4: Application of FYM + vermicompost based on 50 % RDP	20020	25538.00	5518	1.21
T_5: Application of vermicompost + ghanajeevamrutha based on 50 % RDP	23320	28327.50	5008	1.28
T_6: Application of FYM + ghanajeevamrutha based on 50 % RDP	20120	27386.50	7267	1.36
T_7 Application of FYM + vermicompost based on 100 % RDP + *Rhizobium* + PSB	29378	49417.00	20039	1.68
T_8: Application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	31378	54461.00	23083	1.74
T_9 : Application of FYM + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB	29378	50727.50	21350	1.73
T_{10}: Application of FYM + vermicompost based on 50 % RDP + *Rhizobium* + PSB	19549	32585.00	13036	1.67
T_{11}: Application of vermicompost + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	23349	31478.50	8130	1.35
T_{12}: Application of FYM + ghanajeevamrutha based on 50 % RDP + *Rhizobium* + PSB	20149	35505.50	15357	1.76

nutrient rich organics *viz.* vermicompost, ghanajeevamrutha. The crucial role of *Rhizobium* in fixation of atmospheric nitrogen lies in the enhanced supply and translocation of N which influences the development of photosynthetic organs and PSB inoculation, solubilization of the insoluble P through the production of organic acids, accelerating the growth of the native *Rhizobium* population and playing an essential role in nodule formation in black gram. The results confirm to the research findings of Sailaja Kumari and Usha Kumari (2002) in cowpea crop and Wagadre *et al.* (2010) in green gram crop.

However, the application of organic supplements results in slow release of nutrients besides minimizing the loss of nutrients due to increased nutrient absorption as a result of increased cation exchange capacity which leads to a longer period of plant nutrients availability in adequate quantity thereby facilitating the plant for greater absorption of the required nutrients resulting in prominent growth, development and yield components. The organic matter supplementation improves the soil's physical properties such as improved structure, higher porosity, water holding capacity and lower bulk density and chemical properties such as improved soil organic carbon and greater availability of nutrients which promotes overall soil health and crop growth potential on a sustainability basis. Thus, based on the findings of this experiment it can be revealed that application of vermicompost + ghanajeevamrutha based on 100 % RDP + *Rhizobium* + PSB recorded significantly higher gross monetary returns (Rs. 54540/ha), net monetary returns (Rs. 23083/ha) and B:C (1.74) in blackgram production. The higher gross returns, net returns and the benefit cost ratio were obtained due to higher grain yield in T8 treatment over remaining treatments (Sharma *et al.,* 2010). The production cost for the black gram crop was higher with an increased quantity of FYM, vermicompost and ghanajeevamrutham application. So, the intensification of organic manures leads to a substantial and progressive increase of grain yield with a subsequent increase in gross and net monetary returns, which resulted in a higher benefit-cost ratio.

Conclusion

Organic manures plays a vital role in maintaining soil sustainability in the long term as they reduce nutrient losses and improve the organic matter content of the soil. The present study highlights the importance of organic manures for the supplementation of P fertilizers for the yield improvement in black gram and thus interprets that the nutrient availability, uptake and microbial load can be improved with the combined application of different organic manures like vermicompost, ghanajeevamrutha, FYM along with *Rhizobium* and PSB which in turn enhances the soil productivity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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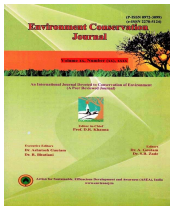

Effect of phosphorus and sulphur levels on growth and yield of lentil (*Lens culinaris* L.)

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ABSTRACT

A field experiment was carried for the duration of *Rabi* 2020-21 at crop research farm of SHUATS, Prayagraj (U.P.).The soil of experimental site was sandy loam in texture, almost impartial in soil reaction (P^H 7.4), specified in randomized block design. It includes of two factors, Phosphorus levels i.e., P_1-(30 kg/ ha), P_2-(40 kg/ ha), P_3-(50 kg/ ha) and Sulphur levels i.e., S_1- (15 kg/ ha), S_2-(20 kg/ ha), and S_3 (25 kg/ ha) which was replicated thrice. Results shown significantly increase in growth parameters viz. , plant height (44.97 cm), number of number of nodules (10.26), dry weight (15.63g), yield attributes viz., number of pods per plant (90.67), number of seeds per pod (1.93), test weight (18.53g) and yield viz., seed yield (1986.66 kg/ ha), straw yield (3450.00 kg/ ha), harvest index (35.98 %).Higher gross returns (₹ 118536/ha), net returns (₹ 84360.50) and B:C ratio (2.46) Therefore, concluded that combination of phosphorus 50 kg/ ha + sulphur 25 kg/ ha recorded significantly in all parameters.

Introduction

Lentil (*Lens culinaris* L.) is one in all the oldest and maximum nutritious leguminous crop. It cultivation back to beginning of agriculture itself. It is used as cowl crop to test the soil erosion in hassle areas. It is mainly used as dal and consumed as whole decorticated or decorticated and split. The cotyledons are orange red in colour. The whole seeded grain which is commonly known as "Masoor" is used in some of the dishes. India occupies 2nd in role in lentil manufacturing with inside the international after Canada and is the 5th maximum critical pulse crop in India in phrases of manufacturing after chick pea, pigeon pea, mungbean and urdbean (Singh *et al.,* 2015). In the country, lentil is cultivated in a place approximately 1.27 million hectares with manufacturing of 0.97 million tonnes and common productiveness of 765 kg/ ha.(Report.,2015-2016). Lentil is a leguminous crop, fixes atmospheric nitrogen via root nodules via way of means of rhizobium bacteria where in atmospheric nitrogen is transformed right into a plant usable form in presence of nitrogenase enzyme. It restores soil fertility and improves soil health. Lentil is known for maximum protein content, that is double than cereals. It is likewise called "A poor man meat" because of cheapest and nutritional protein. It contain 23.25% protein, 59% carbohydrates, 1.8% oil, and lines of iron, calcium, phosphorus and magnesium.

Phosphorus is the important thing detail for a success pulse manufacturing. Phosphorus complements the foundation proliferation and nodulatin in legume crops, will increase dry count manufacturing and seed yield (Sharma and Sharma, 2004, Balyan and Singh, 2005). Phosphorus concerned in lots of plant functions, along with energy storage and transfer, photosynthesis, transformation of sugars and starches, nutrient motion in the plant and switch of genetic traits from one generation to the next. Phosphorus may be an

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element of ATP, nucleic acid, phospholipid, ADP, sugar phosphate, phytin, protein and various co-enzyme.

Sulphur is the fourth predominant nutrient and essential element for plant growth particularly for legumes crops which play an important role in plant metabolism. Sulphur is a component of a few critical amino acids namely cystine, cysteine and methionine. Sulphur is important for boom and improvement, play a key function in plant metabolism, chlorophyll formation needed for improvement of cells and its increases bloodless resistance and drought hardiness.

The productivity of Lentil in U.P. is totally low because of several limitation like nutrient deficiencies in macro (nitrogen, phosphorus and sulphur) and micro (copper, manganese, zinc and iron) nutrients (EnviStats India, 2019), imbalance fertilizer management practices and infestation of great disease and pests furthermore as lack of latest agro techniques like proper sowing time, plant population and inadequate supply of fertilizer and lack of fine seeds etc. Legumes usually require almost equal amount of phosphorus and sulphur. Phosphorus (<12.5 kg/ha) and sulphur (< 10 mg/kg) below critical amount within the soil adversely affect both plant growth and quality of produce. Present study was conducted to study the phosphorus and Sulphur ranges for maximizing growth and yield of lentil in these climatic conditions.

Material and Methods

Site Selection

The experiment entitled "Effect of Phosphorus and Sulphur levels on growth and yield of lentil (*Lens culinaris* L.)" modified into accomplished at some stage of *Rabi*-season 2020, on the Crop Research Farm, Department of Agronomy, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The Crop Research Farm is located at 25°.750 N latitude, 87°.190 E line of longitude (Google-2021) and at an altitude of ninety eight m higher than sea level. The experimental field soil texture changed into sandy loam.

Experimental Design

The experiment was carried out in a randomized block layout and ten treatments has been replicated three times every on KLS 9-3 variety of lentil. The lentil was sown on 7th December 2020 with plant geometry 20×10 cm. The treatments wereT_1-control (N,K), T_2-Phosphorus 30 kg/ ha + Sulphur 15 kg/ ha, T_3-Phosphorus 30 kg/ ha + Sulphur 20 kg/ ha, T_4-Phosphorus 30 kg/ha + Sulphur 25 kg/ ha, T_5-Phosphorus 40 kg/ ha + Sulphur 15 kg/ ha, T_6-Phosphorus 40 kg/ ha + Sulphur 20 kg/ ha, T_7-Phosphorus 40 kg/ ha + Sulphur 25 kg/ ha, T_8-Phosphorus 50 kg/ ha + Sulphur 15 kg/ ha, T_9-Phosphorus 50 kg/ ha + Sulphur 20 kg/ ha and T_{10}-Phosphorus 50 kg/ ha + Sulphur 25 kg / ha.

Statistical analysis

The experimental data recorded for statistical evaluation via way of means of by adopting Fishers approach of evaluation of variance (Anova) as described by Gomez and Gomez (1984). The statistics accrued from the test turned into subjected to statistical evaluation using ICAR WASP software. Critical difference (CD) values had been calculated the 'F' check turned into discovered drastically at 5% level.

Results and Discussion

Growth Parameters

Plant height (cm)

Perusal of data present in Table.1 regarding plant height of lentil was recorded during different stages of crop of growth which turned into substantially better with the utility of Phosphorus 50 kg/ ha + sulphur 25 kg/ ha. During different intervals from 80, 100 DAS to harvest there was significant increase in plant height (42.63 cm, 43.81 cm, and 44.97 cm respectively) with the application of treatment T_{10}.Treatment combination T_9 and T_7 were statistically at par with treatment T_{10}. Above results showed that plant height continued to increase with advancement in crop age and this increase was high during early growth period. However, a slow growth was observed after 80 days of sowing. Increment in plant height might due to stimulation of biological activities in the presence of balanced supply of phosphorus. Similar outcomes on the increased plant height with the increased level of phosphorus application have also been reported by other researchers Maqsood *et al.,* 2000, Barua *et al.,* 2011 and Singh *et al.,* 2016.

Number of nodules per plant

Nodule production per plant was significantly influenced by different phosphorus levels. The highest number of nodules per plant was recorded

Table 1: Effect of Phosphorus and Sulphur levels on Plant height, Number of nodules per plant and Dry weight of lentil.

SN	Treatments	Plant height (cm)			Number of nodules per plant			Dry weight (g/plant)		
		80 DAS	100 DAS	At harvest	40 DAS	60 DAS	80 DAS	80 DAS	100 DAS	At harvest
1	**Control (N,K)**	34.33^{f}	36.26^{f}	37.74^{f}	25.4^{h}	27.2^{g}	6.00^{h}	3.53^{e}	5.23^{e}	7.26^{f}
2	**Phosphorus 30 kg/ha+Sulphur 15 kg/ha**	36.23^{e}	38.02^{e}	39.16^{e}	27.93^{g}	29.70^{f}	6.66^{g}	4.03^{cd}	5.90^{de}	8.86^{ef}
3	**Phosphorus 30 kg/ ha+Sulphur 20 kg/ ha**	36.84^{e}	39.01^{de}	40.48^{d}	28.93^{fg}	30.53^{ef}	7.00^{g}	4.23^{cd}	5.93^{de}	9.46^{e}
4	**Phosphorus 30 kg/ha+Sulphur 25 kg/ha**	37.78^{cd}	39.48^{cd}	40.54^{d}	29.93^{ef}	31.26^{def}	7.73^{f}	4.46^{cd}	7.20^{cde}	10.06^{de}
5	**Phosphorus 40 kg/ha+Sulphur 15 kg/ha**	38.51^{c}	39.82^{cd}	41.38^{cd}	30.6^{ce}	32.06^{cde}	8.2^{e}	4.60^{cd}	7.56^{cde}	10.40^{de}
6	**Phosphorus 40 kg/ha+Sulphur 20 kg/ha**	39.90^{b}	41.08^{b}	42.55^{b}	32.0^{bc}	33.87^{bc}	9.06^{c}	5.23^{b}c	9.16^{bc}	11.80^{cd}
7	**Phosphorus 40 kg/ ha+ Sulphur 25 kg/ha**	41.43^{a}	42.68^{a}	43.83^{a}	32.96^{ab}	35.66^{ab}	9.73^{b}	6.23^{ab}	10.40^{ab}	12.93^{bc}
8	**Phosphorus 50 kg/ ha+ Sulphur 15 kg/ha**	$38.96b^{c}$	40.48^{bc}	42.49^{bc}	31.2^{cd}	33.20^{cd}	8.66^{d}	4.83^{c}	7.86^{bcd}	10.86^{cde}
9	**Phosphorus 50 kg/ ha+ Sulphur 20 kg/ha**	41.58^{a}	42.80^{a}	43.87^{a}	33.06^{ab}	35.93^{ab}	10.06^{ab}	6.73^{a}	12.80^{a}	14.06^{ab}
10	**Phosphorus 50 kg/ha+ Sulphur 25 kg/ha**	42.63^{a}	43.81^{a}	44.97^{a}	34.0^{a}	37.06^{a}	10.26^{a}	6.90^{a}	12.86^{a}	15.63^{a}
	SEm(±)	0.44	0.41	0.39	0.40	0.80	0.13	0.42	0.87	0.72
	CD(P=0.05)	1.32	1.21	1.17	1.20	2.38	0.39	1.24	2.57	2.13

Table 2: Effect of Phosphorus and Sulphur levels on yield and yield components of lentil.

SN	Treatments	Number of pods per plant	Number of seeds per pod	Seed yield (kg/ ha)	Straw yield (kg/ ha)	Harvest index (%)
1	**Control (N,K)**	61.73^{f}	1.33^{g}	995.00^{e}	2085.00^{f}	32.47^{e}
2	**Phosphorus 30 kg/ ha+ Sulphur 15 kg/ ha**	65.00^{ef}	1.48^{f}	1130.00^{d}	2270.00^{ef}	33.32^{cd}
3	**Phosphorus 30 kg/ ha+ Sulphur 20 kg/ ha**	67.46^{e}	1.51^{ef}	1205.00^{cd}	2350.00^{ef}	33.93^{cd}
4	**Phosphorus 30 kg/ ha+ Sulphur 25 kg/ ha**	72.26^{d}	1.63^{de}	1233.33^{cd}	2383.33^{e}	34.11^{bcd}
5	**Phosphorus 40 kg/ ha+ Sulphur 15 kg/ ha**	74.86^{d}	1.70^{cd}	1278.33^{c}	2420.00^{e}	34.56^{abc}
6	**Phosphorus 40 kg/ ha+ Sulphur 20 kg/ ha**	86.16^{b}	1.73^{bcd}	1586.66^{b}	2983.33^{cd}	34.71^{abc}
7	**Phosphorus 40 kg/ ha+ Sulphur 25 kg/ ha**	87.26^{ab}	1.83^{abc}	1871.66^{a}	3368.33^{bc}	35.73^{ab}
8	**Phosphorus 50 kg/ha+ Sulphur 15 kg/ha**	80.53^{c}	1.73^{bcd}	1488.33^{b}	2810.00^{d}	34.62^{abc}
9	**Phosphorus 50 kg/ha+ Sulphur 20 kg/ha**	88.00^{ab}	1.85^{ab}	1895.00^{a}	3405.00^{ab}	35.77^{ab}
10	**Phosphorus 50 kg/ha+ Sulphur 25 kg/ha**	90.66^{a}	1.93^{a}	1986.66^{a}	3540.00^{a}	35.98^{a}
	SEm(±)	1.26	0.05	42.91	106.57	0.59
	CD(P=0.05)	3.76	0.14	127.50	316.63	1.74

Table 3: Effect of Phosphorus and Sulphur levels on economics of lentil.

SN	Treatment combination	Cost of cultivation (₹/ha)	Gross returns (₹/ha)	Net returns (₹/ha)	B:C Ratio
1	**Control (N,K)**	29470.4	61170	31699.6	1.07
2	**Phosphorus 30 kg/ ha+ Sulphur 15 kg/ ha**	32236.7	68980	36743.3	1.13
3	**Phosphorus 30 kg/ ha+ Sulphur 20 kg/ ha**	32686.7	73205	40518.3	1.23
4	**Phosphorus 30 kg / ha+ Sulphur 25 kg/ ha**	33136.7	74798	41661.3	1.25
5	**Phosphorus 40 kg/ ha+ Sulphur 15 kg/ ha**	32756.4	77278	44521.6	1.35
6	**Phosphorus 40 kg/ ha+ Sulphur 20 kg/ ha**	33206.4	95801	62594.6	1.88
7	**Phosphorus 40 kg/ ha+ Sulphur 25 kg/ ha**	33656.4	112261	78604.6	2.33
8	**Phosphorus 50 kg/ ha+ Sulphur 15 kg/ha**	33275.5	89938	56662.5	1.70
9	**Phosphorus 50 kg/ha+ Sulphur 20 kg/ha**	33725.5	113670	79944.5	2.37
10	**Phosphorus 50 kg/ha+ Sulphur 25 kg/ha**	34175.5	118536	84360.5	2.46

at 60 DAS (37.06) with treatment combination T_{10}.However lowest were recorded in control (6.00).Highest number of nodules was recorded during 80 DAS (10.26) with application of phosphorus 50 kg/ ha + sulphur 25 kg/ ha and it was statistically at par with treatment T_9. More availability of phosphorus plays an important role in use of sugar and starch, nucleus formation, cell division, photosynthesis and root growth that improves nodulation (Dhingra *et al.,* 1988). The number of nodules increases as plant grows, and normally reaches maximum at the mid flowering stage. So proper application of phosphorus facilitates the earlier formation of nodules, increasing their which enhances the nitrogen fixation (Gahoonia *et al.,* 2006). Thus phosphorus increases the yield of lentil by stimulating physiological functions and root development that improve nodulation (Sharma and Sharma.2004). Similar results are found with Datta *et al.,* 2013, Pandey *et al.,* 2016, Singh and Singh., 2016.

Plant dry weight (g/ plant)

Plant dry weight (g/ plant) was recorded with different intervals are tabulated Table 1 at various growth stages during plant growth. Dry weight was significantly increased (6.90, 12.86 g/plant) with the treatment combinationT_{10}. The harvest and highest dry weight (15.63 g/ plant) was recorded in treatment T_{10} and lowest was with control (7.26 g/plant). Increment in plant dry weight due to increase in dry weight in photosynthetic ability of different metabolites to various parts which in the end increased the shoot development. These findings are familiar with those of Tophia *et al.,* 2018.

Yield and Yield attributes

Observations regarding the yield and yield attributes viz., number of pods per plant, number of Seeds per pod, Seed yield (kg/ ha), Straw yield (kg/ ha) and harvest index (%) of Lentil were depicted in Table 2**.**

Yield attributes

Number of pods per plant

The data in Table indicate that the pods per plant were significantly affected by the effect of different rates of phosphorus application. The minimum number of pods were found in control plot (61.73). The maximum number of Pods per plant (90.66) were recorded with treatmentT_{10}. Datta *et al.,* (2013) mentioned that number of pods per plant in lentil significantly varied due to different levels of phosphorus. Increase in number of pods per plant because of availability of other nutrients which boosted carbohydrate metabolism and their translocation to reproductive parts of the plant. Phosphorus encourage flowering and fruiting which have stimulated the plants to produce more number of pods and it encourages more number of seeds per pods. These effects had been pronounced with of Maqssod *et al.,* 2000,Togay *et al.,* 2008 and Fatima *et al.,* 2013.

Number of seeds per pod

The average number of seeds per pod have an effect ultimately on the lentil crop. The data in table 2

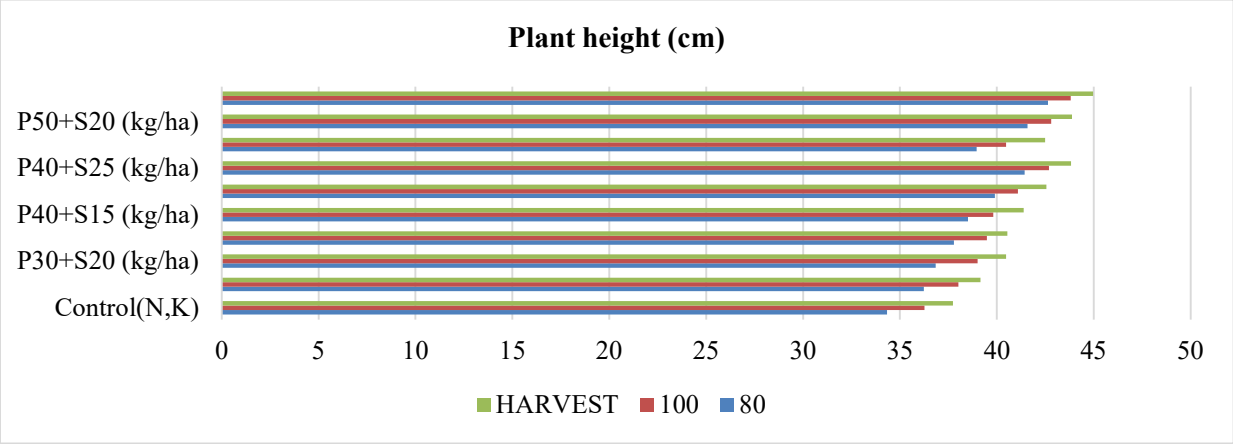

Figure 1: Plant height (cm) of lentil due to different treatment combinations.

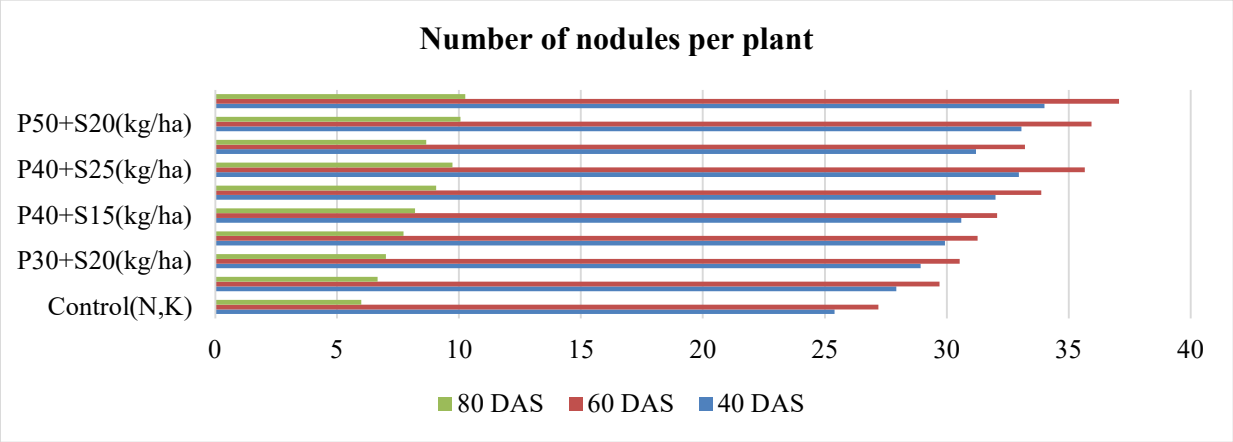

Figure 2: Number of nodules in lentil due to different treatment combinations.

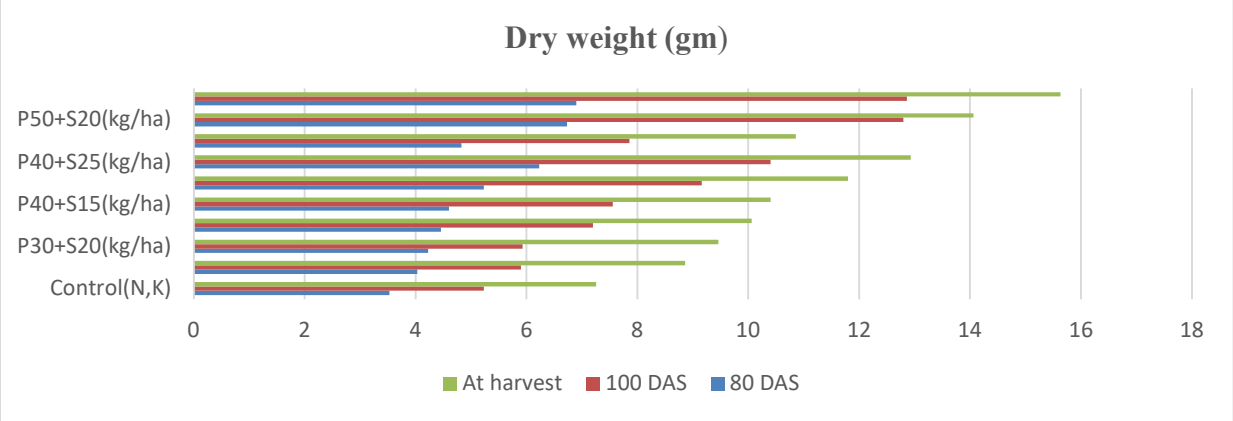

Figure 3: Dry weight (gm) of lentil in different treatment combinations.

reveals that there are significant differences regarding various treatment combinations. Significant growth in number seeds of pod (1.93) have been recorded in T_{10}.However treatment combination T_9 and T_7 are statistically at par with treatmentT_{10}. It is conformed from the above results that due to availability of nutrients by increasing the level of phosphorus increased the number of

seeds per pod. Number of seeds per pod had increased with application of phosphorus and Sulphur, which leads to transfer of photosynthates and its accumulates from growing parts of plant to seeds which make them plump and bold and also effects the seed size and weight. These results were close with Choubey *et al.,* 2013, Sonet *et al.,* 2000 and Upadhyay 2013.

Yield

Seed yield (kg/ ha)

Seed yield is the economic output of different applied treatments as well as the effect of different agronomic practices and environment. The higher supply of phosphorus enhances the grain yield by improving the yield components (Singh *et al.,* 2005).Data related to seed yield was depicated in table 2 higher seed yield was resulted in treatment T_{10} (1986.66 kg/ ha)and lower seed yield recorded in control (995.00 kg/ha). Application of phosphorus improved the nutrient availability which results greater uptake might have increased the photosynthesis and translocation of assimilates to different parts for enhanced growth and yield. In later stages more assimilates are produced than used in growth and development and the excess assimilates are diverted to storage compounds resulting into increase in seed yield. Similar results were observed with Biswas *et al.,* 2015, Sahey *et al.,* 2015, and Chaubey *et al.,* 2019.

Straw yield (kg/ha)

Results associated with straw yield was recorded and tabulated in table 2 revealed that with treatment T_{10} had produced maximum straw yield (3540.00 kg/ ha), and minimum straw yield recorded in control (2085 kg/ha). Numerous straw yield was attained by the enlargement of plant in terms of height, more spread number of branches, maximum dry weight of plant was the result of nutrient uptake. These outcomes had been supported by Singh *et al.,* 2000.

Harvest index (%)

Data related to harvest index represents in table 3.Highest harvest index (35.98 %) was recorded in treatment combination T_{10}.However lowest harvest index recorded in control (32.47%) Increase in harvest index due to better translocation of photosynthesis from growing parts to storage parts which increases the economical yield of the plant. These results are supported by Chaubey *et al.,* 2019 and Shukla *et al.,* 2014.

Economics

Data revealed that experiment economics of lentil was presented in table 3. Cost of cultivation varied from ₹ 29470.40/ha to ₹ 34175.50/ha for different inputs used during research. Among all treatments, highest net returns ₹ 84,360.50/ha, gross returns ₹ 1,18536/ ha and B:C-ratio (2.46) also found in treatment T_{10}.

Conclusion

This study concluded that the lentil variety (KLS 9-3) were found more productive in terms of maximum plant height, number of nodules per plant, dry weight of plant, yield attributes like number of pods per plant, number of seeds per pod and biological yield as well as in B: C ratio. The treatment combination of 50 kg/ha P_2O_5+ 25 kg/ha S was highly effective in terms of all the growth, yield parameters like seed yield (1986.66 kg/ha) and harvest index (35.98%).

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Conflict of interest

The authors declare that they have no conflict of interest.

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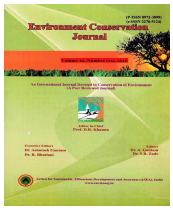

Standardization of harvest maturity of jackfruit (*Artocarpus heterophyllus lam.)* by morpho-physical investigation

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ABSTRACT

An experiment was carried out to investigate the standardization of harvest maturity indicators in Jackfruit (*Artocarpus heterophyllus Lam.*)The mean number of spines/cm^2 was lowest in tree 1 (8.0), the mean metallic sound (hedonic scale) was highest in tree 1 (2.8), the mean fruit length was significantly increasing and reaching its maximum in tree 1 (39.00 cm), and the mean fruit circumference was significantly increasing and reaching its maximum in tree 1 (39.00 cm) (41.48 cm).The experiment's data were considered non-replicated, and the recorded data were statistically analyzed using a one-way ANOVA design in the computer software MS Excel. Considering morphological analysis the characters *viz.,* fruit circumference(39.00 cm), low spine density(8.0), moderate to high spreading of spines, presence of sensible hollow metallic sound could be used as the maturity indices of jackfruit. It is also noted that jackfruit could be harvested after 100 days of fruit set.

Introduction

The jackfruit (*Artocarpus heterophyllus Lam*.) is a tropical and subtropical fruit crop that is grown in tropical and subtropical climates, especially in South and Southeast Asia. The tree is an important part of subsistence and small-scale farmers' farming systems, and the fruit serves as a secondary staple meal as well as a source of income for the impoverished. India has been growing jackfruit since ancient times. It was most likely brought to the East African coast by Arab traders, and it has since spread throughout the tropics (Mijin *et al.,* 2021). In Hindi, the fruit is known as 'Kathal,' and in Kannada, it is known as 'Halasu.' Maturity indices are important for deciding when a given commodity should be harvested to provide some marketing flexibility and to ensure the attainment of acceptable eating quality to the consumer. Maturation is the stage of development leading to the attainment of physiological or horticultural maturity (Mijin *et al.,* 2021). For maturity measurements to be carried out by producers, handlers and quality control personnel they must be simple, readily performed in the field or inspection point, and should require relatively inexpensive equipment. The index should preferably be objective (a measurement) rather than subjective (an evaluation) and ideally the index should be non-destructive There are many different ways for determining maturity. Different maturity or harvest indices have been devised. For these indices to be useful, they must objective, easy to use and interpret, be unambiguous and have generality so that data obtained can be compared between farms, regions and years. Also, they should measure what is important. Attainment of a specific size is one possible index of maturation, but it cannot be used

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alone since fruit size for any variety can be influenced by crop load, climatic conditions, and cultural practices. Fruit shape and/or fullness of cheeks in mango indicate maturity (Rana and marapana , 2019).

Since fruit quality of jackfruit cannot be improved after harvest, selecting the fruits at proper maturity is very much important. Hence, the present investigations will be focusing on harvest maturity indices of jackfruit with the specific objective of assessment of harvest maturity indices by morphological traits (Rana *et al.,* 2018). During the maturation and ripening process, jackfruit undergoes many morphophysical changes. Evaluation of these changes during maturity allows making the best use of jackfruit in different applications. The aim of this study was to determine the variations of morphophysical properties in jackfruit in order to use this knowledge to utilize the most suitable stage to harvest jackfruit.

Material and Methods

A jackfruit orchard was chosen at the College of Horticulture, Kolar, Karnataka. Trees 1–10 were chosen from ten different jackfruit accessions of uniform age. The labelling was finished at the same time as the blossoming. Tagging selected flower buds in all ten trees yielded all of the parameters. The number of spines/cm^2, fruit length (cm), fruit circumference (cm), and hollow metallic sound were recorded as observations for assessing harvest ripeness indices by morphological features. The number of spines/cm^2 was calculated by counting the number of spines on 10 tagged fruits in each tree at 30 day intervals. Every 30 days, the length of the fruit was measured and the average in cm was recorded.

The circumference (cm) of 10 fruits tagged in each tree was measured at 30 day intervals and the average was expressed in centimetres. The metallic sound of ten fruits tagged in each tree was measured on a hedonic scale of 1 to 4, with 1 equaling not sensible and 4 equaling sensible. The study was carried for 120 days. The experiment data was considered non-replicated, and the recorded data were statistically analysed using a one-way ANOVA design in the computer software MS Excel (Rana and marapana ,2019).

Results and Discussion

Number of spines/cm^2

The data in Table 1 show the number of spines per square centimetre in ten jackfruit trees. The number of spines/cm2 of jackfruit varied significantly up to 118 days after harvest. At 30 DAF, there was no significant difference between trees, but T_1 (21.4) and T_3 (23.4) had the highest number of spines/cm^2 (21.4). However, in T_5, it was kept to a bare minimum (19.1), T_{10} (15.2) had the most spines/cm^2 at 60 DAF; followed by T_6 (12.6) and T_2 had the fewest spines/cm^2 (8.5). T_8 (6.8) had the most spines/cm2 at 90 DAF, followed by T_{10} (5.9), and T_2 had the fewest spines/cm2 (4.4). At 104 DAF highest number of spines/cm^2 was recorded in T_8 (5.2) which was followed by T_6 (4.7) and minimum number of spines/cm^2 was found in T_2 (1.4). Observations were taken before harvesting jackfruits of T_2, T_3 and T_5 at 104 DAF. At 110 DAF, highest number of spines/cm^2 was recorded in T_7 (3.7) which was followed by T_8 (3.5) and minimum number of spines/cm^2 was found in T_4 (2.0). Observations were taken before harvesting jackfruits of T_4, T_7, T_8 and T_9 at 110 DAF. At 118 DAF, highest number of spines/cm^2 was recorded in T_1 (2.9) and T_{10} (2.9) and minimum number of spines/cm^2 was found in T_6 (2.1). Observations were made prior to harvesting T_1, T_6, and T_{10} jackfruits at 118 DAF. This is because the surface area of the fruit increased during its growth stage, increasing the distance between spines and resulting in a decrease in the number of spines per square metre. Saha *et al*. (2016) also found that at 100 DAF, the spine number per sq-cm was less than 2, indicating that the spine density of jackfruit was lower in the advanced mature stage. According to Rahman *et al*. (2019), AH Joy-090 (151) had the most dense spine (per five squire centimetre), followed by AH Joy-089 (134), and AH Joy-017 had the least dense spine (per five squire centimetre) (38) (Saha *et al.,* 2019).

Fruit length (cm)

Table 2 shows the results of fruit length measureme nts on ten jackfruit trees. Up to 118 days after harve st, significant disparities in jackfruit fruit length we re detected. At 30 DAF, there was no significant dif ference across trees; however, T_7 (21.98cm) had the longest fruit, followed by T_5 (21.3cm). In T8, thou gh, it was at a bare minimum (18.56cm). T_7 had the longest fruit length (33.67 cm) at 60 DAF,

Table 1: The effect of Number of spines/ cm^2in trees after flowering of jackfruit at different intervals.

Treatment (trees)	Number of spines/ cm^2						
	Number of days						
	30DAF	60DAF	90DAF	104DAF	110DAF	118DAF	Mean
T_1	21.4	11.2	5.3	3.9	3.0	2.9	**8.0**
T_2	19.5	8.5	4.4	1.4	*	#	**8.5**
T_3	21.4	10.4	4.6	3.9	*	#	**10.1**
T_4	20.2	10.0	4.7	4.1	2.0	#	**8.2**
T_5	19.1	9.5	5.5	4.0	*	#	**9.5**
T_6	20.0	12.6	6.6	4.7	2.6	2.1	**8.1**
T_7	20.8	12.3	5.4	4.1	3.7	#	**9.3**
T_8	19.8	11.4	6.8	5.2	3.5	#	**9.3**
T_9	20.1	10.3	5.2	4.3	3.3	#	**8.6**
T_{10}	21.2	15.2	5.9	4.4	3.3	2.9	**8.8**
Mean	**20.4**	**11.1**	**5.6**	**4.0**	**3.1**	**2.6**	
S.E.m±	1.79	1.01	0.62	0.50	0.45	0.37	
CD(5%)	NS	3.00	1.83	1.2	1.32	1.1	

Table 2: The effect of fruit length (cm) in trees after flowering of jackfruit at different intervals.

Treatment (trees)	Fruit length(cm)						
	Number of days						
	30DAF	60DAF	90DAF	104DAF	110DAF	118DAF	Mean
T_1	20.7	28.21	44.45	45.89	47.55	47.21	**39.00**
T_2	19.64	32.61	45.35	46.58	*	#	**36.05**
T_3	20.48	31.82	43.14	45.24	*	#	**35.17**
T_4	18.57	31.4	42.48	43.14	47.31	#	**36.58**
T_5	21.3	29.31	40.86	43.51	*	#	**33.75**
T_6	20.62	29.24	41.12	44.87	46.87	48.95	**38.61**
T_7	21.98	33.67	42.35	43.47	45.76	#	**37.45**
T_8	18.56	28.99	39.76	40.76	44.21	#	**34.46**
T_9	18.71	27.51	39.07	40.19	42.32	#	**33.56**
T_{10}	21.06	32.42	40.82	43.17	45.59	47.08	**38.36**
Mean	**20.16**	**30.52**	**41.94**	**43.68**	**45.66**	**47.75**	
S.E.m±	2.21	1.27	1.21	1.86	1.87	2.13	
CD(5%)	NS	3.79	3.43	5.54	5.60	NS	

Table 3: The effect of fruit circumference in trees after flowering of jackfruit at different intervals.

Treatment (trees)	Fruit circumference (cm)						
	Number of days						
	30DAF	60DAF	90DAF	104DAF	110DAF	118DAF	Mean
T_1	10.04	21.01	53.2	53.66	54.86	56.08	**41.48**
T_2	10.69	31.35	46.76	48.56	*	#	**34.34**
T_3	11.59	27.99	38.87	41.41	*	#	**29.97**
T_4	11.1	25.68	39.96	40.34	42.97	#	**32.01**
T_5	12	23.39	35.87	38.23	*	#	**27.37**
T_6	11.9	23.41	34.47	35.07	35.85	37.23	**29.66**
T_7	11.34	23.52	33.26	36.96	38.17	#	**28.65**
T_8	12.35	23.88	33.93	34.6	37.05	#	**28.36**
T_9	15.43	25.77	36.55	37.19	39.73	#	**30.93**
T_{10}	12.79	24.59	38.85	39.68	40.58	41.98	**33.08**
Mean	**11.92**	**25.05**	**39.17**	**40.57**	**41.31**	**45.09**	
S.E.m±	0.82	0.98	1.35	1.31	1.37	1.71	
CD(5%)	2.32	2.77	3.81	3.69	3.90	5.14	

NS-Non significant
DAF-Days after flowering
***-fruits harvested at 104 days #- fruits harvested at 110 days**
T_1=Tree 1 T_2=Tree 2 T_3=Tree 3 T_4= Tree 4 T_5=Tree 5
T_6=Tree 6 T_7=Tree 7 T_8=Tree 8 T_9=Tree 9 T_{10}= Tree 10

Table 4: The effect of metallic sound in trees after flowering of jackfruit at different intervals.

Treatment (trees)	Metallic sound (Hedonic scale)						
	Number of days						
	30DAF	60DAF	90DAF	104DAF	110DAF	118DAF	Mean
T_1	1.0	2.0	3.5	3.9	4.0	4.0	3.1
T_2	1.0	2.0	3.0	3.1	*	#	2.8
T_3	1.0	1.6	2.6	2.7	*	#	2.5
T_4	1.0	1.6	2.6	2.8	3.4	#	2.6
T_5	1.0	1.6	2.7	3.0	*	#	2.6
T_6	1.0	2.0	2.5	3.1	3.3	4.0	2.7
T_7	1.0	2.0	2.9	3.0	3.1	#	2.7
T_8	1.0	1.7	2.7	3.0	3.3	#	2.6
T_9	1.0	1.7	2.7	3.2	3.4	#	2.7
T_{10}	1.0	2.0	3.0	3.1	3.4	4.0	2.8
Mean	1.0	1.8	2.8	3.0	3.4	4.0	
S.E.m±	0	0.15	0.16	0.5	0.45	0.37	
CD (5%)	0	0.43	0.46	1.3	1.34	1.10	

The degree of metallic sound was quantified through hedonic scale of 1 to 4,
as: 1= not sensible (Absent).
2= slightly sensible,
3= moderately sensible
4= clearly sensible (hollow metallic sound)
***-fruits harvested at 104 days #- fruits harvested at 110 days**

followed by T_2 (32.61 cm), and T9 had the shortest (27.51cm). The length of fruit was highest in T_2 (45.35cm) at 90 DAF followed by T_1 (44.45cm) and minimum fruit length was found in T_9 (39.07cm). At 104 DAF, highest length of fruit was recorded in T_2 (46.58cm) which was followed by T_1 (45.89cm) and minimum fruit length was found in T_9 (40.19cm). Observations were taken before harvesting jackfruits of T_2, T_3 and T_5 at 104 DAF. At 110 DAF, highest length of fruit was recorded in T_1 (47.55cm) which was followed byT_4 (47.31cm) and minimum fruit length was found in T_9 (42.32cm). Observations were taken before harvesting jackfruits of T_4, T_7, T_8 and T_9 at 110 DAF. At 118 DAF, highest length of fruit was recorded in T_6 (48.95cm) which was followed by T_1 (47.21cm) and minimum fruit length was found in T_{10} (47.08cm). Observations were taken before harvesting jackfruits of T_1, T_6 and T_{10} at 118 DAF. The difference in the mean fruit length may be attributed to the difference in their genotypes. Fruit lengths ranged from 20.00 to 47.00 cm, with a mean of 31.40 cm, according to Akter *et al*. (2017) . AHJ05 produced the longest fruit (47.00 cm), foll owed by AHJ19 (46.00 cm), while AHJ04 produce d the shortest fruit (43.00 cm) (20.00 cm). Accordin g to Rana *et al*. (2018), the GM and AM of the soft variety were lower than those of the hard variant, al though both kinds showed a similar trend. As the st age changed from 1 to 4, the GM and AM for soft v arieties increased from 5.15 to 10.11 cm and 5.75 to 11.12 cm, respectively (Saha *et al.,* 2016).

Fruit circumference (cm)

The results pertaining to fruit circumference in ten trees of jackfruit is presented in Table 3. Significant difference was observed with respect to fruit circumference of jackfruit up to 118 of harvest. At 30 DAF, there was significant difference among trees, the maximum fruit circumference was noticed in T_9 (15.43cm) which was followed by T_{10} (12.79cm). However, it was minimum in T_1 (10.04cm). At 60 DAF, highest circumference of fruit was recorded in T_2 (31.35cm) which was followed by T_3 (27.99cm) and minimum fruit circumference was found in T_1 (21.01cm).The circumference of fruit was highest in T_1 (53.2cm) at 90 DAF followed by T_2 (46.76 cm) and minimum fruit circumference was found in T_7 (33.26cm). At 104 DAF, highest circumference of fruit was recorded in T_1 (53.66cm) which was followed byT_2 (48.56cm) and minimum fruit circumference was found in T_8 (34.6cm). Observations were taken before harvesting jackfruits of T_2, T_3 and T_5 at 104 DAF. At 110 DAF, highest circumference of fruit was recorded in T_1 (54.86cm) which was followed byT_4 (42.97cm) and minimum fruit circumference was found in T_6 (35.85cm). Observations were taken before harvesting jackfruits of T_4, T_7, T_8 and T_9 at 110 DAF. At 118 DAF, highest circumference

of fruit was recorded in T_1 (56.08cm) which was followed byT_{10} (41.98cm) and minimum fruit circumference was found in T_6 (37.23cm). Observations were taken before harvesting jackfruits of T_1, T_6 and T_{10} at 118 DAF. The difference in the mean fruit circumference in all the trees may be attributed to the difference in their genotypes. Akter *et al*. (2017) also reported fruit length ranged from 20.00 to 47.00 cm with the mean of 31.40 cm respectively. The longest fruit was obtained from AHJ-05 (47.00 cm) followed by the AHJ-19 (46.00 cm) and shortest fruit in AHJ-04 (20.00 cm). Rana *et al.* (2018) found the GM and AM of the soft variety were lower than those of the hard kind, but both varieties showed a similar pattern. The GM and AM for soft varieties grew from 5.15 to 10.11 cm and 5.75 to 11.12 cm, respectively. As the stage progresses from one to four.

Ruby Khan *et al*. (2010) discovered this using morphological standardized descriptors (IPGRI 2000), and it's the first large-scale in situ assessment of jackfruit diversity in several locales (900 trees) (nine villages). It is expected that jackfruit populations will exhibit genetic diversity reflected in phenotypic variation as a result of various local environmental (i.e., location) and human selection pressures. We anticipate that jackfruit grown on homesteads will be subjected to positive selection pressure for marketable traits, and that the position of a tree reflects its origin storey as much as human selection.

Metallic sound

The result pertaining to metallic sound in ten trees of jackfruit is presented in Table 4. At 30 DAF, there was no characteristic metallic sound heard from the fruits as hedonic scale score was 1. At 60 DAF, slightly sensible hollow metallic was heard from fruits of T_1, T_2, T_6, T_7 and T_{10} as hedonic scale score was 2.0, followed by T_8 and T_9 (1.7). At 90 DAF, moderately sensible hollow metallic was heard from fruits of T_1 (3.5) followed by T_2 and T_{10} (3.0). At 104 DAF, moderately sensible hollow metallic was heard from fruits of T_1 (3.9) followed by T_2 and T_{10} (3.0). Observations were taken before harvesting jackfruits of T_2, T_3 and T_5 at 104 DAF. At 110 DAF, clearly sensible hollow metallic was heard from fruits of T_1 (4.0) followed by T_4, T_9 and T_{10} (3.4). Observations were taken before harvesting jackfruits of T_4, T_7, T_8 and T_9 at 110 DAF. At 118 DAF, clearly sensible hollow metallic was heard from fruits of T_1, T_6 and T_{10} (4.0). Observations were taken before harvesting jackfruits of T_1, T_6 and T_{10} at 118 DAF. The reason behind this could be that in initial stages of growth and maturity of fruit, the bulbs and seeds were not fully developed, thus hollow metallic sound was completely absent because there was more space inside the fruit but with advances in growth and maturity, the bulbs and seeds were fully developed and hence produce clear hollow metallic sound because there was no more space inside the fruit among the trees studied. Saha *et al.* (2016) also reported that no characteristic metallic sound was heard from the fruits of 80 and 90 DAF. It was heard from the fruits of 100 DAF and it was slight to moderately sensible. With the advances of maturity the metallic sound was heard progressively. It was moderately to clearly sensible at 120 DAF and clearly sensible at 130 DAF.

Conclusion

The following conclusions can be drawn based on the aforesaid discussion of results obtained in above investigation. In this experiment, maturity indices were judged upon morphological characters of fruits. The mean fruit length was found significantly increasing and maximum in tree 1 (39.00 cm), followed by tree 6 (38.61 cm) and it was least in tree 9 (33.56 cm). The mean fruit circumference was found significantly increasing and maximum in tree 1 (41.48 cm), followed by tree 2 (34.34 cm) and it was least in tree 9 (30.93 cm). The mean number of spines/cm^2 was maximum in tree 3 (10.1), followed by tree 5 (9.5) and it was least in tree 1 (8.0). The mean number of spines/cm^2 was decreasing significantly but at 30 days, mean number of spines/cm^2 were non significant. The degree of metallic sound was quantified through hedonic scale of 1 to 4, as: 1= not sensible (Absent), 2= slightly sensible, 3= moderately sensible and 4= clearly sensible (hollow metallic sound). At 118 DAF, the characteristic metallic sound was clearly sensible as hedonic scale score was 4.0. The mean metallic sound (hedonic scale) was maximum in tree 1(2.8), followed by tree 2(2.8) and it was least in tree 3(2.5). The

analysis of variance for morphological characters of fruit revealed existence of considerable variation among the genotypes for the characters studied. Fruit circumference, low spine density, moderate to high spine spreading, and the presence of a sensible hollow metallic sound could be used as jackfruit maturity indices based on morphological analysis of the characters. It's also worth noting that jackfruit can be harvested after 100 days of fruit development.

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Conflict of interest

The authors declare that they have no conflict of interest.

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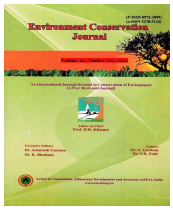

Assessment of certain plant products toxixity against *Sitophilus oryzae* in milled rice grains in coastal Odisha

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ABSTRACT

The rice variety, Jyotirmayee was treated with plant products for the assessment of toxicity towards rice weevil *Sitophilus oryzae* in milled rice grains in the laboratory of Department of Entomology of College of Agriculture under Odisha University of agriculture and technology, Bhubaneswar in coastal climatic condition of Odisha. The toxicity assessment revealed that the average rate of mortality over the time and doses was significantly highest in black pepper with 81.86% mortality which was statistically at par with tobacco (80.37%), turmeric (70.00%) and chilli (68.51%). Theprobit analysis data revealed that at 24 HAT and 48 HAT the lowest LC_{50} value of 0.02 and 0.06% was recorded respectively in black pepper. But at 72 HAT the lowest LC_{50} value of 0.08% was recorded with chilli followed by turmeric (0.14%), black pepper (0.21%), eucalyptus (0.71%) and tobacco (0.95%).

Introduction

The rice weevil *Sitophilus oryzae* has been reported to develop resistance to synthetic insecticides (Benhalima*et al.,* 2004). The increasing serious problems of resistance and residue to pesticides and contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need of effective biodegradable pesticides with greater selectivity. The plant derived chemicals have been used as potential seed protectant (insecticides and antifeedants) often begins with the screening of plant extracts (Pavela, 2007). Plants are rich source of compounds having insecticidal activity (Arnason*et al.,* 1989) and its extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects in insects (Isman, 2006). The plant derived materials are more readily biodegradable, less likely to contaminate the environment and may be less toxic to mammals. Therefore, today researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches.

Material and Methods

For the toxicity test of plant products, 10g of treated rice was taken in each petridish as food materials of rice weevil. Each treatment was replicated thrice. Ten insects per replication were taken in each petridish. In the untreated check (control) same number of insects was tested without treated materials. These petridishes were kept in an incubator at 30 $\pm$ 5° C. Insect mortalities were recorded at 24, 48, and 72 hours after treatment (HAT). Observed mortalities of the insects were corrected by Abbott's formula (1987) and then analyzed by Analysis of Variance (ANOVA). The mean values were separated by DMRT test (Duncan, 1951). LC_{50} values were calculated by Probit analysis in MS excel through Data Analysis Tool Pack. According to Abbott's formula;
Corrected mortality =

$$\text{Corrected mortality} = \frac{\text{(Portion of control group survived)- (Portion of treatment group survived)}}{\text{(Portion of control group survived)}}$$

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Results and Discussion

The toxic effect of the plant products revealed that after 24 HAT highest mortality of *S. oryzae* of 70.00, 76.70 and 83.30 % was recorded with tobacco @ 1.5, 2.0 and 2.5% doses and 73.30, 73.30 and 80.00% with black pepper @ 0.5, 1.0 and 1.5% doses respectively. It was followed by chilli (56.70, 63.30 and 70.00%) and turmeric (60.00, 63.30 and 66.70 %) @ 0.5, 1.0 and 1.5% doses respectively. With fenugreek (6.70, 10.00 and 13.30% respectively) and coriander (3.30, 6.70 and 10.00% respectively) the lowest mortality rates were observed. Same trend was observed at 42 HAT. (Table 1)However Ivbijaro and Agbaje (1986) reported that admixture of dried chilli powder @1.5 g/20 g cowpea caused 46 percent mortality of adult *C. maculatus* within 48h and reduced F1 production by 45 percent which is in support of our results. At 72 HAT highest mortality of 80.00, 86.70 and 96.70% was recorded in black pepper @ 0.5, 1.0 and 1.5% doses respectively which was followed by tobacco with 76.70, 83.30 and 93.30 % at 1.5, 2.0 and 2.5% doses respectively. With turmeric 70.00, 73.30 and 83.30 %; with chilli 70.00, 73.30 and 80.00% and with eucalyptus 43.30, 56.70 and 63.30% mortality were recorded at 0.5, 1.0 and 1.5% doses respectively.

The average rate of mortality over the time and doses was significantly highest in black pepper with 81.86% which was statistically at par with tobacco (80.37%), turmeric (70.00%) and chilli (68.51%). It was followed by Eucalyptus oil (48.89%), castor oil (43.70%), karanja oil (42.98%), chrysanthemum (42.60%), clove (36.68%), cinnamon (32.22%), garlic (28.52%), Tulsi (21.48%) and Bael (20.00%) which was at par with each other. The lowest average mortality was recorded with fenugreek (10.73%) and coriander (7.03%) and was at par with each other.The average mortality of *S. oryzae* was 36.67, 43.33 and 51.10% at 0.5, 1.0 and 1.5% doses. However Yahiya (2004) during his investigation on the efficacy of castor oil at dosages of 0.5 ml, 1.0 ml, 2.0 ml, 3.0 ml and 4 ml/50 g seeds against the longevity of rice weevils (*S. oryzae*) found that longevity of weevils was inversely proportional to the dosages of oils used which is supports our findings.The average rate of mortality over the time and doses was significantly highest in black pepper with 81.86% which was statistically at par with tobacco (80.37%), turmeric (70.00%) and chilli (68.51%). Ntonifor *et al.,* (2010) found that using a dose of 2g/100g of grain of stored cowpea, *P. guineense* caused 97.5% mortality of *C. chinensis* at 3 and 5 DAI. Moreover, Asawalam and Chukwuekezie (2012) evaluated the significant mortality effect (90%) of *S. zeamais* assessed by petroleum ether extract of *C. longa* after 42 days of treatment. These reports further strengthen our findings.

Further Eucalyptus oil (48.89%), castor oil (43.70%), karanja oil (42.98%), chrysanthemum (42.60%), clove (36.68%), cinnamon (32.22%), garlic (28.52%), Tulsi (21.48%) and Bael (20.00%) were at par with each other. Dhakshinamoorty and Selvanarayana (2002) studied the efficacy of some plant materials on the survival of *C. maculatus* infesting stored green gram and reported that at 7 days after treatment the mortality was highest (100%) in castor oil which contradicts from the present findings. The lowest average mortality was recorded with fenugreek (10.73%) and coriander (7.03%) and was at par with each other. Soon *et al.,*(2003) reported that an extract from *Cinnamon umsieboldii* (cinnamon)root bark when used against *S. oryzae* resulted in 100% mortality at 2 days after treatment which contradicts the present findings.

Probit analysis

The calculated probit regression analysis of plant products have been made at 24, 48 and 72HAT. The results of the probit analysis for the estimation of LC_{50} values and their 95% fiducial limits and the slope of the regression lines at 24, 48 and 72HAT for the mortality of *S. oryzae* have also been presented in the Table. The probit analysis data revealed that 24 HAT the lowest LC_{50} value of 0.02% was recorded in black pepper followed by turmeric (0.10%), chilli (0.31%), tobacco (0.83%) and eucalyptus (1.79%). The highest LC_{50} value was reported in garlic (37.86%) and fenugreek (38.06%)(Table 2). After 48 HAT the lowest LC_{50} value was recorded with black pepper (0.06%) followed by turmeric (0.10%), chilli (0.13%), tobacco (0.79%). The LC_{50} value fenugreek was remained down to 25.16% but was still highest among the others (Table 3). But at 72 HAT the lowest LC_{50} value of 0.08% was recorded with chilli followed by turmeric (0.14%), black pepper (0.21%), eucalyptus (0.71%) and tobacco (0.95%).

Table 1: Toxicity test of plant products against rice weevil, *S. oryzae* at 24, 48 and 72 HAT in milled rice grains at different concentrations

Treatment	Conc. (%)	Mortality rate (%)			Average mortality rate	Mortality rate (%) over conc. and time
		24 HAT	48 HAT	72 HAT		
T1 (Bael)	1.5	13.30	16.70	20.00	16.67	20.00[bc]
	2.0	16.70	20.00	23.30	20.00	
	2.5	20.00	23.30	26.70	23.33	
T2 (Chrysanthemum)	1.5	33.30	36.70	40.00	36.67	42.60[b]
	2.0	40.00	46.70	50.00	45.57	
	2.5	40.00	46.70	50.00	45.56	
T3 (Tulsi)	1.5	13.30	16.70	20.00	16.67	21.48[b]
	2.0	16.70	20.00	23.30	20.00	
	2.5	23.30	26.70	33.30	27.77	
T4 (Garlic)	0.5	20.00	23.30	26.70	23.33	28.52[b]
	1.0	23.30	26.70	30.00	26.67	
	1.5	26.70	36.70	43.30	35.57	
T5 (Karanj)	0.5	33.30	36.70	40.00	36.67	42.98[b]
	1.0	36.70	40.00	46.70	41.13	
	1.5	46.70	50.00	56.70	51.13	
T6 (Castor)	0.5	33.30	36.70	40.00	36.67	43.70[b]
	1.0	40.00	43.30	46.70	43.33	
	1.5	43.30	53.30	56.70	51.10	
T7 (Fenugreek)	0.5	6.70	6.70	6.70	6.70	10.73[c]
	1.0	10.00	13.30	13.30	12.20	
	1.5	13.30	13.30	13.30	13.30	
T8 (Turmeric)	0.5	60.00	66.70	70.00	65.57	70.00[a]
	1.0	63.30	70.00	73.30	68.87	
	1.5	66.70	76.70	83.30	75.57	
T9 (Chilli)	0.5	56.70	63.30	70.00	63.33	68.51[a]
	1.0	63.30	66.70	73.30	67.77	
	1.5	70.00	73.30	80.00	74.43	
T10 (Cinammom)	0.5	20.00	26.70	33.30	26.67	32.22[b]
	1.0	26.70	33.30	36.70	32.23	
	1.5	33.30	36.70	43.30	37.77	
T11 (Clove)	0.5	26.70	30.00	30.00	28.90	36.68[b]
	1.0	36.70	36.70	40.00	37.80	
	1.5	40.00	43.30	46.70	43.33	
T12 (Black pepper)	0.5	73.30	76.70	80.00	76.67	81.86[a]
	1.0	73.30	83.30	86.70	81.10	
	1.5	80.00	86.70	96.70	87.80	
T13 (Tobacco)	1.5	70.00	73.30	76.70	73.33	80.37[a]
	2.0	76.70	80.00	83.30	80.00	
	2.5	83.30	86.70	93.30	87.77	
T14 (Coriander)	0.5	3.30	3.30	3.30	3.30	7.03[c]
	1.0	6.70	6.70	6.70	6.70	
	1.5	10.00	10.00	13.30	11.10	
T15 (Eucalyptus)	0.5	36.70	40.00	43.30	40.00	48.89[ab]
	1.0	46.70	53.30	56.70	52.23	
	1.5	46.70	53.30	63.30	54.43	
SEm	-	-	-	-	-	11.77
CD $_{0.05}$	-	-	-	-	-	34.00

HAT – Hours after treatment

Table 2: Relative toxicity (Probit analysis) of plant products treated against rice weevil, *S. oryzae* at 24 HAT in milled rice grains

Plant products	Conc. applied (%)	Regression equations	LC50 value (%)	95% Fiducial limit		Slope ± SE
				Lower limit	Upper limit	
T1 (Bael)	1.5, 2.0, 2.5	y = 1.217x + 3.671	12.33	0.81	1.63	1.22 ± 0.03
T2 (Chrysanthemum)	1.5, 2.0, 2.5	y = 0.833x + 4.444	4.65	-4.29	5.96	0.83 ± 0.40
T3 (Tulsi)	1.5, 2.0, 2.5	y = 1.701x + 3.567	6.94	-2.88	6.28	1.70 ± 0.36
T4 (Garlic)	0.5, 1.0, 1.5	y = 0.451x + 4.287	37.86	-0.33	1.23	0.45 ± 0.06
T5 (Karanj)	0.5, 1.0, 1.5	y = 0.685x + 4.743	2.37	-3.17	4.54	0.68 ± 0.30
T6 (Castor)	0.5, 1.0, 1.5	y = 0.555x + 4.738	2.96	0.18	0.93	0.56 ± 0.029
T7 (Fenugreek)	0.5, 1.0, 1.5	y = 0.799x + 3.735	38.06	-0.002	1.60	0.79 ± 0.06
T8 (Turmeric)	0.5, 1.0, 1.5	y = 0.364x + 5.356	0.10	-0.42	1.15	0.36 ± 0.06
T9 (Chilli)	0.5, 1.0, 1.5	y = 0.726x + 5.374	0.31	-0.88	2.33	0.73 ± 0.13
T10 (Cinnamon)	0.5, 1.0, 1.5	y = 0.845x + 4.403	5.08	-0.33	2.02	0.85 ± 0.09
T11 (Clove)	0.5, 1.0, 1.5	y = 0.790x + 4.627	2.96	-0.69	2.28	0.79 ± 0.12
T12 (Black pepper)	0.5, 1.0, 1.5	y = 0.410x + 5.712	0.02	-3.75	4.58	0.41 ± 0.33
T13(Tobacco)	1.5, 2.0, 2.5	y = 1.974x + 5.164	0.83	-0.93	4.88	1.97 ± 0.23
T14 (Coriander)	0.5, 1.0, 1.5	y = 1.163x + 3.508	19.14	0.82	1.51	1.16 ± 0.03
T15 (Eucalyptus)	0.5, 1.0, 1.5	y = 0.572x + 4.855	1.79	-2.28	3.42	0.57 ±0.22

Table 3: Relative toxicity (Probit analysis) of plant products treated against rice weevil *S. oryzae* at 48 HAT in milled rice grains

Plant products	Conc. applied (%)	Regression equations	LC50 value (%)	95% Fiducial limit		Slope ± SE
				Lower limit	Upper limit	
T1 (Bael)	1.5, 2.0, 2.5	y = 1.065x + 3.843	12.17	0.47	1.66	1.07 ± 0.05
T2 (Chrysanthemum)	1.5, 2.0, 2.5	y = 1.200x + 4.481	2.70	-6.18	8.59	1.20 ± 0.58
T3 (Tulsi)	1.5, 2.0, 2.5	y = 1.525x + 3.745	6.65	-3.04	6.09	1.53 ± 0.36
T4 (Garlic)	0.5, 1.0, 1.5	y = 0.766x + 4.468	4.94	-3.40	4.93	0.77 ± 0.33
T5 (Karanj)	0.5, 1.0, 1.5	y = 0.666x + 4.83	1.80	-3.18	4.51	0.67 ± 0.30
T6 (Castor)	0.5, 1.0, 1.5	y = 0.851x + 4.893	1.33	-2.02	3.72	0.85 ± 0.23
T7 (Fenugreek)	0.5, 1.0, 1.5	y = 0.860x + 3.794	25.16	-3.42	5.15	0.86 ± 0.34
T8 (Turmeric)	0.5, 1.0, 1.5	y = 0.589x + 5.586	0.10	-2.26	3.44	0.59 ± 0.22
T9 (Chilli)	0.5, 1.0, 1.5	y = 0.560x + 5.487	0.13	-2.03	3.15	0.56 ± 0.20
T10 (Cinnamon)	0.5, 1.0, 1.5	y = 0.595x + 4.560	5.47	0.23	0.96	0.59 ± 0.03
T11 (Clove)	0.5, 1.0, 1.5	y = 0.731x + 4.686	2.69	-0.47	1.93	0.73 ± 0.09
T12 (Black pepper)	0.5, 1.0, 1.5	y = 0.801x + 5.969	0.06	0.66	0.94	0.80 ± 0.01
T13(Tobacco)	1.5, 2.0, 2.5	y = 2.189x + 5.22	0.79	-1.53	5.90	2.19 ± 0.29
T14 (Coriander)	0.5, 1.0, 1.5	y = 1.163x + 3.508	19.14	0.82	1.51	1.16 ± 0.03
T15 (Eucalyptus)	0.5, 1.0, 1.5	y = 0.748x + 5.002	0.99	-2.98	4.48	0.75 ± 0.29

Table 4 : Relative toxicity (Probit analysis) of plant products treated against rice weevil *S. oryzae* at 72 HAT in milled rice grains

Plant products	Conc. applied (%)	Regression equations	LC50 value (%)	95% Fiducial limit		Slope ± SE
				Lower limit	Upper limit	
T1 (Bael)	1.5, 2.0, 2.5	y = 0.986x + 3.981	10.78	0.70	1.15	0.97 ± 0.08
T2 (Chrysanthemum)	1.5, 2.0, 2.5	y = 1.183x + 4.570	2.31	-2.25	3.38	1.18 ± 0.22
T3 (Tulsi)	1.5, 2.0, 2.5	y = 1.803x + 3.806	4.59	-5.98	9.58	1.8 ± 0.61
T4 (Garlic)	0.5, 1.0, 1.5	y = 0.882x + 4.598	2.85	-4.78	6.55	0.88 ± 0.44
T5 (Karanj)	0.5, 1.0, 1.5	y = 0.850x + 4.979	1.06	-2.03	3.73	0.85 ± 0.23
T6 (Castor)	0.5, 1.0, 1.5	y = 0.850x + 4.979	1.06	-2.03	3.73	0.85 ± 0.23
T7 (Fenugreek)	0.5, 1.0, 1.5	y = 0.860x + 3.794	25.16	-3.42	5.15	0.86 ± 0.34
T8 (Turmeric)	0.5, 1.0, 1.5	y = 0.860x + 5.74	0.14	-4.59	6.31	0.86 ± 0.43
T9 (Chilli)	0.5, 1.0, 1.5	y = 0.628x + 5.688	0.08	-2.46	3.71	0.63 ± 0.24
T10 (Cinnamon)	0.5, 1.0, 1.5	y = 0.524x + 4.708	3.60	-1.70	2.75	0.52 ± 0.18
T11 (Clove)	0.5, 1.0, 1.5	y = 0.922x + 4.751	1.86	0.70	1.15	0.92 ± 0.02
T12 (Black pepper)	0.5, 1.0, 1.5	y = 1.961x + 6.345	0.21	-8.81	12.73	1.96 ± 0.85
T13(Tobacco)	1.5, 2.0, 2.5	y = 3.394x + 5.074	0.95	-9.52	16.31	3.39 ± 1.016
T14 (Coriander)	0.5, 1.0, 1.5	y = 1.479x + 3.578	9.14	-2.07	5.03	1.48 ± 0.28
T15 (Eucalyptus)	0.5, 1.0, 1.5	y = 1.071x + 5.157	0.71	0.57	1.57	1.07 ± 0.04

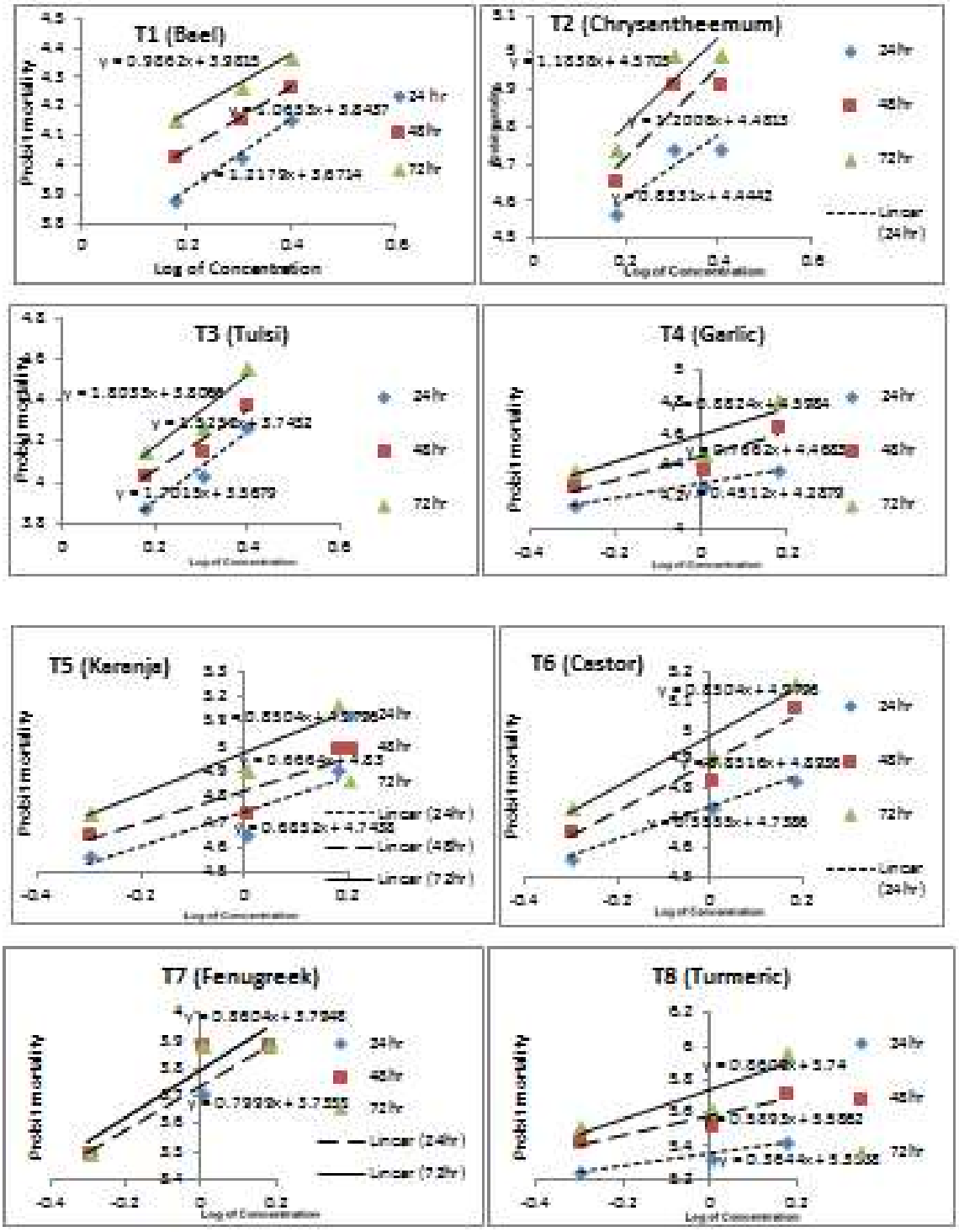

Figure 1: Relative toxicity of plant products (Tobacco, Coriander, Eucalyptus, Garlic, Karanj, Castor, fenugreek, turmeric) against *S. oryzae*at24, 48 and 72 HAT.

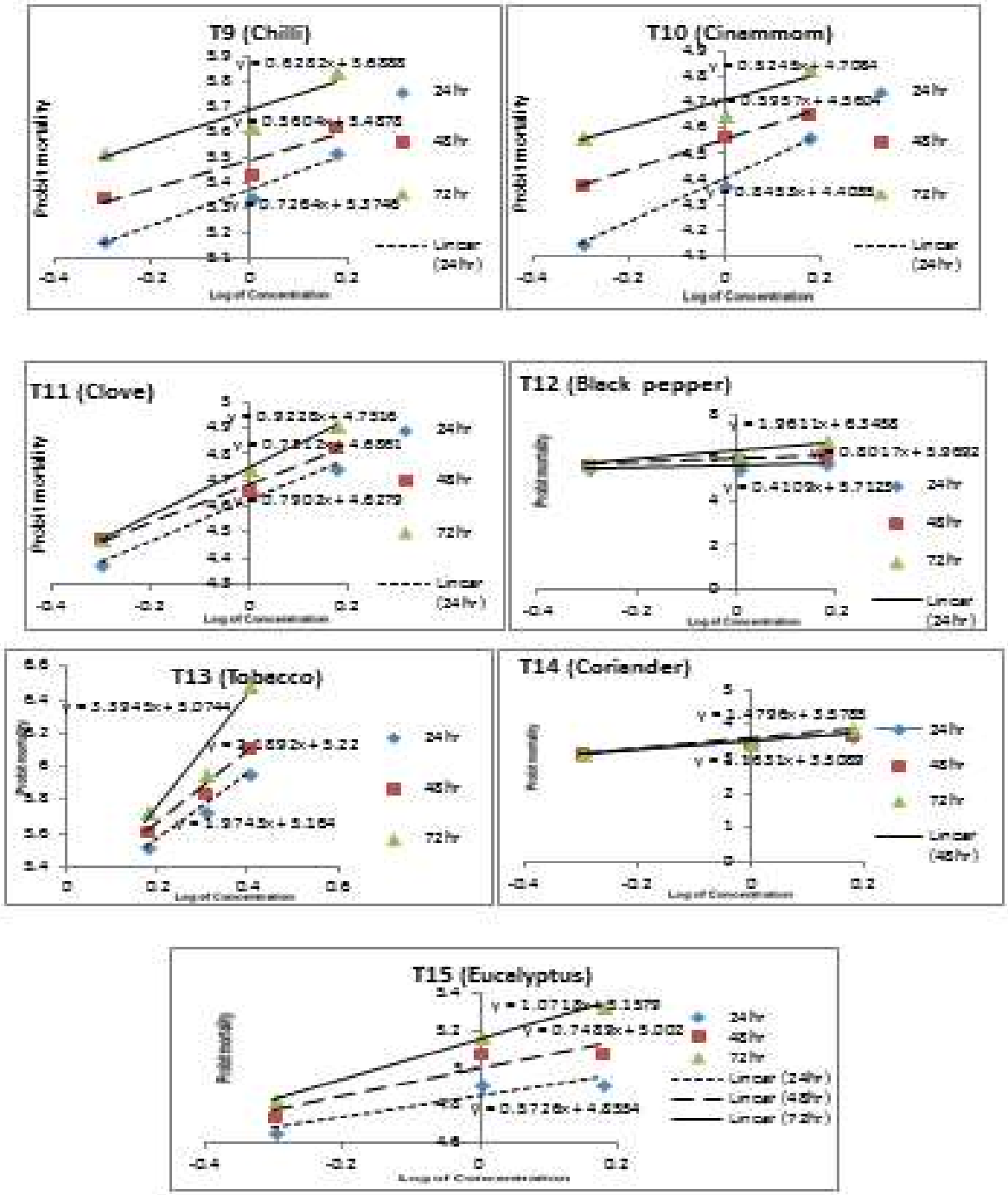

Figure 2: Relative toxicity of plant products (Chilli, Cinnamon, Clove, Black pepper,Tobacco, Coriander and Eucalyptus) against *S. oryzae*at24, 48 and 72 HAT.

Karaj and castor oil had the same LC_{50} value of 1.06% recorded. Clove reported to have the LC_{50} value of 1.86%. Coriander and Bael has a higher LC_{50} value of 9.14 and 10.78% respectively and again fenugreek resulted the highest LC_{50} value of 25.16% which was the highest over the others.

(Table 4).Chaubey (2011) reported that *P. nigrum* (black pepper) essential oils showed significant fumigant toxicity in *S. oryzae* adults with median concentrations (LC_{50}) of 0.58 µL cm^{-1} air respectively which supports our results.At 24 HAT the LC_{50} values of garlic was37.86%, but at 48 HAT it was reduced to 4.94% and at 72 HAT it was 2.85%. However Ragaa *et al.,*(2012) conducted an experiment to evaluate the toxicities of garlic oil against the rice weevil, *S. oryzae* adults and found that the LC_{50} value of garlic oil was 10.81 ml / kg. The oil at LC_{50}'s caused a significant decrease in the mean number of eggs laid by females as compared to the control and completely inhibited adult emergence. Karanj and castor oil had the same LC_{50} value of 1.06% recorded. Clove reported to have the LC_{50} value of 1.86%. Coriander and Bael has a higher LC_{50} value of 9.14 and 10.78% respectively and again fenugreek resulted the highest LC_{50} value of 25.16% which was the highest over the others. But there is no report on the LC_{50} value of these mentioned products.

Conclusion

It is concluded that the average rate of mortality over time and doses was significantly higher in black pepper.

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Conflict of interest

The authors declare that they have no conflict of interest.

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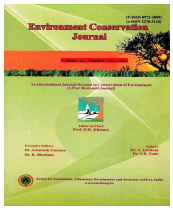

Growth trends of lac production during XII plan *vis-a-vis* XI plan period in Chhattisgarh, India

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ABSTRACT

The study is based on secondary data on lac production during the XI (2007-08 to 2011-12) and XII plans (2012-13 to 2016-17). Some econometric parameters, viz. Minimum, maximum, mean production, growth rate, percentage changes in mean during the XII plan over the XI plan period, and instability were rated. The state of Chhattisgarh, which contributed 30.21 per cent of national production during the XI plan period, decreased during the XII plan to 16.03%. During the XII plan, there is a 39.49 per cent reduction in the mean value. The negative growth rate recorded during the XI plan of 25.17 per cent decreased to 4.32 per cent during XII plan. The district-related percentage change in the mean from the XI to the XII plan showed that only the Bastar district recorded an increase of 39.18 per cent. The mean value decreased in the rest of the districts. Highest decline was recorded in Rajnandgaon (-72.23 %) followed by Raipur (-57.06%), Korba (-46.55%), Dhamtari (-41.97%), Bilaspur (-40.06%), Bastar (-39.18%), Durg (-34.05%), Kanker (-28.89 %), Janjgir-Champa (-22.44 %), Mahasamund (-17.61%), and Surguja district (-7.65%). In respect of instability in production during the XII plan, only Bastar and Janjgir districts recorded lower instability than states record of 14.85 per cent. Crop wise lac production data for different lac growing districts were also analyzed to assess major suffered districts during XI and XII plan periods in Chhattisgarh.

Introduction

Lac resin, the only one of its kind from the animal kingdom, is an example of such a well-exploited system. The lac insect's resinous protective layer is employed for human benefit in the form of paint, varnish, cosmetics, and nutraceuticals, among other things. Few insects, such as the lac insect (*Kerria lacca*), spend their entire lives on a specific host plant. Being sap-sucking insects, they cause minimal direct tissue destruction by using a specialized mouth part, the stylet, to drain sap from the phloem sieve elements of the plant vascular tissue. Though massive infestations of the lac insect can create a chronic lack of photosynthetic energy and severely limit the host plant's development potential, a well-managed population can be used to produce natural goods without harming the host plant. Lac insects, as well as lac-host plants, influence the quantity and quality of the lac produced. Also, there is a high degree of variability in resin producing efficiency of lac insects as well as of host plants which can be exploited for selection/evolution of high yielding varieties. With better management of lac insects and the host plants, the quantity and quality of the lac can be significantly improved. Lac is a key source of income for forest and sub-forest people (Jaiswal *et al.*, 2006). Recently the impact of scientific lac cultivation on lac production in India has been

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reported by Jaiswal *et al* (2020). The potential demand for lac is known about its use in various industries. The principal lac host plants in India include *Schleichera oleosa, Ziziphus mauritiana,* and *Butea monosperma.* Of the two types of lac, *S. oleosa* is most suited for *kusmi* lac while *B. monosperma* for *rangeeni* lac. *Z. mauritiana* is suitable for both *rangeeni* and *kusmi* lac but only during a specific season. They are exploited for their commercial products, such as resin, dye, and wax, and their use in a variety of sectors, including food, pharmaceuticals, cosmetics, paints, and varnishes (Mohansundaram *et al.* 2022). Due to economic value, the forest dwellers protect these trees for their sustainable livelihood. There are two crop cycles in a year from each lac insect strain, *Rangeeni* summer crop starts in October-November and mature in June-July, covering eight months' period. *Rangeeni* rainy crop starts in June-July and matures in October-November, covering four months only. Similarly, *kusmi* summer crop starts in January- February and the crop mature in June-July. *Kusmi* winter crop starts in June- July and mature in January-February. Several studies have been carried out on growth analysis of lac production during past years (Jaiswal *et al.*, 2011a, b, 2012, Jaiswal and Singh, 2014d). Besides this, lac crop production estimates were also made through correlation and regression studies. Chhattisgarh state remained forest-rich and known for its substantial minor forest produce. Production of lac is also one of the important livelihood activities mainly by forest dwellers. The potential and performance assessment of different districts in respect of lac production will help develop a strategy to enhance livelihood opportunities in the state. Considering this in view, crop-wise and district wise secondary data on lac production have been analyzed for XI and XII plan period and assessed the trend.

Material and Methods

Secondary lac production data for the years 2007-08 to 2011-12 and 2012-13 to 2016-17 were gathered from published sources such as the Annual Lac Bulletin, Directorate of Lac Development, Ranchi; Lac Bulletin, Indian Lac Research Institute, Ranchi (Pal *et al.*, 2006, 2007, 2008, 2009, 2010a, 2010b, 2011); and various issues of "Lac, Plant, Resins and Gums Statistics: At a Glance" (Pal *et al.*, 2012, 2013; Yogi *et al.*, 2014, 2015, 2017, 2018, 2020). Eleven major lac growing districts of Chhattisgarh state namely Bastar, Bilaspur, Dhamtari, Durg, Janjgir-Champa, Kanker, Koba, Mahasamund, Raipur, Rajnandgaon, Surguja and a few other districts categorized as others have been covered under the study. Minimum, maximum, mean values, growth rate (simple) and instability in lac production were considered as standard parameters for each district and crop-wise compound annual growth rate (CAGR) of lac production have been calculated as per the standard procedure. CAGR has been calculated by the adoption of procedure followed by Jaiswal *et al* (2020). The percentage change in mean value from XI plan to XII plan period was also calculated. The instability in production was calculated in terms of percentage by adopting the formula (Instability = (Standard deviation/mean) X 100).

Results and Discussion

According to data, the average state production under the XII plan was 2971 tonnes per year. During these five years, the lowest annual production was 2336 tonnes and the highest was 3381 tonnes. The average production during the XI plan, was 4908 tonnes. It means that production fell by 39.49% compared to the XI plan. During XII plan period amongst different districts, the highest mean production was recorded in Korba district followed by Kanker, Bilaspur, Raipur, Bastar, and others. The state's average output is about 12% lower than the highest amount ever recorded. It means at least this much production can be achieved easily by some key interventions. Minimum production value indicated that this much production is ensured without much effort. As evident, Korba district alone contributes around 30.02 per cent of state lac production followed by Kanker (21.37%). Thereby about half of the state's lac production is from only these two districts. The other districts which contributed in a major way include Bilaspur (6.90%), Raipur (6.20%), Rajnandgaon (5.49%), Bastar (4.54%), Surguja (4.23%), Janjgir-Champa (4.07%), and others. The districts which increased their share in-state production during the XII plan include Bastar, Janjgir-Champa, Kanker, Mahasamund and Surguja. Districts that share almost the same quantity during the XII plan include Bilaspur, Dhamtari and Durg. Districts that reduced their

Table 1. District wise mean (tons), per cent share and change in lac production during XII plan *vis-a-vis* XI plan periods in Chhattisgarh state

Districts	Plan period	Attributes	*Rangeeni*			*Kusmi*			Total
			Summer	Rainy	Total	Summer	Winter	Total	
Whole state	**XI**	**Mean**	1823	870	2694	1164	1050	2214	4908
	XII	**Mean**	736	560	1296	656	1019	1675	2971
	XII	**% Share**	25	19	44	22	34	56	100
	XI-XII	**% Change**	-60	-36	-52	-44	-3	-24	-39
Bastar	**XI**	**Mean**	7	5	13	45	39	84	97
	XII	**Mean**	19	11	30	33	72	105	135
	XII	**% Share**	14.07	8.15	22.22	24.44	53.33	77.78	100
	XI-XII	**% Change**	157	112	138	-27	85	25	40
Bilaspur	**XI**	**Mean**	199	91	290	25	27	52	342
	XII	**Mean**	20	12	32	5	16	18	48
	XII	**% Share**	41.67	25.00	66.67	10.42	33.33	37.50	100
	XI-XII	**% Change**	-90	-87	-89	-80	-41	-65	-86
Dhamtari	**XI**	**Mean**	40	25	65	58	50	108	173
	XII	**Mean**	40	24	64	37	38	75	139
	XII	**% Share**	28.67	17.29	45.97	26.66	27.38	54.03	100
	XI-XII	**% Change**	-1	-4	-2	-36	-24	-31	-20
Durg	**XI**	**Mean**	23	14	37				
	XII	**Mean**	13	11.4	24.4				
	XII	**% Share**	53.28	46.72	100				
	XI-XII	**% Change**	-43	-19	-34				
Jang-Champ	**XI**	**Mean**	62	26	88	40	28	68	156
	XII	**Mean**	49	17	66	21	34	55	121
	XII	**% Share**	40.50	14.05	54.55	17.36	28.10	45.45	100
	XI-XII	**% Change**	-21	-35	-25	-48	21	-19	-22
Kanker	**XI**	**Mean**	50	53	103	410	380	790	893
	XII	**Mean**	40	52	92	235	308	543	635
	XII	**% Share**	6.30	8.19	14.49	37.01	48.50	85.51	100
	XI-XII	**% Change**	-20	-2	-11	-43	-19	-31	-29
Korba	**XI**	**Mean**	830	347	1177	265	195	460	1637
	XII	**Mean**	237	192	429	159	304	463	892
	XII	**% Share**	26.57	21.52	48.09	17.83	34.08	51.91	100
	XI-XII	**% Change**	-71	-45	-64	-40	56	1	-46
Mahasamund	**XI**	**Mean**	70	39	109	13	12	19	108
	XII	**Mean**	40	51	91	8	11	19	110
	XII	**% Share**	36.23	46.20	82.43	7.43	10.14	17.57	100
	XI-XII	**% Change**	-43	31	-17	-37	-7	2	2
Raipur	**XI**	**Mean**	42	28	70	189	170	359	429
	XII	**Mean**	16	9	25	49	92	141	166
	XII	**% Share**	9.63	5.54	15.16	29.48	55.35	84.84	100

	XI-XII	**% Change**	-62	-67	-64	-74	-46	-61	-61
Rajnandgaon	**XI**	**Mean**	296.00	156.00	452.00	74.00	61.00	135.00	587
	XII	**Mean**	72	44	116	17	30	47	163
	XII	**% Share**	44.17	26.99	71.17	10.43	18.40	28.83	100
	XI-XII	**% Change**	-76	-72	-74	-77	-51	-65	-72
Surguja	**XI**	**Mean**	88	40	128				
	XII	**Mean**	82	44	126				
	XII	**% Share**	65.02	34.98	100				
	XI-XII	**% Change**	-7	10	-2				
Others	**XI**	**Mean**	110	44	154	45	56	101	255
	XII	**Mean**	68	58	126	39	62	101	227
	XII	**% Share**	29.90	25.51	55.41	17.33	27.26	44.59	100
	XI-XII	**% Change**	-38	32	-18	-12	11	0	-11

share during the XII plan in the states total production include Korba, Raipur and Rajnandgaon. A comparison of annual average production of both plan periods indicated a sharp decline in all districts studied except in Bastar which registered a 39.18 per cent increase. The highest decline was observed in Rajnandgaon (-72.23%) followed by Raipur (-57.06%), Korba (-6.55%), Dhamtari (-41.97%), Bilaspur (-40.06%), Durg (-34.05%), Kanker (-28.89%), Janjgir-Champa (- 22.44%) and Mahasamund (-17.61%). The state as a whole produced around 44 per cent *rangeeni* and 56 per cent *kusmi* lac. Surguja and Durg districts may be considered as purely *rangeeni* crop growing districts while other districts produced both *rangeeni* and *kusmi* both lac. Bilaspur, Janjgir-Champa, Mahasamund and Rajnandgaon are the districts where the share of *rangeeni* lac is more than half and thus may be considered as *rangeeni* dominant districts. (Table1). The state recorded 14.85 per cent instability in production during the XII plan. Only Bastar (8.67%) and Janjgir-Champa (7.94%) districts registered lower instability than the state average. The rest of the districts registered higher instability. Durg district recorded the highest (43.32%) instability in production followed by Mahasamund (32.61%), Raipur (29.42%), Surguja (24.39%) Dhamtari (24.26%), Kanker (21.15%), Bilaspur (19.95%), Rajnandgaon (19.83%) and Korba (16.93%). The state registered negative growth of 4.32 per cent per annum in lac production during the XII plan. Both of the major lac producing districts, Korba and Kanker, saw negative growth of 6.57 and 9.42 percent per year, respectively during XII plan. These negative growths impacted a substantial downfall in overall production in the late years of the XII plan. During XII plan Dhamtari is the only district that registered a positive growth of 19.74 per cent per annum. The growth rate in Janjgir-Champa remained more or less stable (0.43%). The highest negative growth was recorded in Kanker district followed by Korba, Mahasamund, Rajnandgaon, Raipur, Durg, Bilaspur, Surguja and Bastar. The districts which registered a higher negative growth rate than the state (-4.32%) during the XII plan include Kanker, Korba, Mahasamund and Rajnandgaon. Overall, the declining trend in the state lac production which was very fast during the XI plan (-25.17% per annum) slowed down during the XII plan (-4.32% per annum). The same pattern of improvement was recorded in all districts except in Bastar where, a more or less stable figure registered during the XI plan, showed a declining trend during the XII plan (Table 2.). Summer and winter crop of *rangeeni* contributes 25 and 19 per cent of total lac produced in the state. All districts except Dhamtari showed a negative growth rate for *rangeeni* lac production during the XII plan. The highest negative growth rate for *rangeeni* lac was recorded in Raipur district followed by Mahasamund, Korba, Rajnandgaon, Bilaspur, Kanker, Durg, Janjgir-Champa, Surguja and Bastar district. Raipur, Mahasamund, Korba, Rajnandgaon, Bilaspur and Kanker showed higher

Table 2. Compound annual growth rate % for lac production during XII plan *vis-a-vis* XI plan periods in Chhattisgarh State

Districts	Plan period	*Rangeeni*			*Kusmi*			Total
		Summer	Rainy	Total	Summer	Winter	Total	
Whole State	**XI**	-32.51	-30.71	-31.91	-15.91	-18.54	-16.92	-25.17
	XII	-6.33	0.62	-3.43	-9.53	-2.56	-5.56	-4.32
Bastar	**XI**	44.61	34.93	40.51	-2.09	-9.13	-5.60	-0.61
	XII	0.77	-3.97	-0.69	-8.90	2.92	-1.89	-1.16
Bilaspur	**XI**	-32.65	-33.60	-34.02	-21.08	1.71	-8.50	-29.92
	XII	-12.74	6.49	-4.58	2.26	-0.80	0.20	-2.84
Dhamtari	**XI**	-18.77	-38.30	-26.82	-27.23	-50.79	-37.66	-33.40
	XII	28.97	17.46	24.39	22.32	17.79	17.01	19.74
Durg	**XI**	-20.57	-32.10	-25.49	-	-	-	-25.49
	XII	-9.34	4.14	-3.04	-	-	-	-3.04
Janjg.-Champa	**XI**	-12.59	-27.14	-17.46	-12.27	-13.96	-13.15	-15.63
	XII	-4.59	5.92	-2.10	-11.34	16.65	4.10	0.43
Kanker	**XI**	-24.03	-16.40	-19.77	-14.87	-21.62	-17.98	-18.38
	XII	-5.71	-4.29	-4.26	-10.93	-10.29	-10.59	-9.42
Korba	**XI**	-37.90	-36.65	-37.37	-6.70	-4.97	-5.04	-27.47
	XII	-11.60	-5.05	-8.77	-4.70	-4.63	-4.64	-6.57
Mahasamund	**XI**	-24.21	-4.59	-16.40	-34.02	-35.48	-34.80	-19.68
	XII	-11.60	-5.05	-8.77	-4.70	-4.63	-4.64	-6.57
Raipur	**XI**	-15.86	-12.59	-14.40	-17.62	-16.17	-16.68	-16.47
	XII	-25.10	-24.21	-24.52	33.51	-5.11	1.73	-3.84
Rajnandgaon	**XI**	-38.30	-47.73	-41.76	-43.10	-49.88	-46.17	-42.83
	XII	-10.70	-2.21	-7.63	-7.67	6.49	0.00	-5.63
Surguja	**XI**	-26.19	-8.38	-21.15	-	-	-	-21.15
	XII	0.85	-7.36	-1.92	-	-	-	-1.92
Others	**XI**	-32.10	-8.16	-25.10	-8.94	-11.34	-10.30	-19.73
	XII	4.73	4.55	5.06	-22.92	-10.98	-15.45	-3.72

negative growth than state average during the XII plan. Dhamtari is the only district that registered negative growth of high magnitude (-26.82%) during the XI plan but showed positive growth to the tune of 24.39 per cent per annum during the XII plan. This positive growth rate was witnessed in both summer and rainy crops. Similarly, Raipur is the only district where the negative growth rate is higher during the XII plan than the XI plan periods and both summer, as well as rainy crop, was affected substantially. In all other districts, the negative growth rate for *rangeeni* lac production is lower than the XI plan period, indicating improvement in terms of growth rate.The highest positive growth rate for *kusmi* lac production during the XII plan was recorded in the Dhamatari district followed by Janjgir-Champa and Raipur. But highest negative growth was recorded in Kanker district followed by Korba, Mahasamund and Bastar district. More or less stable production was recorded in Bilaspur and Rajnandgaon districts. Districts that registered negative growth rate during the XI plan but showed positive growth rate during the XII plan include Bilaspur, Dhamtari, Janjgir-Champa and Raipur. In all other districts, the negative growth rate is lower during the XII plan when compared to the XI plan. A comparison of data on the mean value for the XI and XII plan indicated that there is a 39 per cent reduction in lac production during the XII plan. The reduction in the mean value for *kusmi* and *rangeeni* was to the tune of 24 and 52 per cent respectively. Summer crop of *rangeeni* (-60%) and *kusmi* (-44%) both declined substantially in comparison to *rangeeni* rainy (-36

%) and *kusmi* winter crop (-3%). Districts that registered substantial decline (>state figure of -52%) in the mean value of *rangeeni* lac production during XII plan include Bilaspur, Korba, Raipur and Rajnandgaon. Dhamtari, Durg, Janjgir-Champa, Kanker, Mahasamund, Surguja registered a lower per cent decline than the state's average. However, there is a substantial increase of 138% in *rangeeni* lac production during the XII plan vis-à-vis XI plan. In respect of *kusmi* lac, Bastar, Bilaspur, Dhamtari, Kanker, Raipur and Rajnandgaon registered higher decline than the state mean value (-24 %) during XII plan but other districts which recorded lower decline include Janjgir-Champa, Korba, and Mahasamund. In respect of *rangeeni*-summer crop, districts which record higher decline, in comparison to the rainy crop during XII plan include Bilaspur, Korba, Rajnandgaon and Mahasamund. This indicated possibility of high-temperature mortality in these districts. Saha and Jaiswal (1993a) evaluated lac production growth and instability and found a negative growth rate of 3.6 percent over a period of 30 years (1960-1961 to1989-1990). Jaiswal and Saha (1998) performed a decade-by-decade growth analysis of lac output over a 65 years' period and found that growth rates were negative except in the thirties, fifties, and nineties, with the highest growth rates in the sixties. Jaiswal *et al.* (2022) and Kumar *et al.* (2022) evaluated the growth analysis of lac production in Odisha and West Bengal and showed its changed pattern in these states of India. Jaiswal *et al.* (1999) found that the increase rate of lac production does not equal domestic consumption in all states except in Madhya Pradesh. Aside from farmer interest and the quantity of host trees used, lac output is also influenced by scientific methods, as well as abiotic and biotic variables present during the crop development stage. The contribution of rainy season *rangeeni* lac to total *rangeeni* lac is roughly 35%, and the crop's output is mostly in the form of broodlac (seed) collected in October-November, but scraped lac reaches the market in December. The dramatic drop in *rangeeni* rainy season yield compared to summer crop reflects the need for better management practises, since the crop is vulnerable to heavy rain during larval emergence in July, as well as pest and disease incidence. It was also recorded that in many parts of Chhattisgarh, trees located on borders of paddy field is harvested after paddy harvesting in order to avoid losses of paddy crop due to felling of branches in the form of broodlac. A comparison of per cent change in mean value between *kusmi* summer and *kusmi* winter crop indicated increased mean values for *kusmi* winter crop in Baster, Janjgir-Champa and Korba districts only. In other districts decline in mean value is for both *kusmi* winter and summer crops. However, in respect of *kusmi* summer lac crop, districts that recorded a higher rate of decline, in comparison to winter crops during the XII plan include Bilaspur, Dhamtari, Kanker, Raipur and Rajnandgaon (Table 1). The lac crop is vulnerable to weather condition as it is produced by living lac insect. Dry weather during summer crop and high humidity for prolonged period during rainy season resulted mortality of insect resulting decreased yield. The production is also affected if inoculation of lac crop is done with relatively less quantity of broodlac. It normally happened when there is shortage of broodlac due to one or other reasons especially high price during inoculation period (Bhattacharya *et al.*, 2016). The declining per cent in mean values and negative growth rate is a serious issue and needs to be addressed quickly, as also this is one of the important sources of livelihood especially for forest dwellers in the state.

Conclusion

Lac farming should be promoted by the government and other line departments for better livelihood and to prevent deforestation. The possibility should be also explored for loans and subsidies on the line of agriculture so that sustainable lac production is achieved by the state. Action may also be initiated for intensive farming of lac by planting trees on the line of sericulture. Besides this, border planting of *Ziziphus mauritiana* on paddy fields is also suggested, which will increase *kusmi* broodlac production and ultimately enhance the availability of *kusmi* broodlac for inoculation of more trees of *Schleichera oleosa* during the summer season. Loans and subsidies for lac producers on the agricultural line, as well as the implementation of scientific lac cultivation methods, will help to boost production, productivity, and employment opportunities in forest and sub-forest areas.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Mathematical modelling for the phosphate and nitrate carrying capacity of dams in Uttarakhand

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ABSTRACT

The Himalayan State of Uttarakhand has abundant natural water resources and 98 Hydro Electric Power Project (HEP's) have been constructed, 25 are under construction and, 336 are planned for the future. The water bodies of these HEP's can also be utilized for other purposes besides electric power generation. To conserve the endemic aquatic biodiversity, it is necessary to understand the phosphate and nitrate dynamics of these water bodies. As there are several HEP's on a single river and the human population around them, water bodies have changed drastically during the last decade. In this study, we have calculated the phosphate and nitrate load-carrying capacity of six dams in the Uttarakhand state of India using the Vollen-Weider mathematical model modified by Dillon, Rigler and Beveridge. We have also measured the phosphate & nitrate content of these water bodies to confirm if our modelling methods confirmed with actual finding of sampling sites. The phosphate and nitrate carrying capacity of these six dams were found to be in the range of 0.155 mg/l to 0.557 mg/l and 0.6 mg/l to 1.3 mg/l. To the best of our knowledge, this is the first study in Uttarakhand that addresses the phosphate and nitrate carrying capacity using a mathematical model.

Introduction

Phosphate and nitrate are useful nutrients, but they can cause difficulties in our water environment if they get excessively concentrated. In aquatic ecosystems, phosphorus is commonly referred to as the "limiting nutrient," meaning that the amount of this nutrient available determines the rate at which algae and aquatic plants are produced. Phosphate is the most frequent type of phosphorus used by biological organisms, and it is essential for the creation of DNA, cellular energy, and cell membranes (and plant cell walls). While largest source of nitrate in water body is decomposing legumes, but high nitrate concentrations can also be created by human or animal wastes and fertilizer run-off. Although it is necessary for plant growth, excessive amounts in water can hasten eutrophication (a decrease in dissolved oxygen in water bodies caused by an increase in minerals and organic materials) in water bodies (Hampel *et al.,* 2018; LeMoal *et al.,* 2019). Soil erosion is a key

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source of phosphorus in water bodies (Rickson, 2014). However, because phosphorus and nitrate is found in small amount in nature, even small increases can have a deleterious impact on water quality and biological circumstances.

In aquatic systems, the phosphorus is mainly deposited in sediment showing its affinity towards particles (Saraswathy*et al.,* 2019). The majority of phosphorus in sediment is integrated into sediment organic matter (Holtan *et al.,* 1988). There are various physicochemical properties of sediments that control the trapping and release of phosphorus into the water like hydrological dynamics, and biological activity (Orihel *et al.,* 2017). The hydrodynamics of the dam advance the formation of inner sedimentary phosphorus load; hence 12% of the global aqueous phosphorus is conserved in it (Orihel *et al.,* 2017). Surface waters in most comparatively uncontaminated lakes have been found to have 0.03 to 0.09 mg/l phosphate (Fadiran *et al.,* 2008). Phosphate levels as low as 0.08 mg/l in water bodies, on the other hand, can induce excessive or bothersome growths of algae and other aquatic plants during the spring when nutrients are cycling to the surface. Because flowing water are less vulnerable to rapid or cultural eutrophication, a phosphate concentration of less than 0.3 mg/l is the intended target for them. The maximum nitrate content in the water that can be tolerated is 10 mg/l, anything above can have negative consequences and contribute to nitrate water pollution (Camargo *et al.,* 2005). The depth and kind of water body, as well as the type of soil, land use, and the age of the groundwater, all influence the contamination of water bodies (Canada, 1997). It's critical to comprehend how these numerous aspects will affect the poisoning of various water sources. In general, nitrate content drops as depth increases, making surface water significantly more vulnerable to nitrate pollution (Almasri and Kaluarachchi, 2004). Nitrate toxicity levels are also significantly lower in larger bodies of water, but they are much higher in smaller and shallower bodies of water because nitrate concentrations are simpler to build up (Camargo *et al.,* 2005).

Dam construction and the dam itself have variable impact- social, economical, geographical and impacts on water quality, climate, flora and fauna. Various man-made pressures are associated with water bodies. These activities can be at the local level (drainage & local activities), at the regional level (energy demands, drinking water, commercial activities, etc.) and, at the global level due to climate change (Richardson *et al.,* 2018). Of these, the effect caused at the local and regional level had the highest impact on the structure and function of water bodies (Maberly *et al.,* 2020). Water biodiversity is highly affected by these anthropogenic pressures (Zhang *et al.,* 2019). With growing water stress and energy demand, the number of dams has increased and will continue to increase in the future too. The construction of dams greatly modifies the ecological functioning of the water body system. The natural flow of water is interrupted by the dams, causing a reduction in the velocity of water and expanding the residence time of water. Due to this the stream competence is restricted in reservoirs, encouraging the accumulation of sediment. Moreover, the fine fraction of sediments are present in the surrounding dam (López *et al.*,, 2016), increasing nutrient levels (Le Faucheur *et al.,* 2016). Since aquatic ecosystems have a limited capacity to remove incoming phosphorous and nitrogen load, they need intervention to limit nitrate threat.

The Uttarakhand state of India is located in the Himalayan region of North India and has a total area of 53483 sq. km. of which 38000 sq. km. is forest area and 4060 sq. km. area is covered by glaciers (India state of forest report 2019). Because of abundant water resources, the state started on an ambitious journey of hydropower regeneration. Uttarakhand has a total of 98 existing hydropower projects (HEPs), with a total installed capacity of close to 3600 Megawatt (MW) and 25 projects with 2376.3 MW capacities are under construction in Uttarakhand (Uttarakhand Jal Vidyut Nigam Limited UJVN Limited). According to reports, a total number of 459 dams are existed or under construction and are planned for the future within 300 miles radius in Uttarakhand (Uttarakhand Jal Vidyut Nigam Limited UJVN Limited). Some projects have suffered damage due to the flash flood disasters in Uttarakhand (Uttarakhand Jal Vidyut Nigam Limited UJVN Limited), the most recent one being the Rishi Ganga project in 2021. The astounding number of HEPs gives us an idea about the level of anthropogenic activities on water bodies. Hence, it is necessary to understand the phosphate

and nitrate dynamics of water bodies. We have calculated the phosphate and nitrate load capacity using a mathematical model and also measured phosphate and nitrate for selected water bodies. More comprehensive monitoring of phosphate and nitrates in water bodies accompanied by the implementation of water policy regulations will help in combating phosphate and nitrate threat to water quality.

Material and Methods

Study sites:

For the present comparative study, we selected six study sites that are already constructed dams in Uttarakhand viz. Nanak Sagar, Koteshwar, Ramganga, Srinagar, Tehri, and Maneri (Figure 1). The state of Uttarakhand is recognized for its various rivers, sacred temples, and places, located on the banks of rivers. These rivers originate from glaciers of the western Himalayas situated in the borders of India, Nepal, and China.

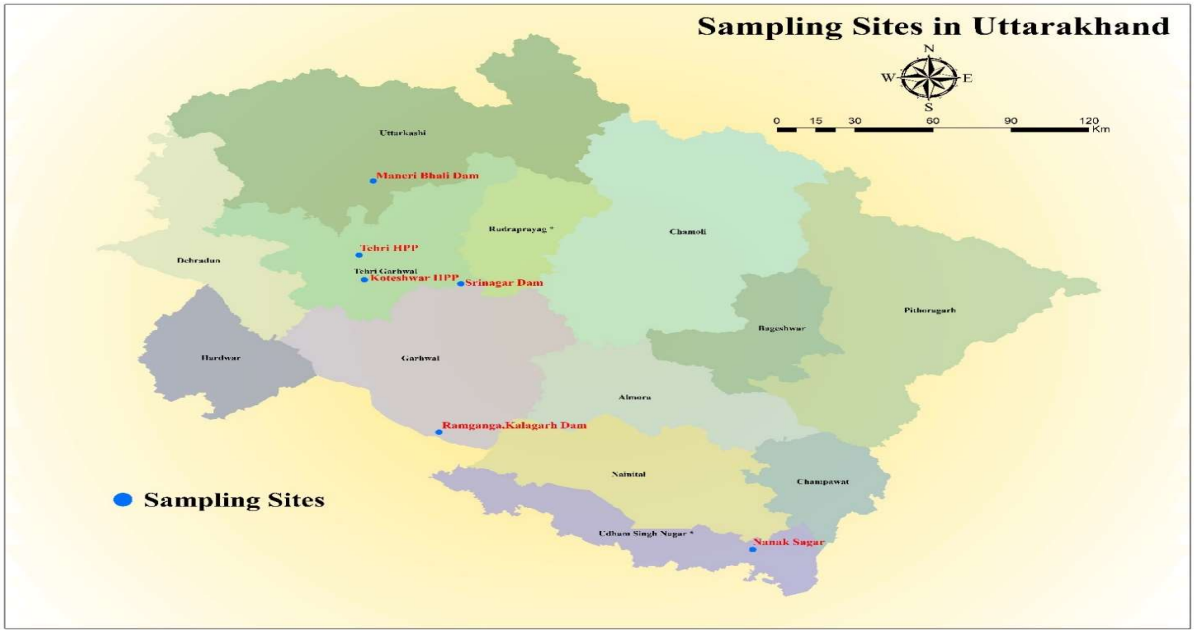

Figure 1: Map showing the location of six dams (a to f). (a) Nanak Sagar dam, (b) Koteshwar dam, (c) Ramganga dam, (d) Srinagar dam, (e) Tehri HPP, and (f) Maneri dam.

In this paper, we used several parameters, the length of the dam, gross storage capacity, area of the dam, effective storage capacity, and the variation in concentration of phosphate [P]i for the mathematical calculation of total [P]i carrying capacity. The data used for calculation in the mathematical model were retrieved from the National Register for Large Dams (NRLD). The data were obtained from government websites/online portals.

Carrying capacity analysis

The carrying capacity analysis of all the dams was done by analyzing the quality of water and by calculating the capacity of pollution load in the dams for the activity of aquaculture. The tools used for the estimation of water quality along with the standard as in Methods for Examination of Water and Wastewater (America Public Health Association 1992).

Data analysis of physicochemical characteristics

The main reason to study the chemical, physical parameters of water is to examine its nutrient status. Since the water has suspended and dissolved materials in many amounts its chemical and physical parameters differ along with its biological properties (Table 1). There are other reasons also due to which water is affected like pollutants and acts on elements present in water e.g. pH, TDS, Turbidity, Alkalinity, Phosphate, Nitrate, etc. (Table 3). Without the knowledge of the chemistry of water, it is not possible to understand the biological phenomenon fully.

Table 1: General overview of six dams of Uttarakhand

Name of Dam	Nanak Sagar	Koteshwar HEP	Ramganga	Srinagar	Tehri HPP	Maneri
Longitude of dam	$79^0$45' E	$78^0$29'52" E	$78^0$45' E	$78^0$49"20.41" E	$78^0$28'44" E	$78^0$32' E
Latitude of dam	$28^0$45' N	$30^0$15'36" N	$29^0$31' N	$30^0$14'31.28" N	$30^0$22'41" N	$30^0$44.5' N
Length of Dam (m)	19200.00	300.50	715.00	248.00	27980.00	13.70
Gross storage capacity (10^3m^3)	209000.00	889000.00	244960.00	78000.00	3540000.00	600.00
Reservoir Area (10^3m^2)	50000.00	3022.00	19720.00	4500.00	44000.00	180.00
Effective storage capacity (10^3m^3)	200550.00	35000.00	218770.00	8000.00	2615000.00	510.00
Designed spillway capacity (m^3/sec)	1600	13290	8467	19200	13000	5000

Source: National Register of Large Dams | Central Water Commission, Ministry of jalshakti, Department of Water Resources, River Development and Ganga Rejuvenation, GoI (cwc.gov.in).

Statistical analysis

In this section, we performed statistical analysis to calculate the mean, standard deviation, and standard error from samples obtained from six dams in the months of April, August, and December 2021. The obtained results are discussed in Table 2, and the numerical mean values of the phosphate and nitrate levels have been used in the mathematical modeling. The phosphate and nitrate Q-value curve for generating a water quality index, as shown in (Figure 2), illustrates the impact of phosphate and nitrate levels on water quality. The q-value curve for phosphate and nitrate, which is used to calculate a water quality index, shows that water quality degrades rapidly as phosphate and nitrate concentrations rise.

Mathematical model

To define the carrying capacity, the Vollen-Weider model has been used in this research which has been modified by Dillon &Rigler.

- Considering the P concentration (mg m^{-3}) in water as a function of the annual P load (L_f, in mg m^{-2} $year^{-1}$), the P retention coefficient (R_f), average depth (z, in meters), and water flushing rate (ρ, in years),
- The amount of phosphate total endured forever by sediment (x),
- The proportion of dissolved total phosphate can be holdout by the sediment (R).

In the fish production carrying capacity analysis model made by (Dillon and Rigler 1974), there are total 5 steps:

➢ **Step 1:**

The capacity of aquatic for fish in accepting phosphorus or total P concentration ($\Delta\,[P]$) is the mean of the phosphate level in the dam (Table 2).

➢ **Step 2:**

Next, we determine R for the dam, where R is the P retention coefficient from the study by (Larsen and Mercier 1976), with modifications by (Canfield and Bachmann 1981).

To calculate the value of R we have used the equation.

$$R = \frac{1}{1+0.747*\rho^{0.507}} \quad (1)$$

➢ **Step 3:**

The amount of phosphate produced by fish is calculated by

$$R_f = x + [(1-x)*R\,]. \quad (2)$$

➢ **Step 4:**

Next, calculate the pollution load capacity of fish in the dam is given by the equation.

$$L_f = \frac{\Delta[P]*z*\rho}{1-R_f}, \quad (3)$$

where Δ[P] is mean phosphate level in dam, z is the depth of dam

$\left(z = \frac{V_G}{R_a}, \text{V}_\text{G} \text{ is the total volume and } \text{R}_\text{a} \text{ is the area of the dam}\right)$

and ρ is the flushing rate

$\left(\rho = \frac{S_w}{V}, \text{ } \text{S}_\text{w} \text{ is the spillway capacity and V is the effective storage capacity of dam}\right)$.

➢ **Step 5:**

The total carrying capacity of fish production in the dam allowed maximum pollution load can be determined by

$$TC_{cap} = L_f * A, \quad (4)$$

Where A is the area of the dam (Bueno *et al.,* 2017).

Note: The same analysis has been performed for nitrate pollution load capacity (Replacing Δ[P] by Δ[N] in Step 1).

Results and Discussion

In this study, a total of six dams were taken and their maximum pollution load capacity (Ton. year^{-1}) was calculated based on the amount of phosphate total endured forever by sediment (which is presented by x), where the value of x is around 0.45 to 0.55 (Warningsih *et al.,* 2016). The Srinagar dam was found to have the largest phosphate pollution load capacity, indicating that it can contain up to 7340.14 tonnes of phosphorous waste every year. Warningsih (2016) found that the phosphate load capacity of the koto panjang reserviour is 225.933,851 tonnes per year, while the highest nitrate pollution load capacity was found in the koteshwar dam, indicating that the capacity of the koteshwar dam to hold nitrogenous waste is maximum 31105.90 tonnes per year.The pH of water is an important water quality indicator as it acts as a major factor in most chemical and biological reactions. The pH of the all six dams observed in the range of 7.58-7.81. Total dissolved solids (TDS) content has long been used as a measure of aquatic ecosystem productivity. Higher levels of TDS in bodies of water are generally harmful to aquatic life. TDS alters the mineral composition of water, which is critical for many species' existence. In addition, dissolved salt can dry aquatic animals' skin, which can be lethal. It can raise the temperature of the water, making it uninhabitable for many species. The TDS of all six recorded in the range of 48 mg/l-102 mg/l (Table 3). The turbidity of water is a measurement of how clear it is. High turbidity indicates that there are many particles suspended in the water that prevent light from passing through. The turbidity recorded all dams are in the range from 0.28 mg/l- 0.42 mg/l. A water body with a high alkalinity level has more calcium carbonate, or $CaCo_3$, which can reduce the acidity of the water. Alkalinity and water hardness are comparable in that they both arise from natural sources. Water moves through rocks (and picks up minerals as it does so) on its way when limestone and dolomite dissolve in water, one half of the molecule is calcium or magnesium (the “hardness”) and the other half is the carbonate (the “alkalinity”). The data on total alkalinity of water samples of six dams is given in (Table 3). The value of total alkalinity was found in all dams ranges from Tehri dam 22 mg/l- 64 mg/l. Hardness observed in all dams ranges from 28 mg/l-86 mg/l. Phosphate amount from the reservoir site is an important factor to determine as it is released due to the decomposition of aquatic vegetation. Phosphate value is minimum in the month of winters as it is utilized immediately by the overgrowth of phytoplankton. (Table 3) shows the levels of phosphate in different dams of Uttarakhand (Raveendar *et al.,* 2021; Singh *et al.,* 2020). The phosphate value recorded in all dams are in the range of 0.155 mg/l-0.557 mg/l. The data of Nitrate concentration of water samples of six different sites of the dam is given in (Table 3). Because of the significant use of algal groups and a low source of nitrate, the lowest concentration of nitrate was found in autumn.The nitrate value recorded in 0.6 mg/l-1.2 mg/l. Sulfur is used by aquatic organisms, and lower quantities have a negative impact on algae development. Sulfate is the most frequent type of sulphur found in well-oxygenated waters. Algal growth will not develop if the sulphate concentration is less than 0.5 mg/l. SO_4 value recorded in six dams are from 7 mg/l-14 mg/l. The F value observed from 0-0.14 mg/l. The occurrence of chloride (CL) is a major cause of water pollution that arises when salts from the soil are leached into water bodies. Although chlorides have only little impact on living organisms, their excessive consumption might cause considerable harm or poisoning. CL value of all six dams ranges from 1.3 mg/l-4.9 mg/l (Table 3).

The use of the hydrodynamic model along with factorial bioenergetics models supports estimating the waste load and regulates the values used to calculate the carrying capacity of the reservoir for the production of fish. Total carrying capacity is directly proportional to the amount of phosphate and nitrate in the dam which means the total carrying capacity (TCcap) of any dam is depends on the value of phosphate and nitrate available in the water of the dam.

Table 2: Statistical analysis of samples collected from six different dams in 2021

Phosphate						
MONTH	**NANAK SAGAR**	**KOTESHWAR**	**RAMGANGA**	**SRINAGAR**	**TEHRI**	**MANERI**
April	0.237	0.155	0.453	0.557	0.192	0.394
August	0.239	0.156	0.455	0.557	0.192	0.395
December	0.241	0.154	0.457	0.556	0.192	0.393
Mean	0.239	0.155	0.455	0.557	0.192	0.394
SD	0.002	0.001	0.002	0.0006	0	0.001
SE	0.0011	0.0005	0.0011	0.0003	0	0.0005
Nitrate						
April	1	1.2	1	1.2	0.7	0.65
August	0.9	1.4	1.2	1.4	0.8	0.6
December	0.8	1.3	1.4	1	1.05	0.55
Mean	0.9	1.3	1.2	1.2	0.85	0.6
SD	0.1	0.1	0.2	0.2	0.18	0.05
SE	0.0577	0.0577	0.1154	0.1154	0.1039	0.0288
pH						
April	7.58	7.79	7.65	7.64	7.82	7.77
August	7.57	7.78	7.63	7.68	7.81	7.76
December	7.59	7.8	7.61	7.66	7.8	7.78
Mean	7.58	7.79	7.63	7.66	7.81	7.77
SD	0.01	0.01	0.02	0.02	0.01	0.01
SE	0.0057	0.0057	0.0115	0.0115	0.0057	0.0057
TDS						
April	102	49	76	88	42	63
August	101	48	78	85	45	64
December	103	47	80	91	39	62
Mean	102	48	78	88	42	64
SD	1	1	2	3	3	1
SE	0.5773	0.5773	1.1547	1.7320	1.7320	0.5773
Turbidity						
April	0.4	0.39	0.39	0.42	0.28	0.31
August	0.45	0.36	0.4	0.43	0.27	0.31
December	0.35	0.33	0.38	0.41	0.29	0.31
Mean	0.4	0.36	0.39	0.42	0.28	0.31
SD	0.05	0.03	0.01	0.01	0.01	0
SE	0.0288	0.0173	0.0057	0.0057	0.0057	0
Alkalinity						
April	64	26	38	36	22	28
August	63	28	38	35	21	28
December	65	24	38	37	23	28
Mean	64	26	38	36	22	28
SD	1	2	0	1	1	0
SE	0.5773	1.1547	0	0.5773	0.5773	0
Hardness						
April	86	33	63	64	26	43
August	85	35	61	68	30	45
December	87	34	62	66	28	44
Mean	86	34	62	66	28	44
SD	1	1	1	2	2	1
SE	0.5773	0.5773	0.5773	1.1547	1.1547	0.5773
SO4						
April	15	ND	11	10	ND	6
August	14	ND	9	11	ND	7
December	13	ND	7	12	ND	8
Mean	14	ND	9	11	ND	7
SD	1	ND	2	1	ND	1
SE	0.5773	0	1.1547	0.5773	0	0.5773
F						
April	0.12	ND	0.14	ND	ND	ND
August	0.11	ND	0.12	ND	ND	ND
December	0.13	ND	0.16	ND	ND	ND
Mean	0.12	ND	0.14	ND	ND	ND
SD	0.01	ND	0.02	ND	ND	ND
SE	0.0057	0	0.011547005	0	0	0
CL						
April	5	2	2.8	2.9	1.3	2.4
August	4.9	1.9	2.9	2.9	1.3	2.3
December	4.8	1.8	3	2.9	1.3	2.2
Mean	4.9	1.9	2.9	2.9	1.3	2.3
SD	0.1	0.1	0.1	0	ND	0.1
SE	0.0577	0.0577	0.0577	0	0	0.0577

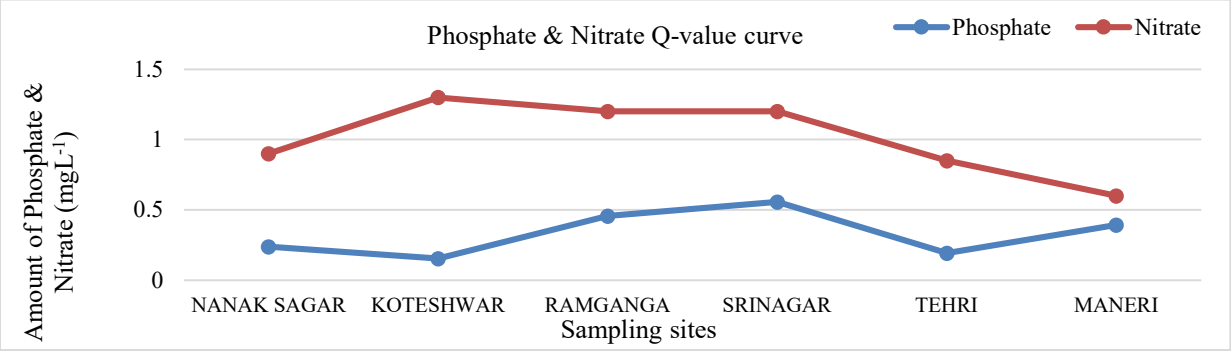

Figure 2: Amount of Phosphate and Nitrate in six dams of Uttarakhand

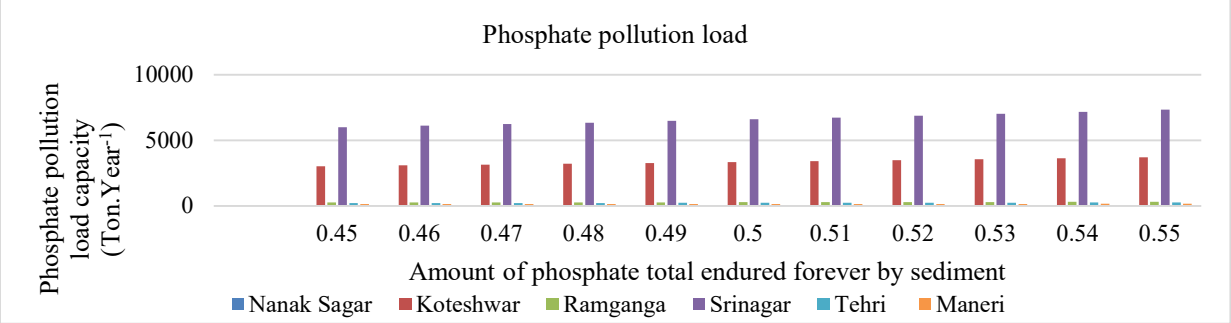

Figure 3: The maximum phosphate pollution load in six dams of Uttarakhand

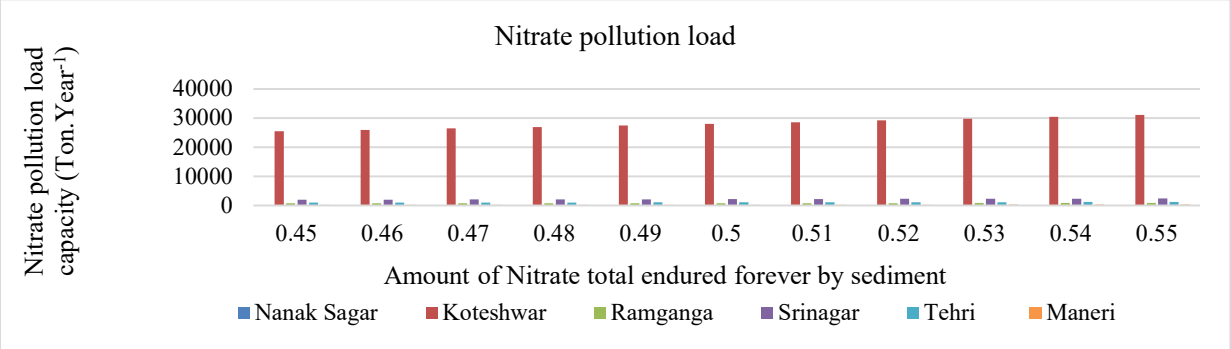

Figure 4: The maximum nitrate pollution load in six dams of Uttarakhand

Table 3: Average yearly analysis of different physicochemical parameters of six dams of Uttarakhand

Parameters	Nanak Sagar	Koteshwar HEP	Ramganga	Srinagar	Tehri HPP	Maneri	Standard
pH	7.58 ± 0.01	7.79±0.01	7.63± 0.02	7.66± 0.02	7.81± 0.01	7.77± 0.01	6-9
TDS (mg/L)	102± 1	48± 1	78± 2	88± 3	42± 3	64± 1	1500
Turbidity (NTU)	0.40± 0.05	0.36± 0.03	0.39± 0.01	0.42± 0.01	0.28± 0.01	0.31± 0.01	Below 1 NTU
Alkalinity (mg/L)	64± 1	26± 2	38± 1	36± 1	22± 1	28± 1	500
Hardness (mg/L)	86± 1	34± 1	62± 1	66± 2	28± 2	44± 1	500
Phosphate (mg/L)	0.239± 0.002	0.155± 0.001	0.457± 0.002	0.557± 0.0006	0.192± 0.001	0.394± 0.001	2
NO_3 (mg/L)	0.9± 0.1	1.3± 0.1	1.2± 0.2	1.2± 0.2	0.85± 0.18	0.6± 0.05	10
SO_4 (mg/L)	14± 1	ND	9± 2	11± 1	ND	7± 1	400
F (mg/L)	0.12± 0.01	ND	0.14± 0.02	ND	ND	ND	1.5
CL (mg/L)	4.9± 0.1	1.9± 0.1	2.9± 0.1	2.9± 0.1	1.3± 0.1	2.3± 0.1	250

The availability of phosphorus in the water was significantly higher after fish mass mortality and harmed the water quality of Maninjau lake in Indonesia (Syandri *et al.,* 2017). In a case study of upper Itchen, (UK), some researchers monitored phosphorus for water quality management. It was suspected that recently increased phosphorus concentration was found in environmental degradation (Fones *et al.,* 2020).

A day after Typhoon Lekima swept across the coastal parts of Penang, Malaysia, in August 2019, mass fish mortalities were documented, resulting in massive losses among fish farmers. After the typhoon, the results showed abnormally low dissolved oxygen and high quantities of nitrate, nitrite, and chlorophyll a (Aileen *et al.,* 2021). In a pond environment in a rural setting in Central Kenya, the use of raw animal manure, high fish stocking density, high nitrates and nitrites, and high ammonia levels are all possible risk factors for fish mortality and the acquisition of infectious diseases (Wanja *et al.,* 2020).

Conclusion

By the vital examination of we analyze that for phosphate, Srinagar dam has the highest total carrying capacity (TC_{cap}), and for nitrate, Koteshwar dam has the highest total carrying capacity (TC_{cap}). As this is a huge number, it means that even if we operate at half the maximum value, there is huge potential still available to develop commercial fisheries at these locations. Large numbers of hydroelectric power. Earlier water was flowing but now flow pattern has changed because of dams. These water bodies can be used but phosphate level is critical for an activity like commercial fisheries.

All of the water quality parameters measured in this study were within acceptable limits for fish survival and growth. Maintaining nutrient levels in the dam through extensive aquaculture is critical to ensuring that product output does not suffer from deficient symptoms. In the hilly areas of Uttarakhand, there is a pressing need to switch to a different form of agriculture to increase productivity and create a distinct brand. Apart from hydropower generating and drinking water utilities, these reservoirs provide an excellent chance and area for adopting this type of agriculture and adding a new value to itself. Because of the well-connected highways surrounding the dam, any stakeholder or business may simply convey products into the market at a higher profit margin. It is high time for stakeholders and the government to take advantage of this tremendous resource to boost the economy and employment possibilities in and around the dam. Gradually, this might significantly reduce migration out of the area. Further study and development in this subject are critical for agro capitalism to flourish in Uttarakhand's migratory regions, which could be critical to the state's economic growth. As a result, embracing culture-based fisheries and minimizing intensive human activities could boost fish productivity of dam. This research will aid policymakers in the development of a reservoir management strategy. It was advised that the Ministry of Fisheries and Aquaculture Development and the competent authorities implement regulations of farming activities adjacent to the dam to ensure good water quality for the survival and growth of fish. Regularly monitoring the dam water quality will go a long way toward ensuring that aquatic resources are conserved and used sustainably. The government of Uttarakhand's efforts to expand domestic fish output through its "Aquaculture for food and jobs" initiative will be aided by the upkeep of water quality in dam.

Conflict of interest

The authors declare that they have no conflict of interest.

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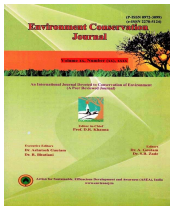

Morpho-quantitative and biochemical characterization of Chia (*Salvia hispanica* L.) seeds to understand its benefits and to increase its adaptability

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Chia

ABSTRACT

Chia (*Salvia hispanica* L.), of the mint family Lamiaceae, is one of the most highly nutritious crops in the world. It has a high economic value in both national and international markets. The present study was carried out with the prime objective of assessing Chia's morphological, biochemical, and nutritional characterization. An average yield of Chia (784 kg/ha) from the field experiment was observed. The biochemical studies showed the presence of higher amounts of carbohydrates, phenols, flavanols and antioxidants. The seed protein content of Chia was evaluated, and it was found to be 183 mg/g, which was greater than other major crops like wheat, rice and maize. The Carbohydrate content (371 mg/g) was also high in Chia seeds. High amounts of phenols (1.29 mgGAE/g) and flavonoids (0.48 mg/g) in Chia seeds were also observed. The mineral content estimated by ICP-OES showed the presence of micronutrients like Fe (11.7 mg/100g), Mg (335 mg/100g), Mn (5.97 mg/100g), Zn (12.01 mg/100g), Cu (1.94 mg/100g), Ca (397.78 mg/100g), Na (42.15 mg/100g) and K (605.83 mg/100g). The FTIR analysis showed the presence of the functional groups, and high peak banding was found related to protein, pectin (polysaccharides), PUFA (fatty acids), lipids etc. The HPTLC analysis indicated the presence of Gallic acid. Thus the present study unveils that the seeds of the Chia crop are a rich source of different essential elements. Hence this pseudo-cereal Chia can be used to provide good food supplements. As this is a newly introduced crop in India, there is very less study on the crop. To utilize the benefits of this crop, further research in various aspects to increase the environment adaptability and yield should be done.

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Introduction

Chia (*Salvia hispanica* L.) is a herbaceous plant belonging to the order Lamiales, the mint family Lamiaceae and the genus Salvia. The genus name Salvia is derived from the Latin word called "Salvare," which in Latin means "to heal" or "to be safe and unharmed," referring to the medicinal properties of the genus.

This genus's origins have been traced back to Afghanistan and Soviet Central Asia. Mexico has approximately about 250 species (Jamboonsri *et al*., 2012). Chia seeds have been used as a food source since 3500 B.C. Chia seeds are consumed as the main grain alone or in combination with other cereals, and they are also used for a variety of medicinal purposes. Chia is a pseudo-cereal, raised for its edible and highly nutritious seeds. It is being called a "Superfood" by the nutritional community due to its high nutritional value. Under ideal agronomic conditions, the plant can produce 500-600 kg of seed per acre (Ullah *et al*., 2016).

The high nutritional and pharmacological value of Chia seed (Munoz *et al.,* 2013) has increased interest among researchers in exploring opportunities for the species to be grown outside of these areas. Compared to other oilseeds or cereal seeds, Chia seeds have a 19-23% high protein content. Methionine and cysteine are particularly abundant in the seed flour protein fraction (Ixtania *et al.,* 2008). For adding to the health benefits of Chia seeds, one cup contains 34 to 40g of fiber, which equals 100% of the daily recommendation for adults to reduce the risk of coronary heart disease. Chia seeds are also a source of antioxidants such as caffeic acid, chlorogenic acid, kaempferol, and quercetin, providing many significant health benefits. Besides this, it reduces insulin resistance, improves blood sugar level, reduces inflammation, and provides vital nutrients for bone health (Webmd, 2022). In addition, plenty of macronutrients like phosphorous and micronutrients like copper, iron, manganese and molybdenum are also found in Chia seeds (Beltan-Orozco and Romero, 2003).

Therefore, this study aims to conduct morpho-quantitative analysis, phytochemical analysis and antioxidant activity analysis of Chia seeds to understand the crop's benefits and increase its environment adaptability.

Material and Methods

Collection of plant materials and Morpho-quantitative trait assessment

Chia Seeds were collected from the All India Coordinated Research Network on Potential Crops (AICRN on Potential Crops), OUAT, Bhubaneswar. The field experiment was carried out at its experimental station. The seeds were sown in the field for recording the morphological observations during the *Kharif* season of 2020 with a spacing of 45x10 cm having a plot size of 5.0 sq. meter. All the recommended agronomic practices were implemented in raising a good crop and were harvested. Observations were recorded taking five random plants selected from the middle rows of the plot for nine morpho-quantitative traits from days to flower to days to attain maturity and 10ml seed weight, which were recorded on a plot basis and from a random sample of the plot, respectively and flowering days were recorded considering all the plant present in the plot.

Preparation of seed extract and Biochemical analysis

The ground sample (1.5 g) was extracted using an environmental shaker after adding 20 ml of 80% methanol placed under room temperature for a night and centrifuged for 15 min at 8000 rpm and a temperature of 4°C. The supernatant was collected and dried. The weight of Chia seed extract was measured and then made up with methanol at 1mg/ml (Beltran-Orozco *et al.,* 2020). Di-acid digestion for mineral estimation was carried out using a 3:2 mixture of HNO_3:$HClO_4$. As the sample was high in fats/oils, pre-digestion using 10 ml HNO_3 /g sample was carried out to avoid explosion (Zasoski and Burau, 1977). The carbohydrate content was obtained using the Anthrone method, whereas protein content was calculated using the Lowry method. The total phenolic content of the Chia seed extracts was determined by comparing them with the standard antioxidants like Gallic acid according to the Folin–Ciocalteu method (Gamez-Meza *et al.,* 1999).

One ml Folin–Ciocalteu reagent was added to the extract. Then 2 ml of 10% Sodium carbonate was added after 5 mins and kept aside at room temperature for 2 hrs. Chia seed extracts and standards readings were measured at 660 nm against distilled H_2O using a UV

spectrophotometer. A calibration graph of the standard Gallic acid is prepared, and the results were calculated using it and expressed as Gallic acid equivalents (GAE mg/g). For protein extraction, two grams of seed powder were homogenized in 5ml of 10% TCA using a pre-chilled mortar pestle. The content was incubated overnight at 4ºC and centrifuged at 8000 rpm for 10mins. First, pellets were washed to remove the pigments with 2ml of 100% acetone. Next, pigment-free pellets were washed with 80% ethanol first and then by ethanol: chloroform (3:1) and washed by ethanol: ether (2:1) to eliminate the phenolic compounds.

Washed pellets were lyophilized and suspended in protein extraction buffer and boiled for 2mins, centrifuge at 12000 rpm for 10mins. The supernatant was taken for quantitative analysis. First, the total flavonoid content was measured by using the colorimetric method (Zhishen *et al.,* 1999) with some modifications. Here, 500 µL of chia seed extract was combined with 1000 µL of distilled water in a test tube, and then 75 µL of sodium nitrite ($NaNO_2$) 5% solution was added. After 5 minutes, 150 µL of a 10% $AlCl_3$ solution was added, and the mixture was agitated for another 5 minutes before adding 500 µL of 1M NaOH and 775 µL of distilled water. The absorbance of the sample was recorded immediately at a wavelength of 510 nm in a spectrophotometer and compared with a catechol calibration curve. The results were determined by calculating as gms of catechol equivalent in a kg of dry sample (g/kg equivalent dry sample).

The analysis was done according to the method described by Shimada (1992) for estimating antioxidant activity. The approach was based on the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging principle. First, the standard of BHT (synthetic antioxidant) was prepared at 500µg/ml to compare the antioxidant activity. Next, solutions of the Chia seed extracts and synthetic antioxidant BHT used in the study were prepared in methanol at concentrations of 50, 100,150 and 200 µg/mL and free radical scavenging activities were determined. Then, 1ml 0.004 % DPPH was added to the Chia seed extract and BHT standard solutions and mixed. After 30 minutes in the dark, the absorbance of each mixture was measured at 517 nm against a methanol blank. For calculation, the following formula was used.

$$\textbf{Antioxidant activity} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{absorbance of control}}$$

For mineral content estimation, the digested Chia seed sample volume consisted of distilled water to 50ml. The solution of the sample was then filtered with the help of Whatman no 42 filter paper. Finally, this digested Chia seed sample was used to calculate the amount of Fe, Mg, K, Ca, Mn, Zn, Na and Cu using the ICP-OES unit.

For FTIR analysis, the plant extract was made by centrifuging 2 gm of ground seed with 20ml of methanol, and the supernatant was used. The absorbance was taken at the wavenumber range of 4000-400 cm^{-1}. The Gallic acid content determination was done (Kaya *et al.,* 2012). The reference standard used in the present study was Gallic acid (1mg/ml). All Chia seed extract samples were taken at a 1 mg/ml concentration. The 200 ml of mobile phase was taken in a ratio of 5:4:1containing toluene, ethyl acetate and formic acid. The TLC plate of analytical chromatography grade of 10 x 10 cm was used. Plates were evaporated to remove excess humidity at 70°C in a hot air oven for 10 minutes. 10µl each of sample and reference standard were spotted on silica plate and bands were observed by UV light at 254 nm.

Results and Discussion

The phenotypic study (Table 1) revealed quantitative characters like plot yield (0.279 Kg/plot), yield (7.84 qt/ha), 50% flowering (72 days), days of maturity (102 days), plant height (109.8 cm), no. of branches (13.2), no. of secondary branches (14), length of inflorescence (19.2 cm), length of primary inflorescences (9.2 cm), length of secondary inflorescence (2.8 cm), leaf length (7.49 cm) and leaf breadth (4.18 cm). The 10 ml seed weight was also calculated by measuring the number of seeds that could be packed in a 10 ml beaker, which was 6.05g. The present investigation revealed that phenolic compounds are present in high concentrations in seeds that provide antioxidant properties to the seeds. The seed protein content of Chia was evaluated and was found to

have significant protein content (183 mg/g). Carbohydrate content (371 mg/g) was also high in Chia seeds. Also, the phenol content (1.29 mg/g) and flavonoid content (0.48 g/100g) were high when compared to other cereals (Table 2).

Table 1. Morpho-quantitative data of Chia

Sl. No	Parameter	Mean value
1	Plant height(cm)	109.80
2	No. of branches	13.20
3	No. of secondary branches	14.00
4	Length of leaf(cm)	7.49
5	Breadth of leaf(cm)	4.18
6	Length of the main inflorescence(cm)	19.20
7	Length of primary inflorescence(cm)	9.20
8	Length of secondary inflorescence(cm)	2.80
9	10ml seed wt(g)	6.05
10	Yield/plant(g)	3.53

Table 2. Quantification of different biochemicals present in Chia seeds

Sl. No.	Parameter	Content
1	Carbohydrate(mg/g)	371
2	Protein(mg/g)	183
3	Phenol(mg/g)	1.29
4	Flavonoid (mg/g)	0.48

For antioxidant scavenging activity, different concentrations of Chia seed extracts were used in the DPPH assay and were compared with BHT (a synthetic antioxidant). The activities were as follows for 50µL (70.4%), 100 µL (71.9%), 150 µL (72.8%) and 200 µL (75.3%). The maximum activity was found when 200 µL of Chia seed extract was used (Table 3). The constituents were evaluated, and the findings are shown in Table 4.

From the plot data collected from the experimental station of AICRN on Potential Crops in the *Kharif* season of 2020, the yield per hectare was 784 kg/ha. The studies performed in Argentina have shown yields of 606 to 1400 kg/ha (Lobo *et al.,* 2011), whereas the studies conducted in Mexico showed a yield of 1200 kg/ha (Bochicchio *et al.,* 2015). The results of field experiments on Chia conducted in Germany gave yields of 618.39 to 1171.33 kg/ha (Grimes *et al.,* 2019). The biochemical analysis showed the presence of a high quantity of phenols in Chia seeds. The results showed the content of 1.29 mgGAE/g phenol in the Chia seeds. Furthermore, scientists researched the Chia seed chemical constituents and found the presence of phenols 0.97mg GAE/g seed sample (Beltan-Orozco *et al.,* 2020).

Martinez-Cruz and Paredes-Lopez (2014) calculated the amount of phenols in Mexican Chia as 1.63 mg GAE/g of seed sample. They recorded a total average of 0.66 to 0.9mg GAE/g for chia seeds cultivated in Mexico. In the present study, the amount of flavonoids in the Chia seed sample was calculated as 0.48 mg/g. The study revealed 0.36 mg/g of flavonoids in Chia seed samples (Beltran-Orozco, 2020). Scapin *et al.,* 2016, calculated the total flavonoids present in Chia seed extracts as 0.16 mg/g. In the present study for antioxidant scavenging activity, different concentrations of Chia seed extracts were used in the DPPH assay and were compared with BHT (a synthetic antioxidant). Maximum scavenging/inhibition activity was found when 200 µL of Chia seed extract was used and was about 75.3%. As a result of these findings, the chia seed sample had a high content of Fe (11.78 mg/g), Mg (>335 mg/g), Mn (5.97 mg/g), Zn (12.01 mg/g), Cu (1.94 mg/g), Ca (397.8 mg/g), Na (42.15 mg/g) and K (605.8 mg/g). Chia seeds showed a high quantity of iron, potassium and magnesium. In cells and body fluids, potassium regulates heart rate and blood pressure. When it comes to mineral abundance, magnesium was ranked sixth. There are two forms of this divalent cation in plants: bound and unbound. Magnesium content could not be calculated as magnesium concentration was saturated in the present Chia seed sample. Hence, it can be more than 335 mg/100g of Chia seeds. The transmittance bands show the functional groups present in the Chia seed sample. A better understanding of the chemicals present in Chia and their molecular analysis was carried out with the help of Fourier transform infrared (FTIR) technology. The bands were analyzed with the functional reference groups and identified the presence of different compounds in the Chia seed sample. The FTIR spectrum presented bands between 3700 cm^{-1} and 3000 cm^{-1},

Table 3. Antioxidant activity of chia seed extract.

Volume of sample (µL)	Chia absorbance	BHT absorbance	Scavenging activity (%) of Chia	Scavenging activity (%) of BHT
50µL	0.495	0.185	70.4	88
100µL	0.470	0.162	71.900	90.3
150µL	0.455	0.156	72.8	90.6
200µL	0.413	0.134	75.3	91.9

Table 4. Estimated mineral content (mg/100g) Chia seed sample.

Sl. No	Mineral	mg/100g-present study
1	Fe(238.204)	11.78
2	Mg(285.213)	>335
3	Mn(257.610)	5.97
4	Zn(206.200)	12.01
5	Cu(327.393)	1.94
6	Ca(317.933)	397.8
7	Na(589.592)	42.15
8	K(766.490)	605.8

N.B. Values within parenthesis represent absorbance in nm

Table 5. FTIR spectra of Chia seed and their corresponding annotations

Wavenumber (cm^{-1})	Transmittance (%)	stretches	Class of compounds	Intensity
3308.89	71.67	≡C-HO-HN-H	Alkynes	strong, sharp
			Amides	Weak to medium
			Alcohols	strong, broad
			Carboxylic acids	strong, broad
2943.86	79.07	C-H O-H	Alkanes	strong
			Alkyls	strong
			Carboxylic acids	strong, broad
2831.93	79.68	O-H	Carboxylic acids	strong, broad
2522.26	97.69	O-H	Carboxylic acids	strong, broad
1449.05	83.91	C=C, C-H	-CH2, Ester	
1115.59	88.45	C-FC-O-C	R-F(Alkyl fluoride)	very strong
			C=C-H2-OH	medium to strong
			C=C-CH(R-OH)	
			C=C-CRR'-OH	
1022.44	22.4	=C-O-C	Ether	Med to strong
			R-F	very strong
625.72	71.69	C-Br ,≡C-H	R-Br (Alkyl bromide)	strong
			Alkynes	strong, broad

Table 6. Rf values, Max % and Area % of Chia and Gallic acid

Sample	Start Rf	EndRf	Max%	Area%
Gallic acid	0.33	0.56	52.19%	60.82
Chia	0.41	0.53	6.23%	8.72

and the peaks between these bands may be related to the stretching of the O-H group's vibrations from hydroxyls of polysaccharides and proteins. The band at 3308 cm^{-1} shows amide stretch showing the presence of proteins (Table 5). A mobile phase with toluene, ethyl acetate and formic acid in the ratio of 5:4:1, respectively, was used in developing the HPTLC profile. Individual peaks were scanned at 254 nm to determine the Rf value and area unit (AU). The Rf values (Table 6) of the band for reference standard were: Gallic acid at Rf 0.33 – 0.56.

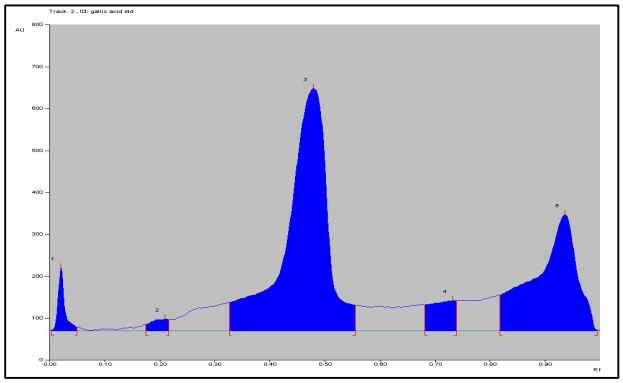

Figure 1. Graph showing the peak for Gallic acid.

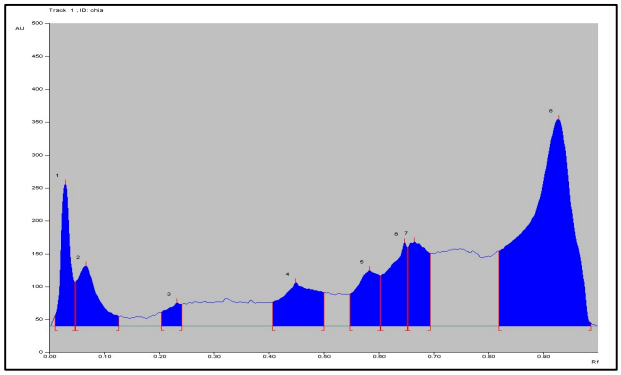

Figure 2. Graph showing the peak in the Chia seed samples comparable to the gallic acid peak.

The chemical components in the Chia sample extracts were detected by comparing bands in the sample with those of a reference standard on the same TLC plate. The HPTLC graph showed that Gallic acid was present in the Chia seed extract (Figure 1). Peak 3 of gallic acid was in the Rf range of 0.33 and 0.56, and a peak was obtained in the chia seed sample in the range of 0.41 and 0.53, which comes in the range of Gallic acid (Figure 2). So it was ascertained that the presence of phenols in the Chia seed extracts (the peak 4 in the Chia

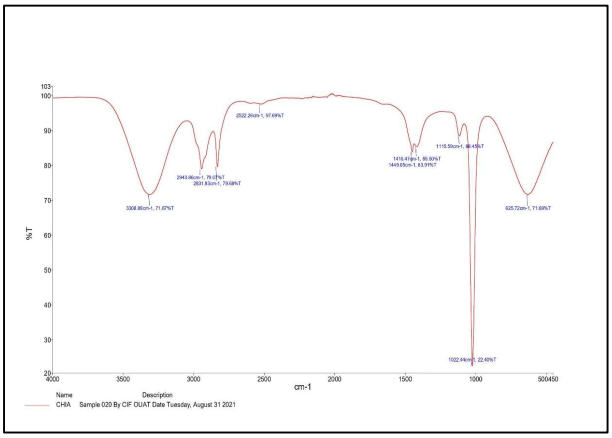

Figure 3. Spectral graph of functional group analysis of Chia seed extract.

sample was considered the Gallic acid). The -C-H stretches of aromatic rings and the methyl group signifying fat content were linked to the broad band at 2943.86 cm^{-1}. Transmittance at 1449.05 cm^{-1} shows the bending vibration of CH_2 groups (lipid) and ester carboxyl stretches of uronic acids in seed polysaccharides. The FTIR shows the presence of C≡C bonds in high intensity that shows the presence of PUFA's (Polyunsaturated fatty acids) like alpha-linolenic acids. The high unsaturation in the spectra was caused due to alpha-linolenic acid present in the sample. The peak at 1115.59 cm^{-1} shows the presence of C-O-C (triglyceride ester linkage). At 1449 cm^{-1}, it shows the peaks for methyl esters in pectin. The FTIR results show the presence of high amounts of polysaccharides, lipids, triglycerides, polyunsaturated fatty acids (PUFA), and pectin (Table 5, Figure 3). At 1449 cm^{-1}, it shows the peaks for methyl esters in pectin. The FTIR results show the presence of high amounts of polysaccharides, lipids, triglycerides, polyunsaturated fatty acids (PUFA), pectin etc. HPTLC technique was highly used in pharmaceutical industries to separate new promising pharmaceutical compounds (Maripandi *et al.,* 2010). The HPTLC chromatogram of the seed extracts of Chia shows the presence of Gallic acid. The HPTLC profile was developed using a mobile phase/solvent system as toluene: ethyl acetate: formic acid (5:4:1). Peak 3 of gallic acid was in the Rf range of 0.33 and 0.56. A peak was observed in the chia seed sample in the range of 0.41 and 0.53, which comes in the range of Gallic acid, indicating the presence of the phenols in the

Chia seed extracts. Chia seeds' DPPH radical scavenging activity was 68.83 per cent (inhibition %). There was a strong antioxidant capacity because of the abundance of phenolic chemicals (Martinez-Cruz and Paredes-Lopez, 2014; Dash *et al.,* 2022). Chia seeds had a higher percentage of inhibition than the standard drug at 500 µg/ml (92% and 67%, respectively), as found by Dugganaboyana *et al.,* 2016. Biochemical analysis of the Chia seed showed the presence of high concentrations of protein, carbohydrates, phenols and flavonoids. The seed protein content of Chia was evaluated and was found a significant amount of protein. The protein content was 183 mg/g, i.e., 18.3%. Kasuya *et al.,* 2012 calculated 15-25% of the protein in Chia seeds. In comparison to other cereals, chia seeds have a protein level of 17 per cent (for example, in corn, the amount of protein content was 9.4 per cent, rice was 6.5 per cent, quinoa 14.1 per cent, and in wheat 12.6 per cent) (Knez *et al.,* 2019). Chia seeds' carbohydrate amount was 371 mg/g, i.e., 37.1%. The study conducted by Dugganaboyana *et al.,* 2016 showed that Chia seeds had 349.2 mg/g or 34.92 % of carbohydrates in Chia seeds. Chia seeds contain 42% of carbohydrates in them. Micronutrient elements present in Chia were evaluated after the wet digestion process (digestion using nitric acid and perchloric acid), followed by spectrometry by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (USDA, 2018; Sahoo *et al.,* 2020). The different elements were analyzed, and the results were presented for the Chia seed powder was employed in this investigation.

Conclusion

Based on the current investigation, it is observed that the local collection of Chia seeds showed good adaptability with a significant quantity of yields. It is also found that the mineral content is higher with good sources of Fe, Mg, Mn, Zn, Cu, Ca, Na and K. Protein and carbohydrate content are also high. Chia crop is rich in phenols, flavonoids and antioxidant activity, which gives this crop a high medicinal value. The presence of high essential polyunsaturated fatty acids and polysaccharides in Chia is also observed. With good market potential and nutritional and medicinal value, this crop can be preferred to be grown on a commercial scale. The diversity, along with the amount of nutrient composition in chia seed, can help to have a healthy diet and add value to the preparation of products. However, in vivo and clinical studies on the safety and efficacy of chia seed are still limited. Although the presence of active ingredients in chia seed warrants its health benefits, the safety and efficacy of this medicinal food or natural product need to be validated by scientific research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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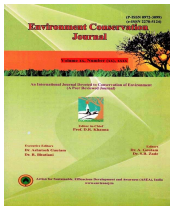

Studies of genetic correlation and path coefficient analysis between resistance to brown spot disease and yield related traits in rice (*Oryza sativa* L.)

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ABSTRACT

Brown spot disease in rice is caused by *Cochliobolus miyabeanus* (Anamorph: *Bipolaris oryzae* (Breda de Haan) Shoemaker, 1959 (Synonyms: *Helminthosporium oryzae*). It causes significant losses by affecting both economic yield and grain quality. Though, it is a minor disease in most of the parts of the world but the historical famines like Krishna Godaveri Delta famine and Bengal famines and huge crop losses in a number of incidences as in Guyana and Nigeria renders it as a potential threat to rice crop and adverted the requirement of efficient, sustainable and economical strategies to cope with the pathogen. In this context, availability of resistant sources against the pathogen is a noteworthy alternative for disease management. Realising the importance of resistant sources, the present research investigation was undertaken to study association between resistance to brown spot disease and yield attributing traits in rice via correlation studies and path analysis to identify high yielding resistant lines for brown spot disease in rice. In this study disease resistance expressed in terms of AUDPC showed negative correlation with yield and yield attributing traits and direct negative effect on yield. Thus, AUDPC can be utilised as a selection parameter for developing improved cultivars with higher grain yield and lower susceptibility towards the brown spot pathogen.

Introduction

Rice (*Oryza sativa L.*) is the most preferred staple food crop of more than half of the global population(Li *et al*., 2014) It supplies 20-80% of dietary energy and 12-17 % of dietary proteins for Asians. It is a semi-aquatic annual grass native to tropical Asia. In India it has the largest area under cultivation and highest production among grain crop. In India approximately 43.19 million hectares area is under the cultivation of rice from which 115.63 million tons of grain is produced with average productivity of 26.77 Q /ha (3rd Adv. Est. 2018-19, Annual Report, DAC&FW). A well-managed crop with adequate irrigation, nutrient and crop health management yield average 2-3 t/ha.

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However, its yield potential is adversely affected by diseases, insect-pest and weeds. Among these, fungal diseases especially brown spot is a potential threat to rice crop with respect to its production and productivity. The disease is caused by *Cochliobolus miyabeanus* (Ito and Kuribayashi,1927) (Synonyms: *Helminthosporium oryzae*). The pathogen infects the coleoptiles (causing blighting), leaves (forming oval, dark brown to purplish-brown spots ultimately killing the leaf) and even the seeds which are badly damaged at the flowering to milk stages than at the soft dough or mature stages (Ou, 1985) . The disease is of great importance on ground of economic significance and have a historical context as well in form of two major epidemics in India, the first in 1918–19 in the Krishna Godavari delta and the second, the Great Bengal Famine during 1942 (Ghose *et al*., 1960). These epidemics were a results of heavy crop loss upto 90 % (Ghose *et al.,* 1960; Sunder *et al*., 2005) in associated area due to large scale devastation by the pathogen in absence of suitable management practises. Among a number of alternatives, use of resistant sources against the pathogen is considered to be the most sustainable and economical method of disease management. However, it is often reported that the field resistance in commercial varieties of a crop is not durable and liable for breakdown on account of fast evolving pathogen like *Cochliobolus miyabeanus.* To cope with this, it is imperative that the resistant sources should be diverse and should be strategically employed to prevent the speedy spread of pathogen or to trap it in limited cropped area. In this regard, the available germplasm resources should be screened against the pathogen and evaluated for higher yield characteristics to identify high yielding resistance genotypes. Further, on ground of being a polygenic trait, yielding potential of a genotype is highly influenced by environmental factors (Khatab *et al.,* 2016). The component traits also have direct and indirect effect via other traits on the final yield. These effects could be identified and estimated following association studies involving correlation and path analysis (Jaiswal *et al*., 2019). Path analysis is employed to disentangle the direct and indirect influences of component traits on grain yield and also quantifies the inter relationship among the various component traits. The inferences derived based on the results of association studies provides an obvious understanding in selection of component trait to be used for direct and /or indirect selection, if required. Keeping these in view, the present investigation is carried out with the objective of studying the associations between resistance to brown spot disease and yield attributing characters in rice.

Material and Methods

The present research was carried out at Rice Research Farm, Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar on 300 genotypes of rice along with three check varieties namely Rasi (resistant check), IR-64 (moderately resistant check) and Pankaj (susceptible check) in augmented design during *kharif* season 2019 and *kharif* season 2020. The experimental plot was subdivided into 12 blocks with replicated checks and unreplicated test genotypes. The entries were raised in nursery and transplanted in the main field 30 days after sowing at a spacing of 20cm x15cm. All the recommended package of practices were followed along with necessary prophylactic plant protection measures to raise a good crop. The fungal suspension was used for spraying the crop for artificial inoculation in controlled conditions while in the field the susceptible check was used as infector row. Moreover, the experimental location is a hotspot for brown spot infection. Disease scoring was done visually on 10 individual plants following Standard Evaluation System for Rice (SES), IRRI, 2013for brown spot at grain filling stage. Data on yield and yield attributing traits were recorded and subjected to statistical analysis using OPSTAT 2020 for studying the association between disease resistance and yield. The correlation coefficients between variables under study was calculated following Johnson *et al.* (1955) and the Path coefficient analysis was carried out following Wright (1921) and Dewey and Lu (1959).

Results and Discussion

Correlation Analysis

Association between two or more traits in terms of degree and direction can be defined by correlation. Table 1 represents the genotypic (above diagonal)

and phenotypic (below diagonal) correlation among different traits under study. AUDPC showed significant and positive phenotypic and genotypic correlation with days to 50% flowering (0.93 & 0.80), days to physiological maturity (0.96 & 0.83) and plant height (0.87 & 0.65) while significant but negative correlation with panicle length (-0.95 & -0.80) number of effective tillers per plant (-0.67 & -0.51), number of grains per panicle (-0.73 & -0.57), test weight (-0.89 & -0.73) and yield (-0.48 & -0.37) (Fig. 1).

Path analysis

Phenotypic Path Matrix

In phenotypic path matrix (table 2) highest direct positive effect on grain yield was shown by number of grains per panicle (0.59) followed by number of effective tillers per plant (0.45; similar to Singh *et al.*, 2018) and test weight (0.36) while the highest direct negative effect was shown by days to physiological maturity (-0.36) followed by AUDPC (-0.13), panicle length (-0.09) and plant height (-0.013; similar to Kumar *et al*., 2018). The highest positive indirect effect was shown by number of grains per panicle (similar to Prakash *et al.,* 2018) via test weight (0.42) while the lowest positive indirect effect was shown by days to 50 % flowering via number of effective tillers per plant (0.02). Further, the highest negative indirect effect on grain yield was shown by days to physiological maturity via days to 50 % flowering (-0.35) while the lowest negative indirect effect was shown by plant height via number of effective tillers per plant (-0.004).

Table 1: Estimates of Genotypic and Phenotypic Correlation co-efficient between yield and its related trait

Traits	Days to fifty per cent flowering	Days to physiological maturity	Plant height (cm)	Panicle length (cm)	Effective tillers per plant	Grains per panicle	Test weight (g)	AUDPC	Grain yield per plant (g)
Days to fifty per cent flowering	1.00	0.99***	0.92***	0.88***	-0.52***	-0.59***	-0.85***	0.93***	-0.31**
Days to physiological maturity	0.99***	1.00	0.92***	0.91***	-0.57***	-0.65***	-0.88***	0.96***	-0.37**
Plant height (cm)	0.80 ***	0.80***	1.00	0.83***	-0.47***	-0.48***	-0.76***	0.87***	-0.22**
Panicle length (cm)	0.78***	0.82***	0.71***	1.00	0.62***	-0.68***	-0.83***	-0.95***	0.43***
Effective tillers per plant	-0.43***	-0.46***	-0.34**	0.52***	1.00	0.85***	0.80***	-0.67***	0.90***
Grains per panicle	-0.52***	-0.57***	-0.40***	-0.57***	0.65***	1.00	0.88***	-0.73***	0.92***
Test weight (g)	-0.72***	-0.75***	-0.57***	-0.71***	0.60***	0.70***	1.00	-0.89***	0.72***
AUDPC	0.80***	0.83***	0.65***	-0.80***	-0.51***	-0.57***	-0.73***	1.00	-0.48***
Grain yield per plant (g)	-0.27**	-0.32**	-0.19**	0.37**	0.79***	0.83***	0.65***	-0.37**	1.00

***Significant at 5%, **1% and ***0.1 % probability levels. Phenotypic correlation above diagonal; Genotypic correlation below diagonal**

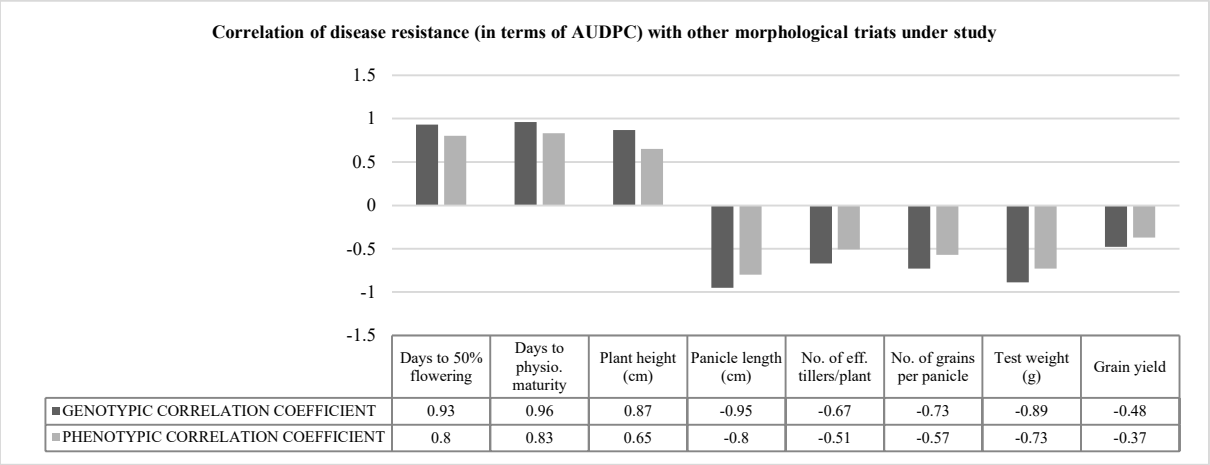

Figure 1: Correlation of disease resistance (in terms of AUDPC) with other morphological traits under study.

Table 2: Estimates of Phenotypic(P) and Genotypic (G)matrix of direct and indirect effects on grain yield per plant

Traits		Days to fifty per cent flowering	Days to physiological maturity	Plant height (cm)	Panicle length (cm)	Effective tillers per plant	Grains per panicle	Test weight (g)	AUDPC	Correlation with Grain yield per plant (g)
Days to fifty per cent flowering	**P**	**0.05**	0.05	0.04	0.04	0.02	0.03	0.04	0.04	-0.27
	G	**4.10**	4.08	3.78	3.61	2.15	2.44	3.47	3.84	-0.31
Days to physiological maturity	**P**	-0.35	**-0.36**	-0.28	-0.29	-0.16	-0.20	-0.27	-0.29	-0.32
	G	-5.74	**-5.76**	-5.31	-5.24	-3.27	-3.76	-5.07	-5.52	-0.37
Plant height (cm)	**P**	-0.01	-0.01	**-0.013**	-0.01	-0.004	-0.005	-0.007	-0.008	-0.19
	G	-0.19	-0.19	**-0.21**	-0.17	-0.10	-0.10	-0.16	-0.18	-0.22
Panicle length (cm)	**P**	-0.07	-0.08	-0.07	**-0.09**	-0.05	-0.05	-0.07	-0.07	0.37
	G	-0.16	-0.17	-0.15	**-0.18**	-0.11	-0.12	-0.15	-0.17	0.43
Effective tillers per plant	**P**	0.19	0.20	0.15	0.23	**0.45**	0.29	0.27	0.23	0.79
	G	0.10	0.11	0.09	0.12	**0.19**	0.16	0.15	0.13	0.90
Grains per panicle	**P**	0.30	0.34	0.24	0.34	0.38	**0.59**	0.42	0.34	0.83
	G	0.62	0.67	0.49	0.70	0.88	**1.04**	0.91	0.75	0.92
Test weight (g)	**P**	0.26	0.27	0.21	0.26	0.22	0.25	**0.36**	0.26	0.65
	G	0.49	0.51	0.44	0.48	0.46	0.51	**0.57**	0.51	0.72
AUDPC	**P**	-0.10	-0.11	-0.08	-0.10	-0.07	-0.07	-0.09	**-0.13**	-0.37
	G	-0.39	-0.40	-0.36	-0.39	-0.28	-0.30	-0.37	**-0.41**	-0.48

Bold diagonal values indicates the direct effect; R^2 (P) = 0.8989 RESIDUAL EFFECT = 0.3179; R^2 (G) = 1.0061 RESIDUAL EFFECT =0.0781

Genotypic Path Matrix

Genotypic path matrix (table 2) showed highest direct positive effect was shown by days to 50% flowering (4.10; similar to Ratna *et al.,* 2015) followed by number of grains per panicle (1.04), test weight (0.57) and number of effective tillers per plant (0.19) while days to physiological maturity (-5.76), AUDPC (-0.41), plant height (-0.21) and panicle length (-0.18) showed direct negative effect. Bhadru *et al.,* (2011) and Chandra *et al.,* (2009) reported positive direct effect of days to 50% flowering and Eidi kohnaki *et al.,* (2013) and Kiani and Nematzadeh (2012) found the positive direct effect and significant positive correlation coefficient between productive tillers/plant and grain yield/plant which also supported the present finding. Thus, number of grains per panicle and test weight were identified as major contributors toward yield enhancement and can be used as the major selection indices for identifying high yielding resistant lines. The highest positive indirect effect on yield (similar to Jaiswal *et al.,* 2019) was shown by days to 50% flowering via days to physiological maturity (4.08) while the lowest positive indirect effect on yield was shown by number of effective tillers per plant via plant height (0.09). The highest indirect negative effect was shown by days to physiological maturity via days to 50 % flowering (-5.74) while the lowest indirect negative effect was shown by panicle length via number of effective tillers per plant (-0.11).

Conclusion

In this study, disease resistance expressed in terms of AUDPC showed negative correlation with yield and yield attributing traits. Further it showed direct negative effect on yield. It also showed indirect negative effect on yield via other traits under study. This is evident from the fact that a genotype that is less affected by pathogen has lower value of AUDPC and is expected to show better growth and development at all the critical stages of plant growth thus, exhibit higher yield. So, a negative correlation is obtained between AUDPC and yield and yield attributing traits. Thus, AUDPC could be used as a selection parameter of developing improved cultivars with higher grain yield and lower susceptibility towards the brown spot pathogen. The salient findings of this study establish a negative association between AUDPC and yield. Hence, a line with lower AUDPC value would be expected to be resistant to the pathogen and have higher yield.

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Conflict of interest

The authors declare that they have no conflict of interest.

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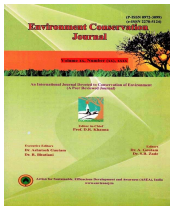

Sulphur and its significance in higher pulse production

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ABSTRACT

Sulphur is one of the emerging plant nutrients, required for pulse crops. After nitrogen, phosphorous and potassium, it is forth key nutrient for plant nutrition. It is taken up by the plants in the form of sulphates form from the soil. The factors that are responsible for the wide spread deficiency of sulphur are excess use of high analysis fertilizers, inadequate use of crop residue, high yielding varieties of crops and its removal of sulphur by the crops. Sulphur plays a pivotal role in overall pulse production, by synthesis of sulphur containing amino acids, enhancing protein content, nodule formation and plant biomass. However, the requirement of sulphur for effective crop production is not showing promising trend. Comparing the sources of sulphur fertilizers, gypsum showed its superiority by producing high grain and straw yield in pulses. In some of the field experiments on pulses, addition of sulphur @30kg and 40kgof S/ha along with the recommended dose, increased the growth (plant height and number of branches) and yield and quality parameters (grain yield and protein content). This review highlights the different response of crops to Sulphur application, sources, uptake and its interactions with other nutrients for profitable crop production. Moreover, it provides new insights to revisit the significance of sulphur in higher pulse production.

Introduction

Pulses are the leguminous crops, which are harvested mainly for dry seed purpose. The most commonly consumed pulses are green gram, chick pea, lentil, dried beans, peas and grams etc. These are the distinguished plants having high protein contents with the excellent dietary fiber content and other complex carbohydrates. Pulses are regarded as a curator in maintaining soil fertility, by virtue of which it fixes the atmospheric nitrogen through root nodules in soils. Although, India is the largest producer (25%) as well consumer (27%) of pulses yet it has to import 14% of pulses from abroad for sustenance of her burgeoning population. Pulses accounts for about 20% area under food grain (Mohanty, S and Satyasai, 2015). It is worth noting that the production of pulses per area is not satisfactory. The current demand for pulses is about 20 to 25 million tons and will be rising eventually in further upcoming days. There is need to bring more area under pulse production and augment the production. India is in foremost position is chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), urd bean (*Vigna mungo*) and green gram (*Vigna radiata*) production. In our country, gram is the most prevalent pulse crop, which is contributing around 40% of its total production followed by pigeon pea and urdbean. The top most pulse growing states includes Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh and Karnataka (Mohanty and Satyasai, 2015). Pulses can be grown either as a kharif or rabi crop and can act both as a fodder for livestock and green manure for the next crop in sequence by enriching the soil. Factors responsible for sulphur deficiency include excess use of high analysis fertilizers that are free from sulphur, multiple and intensive cropping systems, reducing use of the organic manures (Khurana *et al.* 2003, Shivay *et al*. 2014) leaching

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losses in coarse textured soils (McNeill *et al.,* 2005). The deficiency of sulphur is not only prevalent in India but it is global (Tripati *et al*., 2003).

Sulphur as a nutrient

Among the major nutrients, sulphur is said to be the fourth important nutrient, which is mostly required for leguminous and cruciferous crops. Sulphur is treated as a mandatory nutrient, for its role in development and metabolism of the crop plants (Vidyalakshmi *et al*., 2009). Sulphur plays a crucial role in forming sulphur containing amino acids viz., methionine, cystine and cysteine, protein synthesis and root nodule formation. It is present in top most layers of the soil and shows decreasing trend with increase in depth. Sulphur has an important place in growth, production, development and yield attributes in legumes and oilseeds. There exit a greater variation in distribution of total sulphur present in the soil, due to the difference in soil characteristics. It is now recognized that sulphur, being a limiting factor, affecting production of crops in semi-arid tropical regions, covering around 73 million hectares of vertisols and other associated soils in India (Rao and Ganeshamurthy, 1994). Pulse production can be drastically increased to a greater extent, by the application of this nutrient. Though sulphur is not an integral part of chlorophyll yet its deficiency leads to chlorosis. Higher sulphur fertilizer requirement is seen in different regions of Asia. In soils, sulphur is obtained from sulphur containing minerals and from plant and animal residues.

Total /available sulphur in soil

The most common sources of sulphur are elemental sulphur, sulphide and sulphate minerals. In soils, the total sulphur content is ranging from 30 to 400mg kg^{-1}. Out of this, only a minute proportion is made available to the growing plants. The critical deficiency value of available sulphur is taken as 10 mg S kg^{-1}. The sulphur is absorbed by the plant roots, from the soil, in sulphate form (SO_4^{2-}) (Schoenau and Mahli, 2008). In general, the available sulphur varies with respect to different soil types and its results are compiled by Hedge *et al.* (1980) stating that more than 10% of total sulphur is taken as an available sulphur in hilly red and alluvial soils, where paddy is main crop. Mahto *et al.* (1992) confirmed that the alkaline soils of Chota Nagpur region of Bihar contained higher amounts of available sulphur whereas acidic soils contained highest amount of total sulphur. Singh *et al*. (1993) recorded that the soluble sulphur is present in small proportions compared to total sulphur that resulted in 60% deficiency in soils of Chota Nagpur region particular in coarse textured soils where leaching loss of SO_4^{-2} - S is prominent.

Sulphur exists both in both organic and inorganic forms in soil, depending on different factors such as soil texture, organic matter, pH, calcium carbonate and other soil properties (Dhamak *et al.,*2014). Organic form of sulphur is >90%, which is present in humus and other crop residues (Freney,1986). The inorganic sulphur includes pyrites (FeS_2), Gypsum ($CaSO_4.2H_2O$), elemental sulphur and SO_4^{2-}. In Alluvial soils, the total sulphur contributes about 30% in organic forms as against 70% in Mollisols of Tarai region in India (Singh *et al.,* 2015). In general, the sulphur content is low in tropical soils owing to a lesser amount of organic matter, parent material and leaching losses (Olson and Engelstad 1972; Rego *et al.,* 2007). It is estimated that 8kg of sulphur is needed to produce one ton seed of pulses (Jamal *et al.,* 2010).

Sulphur content in plants

Plants take sulphur in SO_4^{2-} forms, which is stored in xylem tissues and vacuoles of the plant cells, in an aqueous solution. But it is present in the plant tissues as S^{2-} (sulphide) or SH^- (thiol or sulfhydryl group) in different organic molecules along with the proteins. Comparing S concentration in grain and straw, Aulakh *et al*. (1985) found that, the grains contain more S content and cruciferous showed wider variation than pulses. Reddy *et al*. (1988) stated that, in cereals 0.16% - 0.25% is the optimal range of S and below 0.20% is referred as a sub optimal range. Khurana and Bansal (2007) established the critical limit of Sulphur in whole shoot in moong to be 0.23%.

Response of pulses to sulphur application

Results of several experiments showed that the yield response of sulphur application differed in magnitude in different pulse crops. A field experiment, conducted on loamy typic soils of Uttar Pradesh by Tripathi *et al.* (1997) in chickpea crop, showed a significant response to 40 kg S ha^{-1}. Ram and Dwivedi (1992) stated that sulphur application increased the yield of chickpea to tune of 2.13 tons ha^{-1} over control in first and second year when sown in sulphur deficient soil. In black gram, the

grain and straw yield increased up to 20% over control, due to sulphur application in 1992 and 1993 respectively (Dwivedi *et al.,* 1996).

Singh *et al*. (1997) noticed that the application of sulphur not only improved grain and straw yield but also the nodule formation and plant biomass. Application of sulphur up to15 kg ha^{-1} increased the bacterial population but beyond that it decreases both the fungal and actinomycetes population. On the basis of availability coefficient ratio (ACR), among all the sulphur carriers, the response of gypsum was found to be highest whereas the elemental sulphur was least, with respect to grain and straw yield. There has been a drastic increase in the total number of nodules and active nodules by the application of sulphur up to 20 kg ha-1 (Ganeshamurthy and Reddy, 2000). The importance of sulphur showed a remarkable increase in pod length, number of pods per plant and grains count, when applied at the rate of 60 kg/ha to black gram (Patel *et al.,* 2018). Sulphur application not only increased the nodule formation in black gram (Khandkar *et al.,* 1985) but also involved in forming a nitrogenase enzyme, that fixes nitrogen in legumes (Saraf, 1988; Scherer *et al.,* 2006). Khurana *et al*. (2004) concluded that significant increase in grain yield of moong was obtained at and above application of 20 kg S ha-1, when grown in coarse textured alluvial soil.

In green gram and pea, sulphur application showed a significant response to total chlorophyll content (Poorani, 1992; Spencer *et al.,* 1990). Addition of sulphur at the rate of 40 kg ha^{-1} improved the plant height, number of branches, pods per plant and 1000 gram weight in green gram crop. Sulphur is well known for its role in enhancing the quality of pulses. In summer green gram, when S applied at the rate of 30 kg ha^{-1} showed a maximum plant height, leaf area index, increase in the number of branches and dry matter content, when compared to other levels (Sitaram, 2010). An experiment was conducted by Patel *et al.* (2013) by employing three different levels of sulphur through gypsum (0, 20, 40 kg ha^{-1}) in the presence and absence of phosphorus and bio-fertilizers, only 40 kg S ha^{-1} was required to be applied to chick pea crop for getting maximum branches per plant, number of nodules, plant spread, dry matter, protein content, seed yield along with the highest net realization. Further application of 40 kg S along with the P_2O_5 ha gave a maximum grain yield than sulphur alone. Hence it is proved that phosphorus and sulphur have a combining effect on the productivity of pulses.

In a cropping sequence involving mung-bean--raya (*Brassica juncea*), Khurana and Bansal (2007) observed that sulphur applied @ 20 kg ha^{-1} to each crop or 40 kg ha^{-1} to the first crop (mung bean) was sufficient to obtain optimum yields of both the crops. Khurana *et al.* (2008) while describing sulphur nutrition of various crops in Indo Gangaetic Plains of South Asia observed that for normal yields, the crops with high sulfur requirements (like oilseed, pulses, groundnut, alfalfa , garlic and onion) need 20 to 45 kg sulfur ha^{-1}. Crops with medium sulfur requirements need 15 to 35 kg sulfur ha^{-1}. Results of several experiments pertaining to responses of pulse crops summarized by Tandan and Messick (2007) under field condition is given in table 1.

Table 1: Average responses of crops to S application under field conditions (Tandon and Messick, 2007).

Pulse Crop	No of field experiments	Average rate of S application kg S ha^{-1}	Response to S kg grain kg S^{-1}
Chickpea	6	85	5.3
Pigeonpea	8	36	8.9
Black gram	9	30	5.4
Green gram	6	40	3.3

Effects of different sources of sulphur in Pulses

Sulphur is made available to the crop, through various sources such as gypsum, pyrite, elemental sulphur, single super phosphate, ammonium sulphate and, potassium sulphate. As the plants take sulphur in sulphate form, only the sulphate- S containing materials are said to be suitable for neutral to slightly alkaline soils. In coarse textured soils, the deficiency of sulphur occurs more often (Aulakh, 2003). Hence the sulphate -S becomes more susceptible to leaching losses in such soils. Under such conditions, the response of gypsum is higher compared to any other source. Gypsum, being sparingly soluble, it can supply sulphur to the crops for longer duration. It has been reported that

gypsum application gave an excellent results regarding various growth factors (plant height, leaf area index, production of dry matter and number of branches/plant and yield (number of pods per plant, number of seeds per pod, grain and hulm) parameters in black gram (Patel *et al.*, 2018).

Banik and Sen Gupta (2012) reported that, when 30 kg SSP is applied along with the recommended dose of N,P and K. They obtained a maximum seed yield compared to other sources in green gram. The available SO_4^{2-} - S, present in gypsum is the main reason behind increasing the grain and straw yield when compared to pyrite and elemental sulphur. In summer moong (*Vigna radiata L.),*Singh and Chibba (1991) found while comparing different S sources viz ammonium sulphate, super phosphate, gypsum , elemental suphur and pyrite respectively that there was significant response to sulphur application for maize and wheat crops grown on S deficient soil irrespective of the sources. For maize ammonium sulphate, super phosphate, gypsum and elemental suphur were at par in their performance. But in case of wheat ammonium sulphate was most efficient followed respectively by super phosphate, elemental suphur and pyrite/ gypsum.

Dhillon *et al*. (1978) studied the effect of three carriers of sulphur namely gypsum, elemental sulphur and ammonium sulphate with three levels of sulphur (0,5 and 10 ppm) on soybean in a pot experiment. Results showed that efficacies of ammonium sulphate, gypsum and elemental S were in order of highest, intermediate and least respectively. Large quantities of indigenous S sources such as mined gypsum, pyrite and by-product phospho- gypsum are available in the country. Research efforts have been directed to evaluate suitability of these indigenous S sources as sulphur fertilizer in soils and crops of eastern India. Basal soil application of gypsum and phosphor-gypsum were found to be superior than that of pyrites. However, pyrites resulted in higher crop response on residual sulphur in various cropping systems (Singh and Singh, 2016).

From the compilation of results of various experiments, it is inferred that in calcareous soils, non calcareous soils and acid soils, elemental S was about twice, 80% and 50% as effective as gypsum respectively.

Uptake of sulphur in Pulses

Although the sulphur uptake ranges from 5 to 30%, but in general it varies from 9 to 15% as that of nitrogen. In crops like mustard, the uptake of sulphur is very high and almost equal to one third of nitrogen. More often it is seen that crop absorbs sulphur similar to that of phosphate. To produce 1 kg of grain, the uptake of sulphur for pulses is 8 kgs (range 5-13), is 12 kgs(range 5-20) and for cereals it is 3 to 4 kgs(range 1-6) (Tandon and Messick, 2002). Higher the requirement of sulphur, higher will be the crop yield. Results of several experiments pertaining to S uptake of pulse crops summarized by Tandon and Messick (2007) under field condition is given in table 2.

Table 2: Average Sulphur uptake by pulse crops.

Pulse Crop	Yield (kg/ha)*	Sulphur uptake, kg/ha
Chickpea	1500	6
Pigeonpea	1200	9
Black gram	600	5
Green gram	870	7
Lentil	2000	9

***Several Indian publications Tandon and Messick (2007)**

Among the different sources of sulphur, the uptake of sulphur was found to be high where gypsum was used followed by super phosphate, pyrites and press mud. Ram and Dwivedi (1992) recorded that, by applying sulphur to chickpea, the uptake of N,P,K and S was higher than the control In black gram and lentil crop, the S content and uptake increases with the increase in the pyrite levels as reported by Singh *et al.* (1992).

Quality and nutritional aspects of Sulphur in Pulses

Review of many authors indicated that the sulphur has gained a greater importance in pulse production by increasing the seed yield and protein content. Pulses contains on an average 20-25% proteins It is seen sulphur not only enhanced yield but also protein content along with the sulphur containing amino acids, in the crops like chick pea and green gram etc. Tiwari (1995), while working on the rain fed soils of Eastern UP reported that application of 40 kg elemental S ha^{-1} increased seed yield and protein content of lentil by 6.5%. But 80 kg ha^{-1} elemental S is required to increase protein content in green gram and chick pea to the tune of 3.5 and

2.7 % respectively. In West Bengal, 45 kg S ha^{-1} enhanced protein content by 12% in black gram (Sen, 2006). In Jharkhand, residual effect emanating from the earlier application of 80kgSha^{-1} recorded 13% increase in protein of black gram (Singh *et al.*, 1998).

Dhillon and Dev (1980) stated that, sulphur application in soyabean improved significantly the proportion of sulphate-S and protein-S. But sulphur as a whole is not utilized by the plants for protein content. Only the requisite amount of S is required for good quality grain. So there is no need of excess S application so as to prevent the accumulation of SO_4^{2-} -S in plants. In groundnut and mustard, S application caused exceptional increase in protein, oil and methionine content (Singh *et al*.,1970). Aulakh and Sharma (2005) reported that the application of sulphur showed significant beneficial effects on yield as well as protein in some pulse crops. When 60 kg of sulphur per hectare is applied to sesame crop, 11% increase is observed in protein and oil content. It also resulted in increase in 11% in oil and 5% in protein content in the succeeding mustard crop (Singh and Tiwari, 1985). For adequacy of sulphur, nitrogen to sulphur ratio is considered as a good indicator in plants. The ratio of N:S ranges from 13 to 17 in green gram.

Sulphur interaction with the other nutrients

Sulphur when applied with the other nutrients, its effects can be amplified or diminished. However, it is fascinating to know that the nature of two nutrients in green house differ from the field conditions. For example, under greenhouse conditions, antagonistic effect was recorded by the combined application of S and P on yield, uptake and protein content in green gram, whereas the deleterious effects were recorded only at a maximum rate under field conditions. It was found that, the combined application of P and S enhanced the nodulation activity. Sulphur's effects might be enhanced or decreased when coupled with other nutrients. For diverse legume crops, it exhibited a synergistic impact for N, K, Ca, and Mg and an antagonistic effect for P, Mg, and Mo (Abdin *et al*., 2003). In genotypes of chickpea - modulating and non-modulating, the sulphur deficiency resulted in decrease in accumulating N^{15} in the root and shoot portion in both genotypes, thereby decreasing the accumulation of K^+, Fe^{2+} and Zn^{2+} while increasing Mg^{2+} and NO_3^{2-}(Badruddin and Karmokee, 2001). The key elements N-S interactions are regulated by NO_3^{2-} and SO_4^{2-} uptake, coupled with regulation of nitrate reductase and variations in the level of O-acetyl-ser.

Conclusion

Sulphur is the essential plant nutrient for synthesis S containing amino acids like cysteine and methionine. Different researches revealed that, sulphur fertilization to pulse crops showed higher increase in crop yield and protein content. There are different sulphur fertilizers in India like sulphur bentonite, gypsum, elemental sulphur and pyrite etc. Of all the inorganic sources of sulphur, gypsum is referred as one of the most economical and effective to pulse crops. Its interactions also showed synergistic and antagonistic effects with other nutrients. Moreover, sulphur is also available in association with the other nutrients like N (ammonium phosphate and ammonium sulphate) P, (SSP) K, (potassium sulphate and potassium magnesium sulphate) and zinc sulphate. The demand for optimum crop yield can be achieved by optimizing the availability of sulphur in required amounts. Hence to strengthen the production of pulses S may be recognized as a key element thereby making it a part of nutrient management.

Conflict of interest

The authors declare that they have no conflict of interest.

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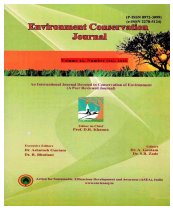

Morphological characterization of maize (*Zea mays* L.) hybrids under excessive soil moisture stress

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ABSTRACT

A critical assessment of 32 maize hybrids with two replications for excessive soil moisture stress (ESM) was carried out during *Kharif* 2019-20. The plants were exposed to waterlogging stress for 12 days at the flowering stage by maintaining a water level of 3-5 cm. High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were attained for maize plants with adventitious roots and senescence percentage after stress. High heritability along with high genetic advance was determined for number of plants with adventitious roots, senescence percentage, plant height and 100 kernel weight. Plant yield depicted a highly significant positive genotypic and phenotypic correlation with plant height, ear height, number of plants with adventitious roots and number of kernels per row, along with a significant negative correlation with senescence percentage. Kernels per row and plant height manifested the highest positive direct effect on plant yield at phenotypic and genotypic levels, respectively, reflecting that the characters can be considered for plant selection under ESM stress.

Introduction

Maize (*Zea mays* L.) or "The queen of cereals" originated from the Andean region of South America. It is a tall, monoecious, cross-pollinating, diploid cereal (2n=2x=20) of family Poaceae, domesticated from a wild grass teosinte by the indigenous people in Mesoamerica from ancient times. Maize is the third predominant cereal after rice and wheat globally. It is cultivated from 58^0N latitude in Canada and Russia to 40^0S in South America, from altitudes higher than 3000m to regions lying below sea level and in areas receiving 250 mm to more than 5,000 mm of annual rainfall (Dowswell *et al.,* 1996). Its versatility and wider adaptability than rice and wheat have led to greater adoption among the cultivators. The total global production was around 1.15 billion tonnes in 2018 (FAOSTAT, 2018). The United States is the leading producer with about 392.5 million tonnes of production per year. USA contributes 34.2%, followed by China and Brazil with 22.4% and 7.2% of the total production respectively (FAOSTAT, 2018). In India, maize is cultivated in almost all agro-ecological regions. India produces about 27.8 million tonnes of maize per year which is about 2.4% of the total global production (FAOSTAT, 2018). The primary maize growing states are

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Andhra Pradesh (20.9%) followed by Karnataka (16.5%), Rajasthan (9.9%), Maharashtra, Bihar, Uttar Pradesh, Madhya Pradesh and Himachal Pradesh (Kavita *et al.,* 2018). These states contribute to more than 80% of the total maize production in the country. The average productivity in India is 2.43 t/ha against the global average of 4.92 t/ha. The reasons for its low productivity include both biotic and abiotic constraints. It is estimated that maize yield is reduced by around 25% due to biotic agents (Kaul, 2011). The most common pests include corn earworm, stem borer and termites. Acute water availability in form of drought and excess soil moisture (ESM), is the predominant abiotic constraints limiting the production and productivity of maize. Abiotic stresses are an integral part of any agro-ecosystem. Drought is the chief major factor for lower productivity in the Asian regions followed by ESM stress, which may be caused by flooding, waterlogging, or high water table (Sahoo *et al.,* 2020). More than 15% of the total maize growing areas are affected by floods and water-logging problems in South Asia alone. In India, out of the total 6.6 million ha of the area under maize, about 2.5 million ha is subjected to ESM stress, leading to an annual average loss of 25-30% of the total national production (Zaidi *et al.,* 2004). Further, most of the coastal regions receive higher rainfall due to prolonged low pressure, leading to waterlogging at critical stages of crop growth. Therefore, screening and development of genotypes tolerant to ESM stress can prevent a huge loss in corn production. Considering all these facts, the following investigation was carried out with the objective of identifying the important morphological traits that can be considered for isolating waterlogging tolerant genotypes and to isolate some genotypes that can withstand ESM stress at flowering stage. Genetic variability is a prerequisite of any breeding programme, and it aids in the selection and development of economically important plant species. A critical analysis of genetic variability is essential for developing cultivars to supplement human needs. The selection efficiency can be maximized for certain traits using the estimates of genetic parameters. These components allow a breeder to recognise the nature of the gene action involved in controlling the quantitative traits and evaluate the effectiveness of different breeding methods to obtain higher genetic gain. The genetic estimates in the form of variance, coefficients of variation, heritability, genotypic, phenotypic and environmental correlations help the scientific community to know the magnitude of a population's genetic variability, deducing inter-relationships between traits, thus assisting in the plant selection process. Further, the study of direct and indirect effects of traits on a basic variable provides a better picture of the correlation between plant yield and other yield contributing traits, facilitating the plant selection process.

Material and Methods

Experimental details

The present investigation was carried out using 32 single cross maize hybrids, with two replications laid out in Randomized Complete Block Design (RCBD). The research was conducted at the EB-II section of the Department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar during *kharif* 2019-20. The experimental plot is located at about 64 km west of Bay of Bengal, at 20°15'N latitude; 82°52'E longitude and at an altitude of 25.9 m above the mean sea level. This region comes under humid and sub-tropical climatic zone of Odisha and receives an average annual rainfall of 1628 mm. Each hybrid was sown in a single line of 3 m row length maintaining a spacing of 60cm x 25cm after thinning. Waterlogging stress was imposed by flooding the field at the beginning of the flowering stage for 12 days. Earthen bunds were provided to maintain a continuous water level of 3-5 cm.

Morphological analysis

Twenty plants were evaluated plot-wise for both the replications and for different traits like days to 50% tasseling (DT), days to 50% silking (DS), days to 75% dry husk (DH), shelling per cent (S) and the number of plants with adventitious roots (after stress) per plot (AR). The mean value was then recorded. The root portion was marked with blue paint before stress to observe the growth of adventitious roots after stress (Figure 1). Five randomly selected plants were observed for leaf senescence per cent after stress (SP), plant height (PHT), ear height (EHT), length of cob (LC), cob girth (GC), number of kernel rows per cob (KR/C)

and number of kernels per row (K/R). The angular transformed values for leaf senescence per cent after stress (SP) was considered for statistical analysis. For seed yield, ears from all the plants in each plot were weighed. 100 kernels were manually counted and weighed to measure 100 kernels weight (KW). The moisture content of the kernels was determined by 'Steinlight Moisture Meter'. Fresh ear weight per plot at harvest (at 15% moisture) was then calculated using the following formula:

$$\text{Shelled weight} = \frac{\text{Fresh weight of cobs x Shelling percentage}}{100}$$

$$\text{Moisture corrected yield} = \frac{\text{Shelled weight x (100} - \text{moisture \%)}}{85}$$

$$\text{Grain yield (GY) (q/ha)} = \frac{\text{Moisture corrected yield x 10,000}}{\text{Area of the plot}}$$

Statistical Analysis

The collected data was analyzed to estimate variability among hybrids (Dash *et al.,* 2022) phenotypic and genotypic coefficient of variation (%) (Awad-Allah *et al.,* 2022), broad-sense heritability (Nishad *et al.,* 2022) and the expected genetic advance due to selection (Sasipriya *et al*., 2022). Further, the genotypic and phenotypic correlation between the associated traits and yield (Al-Jibouri *et al.,* 1958, and Pooja *et al.,* 2022) and path co-efficient analysis was carried out (Kumar *et al.,* 2022; Dash *et al.,* 2022; Sasipriya *et al*., 2022, and Nishad *et al.,* 2022) under water logging condition.

Results and Discussion

Study of genetic variability

The results from ANOVA (Table 1) suggested that all the traits were highly significant at 1% level of significance except length of cob which was significant at 5% level of significance. The results reflected the use of diverse base population for deriving such hybrids. For most of the traits, the CVe value (Table 2) was low which depicted good precision in experiment. However, this value was in the moderate range for number of plants with adventitious roots after stress and yield which represented higher influence of environment on such traits. Similar studies were performed in maize (Lakshmi *et al.,* 2018) under water logging condition.

Phenotypic and genotypic coefficient of variation

The study of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is not only important for analyzing the amount of phenotypic and genotypic variations among various traits but also useful in predicting the scope of improvement through selection. High value of PCV and GCV is desirable as it represents higher variability in genotypes. The GCV estimate is considered more and is most commonly used for plant selection (Bello *et al.,* 2012). The results (Table 3) depicted that, other than yield, the PCVs were slightly higher than GCVs for all other traits. This report portrayed that the environmental impact was low for expression of most of the traits except yield. High value of PCV and GCV were obtained for number of plants with adventitious roots after stress and senescence percentage after stress, while traits like plant height, ear height, kernels per row and 100 kernel weight had moderate values. High GCV estimates indicated low amenability of a trait to environmental fluctuations (Hefny, 2011). This reflects that such characters can be considered for selection of plants. Similar inference and suggestions were also proposed in previous studies for plants under ESM stress (Lakshmi *et al.,* 2018) and under normal condition (Bello *et al.,* 2012). Days to 50% tasseling, days to 50% silking, days to 75% dry husk, length of cob, girth of cob, kernel rows per cob and shelling percentage had low PCV and GCV, which revealed low genotypic variability among the hybrids for these traits which are similarly to the previous reports (Sravanti *et al.,* 2017; Siluveru *et al.,* 2015; Bartaula, 2019, and Mallikarjuna *et al.,* 2011) available for maize.

Heritability and genetic advance

The estimates of heritability aid the breeders in allocating resources necessary to effectively select for desirable traits and to achieve maximum genetic gain with less time and resources (Smalley *et al.,* 2004). Heritability was classified as low (below 30%), medium (30-60%) and high (above 60%) (Sasipriya *et al*., 2022). High broad sense heritability was observed for senescence percentage (after stress), number of plants with adventitious roots (after stress), days to 50% tasseling, days to 50% silking, 100 kernel weight, days to 75% dry husk, kernels per row, plant height, ear height and girth of cob. The measure of high heritability

Table 1: Analysis of variance for 14 traits in 32 maize hybrids

SN	Character	Source	d.f	S.S	M.S.S	F- value
1	Days to 50% tasseling (DT)	Replication	1	1.266	0.391	0.548
		Genotype	31	137.859	4.419	6.196**
		Error	31	17.234	0.713	
2	Days to 50% Silking (DS)	Replication	1	1.266	1.266	2.277
		Genotype	31	127.984	4.129	7.426**
		Error	31	17.234	0.556	
3	Days to 75% Dry Husk (DH)	Replication	1	0.391	0.391	0.326
		Genotype	31	193.484	6.241	5.214**
		Error	31	37.109	1.197	
4	Plant Height (PHT)	Replication	1	93.123	93.123	0.313
		Genotype	31	38658.830	1247.059	4.193**
		Error	31	9219.387	297.400	
5	Ear Height (EHT)	Replication	1	33.785	33.785	0.584
		Genotype	31	7579.306	244.494	4.227**
		Error	31	1793.240	57.846	
6	Number of plants with Adventitious Root (AR)	Replication	1	7.563	7.563	2.915
		Genotype	31	1155.750	37.282	14.368**
		Error	31	80.438	2.595	
7	Senescence per cent (after stress) (SP)	Replication	1	0.281	0.281	1.837
		Genotype	31	3369.236	108.685	709.901**
		Error	31	4.746	0.153	
8	Length of cob (LC)	Replication	1	2.800	2.800	1.644
		Genotype	31	104.773	3.380	1.984*
		Error	31	52.796	1.703	
9	The girth of Cob (GC)	Replication	1	0.222	0.222	0.644
		Genotype	31	43.974	1.419	4.114**
		Error	31	10.689	0.345	
10	Number of Kernel rows per Cob (KR/C)	Replication	1	0.276	0.276	0.379
		Genotype	31	60.144	1.940	2.668**
		Error	31	22.544	0.727	
11	Number of Kernels per row (K/R)	Replication	1	16.134	16.134	3.248
		Genotype	31	679.904	21.932	4.416**
		Error	31	153.972	4.967	
12	100 Kernel weight	Replication	1	5.018	5.018	1.261
		Genotype	31	825.078	26.615	6.690**
		Error	31	123.332	3.978	
13	Shelling % (S)	Replication	1	0.004	0.004	0.330
		Genotype	31	0.884	0.029	2.379**
		Error	31	0.372	0.012	
14	Yield (t/ha)	Replicatin	1	2.551	2.551	1.688
		Genotype	31	128.843	4.156	2.749**
		Error	31	46.868	1.512	

***significant at 5% level of significance (1.82); **significant at 1% level of significance (2.35)**

indicated that the environmental impact was minimal on such traits (Ogunniyan and Olakojo 2014). Therefore, the breeders may perform superior selection of genotypes based on the phenotypic performance of these traits (Sravanti *et al.,* 2017; Siluveru *et al.,* 2015; Bartaula *et al.* ,2019; Khan *et al.,* 2018; Mallikarjuna *et al.,* 2011; Bello *et al.,* 2012). Genetic advance exhibits the extent of genetic gain acquired by a trait under a specific selection pressure. The values of genetic

advance as percentage of mean population ranged from 1.30% to 79.52%. High genetic advance as percentage of mean (GAM) was estimated for number of plants with adventitious roots after stress, senescence percentage after stress, plant height, 100 kernel weight and yield (Sasipriya *et al*., 2022). High genetic advance along with high heritability estimates offers the most satisfactory condition for selection (Sasipriya *et al*., 2022). This indicates prevalence of additive genes in these traits and further portrays definitive crop improvement through selection of such traits is easier. Considering this delineation, traits like number of plants with adventitious roots after stress, senescence percentage after stress, plant height and 100 kernel weight can be examined for selection of plants under ESM stress. Similar results were also reported for senescence percentage after stress under ESM stress (Lakshmi *et al.,* 2018; Sravanti *et al.,* 2017; Bartaula *et al.,* 2019; Khan *et al.,* 2018).

Character Association

The knowledge of the degree of association between yield and its component traits and within the component characters themselves, can improve the selection efficiency in plant breeding. The correlation studies (Table 4) reflected that the genotypic correlation coefficients were higher than phenotypic correlation coefficients for most of the characters under study. This indicated a strong inherent relationship among the characters studied, which was majorly regulated by genetic causes (Lakshmi *et al.,* 2018). This further suggested that, predominance of environmental components might have suppressed the expression of traits at phenotypic level (Lone *et al.,* 2010). Similar results were also acquired previously (Lone *et al.,* 2010; Pavan *et al.,* 2011; Begum *et al.,* 2016; Usha Rani *et al.,* 2017, and Lakshmi *et al.,* 2018). Under ESM stress, plant height showed highly significant, positive correlation with yield at both phenotypic and genotypic levels. Some hybrids (ZH17367, ZH17375) exhibited greater biomass accumulation under ESM stress with plant height greater than 200 cm. This was in accordance with the previous findings (Lizaso and Riche, 1997; Lone *et al.,* 2010). This relationship between plant height and yield was due to higher biomass accumulation because of greater plant height, which led to higher yield (Begum *et al.,* 2016, and Nzuve *et al.,* 2014). Yield and number of plants with adventitious roots (after stress) were positively correlated. This suggested that plants bearing adventitious roots assisted in avoiding lodging and facilitated oxygen availability to submerged roots (Lone *et al.,* 2010) which led to an overall higher yield. Under normal soil conditions, this trait shows poor expression, but, there is significant increase in this traits (Shah *et al.,* 2012, and Zaidi *et al.,* 2007) under waterlogging environment.

Scientists inferred similar significant correlations and further emphasized in considering this trait as selection criteria for screening maize genotypes for ESM stress tolerance (Lakshmi *et al.,* 2018). Length of cob and yield exhibited positive correlation under water-logging (Lone *et al.,* 2010). The girth of cob and number of kernels row per cob had significant positive correlation with yield at phenotypic level. Moreover, kernels per row and 100 kernel weight under ESM stress, delineated positive correlation with yield. Such relationships were also depicted by plants grown under normal soil moisture regime (Pandey *et al.,* 2017; Belay N, 2018; Pavan *et al.,* 2011; Kumar *et al.,* 2014, and Lakshmi *et al.,* 2018) under ESM conditions. Yield and senescence percentage (after stress) manifested highly negative correlation. Lowest senescence percentage after stress was observed for the hybrids ZH17446 and VH15676. Scientists proposed that corn genotypes with three or less dead leaves during ESM stress exhibited higher biomass as well as yield grain (Campbell *et al.,* 2015 and Shah *et al.,* 2012). Foliar senescence was also approved to be the most common and immediate symptom of water-logging (Shin *et al.,* 2016). Further, days to 75% dry husk and days to silking correlated to yield which was partly in accordance with the findings of Lakshmi *et al.* (2018) under ESM stress.

Path coefficient studies

Plant selection based on correlation coefficients is often misleading due to the impact of third factor in the correlation between two variables. The most beneficial selection criteria was the traits with high positive correlation along with high direct effects (Pavan *et al.,* 2011). The genotypic path coefficient was more useful to a breeder than phenotypic path coefficients with regard to more effective selection. Considering this, both genotypic (Table 5) as well as phenotypic (Table 6) path coefficient analysis

Table 2: Mean performance of 32 Maize hybrids for 14 characters

Sl. No.	Hybrids	DT	DS	DH	PHT (cm)	EHT (cm)	AR	SP (%)	LC (cm)	GC (cm)	KR/C	K/R	KW (g)	S (%)	Y (t/ha)
1	VH19559	55.5	56.5	88.0	175.4	83.9	8.0	19.90	16.59	13.67	14.0	29.33	30.35	85.00	7.38
2	ZH17368	53.0	54.0	85.0	215.0	99.1	5.5	31.11	16.02	14.41	13.0	25.30	34.31	88.30	7.64
3	VH16921	52.5	54.0	87.0	152.7	70.2	5.5	15.83	18.32	13.13	14.4	34.10	28.88	87.30	6.26
4	VH19487	56.0	56.0	88.5	189.0	91.5	13.5	19.76	17.48	16.42	16.4	32.40	28.11	85.95	7.41
5	VH15676	57.0	57.0	87.5	189.1	95.1	9.5	11.84	15.33	14.77	14.0	31.30	26.85	85.45	6.69
6	ZH17814	53.0	54.0	85.0	193.3	92.3	8.0	18.86	16.29	14.09	13.8	32.70	27.94	85.15	6.70
7	ZH161418	51.5	53.5	83.5	126.4	59.2	9.0	19.66	14.78	13.21	13.2	24.80	29.77	85.85	5.83
8	ZH161384	53.5	55.5	85.5	121.3	59.9	5.0	19.79	13.81	15.17	12.0	27.70	23.95	82.50	4.37
9	VH131019	54.5	56.5	91.0	177.5	78.9	5.5	15.31	18.45	15.25	14.4	37.00	29.52	87.10	6.48
10	ZH17463	56.0	58.0	89.0	164.7	79.2	11.5	15.70	16.65	13.70	13.6	30.90	28.99	84.35	6.37
11	ZH17820	55.5	57.5	87.5	154.1	76.3	7.5	20.47	14.79	15.70	12.0	29.90	25.98	82.95	4.73
12	ZH17446	55.0	57.0	90.0	181.6	88.3	15.0	11.21	16.06	15.03	14.0	31.50	29.71	87.40	8.43
13	ZH17362	56.0	57.0	86.5	196.0	87.8	11.5	13.78	16.03	14.20	14.0	28.60	34.34	85.00	6.43
14	ZH17815	55.5	56.5	88.5	180.1	85.8	5.5	21.84	15.79	14.26	14.0	34.10	24.49	83.00	7.91
15	ZH17470	55.5	56.5	89.0	146.6	74.1	7.5	15.84	15.07	14.09	16.0	24.00	30.16	83.75	5.03
16	ZH17835	55.0	56.0	87.5	161.4	78.2	7.5	19.88	14.32	14.83	15.2	28.20	27.39	85.95	5.10
17	ZH161484	56.0	57.0	88.0	172.3	87.4	15.5	15.64	17.15	15.53	13.6	35.70	27.37	83.35	7.23
18	VH133157	55.0	56.0	86.0	203.2	99.8	14.5	13.75	15.74	14.13	13.2	29.40	29.39	85.10	7.76
19	ZH161054	54.5	56.5	87.5	156.9	74.0	17.0	17.73	16.43	14.17	14.2	26.60	32.73	83.55	5.53
20	ZH17367	54.0	55.0	87.0	208.1	96.7	16.0	13.58	17.81	14.44	12.8	27.70	36.71	84.65	6.53
21	ZH138269	55.5	57.5	87.5	138.2	64.2	11.5	24.51	16.62	13.25	14.4	26.30	29.68	86.15	5.75
22	ZH17509	53.0	55.0	90.0	146.0	70.0	11.5	15.19	16.71	13.22	14.4	32.00	24.88	85.90	4.82
23	ZH17375	57.5	57.5	89.0	206.1	94.7	10.5	14.51	16.56	14.52	14.0	26.30	34.09	86.05	5.63
24	ZH17251	57.0	58.0	88.5	133.3	67.9	13.5	14.85	14.28	14.01	13.8	25.60	28.66	85.95	5.97
25	ZH17793	56.0	57.5	90.0	177.7	79.9	16.0	21.98	17.56	13.80	13.4	27.50	36.07	85.80	6.33
26	ZH17749	53.0	54.0	88.5	144.4	67.4	7.5	22.11	17.75	13.27	13.8	26.10	30.13	83.55	4.38
27	ZH17800	56.5	59.5	90.5	158.1	82.6	3.5	44.99	14.77	14.42	13.6	27.65	23.32	76.30	2.59
28	ZH17833	54.5	57.0	88.0	147.9	76.65	2.5	51.49	14.02	13.66	13.0	25.20	24.22	84.80	3.22
29	ZH17363	53.5	56.0	90.5	144.5	73.0	4.0	63.83	17.43	13.95	13.3	28.85	30.52	87.10	2.99
30	CAH1821*	54.5	56.0	88.5	149.3	76.3	16.0	13.75	16.10	16.12	15.6	26.70	34.09	83.20	7.05
31	BIO 9544*	52.5	54.5	87.0	187.7	86.6	16.0	22.99	18.09	14.09	13.6	27.90	35.42	84.50	7.28
32	OMH 14-27*	54.5	58.0	90.0	173.2	79.6	11.0	14.93	17.31	14.87	15.2	30.8	27.74	86.95	6.33
	G.M.	54.77	56.27	87.98	167.85	80.52	10.6	21.14	16.25	14.36	13.93	29.13	29.55	84.93	6.00
	C.D.	1.73	1.53	2.24	35.34	15.58	3.30	0.62	2.67	1.20	1.74	4.57	4.09	0.22	2.52
	C.Ve	1.54	1.33	1.24	10.27	9.45	16.0	1.13	8.03	4.09	6.12	7.65	6.75	1.17	20.48

*** indicates check hybrids; DT: Days to 50% tasseling; DS: Days to 50% silking; DH: Days to 75% dry husk; PHT: Plant height (cm); EHT: Ear height (cm); AR: Number of Adventitious roots/ stilt/ brace roots bearing plants (after stress); SP: Senescence per cent (After Stress); LC: Length of cob (cm); GC: Girth of cob (cm); KR/C: Number of kernel rows per cob (KR/C); K/R: Number of kernels per row; KW: 100 kernel weight (g); S: Shelling %; Y: Grain yield (t/ha).**

Table 3: Estimates of variability parameters and expected genetic advance for 14 characters

Sl. No.	Characters	Mean	Range	PCV %	GCV %	h^2 (%)	GA (%)	GA % of population mean
1	Days to 50% tasseling (days)	54.77	51.50 - 57.5	2.89	2.55	77.78	2.53	4.62
2	Days to 50% Silking (days)	56.27	53.50 - 59.5	2.72	2.38	76.26	2.40	4.27
3	Days to 75% Dry Husk (days)	87.98	83.50 -91.0	2.19	1.81	67.81	2.69	3.06
4	Plant Height (cm)	167.85	121.30 - 215	16.56	12.98	61.49	35.20	20.97
5	Ear Height (cm)	80.52	59.20- 99.80	15.27	12.00	61.73	15.64	19.42
6	Number of plants with Adventitious roots (after stress) (number)	10.06	2.50 -17.0	44.38	41.39	86.99	8.00	79.52
7	Senescence Percentage (after stress) (%)	21.14	11.21- 63.83	27.40	27.37	99.83	15.16	56.34
8	Length of Cob (cm)	16.25	13.81-18.45	9.81	5.63	32.99	1.08	6.67
9	Girth of Cob (cm)	14.36	13.13-16.42	6.54	5.10	60.89	1.18	8.20
10	Kernel Rows per Cob (number)	13.93	12 .00-16.40	8.29	5.59	45.47	1.08	7.76
11	Kernels per row (number)	29.13	24.00 -37.00	12.59	10.00	63.07	4.77	16.36
12	100 Kernel Weight (g)	29.55	23.32 -36.71	13.23	11.38	73.99	5.96	20.17
13	Shelling Percentage (%)	84.93	76.30 -88.30	1.55	0.99	40.82	0.12	1.30
14	Yield (t/ha)	6.00	2.59 - 8.43	28.04	19.15	46.65	1.62	26.95

Table 4: Phenotypic (rp) and Genotypic (rg) correlation coefficients among 32 maize hybrids for 14 characters

Characters		DT	DS	DH	PHT	EHT	AR	SP	LC	GC	KR/C	K/R	KW	S	Y
DT	r_p	1.000	0.874**	0.450**	0.205	0.275*	0.182	-0.183	-0.159	0.316*	0.196	0.048	-0.105	0.051	0.059
	r_g	1.000	0.871**	0.448**	0.247*	0.377**	0.152	-0.203	-0.302*	0.420**	0.308*	0.091	-0.135	0.155	0.087
DS	r_p		1.000	0.588**	-0.009	0.055	0.089	0.054	-0.189	0.250*	0.094	0.004	-0.184	0.013	-0.119
	r_g		1.000	0.608**	-0.063	0.079	0.044	0.07	-0.372**	0.241	0.048	0.041	-0.296*	-0.054	-0.296*
DH	r_p			1.000	0.004	0.011	0.016	0.137	0.268*	0.168	0.332**	0.237	-0.046	-0.129	-0.128
	r_g			1.000	-0.192	-0.123	-0.01	0.176	0.400**	0.104	0.389**	0.289*	-0.207	-0.322**	-0.398**
PHT	r_p				1.000	0.943**	0.217	-0.223	0.330**	0.231	0.051	0.263*	0.406**	0.274*	0.499**
	r_g				1.000	0.974**	0.336**	-0.277*	0.448**	0.251*	-0.041	0.268*	0.496**	0.498**	0.786**
EHT	r_p					1.000	0.179	-0.15	0.181	0.272*	0.015	0.256*	0.253*	0.221	0.415**
	r_g					1.000	0.318*	-0.187	0.271*	0.435**	0.024	0.249*	0.356**	0.427**	0.759**
AR	r_p						1.000	-0.554**	0.263*	0.167	0.165	-0.002	0.514**	-0.167	0.499**
	r_g						1.000	-0.588**	0.433**	0.24	0.245	-0.03	0.593**	-0.214	0.621**
SP	r_p							1.000	-0.111	-0.197	-0.238	-0.241	-0.198	0.054	-0.554**
	r_g							1.000	-0.168	-0.232	-0.337**	-0.293*	-0.217	0.072	-0.792**
LC	r_p								1.000	0.111	0.221	0.477**	0.475**	0.052	0.341**
	r_g								1.000	-0.477**	0.258*	0.373**	0.550**	0.291*	0.294*
GC	r_p									1.000	0.257*	0.304*	0.042	-0.261*	0.256*
	r_g									1.000	0.134	0.283*	-0.216	-0.555**	0.233
KR/C	r_p										1.000	0.143	0.093	-0.168	0.268*
	r_g										1.000	0.096	-0.01	-0.431**	0.095
K/R	r_p											1.000	-0.268*	0.109	0.337**
	r_g											1.000	-0.416**	0.189	0.464**
KW	r_p												1.000	-0.042	0.405**
	r_g												1.000	-0.199	0.319*

DT: Days to 50% tasseling; DS: Days to 50% silking; DH: Days to 75% dry husk; PHT: Plant height (cm); EHT: Ear height (cm); AR: Number of Adventitious roots/ stilt/ brace roots bearing plants (after stress); SP: Senescence per cent (After Stress); LC: Length of cob (cm); GC: Girth of cob (cm); KR/C: Number of kernel rows per cob (KR/C); K/R: Number of kernels per row; KW: 100 kernel weight (g); S: Shelling %; Y: Grain yield (t/ha)

Table 5: Genotypic path coefficient analysis of 14 component traits on yield.

Characters	DT	DS	DH	PHT	EHT	AR	SP	LC	GC	KR/C	K/R	KW	S	Y (correlation)
DT	-1.006	0.951	-0.307	0.472	-0.461	0.026	0.060	-0.054	0.178	0.165	0.004	0.037	0.021	0.087
DS	-0.876	1.091	-0.417	-0.121	-0.097	0.008	-0.021	-0.066	0.102	0.026	0.002	0.081	-0.007	-0.296*
DH	-0.450	0.663	-0.686	-0.368	0.150	-0.002	-0.052	0.071	0.044	0.208	0.012	0.057	-0.044	-0.398**
PHT	-0.248	-0.069	0.132	1.914	-1.191	0.058	0.082	0.080	0.106	-0.022	0.011	-0.136	0.069	0.786**
EHT	-0.379	0.086	0.084	1.864	-1.223	0.055	0.055	0.048	0.185	0.013	0.010	-0.098	0.059	0.759**
AR	-0.153	0.048	0.007	0.644	-0.388	0.173	0.174	0.077	0.102	0.131	-0.001	-0.163	-0.029	0.621**
SP	0.204	0.076	-0.121	-0.530	0.229	-0.102	-0.296	-0.030	-0.098	-0.180	-0.012	0.060	0.010	-0.792**
LC	0.304	-0.406	-0.275	0.858	-0.331	0.075	0.050	0.178	-0.202	0.138	0.016	-0.151	0.040	0.294*
GC	-0.422	0.263	-0.071	0.480	-0.532	0.042	0.069	-0.085	0.424	0.072	0.012	0.059	-0.077	0.233
KR/C	-0.310	0.053	-0.267	-0.078	-0.029	0.043	0.100	0.046	0.057	0.535	0.004	0.003	-0.059	0.095
K/R	-0.091	0.044	-0.198	0.512	-0.305	-0.005	0.087	0.066	0.120	0.052	0.042	0.115	0.026	0.464**
KW	0.136	-0.323	0.142	0.950	-0.435	0.103	0.064	0.098	-0.092	-0.005	-0.017	-0.275	-0.027	0.319*
S	-0.156	-0.059	0.221	0.953	-0.522	-0.037	-0.021	0.052	-0.235	-0.230	0.008	0.055	0.138	0.165

Genotypic residual effect = 0.061 * Significant at 5% level ** Significant at 1%

Table 6: Phenotypic path- coefficient analysis of 14 component traits on yield.

Characters	DT	DS	DH	PHT	EHT	AR	SP	LC	GC	KR/C	K/R	KW	S	Y (correlation)
DT	**-0.077**	0.117	-0.124	0.076	-0.035	0.033	0.031	0.024	-0.008	0.041	0.022	-0.035	-0.004	0.059
DS	-0.068	**0.134**	-0.162	-0.003	-0.007	0.016	-0.009	0.028	-0.006	0.020	0.002	-0.061	-0.001	-0.119
DH	-0.035	0.079	**-0.276**	0.001	-0.001	0.003	-0.023	-0.040	-0.004	0.069	0.106	-0.015	0.009	-0.128
PHT	-0.016	-0.001	-0.001	**0.372**	-0.121	0.039	0.038	-0.049	-0.006	0.011	0.117	0.135	-0.019	0.499**
EHT	-0.021	0.007	-0.003	0.351	**-0.128**	0.032	0.025	-0.027	-0.007	0.003	0.114	0.084	-0.016	0.415**
AR	-0.014	0.012	-0.004	0.081	-0.023	**0.180**	0.094	-0.039	-0.004	0.034	-0.001	0.171	0.012	0.499**
SP	0.014	0.007	-0.038	-0.083	0.019	-0.100	**-0.169**	0.016	0.005	-0.050	-0.107	-0.066	-0.004	-0.554**
LC	0.012	-0.025	-0.074	0.123	-0.023	0.047	0.019	**-0.148**	-0.003	0.046	0.212	0.158	-0.004	0.341**
GC	-0.024	0.033	-0.046	0.086	-0.035	0.030	0.033	-0.016	**-0.025**	0.054	0.135	0.014	0.018	0.256*
KR/C	-0.015	0.013	-0.091	0.019	-0.002	0.030	0.040	-0.033	-0.006	**0.208**	0.064	0.031	0.012	0.268*
K/R	-0.004	0.001	-0.065	0.098	-0.033	0.000	0.041	-0.071	-0.008	0.030	**0.446**	-0.089	-0.008	0.337**
KW	0.008	-0.025	0.013	0.151	-0.032	0.093	0.033	-0.070	-0.001	0.019	-0.120	**0.333**	0.003	0.405**
S	-0.004	0.002	0.036	0.102	-0.028	-0.030	-0.009	-0.008	0.007	-0.035	0.049	-0.014	**-0.070**	-0.004

Phenotypic residual effect = 0.384; * Significant at 5% level; ** Significant at 1% level

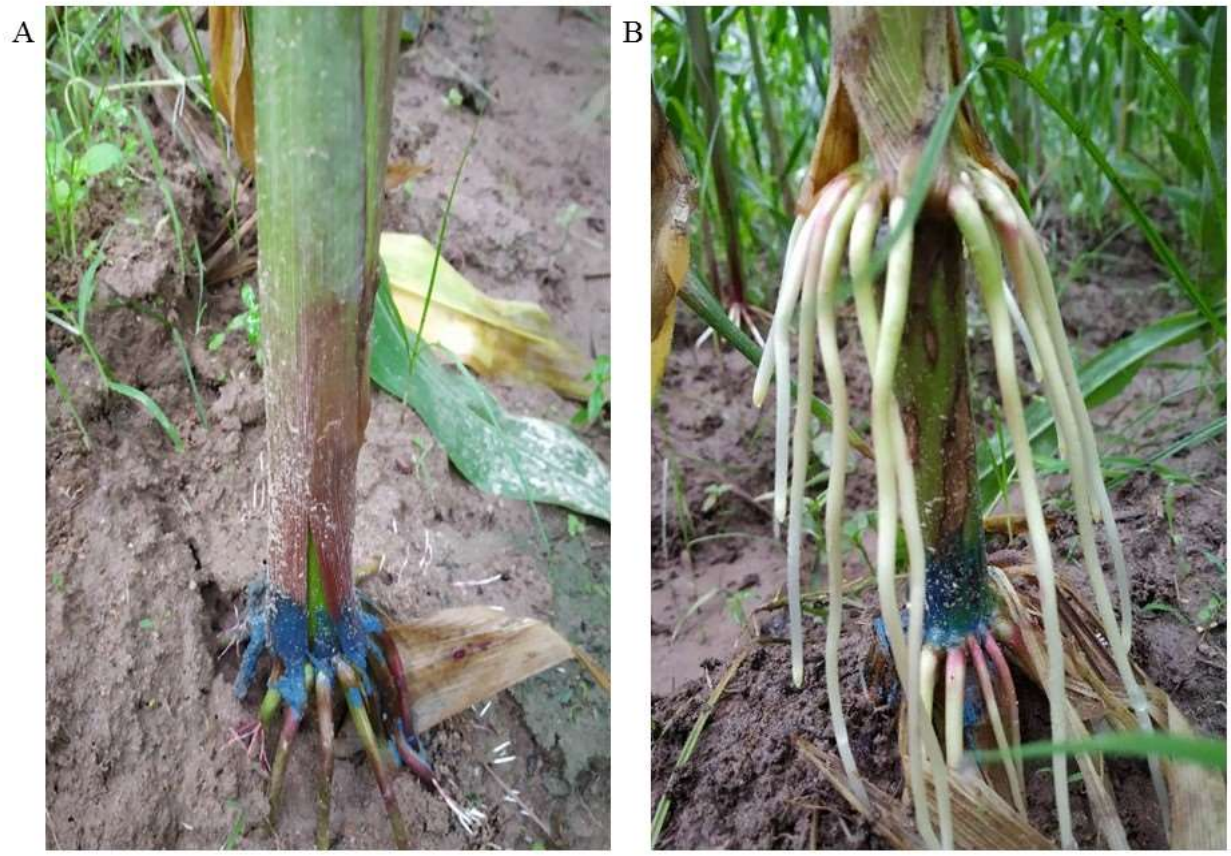

Figure 1: Illustration showing the development of adventitious roots in maize hybrid ZH17367 under excessive soil moisture stress (A: before stress; B: after stress)

was performed. The genotypic path analysis reflected that plant height had highest direct positive impact as well as high positive correlation with yield (Khodarahmpour, 2012) under heat stress that tall plants gave better yield. On the contrary, ear height showed highest direct negative effect but positive correlation with yield via higher positive indirect effects of plant height. Days to silking also depicted positive direct effect on yield, which was counter balanced by negative indirect impact of days to tasseling and days to 75% dry husk leading to an overall negative correlation with yield (Pandey *et al.,* 2017). Kernel row per cob and girth of cob reflected positive direct effect on yield even though their correlation with yield was non-significant. Tulu (2014) also detected similar trend for cob diameter. Number of plants with adventitious roots after stress showed direct positive effect as well as positive correlation with yield. This confers the trait to be an important selection parameter for plants under ESM stress. The low yielding hybrids (ZH17800, ZH17833) showed very poor adventitious root development under stress. The length of cob and shelling percentage also exerted positive direct effect on yield. Kernels per row had highly significant positive correlation and its direct effect on grain yield was positive but low (Begum *et al.,* 2016). Furthermore, senescence percentage after stress had direct negative effect as well as negative correlation with yield similar to the findings of Lakshmi *et al.,* (2018) and Sesay *et al.,* (2017) under ESM stress. Days to tasseling delineated negative direct effect on yield (Begum *et al.,* 2016). Negative selection can be applied for days to 75% dry husk as it had higher negative direct effect and negative correlation with yield. It was noteworthy that 100 kernel weight exhibited negative direct effect on

grain yield but a positive correlation via indirect effect of plant height. This indicated that selection for higher grain yield can be done through indirect selection from the yield components. The residual effect of genotypic path coefficient was 0.061 which indicated that the traits studied were sufficient enough to determine their effect on dependent variable i.e. plant yield. The path coefficient analysis through phenotypic correlation revealed that kernels per row had highest direct positive effect along with positive correlation on yield followed by plant height, 100 kernel weight, kernel row per cob, number of plants with adventitious roots (after stress) and days to silking. Ear height depicted highest indirect effect on yield via plant height. On the other hand, highest direct negative effect on yield was exerted by days to 75% dry husk followed by senescence percentage, length of cob, ear height, days to tasseling, shelling percentage and girth of cob. It was noteworthy that days to silking had highest negative indirect effect on yield via days to 75% dry husk (Patil *et al.,* 2016; Upadhyay *et al.,* 2017).

Conclusion

The results obtained indicate that plants with greater plant height, low senescence percentage after stress, higher number of plants with adventitious roots after stress and greater number of kernels per row were detected to be good indicators of ESM stress tolerance. Considering these attributes, among 32 hybrids, ZH17368, VH19487, ZH17446, ZH17815, and VH133157 were the best performing hybrids under ESM stress. These hybrids were found to withstand 12 days of waterlogging stress at the flowering stage and can be considered for planting in the areas where yield loss due to ESM is prevalent.

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Conflict of interest

The authors declare that they have no conflict of interest.

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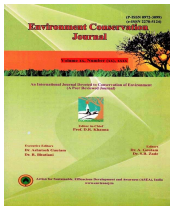

Genetic divergence analysis in Taramira (*Eruca sativa* Mill.) under different environment conditions with special reference to principal component analysis

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ABSTRACT

The field experiment was conducted to identify the principal component among ten morphological one (biochemical) oil content traits of thirty germplasm lines from the All India coordinate research project oilseeds (Taramira) in a randomize block design (RBD) with three replications in each of the test conditions, which were generated using 3 separate sowing dates with fifteen days interval from October 2nd week to November 3rd week (15th October, 30th October & 15th November 2018-19). First three principal components contributed 76.8% proportion of variation with an eigen value more than one (1.329). The largest percent contribution (30.11) to overall genetic divergence was shown by siliqua per plant followed by test weight, number of primary & secondary branches per plant & seed yield per plant. The genotypes were divided into nine groups, with Cluster II having the most genotypes (12), followed by Cluster I with five genotypes. Based on mean value of seed yield, oil content & cluster analysis, eight germplasms with cross combination viz., RTM-1806 X (RTM-314, RTM-1351, RTM-1805, RTM-1810, RTM-1800, RTM-1791, RTM-1815) & RTM-1804 X (RTM-314, RTM-1351) were identified as high yielding which can be widely utilized as a parents in hybridization programme for the development of 9 new diverse varieties/ hybrids for enhanced seed yield as well as oil content.

Introduction

Oilseed crops are the basis of an agricultural economy like India's, and they're mostly grown for oil production across the world with total output of 32.26 million tonnes and area of 25.50 million hectares and average yield of 1265 kg/hectare, India is one of the world's leading oilseed producers (Anonymous, 2019). The toria seed & mustard group of the family Brassicaceae includes Eruca sativa (L.) Miller (Rocket plant or Taramira). This plant's name is derived from the Latin word eruca, which means cabbage (Garg & Sharma, 2014). Taramira is an important oilseed crop suitable to be grown under in drought-prone areas of northwestern India, & it has gained attracted interest as a possible biodiesel crop. Taramira oil is used in medications, cosmetics, & in the manufacturing of grease, plastics, lubricants, soaps, & paints, among other things (Warwick *et al*.,

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2007; Yaniv *et al*., 1998). Salads, cooked veggies, & functional foods are all made with the plant.

Groundnut, soybean, rapeseed-mustard, sesamum, & taramira are the most important oilseed crops farmed in Rajasthan. Due to the native ecological circumstances of Rajasthan & its potential application in bio-diesel generation, taramira has grown in popularity among all oilseed crops. Taramira is produced on 7.63 lakh hectares in Rajasthan, yielding 4.62 lakh kg with an average productivity of 606 kg/hectare (Anonymous, 2019).

Presence of optimal genetic divergence between the parents is a significant pre-requisite for the success of crop improvement initiatives in various crops. Because superior transgressive segregates arise in segregating generations in crossings between genetically diverse parents, the Thus, crossing genetically different parents in any breeding effort can result in a wide range of gene combinations, which can then be used to either exploit heterosis for the development of hybrid varieties or to obtain superior recombinants for the development of pure line varieties. As a result, a preliminary assessment of the genetic diversity available in the breeding materials is a necessary precondition for any breeding program's success (Deep *et al*., 2019). Correlation studies provide trustworthy & relevant information about the type, scope, & direction of any selection process.

In light of the foregoing, the current research was conducted to evaluate the character association & relationship between distinct Taramira germplasm using a clustering & PCA approach in order to find different germplasms suited for the crop's hybridization programme.

Material and Methods

The experimental material included 30 germplasm lines from the All India Coordinated Research Projects, Oilseeds (Taramira), Department of Plant Breeding & Genetics, S.K.N. College of Agriculture, Jobner, as well as two checks, RTM-314 & RTM-1351. The experiment was set up in a (RBD) with three replications in each of the test conditions, which were established by three different sowing dates separated by fifteen days from October 2nd to November 3rd (15th October, 30th October & 15th November 2018-19). Within each replication, the germplasms were distributed at random, with inter & intra row spacing of 30 & 10 cm, respectively.

For 10 agro-morphology & 1 bio-chemical (Oil content), observations were recorded on ten randomly selected competing plants in each replication & in each environment, then the average of all replications of all environments for each character viz., days to 50% percent flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, length of siliqua (cm), siliqua per plant, seed per siliqua, test weight (g), oil content (%) & seed yield per plant (g). The major descriptive statistics such as mean, range, standard deviation & coefficient of variation were calculated using standard methods as described by Panse & Sukhatme (1964) correlation coefficients were calculated using as per the method proposed by Al-jibouri *et al*. (1958). he data were subjected to analysis of genetic divergence through D^2 statistic (Mahalanobis 1936) to quantify genetic divergence as proposed by Rao (1952), while Tocher's method as used to form cluster.

Results and Discussion

The genetic variability available in the base population is the most important prerequisite for selection in any crop improvement effort. For all of the agromorphological & biochemical parameters under consideration, the experiment's pooled analysis of variance indicated significant differences across 30 germplasm (Table 1). Sujatha et al. (2002) investigated the extent of divergence in taramira and found that the majority of yield and its contributing features had considerable variability.

The checks i.e., popular cultivated varieties namely RTM-314 & RTM- 1351 showed superior performance for most of the characters under study. Variety RTM-314 exhibited superiority for days to 50 percent flowering, days to maturity, secondary branches per plant & oil content while RTM-1351 showed good performance for primary branches per plant, length of siliqua, siliqua per plant, seed per siliqua & seed yield per plant. Germplasm RTM-1797 exhibited superior performance for secondary branches per plant, length of siliqua & seed yield per plant (Table 2).

The correlation coefficient, which expresses the intensity of association among a group of characters, is an important biometrical technique

Tabel 1: Pooled ANOVA for seed yield & its component traits in taramira

Source of variation	d.f	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No. of secondary branches per plant	Length of siliqua (cm)	Siliqua per plant	Seeds per siliqua	Test weight (g)	Oil content (%)	Seed yield per plant(g)
Environment	2	1590.28**	1091.24**	633.52**	59.05**	134.40**	3.72**	3117.15**	2.11	0.55**	75.78**	63.68**
Rep in Env.	6	16.31	83.30	40.84	0.18	0.44	0.02	22.10	2.24*	0.08	8.58	0.12
Genotype	29	27.13*	115.85**	77.14*	1.95*	3.26**	0.14*	539.70**	0.65	0.47**	18.18**	1.39**
Genotype x environment	58	15.69**	46.34	38.84**	1.06**	0.60	0.06**	50.50**	16.80*	0.09*	5.38	0.27**
Error	174	8.03	40.22	19.60	0.15	0.38	0.03	24.06	0.24	0.05	5.07	0.15

***Significant at 5% level of significance **Significant at 1% level of significance**

Table 2: mean of seed yield & its components of taramira evaluated under different environments

Gemplasm	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No. of secondary branches per plant	Length of siliqua (cm)	Siliqua per plant	Seeds per siliqua	Test weight (g)	Oil content (%)	Seed yield per plant(g)
RTM-314	56.44	130.67	77.55	6.25	9.44	2.74	70.30	20.58	3.18	39.92	3.76
RTM-1791	54.89	130.00	75.13	5.89	8.23	2.57	62.33	19.38	3.25	38.37	3.37
RTM-1792	54.33	128.33	74.46	5.61	7.73	2.34	51.34	18.73	2.80	36.05	3.01
RTM-1793	49.78	122.22	74.52	5.86	7.54	2.45	50.37	18.73	2.98	36.50	2.98
RTM-1794	53.67	119.89	73.16	5.17	7.42	2.45	53.57	18.62	2.75	35.20	2.96
RTM-1795	50.45	119.45	72.51	6.37	8.00	2.59	60.80	17.75	2.97	38.04	3.37
RTM-1796	50.56	122.44	71.53	5.56	7.92	2.49	56.51	18.08	2.76	35.24	3.14
RTM-1797	51.89	128.44	67.53	6.54	6.77	2.25	56.33	18.18	2.98	36.35	2.73
RTM-1798	54.11	125.89	68.19	6.01	8.09	2.53	59.84	19.05	3.00	37.20	3.35
RTM-1799	51.22	124.78	71.37	5.77	8.03	2.54	62.17	18.40	2.97	37.07	3.32
RTM-1800	55.11	124.67	76.08	6.01	8.55	2.61	62.00	20.14	3.11	38.25	3.88
RTM-1801	54.22	120.56	68.68	5.65	7.51	2.44	45.23	19.16	2.54	36.58	2.73
RTM-1802	51.00	121.33	72.54	5.24	7.07	2.37	52.59	18.40	2.54	35.34	3.02
RTM-1803	51.00	122.00	71.99	5.42	7.94	2.32	54.53	19.17	2.93	34.47	2.87
RTM-1804	52.44	122.55	76.39	5.06	8.36	2.33	48.82	19.05	3.05	36.94	3.05
RTM-1805	52.22	121.00	79.18	5.92	8.54	2.57	66.03	20.14	3.21	38.14	3.78
RTM-1806	54.22	119.55	73.67	4.87	8.15	2.66	59.97	17.97	2.86	35.95	3.15
RTM-1807	51.11	120.33	76.07	5.55	8.11	2.34	53.64	17.86	2.72	36.73	3.14
RTM-1808	53.11	121.33	75.72	5.47	7.41	2.56	41.64	19.17	2.55	35.85	2.93
RTM-1809	52.22	127.11	72.33	5.58	6.95	2.56	55.46	17.86	2.67	35.79	2.87
RTM-1810	51.89	123.33	75.99	5.98	8.49	2.53	65.57	20.03	3.13	38.44	3.93
RTM-1811	54.89	118.34	71.14	4.88	7.89	2.49	49.98	19.38	2.63	36.23	2.94
RTM-1812	53.11	124.00	73.68	5.00	7.67	2.51	54.96	17.64	2.87	35.08	2.98
RTM-1813	52.33	119.22	70.73	5.10	8.19	2.40	53.05	19.38	2.66	35.04	2.85
RTM-1814	54.33	124.89	71.71	5.97	7.12	2.48	48.90	19.38	2.70	35.69	2.87
RTM-1815	55.33	125.33	78.26	6.06	8.03	2.58	59.71	20.14	3.05	39.27	3.91
RTM-1816	55.45	128.45	71.96	5.98	7.14	2.56	38.55	19.49	3.01	35.85	2.85
RTM-1817	51.78	126.11	70.73	6.13	7.35	2.42	56.42	18.40	2.69	36.85	2.93
RTM-1818	53.22	127.45	74.98	5.81	8.04	2.28	50.29	18.73	2.43	36.72	2.82
RTM-1351	52.11	129.33	76.65	6.52	9.00	2.75	72.60	20.80	3.25	39.52	3.99
Mean	52.95	123.97	73.48	5.71	7.89	2.49	55.78	18.99	2.87	36.76	3.18
Minimum	56.44	130.67	79.18	6.52	9.44	2.75	72.60	20.80	3.25	39.92	3.99
Maximum	49.78	118.34	68.68	4.87	6.77	2.25	38.55	17.75	2.43	34.47	2.73
C.V	3.33	3.04	3.29	4.30	4.54	4.49	4.71	4.23	4.63	3.53	7.25
S.E	1.02	2.18	1.40	0.14	0.21	0.06	1.52	0.46	0.08	0.75	0.13

for constructing the selection index. The phenotypic correlations were assessed among eleven traits in 30 Taramira genotypes, & these revealed an intrinsic relationship between any two variables, which might have occurred due to pleiotropic gene action, linkage, or most likely both. The phenotypic correlation coefficients between yield & its component characters are reported in this study (Table 3). The most economic trait i.e., seed yield per plant & oil content showed positive & significant association with plant height, number of primary & secondary branches per plant, length of siliqua, siliqua per plant, seed per siliqua & test weight. Seed yield per plant & oil content also showed positive correlation & were significantly associated with each other. Selection criteria based on these component traits, as well as seed yield, will be more useful as a result. Earlier researchers Yadav & Pandey, (2018), Rauf & Rahim, (2018) and Shinwari *et al*., (2013) noticed a similar type of correlation between yield & yield-related traits.

The mean data of eleven quantitative traits were submitted to principal component analysis in this study, which used a data reductionist technique employing a linear combination of optimally-weighted observed variables to discover the plant features that contribute the most to overall variance. Only three of the eleven PCs had an Eigen value greater than 1.0, indicating 76.8% diversity among 30 genotypes (Table 4). The first principal component (PC1) accounted for 51.3 percent of total variance, whereas PC2 & PC3 contributed 13.5 & 12.1 percent of total variation, respectively. This similar result agreement by Ara *et al*. (2018) and Parvin *et al*. (2019).

Table 5 shows the % contribution of individual characteristics to genetic divergence for all eleven characters. The character siliqua per plant contributed the most to total genetic divergence (30.11%), followed by test weight (22.53), number of primary branches per plant (18.62), number of secondary branches per plant (13.56), & seed yield per plant (13.56). The findings of Renuka Devi *et al*., (2017), Kumari & Kumari, (2017) were in similar direction to the present research findings. Table 6 shows the factor loadings of characters from PCA, which have five components that identify the main characters responsible for the most variability. All of the characteristics contributed to the overall variety in a good way. While the most significant contributions in PC1 were seed yield per plant, oil content, test weight, siliqua per plant, & secondary branches per plant, the most significant contributors in PC2 were seed yield per plant, oil content, test weight, siliqua per plant, & secondary branches per plant. Plant height (0.351), secondary branches per plant (0.329), & seed production per plant all contributed to the PC2 (0.154). siliqua per plant (0.357) made the most contribution to PC3, followed by secondary branches per plant (0.244), & test weight (0.244). (0.159). The goal of principal component analysis is to find the smallest number of components that may explain the most variability out of all the variables, as well as to rank items based on PC scores. Germplasms from clusters separated by a large statistical distance might be used in a hybridization programme to obtain a broad range of diversity among segregates. Cluster VII had the most intra-cluster distance, whereas clusters IV, V, VIII, & IX had none. Similar result was found by Sodani *et al*., (1990); Naznin *et al*., (2015), Doddabhimappa *et al*., (2010) and Chandra *et al*., (2018). Cluster analysis is a data classification approach that allows for the separation of genetic material into several homogeneous groups. It makes it easier to categories genotypes based on morpho-genetic characteristics. Cluster analysis also aids in the reduction of variance within a group while boosting variance between groups, as well as the detection of outliers.

For majority of the characters studied, there was a lot of variance in cluster mean performance. Using Tocher's approach, a hierarchical clustering methodology based on eleven quantitative trait data was able to arrange 30 germplasms into nine groups (Table 7 Fig. 1). Cluster II had the most germplasm (13), followed by cluster I (5), cluster III & cluster VII (3), cluster VI (2), & the other clusters each had one germplasm. Cluster VI & VII (15.66) had the greatest inter-cluster distance, followed by Cluster IV & Cluster VI (14.26), Cluster VI & Cluster IX (13.98), & Cluster V & Cluster IX (13.98). (13.68). Cluster II & III (6.07) & Cluster I & Cluster III (6.22) had the shortest inter-cluster distances. Except for days to 50% flowering (highest in cluster IV) & number of primary branches per plant (highest in cluster IX), Cluster VI had the highest mean value for all yield & component characters, while Cluster IX had the lowest mean value for

Table 3: Phenotypic correlation coefficients for different traits in 30 germplasms of taramira over the pooled environmental data.

Characters	Days to 50% flowering	Days to maturity	Plant height(cm)	No. of primary branches per plant	No. of secondary branches per plant	Length of siliqua(cm)	Siliqua per plant	Seeds per siliqua	Test weight(g)	Oil content (%)
Days to 50% flowering										
Days to maturity	0.184									
Plant height	0.021	-0.002								
primary branches per plant	0.031	0.386**	0.016							
secondary branches per plant	0.111	0.120	0.402**	0.165						
Length of siliqua	0.273**	0.064	0.263*	0.245*	0.312**					
Siliqua per plant	-0.019	0.218*	0.283**	0.391**	0.601**	0.427**				
Seeds per siliqua	0.410**	0.155	0.272**	0.250*	0.447**	0.297**	0.289**			
Test weight	0.107	0.280**	0.253*	0.396**	0.450**	0.456**	0.566**	0.354**		
Oil content	0.120	0.329**	0.431**	0.449**	0.527**	0.378**	0.528**	0.420**	0.489**	
Seed yield per plant	0.064	0.129	0.465**	0.377**	0.616**	0.522**	0.700**	0.436**	0.611**	0.608**

Table 4: Principal components showing the eigen values & proportion of variation.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Eigenvalues	5.644	1.481	1.329	0.688	0.493	0.461	0.372	0.226	0.177	0.071	0.06
Proportion	0.513	0.135	0.121	0.063	0.045	0.042	0.034	0.021	0.016	0.006	0.005
Cumulative Proportion	0.513	0.648	0.768	0.831	0.876	0.918	0.952	0.972	0.988	0.995	1

Table 5: Contribution of different characters towards genetics divergence among 30 taramira germplasm.

	Days to 50% flowering	Days to maturity	Plant height(cm)	No. of primary branches per plant	No. of secondary branches per plant	Length of siliqua(cm)	Siliqua per plant	Seeds per siliqua	Test weight(g)	Oil content (%)	Seed yield per plant(g)
Times Ranked	8	4	12	81	59	5	131	4	98	2	31
Contribution	1.84	0.92	2.76	18.62	13.56	1.15	30.11	0.92	22.53	0.46	7.13

Table 6: Principal component analysis for taramira germplasm accessions – non rotated loadings.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
1	0.129	-0.092	-0.774	0.203	-0.12	0.118	-0.111	0.415	0.179	0.3	-0.01
2	0.186	-0.622	-0.136	-0.151	-0.551	0.03	-0.087	-0.372	-0.037	-0.242	0.159
3	0.267	0.351	-0.07	-0.587	-0.315	-0.498	-0.001	-0.018	0.077	0.251	-0.197
4	0.236	-0.565	0.244	-0.094	0.395	-0.195	-0.115	0.095	-0.143	0.565	0.025
5	0.334	0.329	0.001	-0.057	-0.09	0.465	-0.282	-0.106	-0.618	0.199	0.2
6	0.298	0.113	-0.132	0.647	0.013	-0.528	0.095	-0.323	-0.254	-0.025	-0.064
7	0.331	0.058	0.357	0.29	-0.208	0.296	-0.263	-0.131	0.498	0.175	-0.424
8	0.306	0.009	-0.362	-0.261	0.547	0.234	0.213	-0.475	0.172	-0.132	-0.186
9	0.34	-0.073	0.159	0.033	-0.195	0.203	0.812	0.302	-0.123	0.015	-0.086
10	0.385	-0.088	0.041	-0.08	0.167	-0.112	-0.322	0.471	-0.158	-0.608	-0.274
11	0.392	0.154	0.122	0.027	0.099	-0.088	-0.016	0.089	0.419	-0.104	0.771

Table 7: Constituents of 9 clusters of 30 taramira germplasm

Cluster	No. of germplasm	Germplasm
Cluster I	5	RTM-1805, RTM-1810, RTM-1800, RTM-1791, RTM-1815
Cluster II	13	RTM-1794, RTM-1812, RTM-1796, RTM-1813, RTM-1807, RTM-1803, RTM-1809, RTM-1792, RTM-1818, RTM-1793, RTM-1817, RTM-1814
Cluster III	3	RTM-1798, RTM-1799, RTM-1795
Cluster IV	1	RTM-1811
Cluster V	1	RTM-1806
Cluster VI	2	RTM-314, RTM-1351
Cluster VII	3	RTM-1801, RTM-1808
Cluster VIII	1	RTM-1804
Cluster IX	1	RTM-1797

Table 8: Average intra (bold) & inter-cluster D^2 values for nine clusters in 30 taramira germplasm

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX
Cluster I	**3.58**	9.06	6.22	11.06	9.42	5.45	12.23	7.81	11.11
Cluster II		**4.93**	6.07	6.13	7.76	12.37	7.18	6.29	8.57
Cluster III			**3.2**	8.27	7.42	8.84	9.8	7.37	7.99
Cluster IV				**0**	5.7	14.26	7.57	6.5	12.32
Cluster V					**0**	11.66	11.46	7.35	13.68
Cluster VI						**3.99**	15.66	11.37	13.98
Cluster VII							**5.76**	8.4	10
Cluster VIII								**0**	11.21
Cluk8ster IX									**0**

Table 9: Cluster means of 9 clusters of 30 taramira germplasm from three different environments

	Days to 50% flowering	Days to maturity	Plant height	No. of primary branches per plant	No. of secondary branches per plant	Length of siliqua	Siliqua per plant	Seeds per siliqua	Test weight	Oil content	Seed yield per plant
Cluster I	53.89	124.87	76.93	5.97	8.37	2.57	63.13	19.97	3.15	38.49	3.77
Cluster II	52.19	123.49	72.96	5.54	7.62	2.42	53.2	18.54	2.73	35.75	2.96
Cluster III	51.93	123.37	70.69	6.05	8.04	2.55	60.94	18.4	2.98	37.44	3.35
Cluster IV	54.89	118.34	71.14	4.88	7.89	2.49	49.98	19.38	2.63	36.23	2.94
Cluster V	54.22	119.55	73.67	4.87	8.15	2.66	59.97	17.97	2.86	35.95	3.15
Cluster VI	54.28	130	77.1	6.38	9.22	2.74	71.45	20.69	3.21	39.72	3.88
Cluster VII	54.26	123.45	72.12	5.7	7.35	2.52	41.81	19.27	2.7	36.1	2.83
Cluster VIII	52.44	122.55	76.39	5.06	8.36	2.33	48.82	19.05	3.05	36.94	3.05
Cluster IX	51.89	128.44	67.53	6.54	6.77	2.25	56.33	18.18	2.98	36.35	2.73
Maximum	54.89(IV)	130(VI)	77.1 (VI)	6.54 (IX)	9.22 (VI)	2.74 (VI)	71.45 (VI)	20.69 (VI)	3.21 (VI)	39.72 (VI)	3.88 (VI)
Minimum	51.89 (IX)	118.34(IV)	67.53 (IX)	4.87 (V)	6.77 (IX)	2.25 (IX)	41.81(VII)	17.97 (V)	2.63 (IV)	35.75 (II)	2.73 (IX)

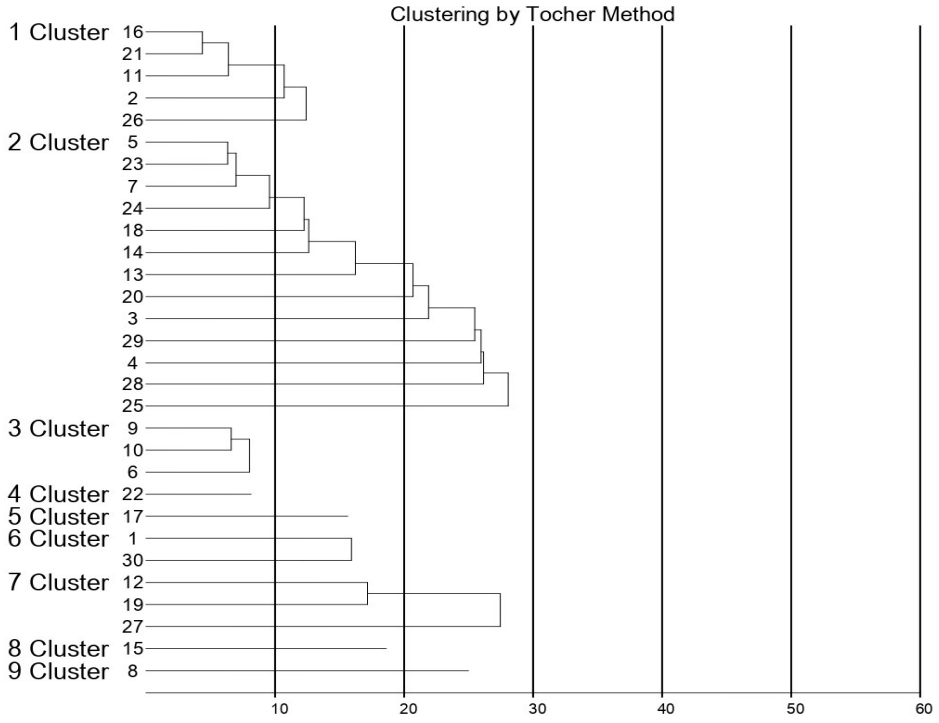

Figure 1: Dendogram of 30 taramira

days to 50% flowering, plant height, secondary branches per plant, length of siliqua, & seed yield per plant (Table 9). This result indicated that the germplasm under investigation was genetically diverse & that the base material included a significant degree of variability indicating that selection to produce new enhanced inbred lines is possible. Seed yield is such a complex & reliable character, crop improvement through indirect selection using these traits would be effective.

Conclusion

Mean sum of square of all characters under investigation showed significant variations i.e., existence of sufficient variability in selected germplasms. Selection of more than one character at a time can done because seed yield have significant positive correlation with its components. Characters i.e., length of siliqua, seed per siliqua, number of primary & secondary branches per plant are responsible to contributes to the total diversity because these characters have high contribution percentage. PC I to PC III contribute 75 % of total genetics divergence so more emphasized should be done in three principle components. High intra cluster distance accounts to higher genetic divergence between clusters & vice-versa. Gemplasm belongs to cluster I, cluster V, cluster VI & cluster VIII are suitable to serve as parents in development of hybrid varieties as these clusters have high inter cluster distance & high cluster mean for seed yield & oil content. Characters *i.e.,* length of siliqua, seed per siliqua, number of primary & secondary branches per plant are responsible to contributes to the total diversity because these characters have high contribution percentage will be exploited for crop improvement in taramira.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ameliorative effect of herbal extracts on lipid profile in albino rats, *Rattus norvegicus* exposed to metanil yellow

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ABSTRACT

Synthetic food colours are used as key component by food manufacturers to increase the consumer acceptance towards food items and beverages as well as for having certain properties like low cost, high colour intensity and more colour stability. These food items and beverages may have more than recommended amount of permitted food colours or some non-permitted synthetic food colours, which may lead to several health problems like disturbances in biochemical parameters, allergic reaction, cancer, mutations etc. Some herbs are having active chemical components and could be used regularly to ameliorate the toxic effect of synthetic food colours. The aim of this study was to analyse the effect of garlic and turmeric extract as a herbal antihyperlipidemic agent in albino rats fed on an azo dye, metanil yellow. The albino rats were divided into four groups (6 rats in each group). Group I (Negative control) fed on normal pellet diet, Group II (Positive control) fed on metanil yellow (MY), Group III fed on MY+ garlic extract and Group IV fed on MY+ turmeric extract. All experimental group fed on normal pellet diet and water ad libitum. Total cholesterol (TC), LDL, HDL and triglycerides (TG) were observed in serum of albino rats from all the groups. The results showed that administration of garlic and turmeric extract raise the level of HDL and lowered the level of LDL, TC and TG in blood serum of albino rats exposed to metanil yellow for 12 and 24 weeks of exposure periods. Garlic was found to be more potent in correcting the lipid profile of metanil yellow fed albino rats in comparison to turmeric extract. However, it has been concluded that both the herbs could be used as antihyperlipidemic agent to avoid health risk in human beings caused by chronic consumption of food colours in different food types consumed daily.

Introduction

Synthetic food colours are being used from last few decades for aesthetic appearance and new trends of food technology. The overall worldwide turnover of food colouring agents is nearly 8000 tons per year and India accounts for only 2% of this output (Das and Mukherjee, 2004). Azo dyes are widely used as colorants in food which can trigger toxic effects such as allergic reactions, biochemical disturbances, tumour formation, and endocrine disruptions. The long-term use of any synthetic colour can cause serious damage to human health. Among the azo dyes, metanil yellow is used in many food processing industry for increasing visual appearance of different kinds of food stuffs like juices, ice creams, sweets, carbonated drinks, etc. It is basically used as yellow colouring factor in food stuffs. Limited amount of azo dyes can be consumed and recommended amounts are mentioned as acceptable daily intakes (ADI) (Das and Mukherjee 2004). The acceptable daily intake (ADI) of metanil yellow given by food and agricultural organization (FAO) was 0-0.3mg/kg b.w and according to Food

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Adulteration act 1954, it was declared as illegal colouring agent in India (Khan *et al.,* 2020). Metanil yellow is found to cause damage to internal tissues (Rahman *et al.,* 2019) and induce changes in biochemical parameters like lipid profile, enzyme activity and oxidative stress, if consumed in more amount than ADI. Herbal extracts are being tested during the last decades to reduce toxic effects induced by food colours as well as other chemicals used in food stuffs. *Allium sativum*, commonly known as garlic, is small perennial herb and could be used to reduce abnormal cholesterol and blood pressure, and as antioxidant to neutralize free radicals (Mahdi *et al.,* 2019). Turmeric has many bio-functional properties including anti-inflammatory, anticancer, antitumour and antilipidemic effects (El-Hack *et al.,* 2021). So, in present study ameliorative effect of garlic and turmeric extract have been studied on the serum lipid profile in young male albino rats exposed to chronic administration of metanil yellow.

Material and Methods

Preparation of Garlic and Turmeric extract

Aqueous extract of garlic was prepared using method of Iwalokun *et al.* (2004) with some modification. Fresh bulbs of garlic (*Allium sativum* L.) were purchased from local markets of Bareilly. 50 gms of cloves were separated, peeled, chopped and homogenized in 200 mL of autoclaved water and the homogenate was then filtered by muslin cloth to give a crude aqueous extract of 250 mg of garlic/mL. Similarly, 50 gms of Rhizome of turmeric have been homogenized in 200 ml of autoclaved water and placed on muslin cloth and squeezed to give a crude aqueous extract of 250 mg of turmeric/mL. The extracts were collected in a sterile vial and stored at 4°C until used.

Experimental animal

Twenty-four males *Rattus norvegicus* with 2 months of age and ± 200g in body weight were used as experimental animals. They were acclimatized for at least 2 weeks before starting the experiment. The animals were maintained under control condition of temperature (22 $\pm 1^0$C), humidity (50±10%) and normal photoperiod (12-hour light /dark cycle). Rats were provided standard dried pellet diet and water ad libitum (Ebrahimi *et al.,* 2015).

Experimental design

Rats were randomly divided into four groups (6 rats in each group). Group I (Negative control) served as untreated control group. Group II (Positive control) received metanil yellow @ 250 mg/kg/day body weight (bw). Group III (MY+GE) were given metanil yellow (at the same rate as in group II) followed by garlic extract @250mg/kg/day bw. Group IV (MY+TE) were given metanil yellow (at the same rate as in group II) followed by turmeric extract @250mg/kg/day body weight. All doses corresponding to 1ml/kg/day bw and injected orally for 12 and 24 weeks. Normal pellet diet and water was given ad libitum to all groups.

Blood collection and assay of lipid profile:

After 12- and 24-weeks blood was drawn from the retro-orbital sinus of rats from each group to obtain serum. The blood was transferred into non heparinized glass centrifuged tube and permitted to clot at room temperature followed by centrifugation at 3500 rpm for 15 minutes (El-Desoky *et al.,* 2017). Blood serum was used for measurement of lipid profile. Analysis of lipid profile was performed by use of biochemical analyser with commercially available Beacon laboratory kit.

Statistical analysis

The statistical analysis was done using SPSS software. Mean and standard deviation were descriptive measures of quantitative data using ANOVA, followed by the post hoc Tukey test for multiple comparisons of mean. P values <0.05 were considered significant.

Results and Discussion

Table 1 and 2 illustrates the changes in serum lipid profile for the control and experimental group of albino rats. Rats administered with metanil yellow (group II) showed significant increase in TC, TG and LDL and decrease in HDL after 12 and 24 weeks of exposure as compared to control. Concentration of serum TC, TG and LDL of rat exposed to MY (group II) were significantly higher by 22.84%, 32.63 % and 68.35% after 12 weeks of exposure period and 76.00%, 91.63% and 164.18% higher after 24 weeks of exposure period respectively as compared to control (group I). The concentration of HDL decreased by 21.64% after 12 weeks of exposure period and 25.61% after 24 weeks of exposure period as compared to control. The altered lipid profile is significantly different in comparison to control ($p<0.05$).

Table 1: Effect of garlic and turmeric extract on lipid profile of albino rats exposed to metanil yellow for 12 weeks.

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (Negative control)	133.36±0.98[a]	117.87±5.36[a]	49.01±1.62[c]	65.29±0.96[a]
Group II (Positive control)	163.83±1.12[d] (+22.84%)	156.34±7.31[c] (+32.63%)	38.40±1.66[a] (-21.64%)	109.92±1.72[d] (+68.35%)
Group III (MY+GE)	140.41±1.13[b] (+5.28%)	132.16±1.47[b] (+12.12%)	44.19±0.95[b] (-9.83%)	78.34±0.74[b] (+19.98%)
Group IV (MY+TE)	151.27±0.54[c] (+13.43%)	138.56±0.88[b] (+17.55%)	40.81±1.02[a] (-16.73%)	83.35±3.05[c] (+27.66%)

Table 2: Effect of garlic and turmeric extract on lipid profile of albino rats exposed to metanil yellow for 24 weeks

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (Negative control)	125.20±1.57[a]	112.64±1.60[a]	44.07±1.00[b]	60.95±1.14[a]
Group II (Positive control)	220.36±0.60[d] (+76.00%)	215.86±1.16[d] (+91.63%)	32.78±1.48[a] (-25.61%)	161.02±1.41[d] (+164.18%)
Group III (MY+GE)	170.25±0.68[b] (+35.98%)	180.75±2.05[c] (+60.46%)	42.44±1.37[b] (-3.69%)	108.78±1.00[b] (+78.47%)
Group IV (MY+TE)	191.17±0.99[c] (+52.69%)	162.07±0.89[b] (+43.85%)	41.86±1.17[b] (-5.01%)	122.99±1.66[c] (+101.78%)

Values are expressed as Mean±SD, Data were analysed by one way ANOVA followed by Tukey's test, P values <0.05 were considered significantly different from control. Value in parentheses represent percent change with respect to control.

Ameliorative effect of garlic extract on lipid profile

When albino rats were supplemented with garlic extract @250mg/kg bw after administration of metanil yellow (group III) then significantly lesser concentration of TC, TG and LDL by 14.29 %, 15.46 % and 28.72% were reported after 12 weeks of exposure period and 22.74%, 16.26% and 32.44% after 24 weeks of exposure period as compared to rats exposed to MY (group II). However, these concentrations of TC, TG and LDL showed recovery and remained only 5.28%, 12.12% and 19.98 % higher after 12 weeks (Figure 1) and 35.98%, 60.46% and 78.47% higher after 24 weeks exposure period as compared to control (Figure 2). The mean conc. of HDL in rats fed MY along with GE showed higher conc. (42.44±1.37) than that in rats fed metanil yellow (32.78±1.48) and became nearly equivalent to that of control (44.07±1.00) after 24 weeks of exposure (Figure 2). All values were found to be significant at $p<0.05$.

Ameliorative effect of turmeric extract on lipid profile

When albino rats were supplemented with turmeric extract @250 mg/kg bw after administration of metanil yellow (group IV) then significantly ($p<0.05$) lesser concentration of TC, TG and LDL by 7.66%, 11.37% and 24.17% were reported in serum after 12 weeks of exposure period as compared to rats exposed to MY (group II). These concentrations in serum were decreased by 13.24 %, 24.93% and 23.62 % in comparison to rats exposed to MY (group II) after 24 weeks of exposure period. However, treatment with turmeric extract showed lesser recovery and concentration of studied parameters remained only 13.43 %, 17.55% and 27.66 % higher after 12 weeks and 52.69 %, 43.88 % and 101.78% higher after 24 weeks exposure period as compared to control (group 1) (Figure1 &2). The mean conc. of HDL in rats fed MY along with TE showed higher conc. than that in rats fed metanil yellow (Group II) i.e., 40.81±1.02 and 41.86±4.74 after 12- and 24-weeks exposure period respectively.

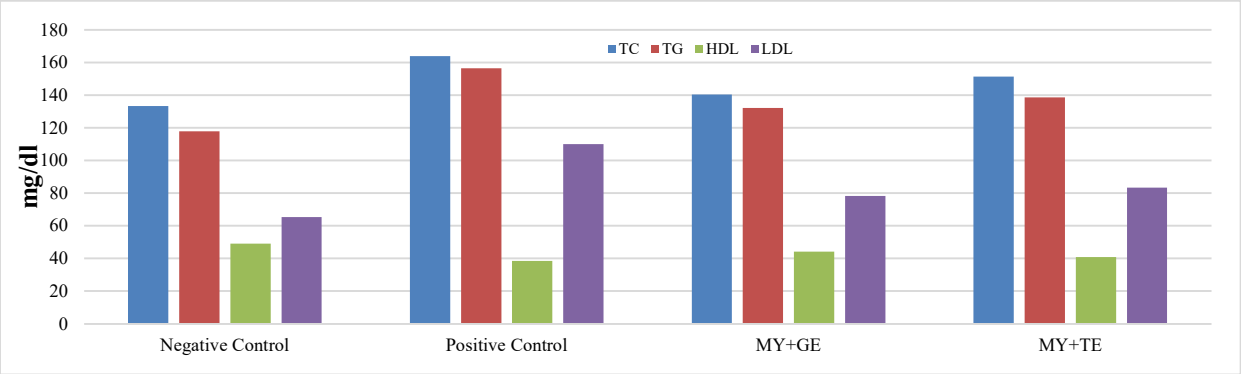

Figure 1: Effect of garlic and turmeric extract (250mg/kg bw) on lipid profile in comparison with negative control and positive control group of albino rats after 12 weeks of exposure.

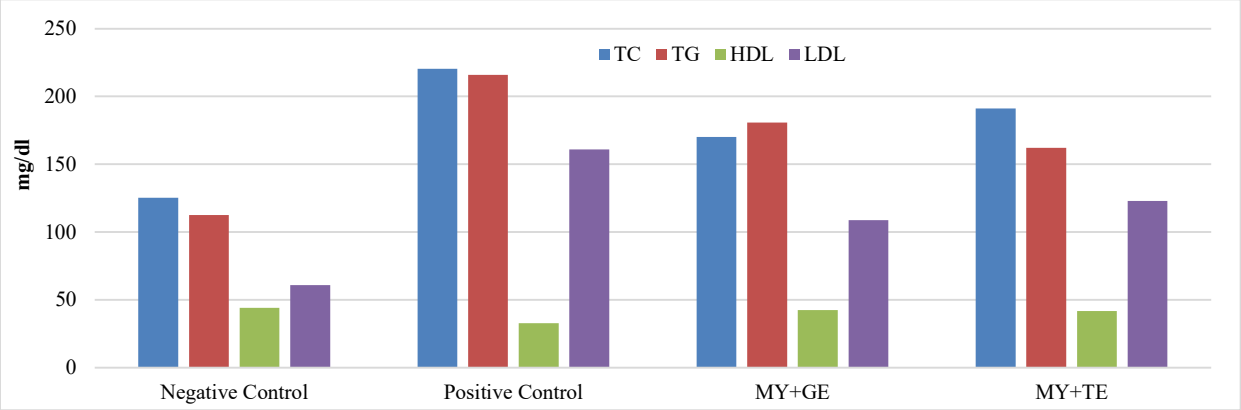

Figure 2: Effect of garlic and turmeric extract (250mg/kg bw) on lipid profile in comparison with negative control and positive control group of albino rats after 24 weeks of exposure.

Altered lipid profile in blood serum observed in rats fed with common azo dye during this study also agree with other studies (Saxena and Sharma, 2015; El-Desoky *et al.,* 2017; Reza *et al.,*2019). Sharma *et al.* (2009) and Bahnasy and Ragheb (2020) reported changes in lipid profile of rats fed with other food colours also viz., sunset yellow and chocolate brown. Significantly greater concentration of TC and TG has also been reported in rats when administered to fast green orally (Turley 2004; Ashour and Abdelaz, 2009). So, changes in lipid profile on exposure to azo dye in albino rats were observed by other workers, which favours the present study. In current study, when aquatic extract of garlic and turmeric (@ 250 mg/kg bw) was given to rats fed with metanil yellow, it successfully normalized concentration of lipid which indicates that these plant extracts were able to reduce toxicity caused by food colours. Other researchers also studied that plant extracts could be effective means for reducing toxicity caused by food colours. Bahnasy and Ragheb (2020) studied ameliorative effect of *Chenopodium quinoa* for toxicity caused by chocolate brown and sunset yellow and observed significant decrease in TC, TG and LDL and significant increase in HDL treated with its extract along with food colour. Yanam *et al.,* (2020) also reported that quinoa could ameliorate the hyperlipidaemic condition induced by high fat diet. Ebrahimi *et al.* (2015) studied that dietary supplementation with garlic extract could be used as remedy for hypercholesterolemia. These data are in the line of present study where turmeric and garlic extract both showed ameliorative effect on lipid profile of albino rats fed on metanil yellow.

Conclusion

In current study, albino rats fed on metanil yellow dye in the diet for 12 and 24 weeks showed increase

in the serum TC, LDL, TG and decrease in serum HDL. Administration of garlic and turmeric extract in metanil yellow fed albino rats caused significant improvement in their serum lipid profile. Garlic extract is found to be more potent than turmeric in rescuing concentration of serum total cholesterol, low density lipoprotein, high density lipoprotein and triglycerides to values near to those of control. Artificial food colours are consumed by children and adults without realising its bad effect on health. So, it is necessary to evaluate natural herbal products which can be used in diet once daily to get rid of from chronic toxicity caused by food colours.

Conflict of interest

The authors declare that they have no conflict of interest.

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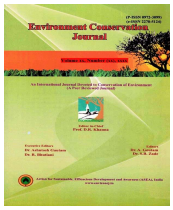

Impact of gamma irradiation on vegetative growth of Gladiolus cv. White prosperity

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ABSTRACT

The present research was carried out at Experimental Farm, Department of Agriculture, Mata Gujri College, Fatehgarh Sahib, Punjab during winter season of 2020-21. The experiment was laid out in randomized block design with seven treatments (Control, 20gy, 40gy, 60gy, 80gy, 100gy and 120gy) and the treatments were replicated thrice. From the experiment it can be concluded that the lower dose (20gy) of gamma irradiation show positive result on growth i.e. maximum plant height (90.03 cm), size of leave (28.44 cm) and take less number of days to sprout (12.65 days) and flowering of Gladiolus cultivar White Prosperity. As the dose of gamma irradiation increases (60gy-120gy), it affect the vegetative characteristic like days taken to sprouting, plant height, number of leaves, size and length of longest leaf, it reduces with the dose increase, but it has no effect on number of sprouts/corm and on size of corm.

Introduction

Gladiolus is a glamorous flower and known for its perfection and known as Queen of the bulbous plants due to its magnificent spikes containing massive form of florets, elegant florets of different shade, attractive shapes, varying in size and marvelous vase life. It is very important ornamental commercial flower cultivated in various parts around the world and most diverse in South Africa, where they originated. Botanically gladiolus belongs to family Iridaceae and propagated through underground corms. Gladiolus improvement through breeding began in England around the beginning of the 18th century. Plant breeding program set a main goal to form variability and to choose the leading recombinants possessing desirable features. Because variations occurred in phenotypic features like Chlorophyll variation in leaves, shade of bloom, shape or size can be easily recognized. Mutation breeding has been successful in ornamental plants. Artificial mutations are used exclusively in mutation breeding. Induced Mutation could be one of the methods for creating hereditary variability.Colchicine therapy, recurrent irradiation, ion beam technology, combined treatment split dosage, space breading, and other unique treatment techniques have been wisely chosen for the effective advancement of novel cultivars (Datta 2012).Gamma rays have shown to be the most successful method of induced mutation, resulting in the creation of one of the many new decorative varieties.

Material and Methods

Present research was carried out during October-March (2020-2021). Experiment was conducted at Research farm, Mata Gujri College, Fatehgarh Sahib, Punjab. Land was brought to a good tilth with the help of plougher and then levelling. Well

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decomposed farm yard manure incorporated in the field at the time of bed preparation. The experiment was laid out in Randomized Block Design (RBD) with three replications. During the month of October, after treatment with gamma rays in the gamma chamber at a distance of 30×30cm, the uniform size (3-5cm) of corms was placed in the beds. Corms of white prosperity variety were exposed to various gamma doses i.e. 20, 40, 60, 80, 100 and 120gy. Total number of plots were 21 and size of each plot was 2×2m and number of plants per plot was 16.

Results and Discussion

Most of the plants who propagated vegetatively and no variation found in them because they are identical to mother plant can also be brought into change with mutation breeding and gladiolus is one of them. Although many varieties in gladiolus created through mutation breeding and gamma rays found to be an effective mutagen among others. Gladiolus is famous for its elegant attractive spike and long lasting keeping quality and that's why cultivated commercially in various regions of India and in foreign countries. Mutation breeding bring change in flower color, size and shape of the plant and create variations in leaves. So on that account present research carried in the Department of Agriculture.Most of the growth parameters significantly influenced because of the application of gamma irradiation at different levels in corms of gladiolus. Numerous doses of gamma irradiation affected the days to sprouting and gave significant results in sprouting. Early sprouting showed in control and 20gy gamma dose. Late sprouting recorded in higher doses (80gy-100gy), as the level of dose increases, the corm takes more time to sprout but it failed to exert any effect on number of sprouts per corm. Less number of sprouts found at higher dose (120gy). An amazing results of gamma dose 20gy found in the number of leaves per corm. Significant results found among various doses of gamma irradiation and also in leaf length and size significant results to be brought because of radiation. As the dose increases the length and especially the width of the leaf decreases and therefore size of leaf is influenced due to application of gamma irradiation.Early sprouting did not respond well to various doses of mutagens, although physical mutagens have an impact on enzyme activity. Enzymes play an important role in numerous plant metabolism processes, resulting in plant growth stimulation, with several growth parameters increasing as gamma irradiation levels increased (Xing *et al*. 2011 on *Catharanthus roseus*, Sahariya *et al*. 2017 on *Gladious hybridus*). Auxin has been shown to play a role in plant development. Growth is stimulated by changes in auxin levels, probably due to inactivation of auxin or destruction of enzymatic activity or due to secondary physical damage, reduction in mitotic activity & chromosomal damage occur or suppression of auxin synthesis (Sathyanarayan *et al*. 2019). At lower doses of gamma irradiation, various growth parameters increased; however as dose of gamma irradiation increased, they decreased. It could be related to increased gibberellins enzyme activity and auxin disappearance of inhibitors, and it could be due to some enzyme activities. The current findings are also experimentally corroborated by previous findings (Kakri *et al*. 2010). Height of plant, leave length and number may have decreased as a result of a drop in the number of vertical cell layers, which resulted in shorter internodes, or a combination of these events. The morphological findings of this experiment corroborated with Patil *et al*. (2010), Singh and Kumar (2013) findings, which stated that most of the growth features were stimulated at lower dosages of gamma radiation. Higher dosages of gamma rays were not shown to be effective in improving plant development in different gladiolus cultivars while lower doses (2 and 3kR) can be effected.

Various researchers found that gamma rays have a significant impact on Gladiolus and other ornamental plants′ growth characteristics. A research conducted by Sathyanarayana *et al*. (2019) on cultivars of *Gladiolus grandiflorus* L. viz. Summer Sunshine, Candy Man, Saffron, Dull Queen and American Beauty, treating corms with gamma rays at 15, 25, 35, 45 and 55gy doses. Cultivars Candy Man and Saffron were found to be influenced by gamma doses. These two kinds have a higher mortality rate than the others. Patil (2011) conducted a detailed investigation on 3 cvs. Eurovision, Nova lux and American beauty in order to create genetic variability. Gamma radiation from

Table 1- Effect of gamma irradiation on different vegetative parameters of Gladiolus cultivar White Prosperity

Treatment doses	Days to sprouting	Plant height (cm)	Number of sprouts per corm	Number of leaves per corm	Length of longest leaf (cm)	Size of longest leaf (cm)
T_1 (Control)	12.50	76.35	1.66	7.98	54.03	28.00
T_2 (20gy)	12.65	90.03	1.47	7.96	54.68	28.44
T_3 (40gy)	13.57	61.46	1.51	6.74	45.70	23.73
T_4 (60gy)	14.43	64.58	1.67	6.94	47.50	24.61
T_5 (80gy)	18.00	41.17	1.42	5.49	37.49	18.48
T_6 (100gy)	14.24	36.42	1.61	5.17	33.65	17.27
T_7 (120gy)	17.78	24.17	1.22	4.86	24.17	12.41
SEm±	0.97	3.84	0.12	0.40	1.87	1.01
$CD_{(0.05)}$	3.00	11.83	NS	1.23	5.75	3.12

1-7kR was used to treat corms. Treatments ranging from 1-3kR of gamma dosages were too low to have an unfavorable impact, and in some cases were even demonstrated stimulating, whereas treatments ranging from 4kr and beyond lowered and slowed most Gladiolus vegetative measures.

Due to doses of gamma rays plant height was affected in the current investigations (Table1). As the doses of gamma irradiation increased, so did the reduction in leaf width. Inhibition or delay in mitosis is thought to be primary cause of growth of plant, leaf size and other vegetative characteristics. The similar thought was expressed by Singh and Kumar (2013), Kumari and Kumar (2015), Sahariya *et al*. (2017). Auxin and DNA synthesis are likely to be involved. The presented experiment findings are likewise consistent with observation of Tiwari *et al*. (2010) who noticed that applying gamma doses to the Gladiolus resulted in lower plant height and shorter leaf length and width. They also discovered that as gamma irradiation doses increased, plant growth in gladiolus declined; however, in the current study, plant height grew at lower doses and decreased at higher doses of gamma rays. Singh and Kumar (2013), Tiwari *et al*. (2018) used gamma doses to treat gladiolus corms and then planted them, finding that different gamma dosages resulted in narrow leaves. At large levels of gamma irradiation, this effect became more pronounced. These findings near to the finding on Gladiolus by Sisodia *et al*. (2015), who found that higher doses of gamma rays resulted in reduced plant growth and a reduction in leaf size in Gladiolus.

Conclusion

The present study concludes that gamma irradiation at a lower dose (20 gy) has a good effect on Gladiolus cultivar White Prosperity growth and flowering. As the dose of gamma irradiation increases, it affects the growth characteristic like height, size of leaf, days to sprout etc.

Conflict of interest

The authors declare that they have no conflict of interest.

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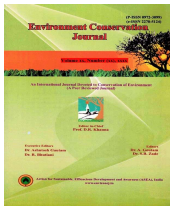

Bioefficacy and economics of certain new molecule of insecticides against Gram pod borer, *Helicoverpa armigera* (Hübner) in chickpea

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ABSTRACT

Gram pod borer (*Helicoverpa armigera*) is a major insect pest of chickpea. The gram pod borer begins to infest at vegetative stage and later feeds on flowers and developing pods. A field investigation was conducted to evaluate the bioefficacy of certain new molecule insecticides against *Helicoverpa armigera* (Hübner) on chickpea during *Rabi* 2020-21 in randomized block design with three replications. The outcomes revealed that the application of Chlorantraniliprole 18.5% SC @ 25g a.i./ha and Cyantraniliprole 10.26% OD @ 60g a.i./ha were established to be most effective treatments and application of Fipronil 5% SC @ 50g a.i./ha was least effective in respect of reduction of *H. armigera* larval population. The maximum yield was recorded in Chlorantraniliprole 18.5% SC @ 25g a.i./ha (14.00 q/ha) followed by Cyantraniliprole 10.26% OD @ 60g a.i./ha (13.73 q/ha) and lowest yield was recorded from Novaluron 75g a.i./ha (10.15 q/ha) treated plot. The economics of different new molecule insecticides indicated that higher benefit cost ratio (BCR) was observed from Lambda Cyhalothrin 30g a.i./ha (7.86:1) followed by Emamectin benzoate 12g a.i/ha (6.75:1) and the lower BCR was recorded from Cyantraniliprole 60g a.i./ha (1.64:1) and Novaluron 75g a.i./ha (1.58:1). Chlorantraniliprole and Cyantraniliprole are newer group of insecticides, which are relatively safer and more effective against gram pod borer as comparison to conventional insecticides and can be used in successful management of this key pest of chickpea.

Introduction

Pulses are dry seeds of plants which belongs to Leguminosae family. Pulses are source of protein, amino acids and have other medicinal properties. Production and consumption of higher amount of pulses are the best way to overcome spread of protein malnutrition in world. In 2016, United Nations General Assembly (UNGA) celebrated as International Year of Pulses (IYP) to generate awareness in food security and several benefits of protein and also about sustainable foods production for small holder farmers (Anonymous, 2016). In India over dozens of pulse crops grown, however Chickpea (*Cicer arietinum* L.) is the third most important pulses crop after dry beans and field pea. It is commonly known as Bengal gram, chana or gram, originated from South Western Asia. It is an important *Rabi* pulse crop of India, and considered as 'King of Pulses' due to its nutritional values and high demand (Bhatt and Patel, 2001). Chickpea highly fix more than 80 per cent of atmospheric nitrogen in association with *Rhizobium* spp. India leads top rank in area and production of chickpea. In India, chickpea occupies 107.21 lakh hectare area and producing 9.02 million tons with 895

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kg/ha productivity (Anonymous, 2020). Madhya Pradesh ranks highest in chickpea production (32.37%) followed by Rajasthan (19.46%), Maharashtra (15.82%), Andhra Pradesh (8.76%) and Uttar Pradesh (6.45%) and these states contributing 82% of total production of country (Naik *et al.,* 2018). Insect pests are one of the major limiting factors for production of chickpea. In India, gram pod borer (*Helicoverpa armigera* Hübner) (Noctuidae, Lepidoptera) is a major pest of chickpea. The gram pod borer begins to infest at vegetative stage and later feeds on flowers and developing pods until crop maturity, where pod borer caused 60 to >90 per cent losses in seeds/grains yield under favourable conditions throughout the India (Anonymous, 2013; Patil *et al*., 2017). Due to the feeding preference of the *H. armigera* larvae on the plant parts that are rich in protein content and reproductive parts of growing plants, e.g. flowers, pods, cotton bales and buds results in a reduction in the crop yield. The Indian farmers mostly rely on insecticides for the management of insect pests' infestation because; agrochemicals are considered as the last recline for management due to their quick knockdown effect. Over dependence on a particular group of chemicals is one of the important reasons for the rapid development of resistance and hazards to the environment and human health, among the several avenues to overcome the insecticidal resistance and environmental problems, replacement with the new molecules of insecticide is one of the important considerations (Gill and Garg, 2014). Keeping these facts in mind the present investigation was planned and conducted to find out the reliable and cost effective source for the management of gram pod borer in chickpea.

Material and Methods

The present experiment was conducted under field conditions at Students' Instructional Farm, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during *Rabi* 2020-21 on chickpea cultivar of PUSA-262 in Randomized Block Design (RBD) with 10 treatments and 3 replications. The unit plot size kept 1.50×2.50m of each with line to line 30 cm spacing and plant to plant spacing 10cm. The observation on *H. armigera* larval population was taken on mean larval population per metre row length basis. The larval population of *H. armigera* was recorded at a day before spraying and 3, 7 and 15 days after application of treatments at each spraying. The Benefit-Cost Ratio worked out for each treatment on the basis of additional return over control in terms rupees and cost of insecticidal spray in each treatment The data obtained were analyzed statistically to compare the treatment effects for randomized block design (Panse and Sukhatme, 1961).

Table 1: Details of different insecticides and their source used in the present investigation

Treatments	Chemical name	Trade name	Strength of pesticide	Dose of Insecticides (g/ml) or Concentration (%) dose/ha	Source of availability
T1	Spinosad	Tracer	45% SC	60g a.i	Dow Agro Science
T2	Chlorantraniliprole	Coragen	18.5% SC	25g a.i.	FMC India Private Limited
T3	Emamectin benzoate	Emagold	5% SG	12g a.i	Alfa Crop Science, Raipur (C.G.)
T4	Flubendaimide	Fame	39.35% SC	60g a.i	Bayer Crop Science Limited, Mumbai
T5	Cyantraniliprole	Benevia	10.26% w/w OD	60g a.i	FMC India Private Limited
T6	Indoxacarb	Isacarb	14.5% SC	60g a.i	Isagro Agrochemicals Private Limited
T7	Lambda Cyhalothrin	Karate	5% SC	30g a.i.	Syngenta Agrochemicals Limited
T8	Novaluron	Rimone	10% EC	75g a.i.	Indofil Industries Limited
T9	Fipronil	Regent	5% SC	50g a.i.	Bayer Crop Science Limited, Mumbai
T10	Control (Water spray)	-	-	500 L	-

Results and Discussion

Bioefficacy of certain new molecule of insecticides against larval population *H. armigera*

The initial count of *H. armigera* larvae revealed that the pest population was distributed homogenously throughout the experimental field a day before application of treatments on the crop during the *Rabi* 2020-21 (Table 1 and Figure 1 & 2).

First spray

Pre-treatment observation was recorded a day before the application of insecticides, which revealed the uniform distribution of pod borer in the field. The data pertaining efficacy of the first spray was obtained and presented in Table 1 and Figure 1 indicates that the population a day before was ranged from 5.11 to 6.00 larvae/mrl. The data obtained from 3 DAS (Days after spray) revealed that reduction in larval population was recorded in all treated plots in comparison to the untreated plot. However, among all the treatments the minimum larval population was found in treatment T_2- Chlorantraniliprole 25g a.i./ha (3.00 larvae/mrl) followed by the treatment T_5- Cyantraniliprole 60g a.i./ha (3.33 larvae/mrl) and highest in treatment T_7- Lambda Chylothrin 30g a.i./ha (4.67 larvae/mrl). The observation recorded at 7 DAS revealed that the minimum population was found in T_2- Chlorantraniliprole 25g a.i./ha (0.78 larvae/mrl) followed by T_5- Cyantraniliprole 25g a.i./ha (0.89 larvae/mrl), and maximum in the treatment T_9- Fipronil 50g a.i./ha (2.44 larvae/mrl). The data noted at 15 DAS depicted that all the treatments were significantly superior to over control and treatment T_2- Chlorantraniliprole 25g a.i./ha (1.11 larvae/mrl) was the most effective treatment recorded the lowest population over control followed by T_5-Cyantraniliprole 60g a.i./ha (1.22 larvae/mrl) and treatment T_9- Fipronil 50g a.i./ha (3.22 larvae/mrl) least effective treatment recorded the highest population over control. The overall mean population of 3, 7 and 15 DAS indicate that all treated plots were significantly outperformed over control. However, among the all treatments minimum larval population was found in T_2- Chlorantraniliprole 25g a.i./ha (1.63 larvae/mrl) and T_5- Cyantraniliprole 60g a.i./ha (1.81 larvae/mrl), which were most effective treatments in reducing the larval populations and T_9- Fipronil 50 a.i./ha (3.22 larvae/mrl) had maximum population.

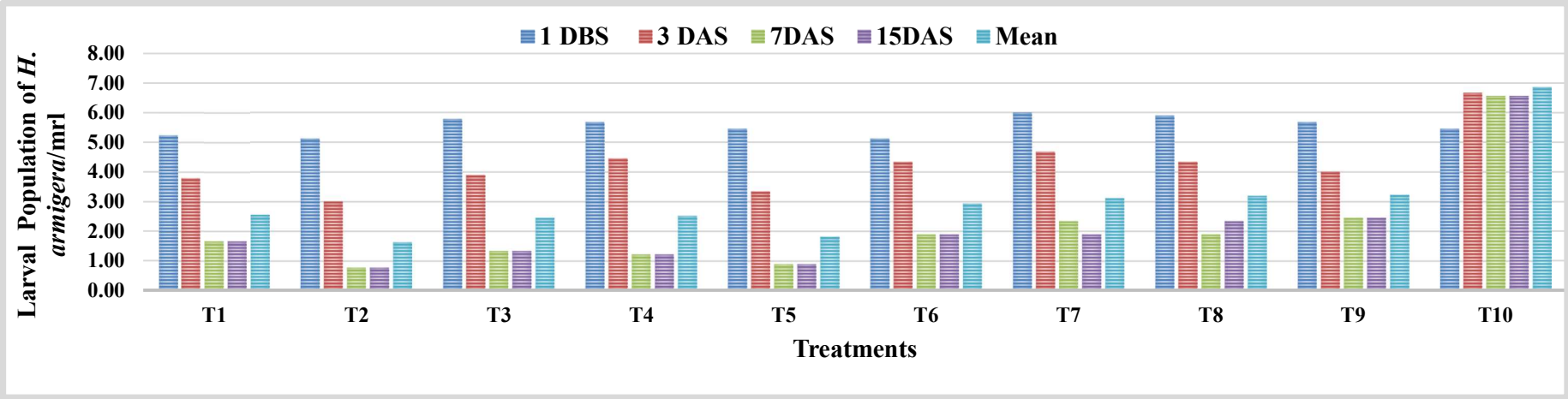
Second spray

The data pertaining population recorded a day before second spray varied in the range of 5.67 to 6.00 larvae mrl^{-1} (Table 1 and Figure 2). The data recorded at 3 DAS revealed that minimum larval population was recorded in T_2-Chlorantraniliprole 25g a.i./ha (3.33 larvae/ mrl) followed by T_5-Cyantraniliprole 60g a.i./ha (3.67 larvae/mrl) and minimum population was found in T_6-Indoxacarb 60g a.i./ha (5.11 larvae/mrl).The data noted at 7 DAS depicted that the lowest population was found in T_2-Chlorantraniliprole 25g a.i./ha (1.33 larvae/mrl) followed by T_5-Cyantraniliprole 60g a.i./ha (1.44 larvae/mrl) and highest population was found in T_9-Fipronil 50g a.i./ha (3.44 larvae/mrl). At 15 DAS that the minimum population was recorded in T_2-Chlorantraniliprole 25g a.i./ha (1.44 larvae/mrl) followed by T_5-Cyantraniliprole 60g a.i./ha (1.56 larvae/mrl) and minimum population was found in T_9-Fipronil 50g a.i./ha (3.56 larvae/mrl). The records on overall insecticidal efficacy revealed that the treatments were statistically superior to control. The overall population after second spraying indicated that treatment T_2-Chlorantraniliprole 25g a.i./ha (1.96 larvae/mrl) was superior to the remaining treatments followed by T_5-Cyantraniliprole 60g a.i./ha (1.15 larvae/mrl), whereas treatment T_9-Fipronil 50g a.i./ha (3.56 larvae/mrl) was least effective treatment after second insecticidal spray. The results are in conformity with the Chitralekha *et al.* (2018) who tested Novaluron 10 % EC @ 375 ml/ha, quinalphos 25 % EC @ 1000 ml/ha, Chlorantraniliprole 18.5 % SC @ 135 ml/ha, Lambda- Cyhalothrin 5 % EC @ 500 ml/ha, and emamectin benzoate 5 % SG @ 220 g/ha against gram pod borer at the population of larvae reached at economic threshold, *i.e*. l larvae/mrl on chickpea. All the treatments had resulted significantly better than untreated control; Chlorantraniliprole (18.5% SC) had the highest per cent larvae reduction compared to control (85.68%). The similar results also reported by Rani *et al.* (2018) who found that Emamectin benzoate 5% SG, Flubendamide 20% WG, Chlorantraniliprole 20% SC, Thiodicarb 75% WP, Indoxacarb 14.5% SC, Novaluron 10% EC were effective against the larval population of *H.*

Table 1: Efficacy of certain new molecule of insecticides against gram pod borer, *H. armigera* infesting chickpea during *Rabi* 2020-21

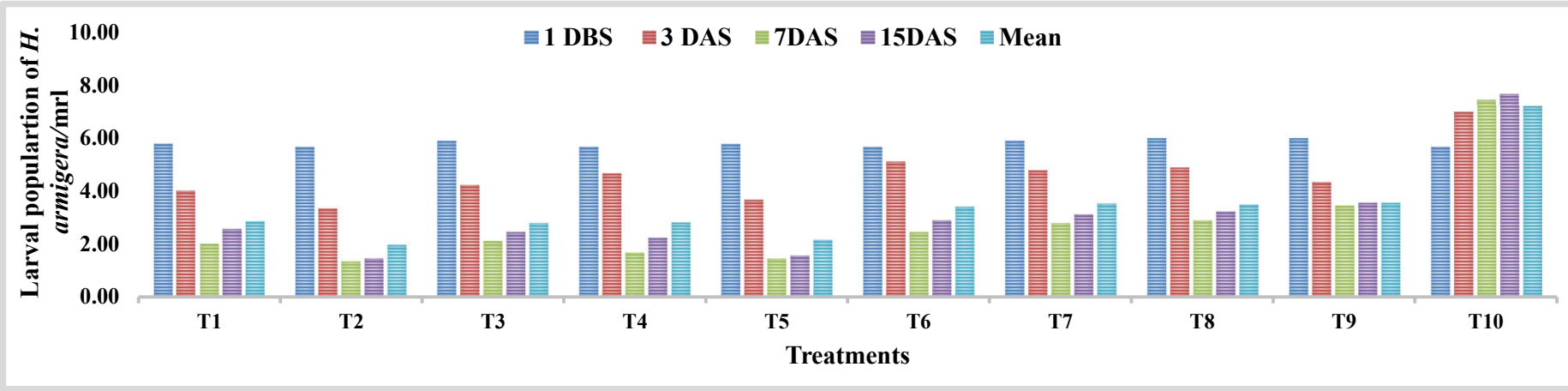
Tr. No.	Treatments	Dose/ha	*Mean larval population of *H. armigera* per metre row length										**Pod damage (%)
			First Spray					Second Spray					
			DBS	3 DAS	7 DAS	15 DAS	Mean	DBS	3 DAS	7 DAS	15 DAS	Mean	
T_1	Spinosad	60g a.i	5.22 (2.39)	3.78 (2.07)	1.67 (1.47)	2.22 (1.65)	**2.56 (1.75)**	5.78 (2.51)	4.00 (2.12)	2.00 (1.58)	2.56 (1.75)	**2.85 (1.83)**	12.00 (20.77)
T_2	Chlorantraniliprole	25g a.i.	5.11 (2.37)	3.00 (1.87)	0.78 (1.13)	1.11 (1.27)	**1.63 (1.46)**	5.67 (2.48)	3.33 (1.96)	1.33 (1.35)	1.44 (1.39)	**1.96 (1.57)**	2.00 (8.13)
T_3	Emamectin benzoate	12g a.i.	5.78 (2.51)	3.89 (2.09)	1.33 (1.35)	2.11 (1.62)	**2.44 (1.72)**	5.89 (2.53)	4.22 (2.17)	2.11 (1.62)	2.44 (1.72)	**2.78 (1.81)**	9.33 (17.79
T_4	Flubendiamide	60g a.i.	5.67 (2.48)	4.44 (2.22)	1.22 (1.31)	1.89 (1.55)	**2.52 (1.74)**	5.67 (2.48)	4.67 (2.27)	1.67 (1.47)	2.22 (1.65)	**2.81 (1.82)**	7.33 (15.21)
T_5	Cyantraniliprole	60g a.i	5.44 (2.44)	3.33 (1.96)	0.89 (1.18)	1.22 (1.31)	**1.81 (1.52)**	5.78 (2.51)	3.67 (2.04)	1.44 (1.39)	1.56 (1.43)	**2.15 (1.63)**	4.67 (12.48)
T_6	Indoxacarb	60g a.i.	5.11 (2.37)	4.33 (2.20)	1.89 (1.55)	2.56 (1.75)	**2.93 (1.85)**	5.67 (2.48)	5.11 (2.37)	2.44 (1.72)	2.89 (1.84)	**3.41 (1.98)**	12.00 (20.27)
T_7	Lambda Cyhalothrin	30g a.i	6.00 (2.55)	4.67 (2.27)	1.89 (1.55)	2.78 (1.81)	**3.11 (1.90)**	5.89 (2.53)	4.78 (2.30)	2.78 (1.81)	3.11 (1.90)	**3.52 (2.00)**	15.33 (23.05)
T_8	Novaluron	75g a.i	5.89 (2.53)	4.33 (2.20)	2.33 (1.68)	2.89 (1.84)	**3.19 (1.92)**	6.00 (2.55)	4.89 (2.32)	2.89 (1.84)	3.22 (1.96)	**3.48 (2.00)**	14.67 (22.52
T_9	Fipronil	50g a.i	5.67 (2.48)	4.00 (2.12)	2.44 (1.72)	3.22 (1.93)	**3.22 (1.93)**	6.00 (2.55)	4.33 (2.20)	3.44 (1.99)	3.56 (2.01)	**3.56 (2.01)**	18.00 (25.10)
T_{10}	Control (Water Spray)	500 L	5.44 (2.44)	6.67 (2.68)	6.56 (2.66)	7.33 (2.80)	**6.85 (2.71)**	5.67 (2.48)	7.00 (2.74)	7.44 (2.82)	7.67 (2.86)	**7.22 (2.78)**	24.67 (29.78)
S. Em±			**0.04**	**0.07**	**0.03**	**0.04**	**0.08**	**0.03**	**0.08**	**0.06**	**0.03**	**0.04**	**(0.63)**
CD at 5%			**-**	**0.22**	**0.11**	**0.12**	**0.24**	**-**	**0.25**	**0.20**	**0.11**	**0.13**	**(1.89)**

Figures in the parenthesis are $\sqrt{x+0.5}$ transformed values, **Figures in the parenthesis are Arcsine transformed values, DBS= Day before spray, DAS= Days after spray, *Mean of three replications



First Spray

Figure 1: Effect of certain new molecule of insecticides on gram pod borer, *H. armigera* during *Rabi* 2020-21.



Second Spray

Figure 2: Effect of certain new molecule of insecticides on gram pod borer, *H. armigera* during *Rabi* 2020-21.

Table 2: Economics of certain new molecule of insecticides during *Rabi* 2020-21

Tr. No.	Treatments	Dose/ha	Quantity of insecticide formulation/ha	Cost of one Spray (labour+ Sprayer+ insecticide)/ha)	No. of sprays	Total cost of spraying /ha	Yield (q/ha)	Additional yield over control (q/ha)	Total return /ha)	Net return /ha	B:C ratio	Rank
T_1	Spinosad	60g a.i.	133mL	4454	2	8908	12.80	5.50	28050	19142	2.14	**VII**
T_2	Chlorantraniliprole	25g a.i.	135mL	3225	2	6450	14.00	6.70	34170	27720	4.29	**III**
T_3	Emamectin benzoate	12g a.i.	240g	1790	2	3580	13.10	5.80	29580	26000	6.75	**II**
T_4	Flubendiamide	60g a.i.	152mL	3907	2	7814	13.30	6.00	30600	22786	2.91	**V**
T_5	Cyantraniliprole	60g a.i.	584mL	6190	2	12380	13.73	6.43	32793	20413	1.64	**VIII**
T_6	Indoxacarb	60g a.i.	413mL	2498	2	4996	12.10	4.80	24480	19484	3.89	**IV**
T_7	Lambda Cyhalothrin	30g a.i.	600mL	854	2	1708	10.27	2.97	15147	13439	7.86	**I**
T_8	Novaluron	75g a.i.	750mL	2810	2	5620	10.15	2.85	14535	8915	1.58	**IX**
T_9	Fipronil	50 a.i.	1000mL	2450	2	4900	10.80	3.50	17850	12950	2.64	**VI**
T_{10}	Control (Water Spray)	500 L	-	-	-	-	7.30	-	-	-		

BCR= Benefit Cost Ratio, Minimum support price of chickpea during 2020-21 = Rs. 51/kg, Labour charge = Rs. 300/day/labour, Sprayer charge: 50/day

armigera. Similarly, Upadhyay *et al*. (2020) also reported that the highest efficacy of insecticide after the spray was found in T_3-Chlorantiniprole 18.5 SC 92g a.i. ha^{-1} (63.05%) and the lowest overall % efficacy was registered in T_8-Acephate 75 WP 750 g a.i. ha^{-1} (30.04%).

Effect of certain new molecule of insecticides on pod damage

The efficacy of insecticides was tested in terms of pod damage in the field trial for the *Rabi* 2020-21 (Table 1). The respective results show that each of the individual treatments was significantly efficient than the control. The best result in terms of minimum pod damage was shown by treatment T_2-Chlorantraniliprole 25g a.i./ha (2.00%) followed by T_5- Cyantraniliprole 60g a.i./ha (4.67%) whereas maximum pod damage was recorded from T_9-Fipronil 50g a.i./ha (18.00%) and T_7-Lambda Chylothrin 30g a.i./ha (15.33%). The results are in conformity with the Upadhyay *et al.* (2020) who found that the lowest pod damage was recorded in the treatment (4.67%) followed by T_6-Flubendamide 39.35 EC 49g a.i./ha (5.33%). Rani *et al.* (2018) reported that application of Chlorantraniliprole in red gram had lowest pod damage caused by gram pod borer.

Effect of certain new molecule of insecticides on yield chickpea

The study made on the effect of insecticidal treatments on yield is shown in Table 2. All treatments showed superior with less pod damage compared to untreated control. Among all treatments the minimum pod damage was 2 per cent with highest yield of chickpea pods (14.00 q/ha) was recorded in T_2-Chlorantraniliprole 25g a.i./ha. The succeeding best treatment was T_5-Cyantraniliprole 60g a.i./ha 13.73 q/ha yield and next best treatment was T_4-Flubendiamide 60g a.i./ha with 13.30 q/ha yield. Among all the treatments T_8- Novaluron 75g a.i./ha produced minimum yield (10.15 q/ha). The results are in conformity with the Upadhyay *et al*. (2020) who found that the highest yield was recorded in the treatment Chlorantiniprole 18.5 SC 92g a.i./ha (17.33 q/ha) followed by Flubendamide 39.35 EC 49g a.i./ha (16.44 q/ha) and Spinosad 45 SC 74g a.i./ha (15.55 q/ha). Rani *et al.* (2018) found that use of Chlorantraniliprole in red gram produced higher yield against gram pod borer.

Economics of new molecule of insecticides in Chickpea

The data pertaining to economics of various treatments are presented in Table 2. The highest net return was recorded from T_2-Chlorantraniliprole 25g a.i./ha (Rs. 27720) and the minimum in T_8-Novaluron 75g a.i./ha (Rs. 8915). The benefit: cost ratio of different insecticides revealed that T_7-Lambda Cyhalothrin 30g a.i./ha (7.86:1) was the most economical treatment followed by T_3-Emamectin benzoate 12g a.i./ha (6.75:1), T_2-Chlorantraniliprole 25g a.i./ha (4.29:1), T_6-Indoxacarb 60g a.i./ha (3.89:1), T_4-Flubendiamide 12g a.i./ha (2.91:1), T_9-Fipronil 50g a.i./ha (2.64:1), T_1-Spinosad 60g a.i./ha (2.14:1), T_5-Cyantraniliprole 60g a.i./ha (1.64:1) and treatment T_8-Novaluron 75g a.i./ha (1.58:1) was least economical treatment. The present findings are in agreement with Upadhyay *et al*. (2020) who reported that Lambda Cyhalothrin was second most economical treatment after Indoxacarb. Meena *et al*. (2018) also found treatment with Indoxacarb (1:9.52) was highly cost effective treatment in chickpea against gram pod borer.

Conclusion

Application of insecticides for the management of insect pests in agriculture ecosystem is one of the most common activities as insecticides provide good control of insect pests in very short span of time. Foliar spray of Chlorantraniliprole 25g a.i./ha and Cyantraniliprole 60g a.i./ha were the most effective insecticides against *Helicoverpa armigera* with minimum larval population, lowest pod damage and highest yield per hectare. Chlorantraniliprole 25g a.i./ha had highest net return while Lambda Cyhalothrin 30g a.i./ha was most cost effective treatment with highest benefit cost ratio. These insecticides belong to newer group, relatively safer and more effective at lower doses against gram pod borer as comparison to conventional insecticides for management of this key pest of chickpea. The information generated in present study can be suitably incorporated in the management strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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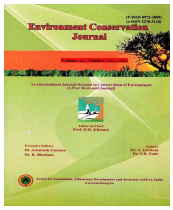

A review on impact of salt stress in soil health and its suitable control measure

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ABSTRACT

Soil salinity is associated with the accumulation of soluble salts in higher concentration deteriorating soil health associated with unfavourable environment for plant growth. It is mostly confined to those regions where there is high temperature and low precipitation, mostly in arid and semi-arid regions. Major factors responsible for soil salinity can be categorised into primary and secondary factor affecting at the spatial and temporal scale. Higher concentration of soluble salts in soil increase the osmotic potential disrupting the movement of water from root to leaf. So, soil salinity is primarily associated with the water stress condition in plants which is a direct impact to plants. Indirectly it interferes with the nutrients absorption which is one of the most important factors for proper plant growth. Plants poses different mechanisms to avoid salt stress condition in soil but maximum of it are an active processes were additional energy must have to spend for it that can impact proper growth and production. The ions primarily responsible for both the soil and plant stress under soil salinity are Na^+ and Cl^- which concentration increases with certain primary and secondary soil salinization factors. So, primary aim to control the impact of soil salinity is to reduce the activity/concentration of both Na^+ and Cl^- from the soil. So, use of the essential nutrients (K^+ and SO_4^{-2}) that has an antagonistic relationship with the salts is a new approach. Due to similar charge and physico chemical properties of K^+ and SO_4^{-2} with toxic ions Na^+ and Cl^- respectively, there lies an antagonistic relationship. Furthermore, SO_4^{-2} of its less toxicity to plants and improve soil pH condition especially in arid and semi-arid region, the combination of K^+ and SO_4^{-2} salt is a good combination to ameliorate the Na^+ and Cl^- toxicity under saline soil.

Introduction

Soil salinization is an important land degradation process that has a significant impact on agriculture restricting crop growth and its production. It refers to the amount of water soluble salts present in soil solution (Rahman *et al.*, 2022). The water soluble salts responsible for soil salinity belongs to neutral salts including calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), chloride (Cl^-) and sulphate (SO_4^{-2}) (Guo *et al.,* 2017). Since neutral salts are involve in soil salinization process, the pH of the soil is always <8.5 but Electrical conductivity (EC) higher than 4 dS m^{-1}. Soil salinization is associated with the accumulation of these soluble salts in higher concentration to the soil making unfavourable environment for crop growth. The soluble salts responsible for soil salinity belongs to essential nutrients except with Na^+ a beneficial nutrient, however with higher concentration of these salts in soil their lies certain negative effect to both soil and plant. Therefore, the problems developed due to soil salinity are primarily related with the salts of Na^+ and Cl^- in soil

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(Li *et al.,* 2019; Rahman *et al.*, 2022; Bhardwaj *et al.*, 2020). Soil salinity level varies from place to place which predominantly depend upon the climatic condition of a region and is mostly confined to those regions where there is high temperature and low rainfall mostly under arid and semi-arid region (Akça *et al.,* 2020). Another important factor for soil salinization turns to be the elevation level of land where lower elevated land has chances of concentrating more level of soluble salts as in coastal region (Celleri *et al.*, 2022). In near future it is expected to affect the world food production more strongly and extensively with increasing distribution of soil salinization. When the salinity level in soil increases more than 60 mM, it is evident that the Na^+ level also increases. Increasing soil salinity has a significant effect on soil properties especially soil structural stability, affecting soil infiltration and permeability and ultimately affecting soil water movement which will impact directly or indirectly to plant growth. But, directly soil salinity will affect the absorption of water by plant roots, impart toxicity and finally retard growth. So, soil salinity is always related with the water stress developed in the vicinity of roots in soil (Arif *et al.,* 2020; Ma *et al.*, 2022).

The absorption of water from soil by plant depends on the potential gradients generated from soil-plant, plant-leaves and leaves-atmosphere. For proper water absorption there must be a decreasing trend of water potential from soil to leaf of a plant. The lowest water potential on leaf surface is achieved due to evapotranspiration processes taking place on the leaf surface where water content is the lowest. This lower water content and potential on plant leaf results in the movement of water from soil to roots through stem and finally to leaf, which maintained a continuum (Goyal *et al.,* 2021). However with higher concentration of soluble salts in soil, the osmotic potential increases reducing the soil water potential of root rhizosphere that breaks the continuum of water potential from root to leaf. An extra energy has to apply by the plant to absorb water against the concentration gradients which results in lower growth of the plant and productivity. Excess amount of soluble salts in the vicinity of the root zone under saline condition will hamper the water absorption by plants resulting in water stress condition (Hussain *et al.,* 2019). The impact of soil salinity can be categorized into direct and indirect effect (Chen *et al.,* 2019). The direct effect includes reduction of water absorption by plant due to disruption of water potential continuum with excess amount of dissolved salts in the soil. Another is the ion toxicity to plant with higher concentration of dissolve salts which is harmful for the proper functioning of a plant. Cl^- toxicity can developed in saline soil and will lead to show certain visual symptoms like leaf burning, dry leaf tissue, stunted growth and in severe cases death of the plant. Indirectly it interferes with the nutrients absorption which is one of the most important factors for proper plant growth. There are certain antagonisms between essential nutrients and the harmful ions during soil salinity (Zörb *et al.,* 2019). With the increase concentration of dissolved salts (mostly Na^+ and Cl^-), Na^+, especially compete (antagonistic effect) with NH_4^+ and K^+; Cl^- with NO_3^- which drastically affect plant growth (Javed *et al.,* 2022). Indirectly soil salinity affects soil structure making soil more dispersed. During the ion exchange process in soil higher concentration of Na^+ ions will replace cations like Ca^{2+} and Mg^{2+} from the clay lattice which actually maintained the soil aggregate stability. Furthermore because of low charge density and higher hydrated ionic radii of Na^+, it will disperse the soil primary particles (sand, silt and clay) making structurally unstable (Choudhary and Kharche, 2018). Thus aggregation of soil particles is drastically affected making soil more dispersed. The dispersed particles (especially silt and clay) on the other hand will block all the pores of soil affecting soil permeability. The infiltration rates of soil drops drastically resulting in water stagnation and higher rate of erosion during rainy season. In dry spell the dispersed soil becomes hard enough and cracks are form which will severely affect the seed germination and root growth.

Plants have different mechanisms to mitigate salt stress and most of it are an active processes were additional energy must spend for it. Some of the important mechanisms include enzyme production in the root cell membrane, removal of excess salts through plant leaves and filtration in the membrane bound organelle vacuoles. The above mechanism to withstand soil salinity requires additional amount of energy that can impact proper growth of the plant

(Zhao *et al.,* 2020). So, now it is high time to find out a strategy to cope up the soil salinity problem. Different strategies to withstand the effect of dissolve salts in saline soil causing water stress and ion toxicity is to reduce the concentration of salts in soil and absorption through roots. If the toxic salts attached in the clay lattice can be removed or the toxic ions in the soil can be replace by some other essential nutrients which doesn't have toxicity effects to both soil and plants, can be another strategies to reduce the effect of salt stress. It has been reported that their lies an antagonisms between the ions (Na^+ and Cl^-) of dissolved salts with some of the essential nutrients/ions (Ca^+, Mg^+ and K^+) (Naz *et al.*, 2021; Wakeel 2013). This antagonistic relationship can be used as a remedial measure to control soil salinity problem. Out of all K^+ because of it luxury consumption properties it is widely used to control the salinity stress in soil and plants.

Major causes of soil salinization

Salts are not an alien to the soil. It is available on the earth crust in the form of primary minerals since our earth is formed. After all most of the salts presences in soil are essential nutrients, its optimum availability to soil is vital for proper plant growth. The only problem in soil salinization is excess deposition of soluble salts to the soil. Any factors that deposit or increase the concentration of salts in soil are responsible for salinization. Different causes of soil salinity can be grouped into four factors (Fig. 1) viz. Geological, climatic, hydraulic and anthropogenic factors depending on how the salts accumulation takes place. However, major factors can be categorised into primary and secondary factor affecting at the spatial and temporal scale (Seydehmet *et al.,* 2018). Different natural phenomenon that determined the salinization process belongs to primary factors (Rafik *et al.*, 2022). So, geological, climatic, hydrological and topographical factors belong to the primary salinization. Whereas different anthropogenic activities like Faulty agricultural practices, canal irrigation, irrigation with saline water, shallow water table, tube well irrigation, industrial waste water and deforestation comes under secondary salinization. Geological factors include volcanic eruption, parent materials, its transportation and deposition. Most of the rocks formed after cooling and consolidation of molten magma are primarily polymineralic and different mineral salts are present in it. After disintegration and decomposition of the rocks and mineral constituents the salts present in it will transfer to soil. The weathered parent materials containing salts will be transported by different agents (wind, water, gravity, snow etc.) and distributed to different places which alter the properties of soil. So, naturally salts are present in every type of soil which concentration depends on the mineral constituents of the parent rocks/materials. However the problem of soil salinity lies with the climatic condition of the region. Although salinization takes place in all climatic regions but generally it confined to the region where there is high temperature and low rainfall. Under this condition evaporation loss of soil water will triggered, resulting more salt to be concentrated on the surface soil. Furthermore, with scarcity of rainfall water movements are restricted making water to stagnant in a particular places and after evaporation more salts will concentrate in the soil. So, naturally saline soils are widespread in the coastal region, arid and semi-arid region of the world. Topography also influences soil salinization directly with the depth of groundwater and indirectly with lateral and vertical infiltration of sea water to the aquifers (Celleri *et al.,* 2022). Topographic condition and saline shallow water table are also the most effective factors responsible for soil salinity built up (Akça *et al.,* 2020). When ground water gets shallower there is an up flow of water containing higher concentration of salts through capillarity to the plant root zone and surface soil. Water logging and soil salinity decreases with increase in elevation along topographical gradients. It is also predicted that the impact of global warming could increase sea level resulting with increase in vertical and lateral infiltration of sea water to aquifers that could enhance soil salinity problem. So, in the low lying region higher concentration of soluble salts coupled with high evaporation rate leads to accelerate soil salinization making more vulnerable to salt stress.

Secondary factor of soil salinization primarily includes land use change, deforestation, climate change and irrigation (Wang *et al.,* 2020; Yang *et al.,* 2019; Bernzen *et al.,* 2019; Moharana *et al.,* 2019). Land use change primarily to increase the agricultural holdings for feeding the growing

population globally is one of the major factors for soil salinization. It has been reported that an extensive and rapid change of ecosystem takes place over the last 50 years, which has not took place in any other time (Hobbs *et al.,* 2009). These changes are mainly related with the land use change for the increasing population globally. Clearing forest for agriculture practices leads to soil health deterioration which ultimately reduces the crop production constantly if not maintained properly. Land use change altered the potential characteristic of the area making more vulnerable to the present climate change. So, understanding the information of land use change is also essential to assess the effect of climate change and environmental impact due to human intervention (Borrelli *et al.,* 2020). In arid and semi-arid region, soil salinity is mostly due to the capillary rise of water from groundwater leading to the upward movement of soluble salts along with water making high concentration of salts in the surface soil. Any land use changes that affect or reduced the hydrological cycle will leads to increase the level of water table accelerating the salinization problem. It can be seen that the salinity problem due to land use change is mostly due to overexploitation of land for agriculture practices (Table 1). So, with increase in agriculture holding more exploitation of natural resources also increases making more vulnerable to soil salinity. And most of the secondary factors relating to soil salinization are due to intensive agricultural practices.

Another important secondary factor for soil salinization includes the present climate change which is a big concern for soil health sustainability and food security. The most important concern for soil health due to climate change is severe exhaustion of soil organic carbon (SOC). By 2050 the content of SOC in soil is predicted to reduce by 14% to 23% (Haj-Amor *et al.,* 2020). For agriculture sustainability and soil health an optimum amount of SOC is essential. And, as far as climate change is concern soil is an important sink for carbon (C) which has an impact on global warming (Rawat *et al.,* 2022). So, the climate change especially with temperature and erratic rainfall will greatly affect soil salinization problem in the near future. Since soil salinization is all about the deposition of salts in the surface soil due to excess evaporation of water containing solutes. So, increase in atmospheric temperature with low rainfall due to global warming, there is likely to increase the salinization problem in near future. It is projected in coastal region to reach the salinity levels more than 4 dSm^{-1} in 2050 (Haj-Amor and Bouri, 2019). Another study using HYDRUS-1D and the global circulation model (MIROC5) shows that the electrical conductivity of the cultivation region of northeast Thailand is predicted to increase up to the average value of 3.31 dSm^{-1} in the year 2081 to 2100 (Yoshida *et al.,* 2021). Different researchers has also predicted to increase the soil salinity level globally which is directly or indirectly related with the climate change/global warming. So, now it is time to get ready for the consequences for our action with some strategies to mitigate the soil salinization problem especially in agriculture sector. Because "everything else can wait, but not agriculture" a remarked by Jawaharlal Nehru (Swaminathan, 1972).

For an intensive agriculture and crop diversification water is a vital component. Large distribution of rainfed agriculture (52 percent of country's net sown area) in India results in need for area expansion under irrigation to increase food security (Bal *et al.,* 2022). However, water requirement for domestic use, industrial requirement and agricultural need increases but the supply of fresh water globally decreases with time (Flörke *et al.,* 2018). There are certain reasons affecting fresh water availability, but among all one of it is the present global climate change (Flörke *et al.,* 2018). So, the progressive requirement of water for different sectors leads to reused and recycled of the available water (Ghernaout, 2018; Phogat *et al.,* 2020). In agriculture with rapid increase in human population, demand for diverse food items increases substantially. Water is one of the most important inputs which are vital for crop production. But the availability of good quality water for irrigation purpose decreases substantially particularly in those regions of arid and semiarid. So, to meet the need of irrigation water, recycling and reused of water is a quiet a common practices. In many part of the globe used of field drainage water has already been started despite of higher concentration of salts present in it (Abou El Hassan and Allam, 2017). The salts concentration increases

with every recycled and reused of water if not treated properly. But to meet the water requirement of today's intensive agriculture, farmers did not concerned about the salt present in the irrigation water and subsequently accelerated soil salinity. Increasing shortage of irrigation water with increasing demand for food production in agriculture makes the farmers use more and more ground water (Pulido-Bosch *et al.,* 2018). Therefore, used of poor quality ground water for irrigation in the surface soil also substantially increase the salt concentration accelerating soil salinity problem.

Impact of soil salinity on plant

Salt is associated with the rocks and its minerals constituents during earth formation. The primary salt mineral present in earth crust is rock salt or halite. During the process of soil formation i.e. the disintegration and decomposition of rocks and minerals, salts are transferred to the soil. So, naturally salts are important constituents of soil and its present in soil is important as salts are nutrient to both plant and animal (Herbert *et al.,* 2015). The only concern is the quantity of salts within a threshold value. If the quantity of salts exceeds a threshold value, only then it will be harmful to the plants. The accumulation of excess salts in soil which eventually affect the crop growth and even death of the plant is due to the abiotic stress developed in soil which is referred to as salt stress (Abdelraheem *et al.,* 2019). Salt stress is one of the limiting factors for crop production especially in arid, semi-arid region and coastal. In salt stress condition the process of osmosis can reverse and water will come out from the plant to soil due to higher concentration of dissolved salts in soil. In this situation plant will loses water and suffer stress with stunted growth. The pathway of water from soil through plant to the atmosphere or the soil-plant-atmospheric continuum (SPAC) will be greatly affected by salt stress condition (Minhas *et al.,* 2020). The potential gradients developed from root through shoot to the shoot will disrupt. The water potential in the soil will dropped resulting in reverse flow of water from plant to soil. So, the impact of salt stress is quiet related with the draught stress, since in both the cases water is the limiting factor for proper growth of the plant (Forni *et al.,* 2017). The main impact of salt stress can be grouped into three broad topic i.e. germination effect, physiological effect and ionic effect (Farooq *et al.,* 2017; Jangra *et al.,* 2022). Seed germination is an emergence of radicles through the seed coat which is a very important stage in a plant life cycle. For a proper germination and early seedling growth a sufficient supply of water in soil is essential for appropriate imbibition of the seed coat for radicle to emerge. During this early establishment stage of plant they are very sensitive to the salt stress either because of osmotic effect reducing water absorption or ionically through the toxic effect of ions causing imbalance in nutrient uptake. Different other reason for salt stress on germination can be suppressed protein content, reduce phosphatase activity, increased soluble sugar, starch, absicic acid and reducing gibberellic acid.

Photosynthesis is one of the most important processes where green plant transformed light energy to chemical energy. It provides 90% of the plant dry matter. However this process is highly sensitive to salt stress with its effect on opening and closing of stomata (stomatal conductance), destruction of chlorophyll pigments and damage of photosystems (PS) reaction in plant (Mahlooji *et al.,* 2018). The extent of stress by salts on photosynthesis depends on the types of salt stress, its concentration, duration of stress, plant species and its age (Ma *et al.,* 2020). The effect of soil salinity on plant photosynthesis is primarily related with the reduction of PSII activities than stomatal reduction (Najar *et al.,* 2019). Under salinity stress condition, relative water content (RWC) of plant decreases with concomitant dropped of leaf water potential (LWP). Accumulations of toxic ions like Cl^- and primarily Na^+ in plant leaves affects cell turgidity which ultimately leads to the closing of stomata (to prevent cell plasmolysis) reducing CO_2 uptake and photosynthetic process. Cl^- toxicity in glycophytes leads to chlorosis and necrosis of leaves which reduces the plant growth. Visual symptoms start from the leaf edges which precede the inner leaf and finally leaf abscission takes place. Cl^- toxicity developed due to soil salinity is not only a thread to food security but it also affects the aesthetic values of ornamental plants leading to decrease its marketable values. The toxicity effect of chloride is more severe than sulphate in saline soil (Irakoze *et al.,* 2022). It has been reported that half the concentration of chloride toxicity is equal to full concentration of sulphate.

Different salts affect crop growth differently (Javed *et al.,* 2022). Even though stress developed due to soil salinity is always associated with the excess amount of NaCl in the soil. Different other salts are also present in salt stress soil of which salts of Na^+, Cl^-, SO_4^{-2}, CO_3^-, HCO_3^-, and BO_3^- are common. It has been reported by different researchers that salinity problem caused by Cl^- and SO_4^{-2} are not similar (Yu *et al.,* 2018; Ahmadi and Souri, 2018; Reich *et al.,* 2017). On different studies conducted on rice, wheat, maize and French beans shows less detrimental effect of SO_4^{-2} salts compared with Cl^- salts on growth and yield. Under solution of same concentration (Isoosmotic concentration), Cl^- salinity is much more toxic than SO_4^{-2} salinity affecting photosynthesis of peanut and causes severe leaf chlorosis reducing its growth. Sweet pepper plant growth is also significantly affected by the presence of Cl^- when compare with SO_4^{-2} salinity reducing its fruit quality and marketable value of pepper (Navarro et al., 2002). The toxicity effect of SO_4^{-2} salinization on sorghum species is comparable or even more than Cl^- toxicity which increases with increase in its concentration due to suppression of K and Mg in the shoot. In a hydroponic culture technique under barley crop SO_4^{-2} salinity is more detrimental than Cl^- under different cultivar used in the study (Datta *et al.,* 1994). Different salts (NaCl, Na_2SO_4 and Na_2CO_3) in soil affect fresh dry matter and water content of the pea plant differently. The toxicity effect of different salts affect pea plant is in the order Na_2CO_3> NaCl> Na_2SO_4 (Abd El-Samad and Shaddad, 1996). In another study the stresses developed by neutral salts (NaCl and Na_2SO_4) and alkali salts (Na_2CO_3 and $NaHCO_3$) on barley crop differ. More severity is under alkali salts than the neutral salts. However when the salinity of a medium increases more than 60 mM, Na^+ concentration also increases slowly in saline stress but sharply under alkali stress soil (Yang *et al.,* 2009). Osmotic stress and ionic toxicity are the general problem in both saline stress and alkali stress soil, in addition to this high pH is associated with alkaline stress leading to soil structure deterioration. In many cities, globally lawn occupies maximum area of garden, backyard, park, even terraces. So, the popularity of turf grass is increasing day after day. For growing turf grass, timely irrigation is a must and understanding the salt effect on turf grass is also crucial to maintain the aesthetic and ornamental benefits. On above that turf grass possess many inevitable functional benefits such as it control soil erosion, maintain ecological balance, reclaim polluted environment, improve soil quality, reduces air and noise pollution. Different salts of CO_3^-, Cl^- and SO_4^{-2} on leaves of turf grass affect different level of injuries (leaf firing). Leaf firing injuries in turf grass is mainly due to high pH caused by CO_3^- salts and Cl^- is primarily involved in reducing the growth than Na^+ in irrigation water (Gao and Chen, 2012).

Mitigation of soil salinity

Different causes of soil salinization has already discussed in the previous sections. All of it has direct or indirect relationships with the climate (temperature and rainfall) of the region. With continuous increase in global population the effect of man-made climate change or global warming are also predicted to increase and worsen in decades to come. There is evidence that climate change will affect the intensity and frequency of rainfall and will also increase the temperature (Guntukula, 2020; Kogo *et al.,* 2021). So, scarcity of rainfall and high temperature due to climate change can increase the soil salinity problem in the near future if we do not control it. Different strategies are there to manage/reduce the effect of soil salinity to plants and it mainly includes three methods, namely eradication, conversion and control of the excess salts present in soil (Shahid *et al.,* 2018; Hayat *et al*. 2020; Hafez *et al.,* 2021). Eradication includes those practices that remove the salts from the soil through drainage or flushing whereas conversion is all about the chemical amendments used to reduce the caustic effect of salts. The reduction of evaporation lost from the soil and growing of salt tolerant crop variety are the important control method. Another method under this category can be use of any essential nutrients that has an antagonistic relationship with the salts present in soil. Due to similar physico chemical properties of Potassium (K) with sodium (Na), calcium (Ca) and magnesium (Mg), there lies an antagonistic relationship (Daoud *et al.,* 2020; Yan *et al.,* 2020). So, the widely used essential element to reduce the effect of salts is K (Table 2). Salts is not an alien to the earth, it is an important constituents of soil

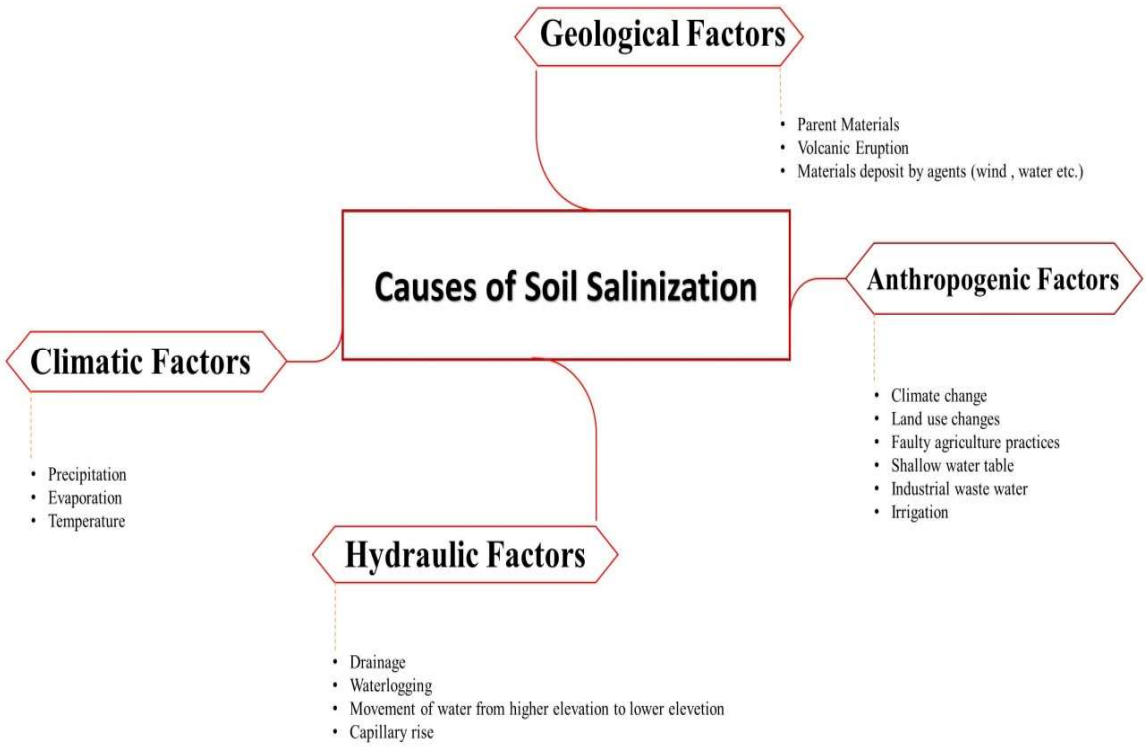

Figure 1: Different factors affecting soil salinization.

Table 1: Impact of different land use changes on soil salinity in different parts of the globe

SN	Land use change		Impact	References
	From	To		
1	Rice	Shrimp farming	Increased soil salinity	Ali (2006)
2	Bare land, fallow land	Intensive agriculture	Irrigation related salinization problem increases	Zewdu *et al.,* 2016
3	Grassland	Crop land	Soil salinization increases	Yu *et al.,* 2018
4	Active paddy land	Abandoned paddy land	Soil salinization increases	Gopalakrishnan and Kumar, 2021
5	Rice farming	Salt water shrimps farming	Soil salinization increases	Islam *et al.,* 2019
6	Date Palm	Bare land	Soil salinization increases	Turk and Aljughaiman, 2020
7	Unirrigated farmland; Grassland	Irrigated farmland; Farmland	Soil salinization increases	Wang and Li, 2013
8	Perennial pasture	Annual pasture	More runoff and salt load in the catchment area	Tuteja *et al.,* 2003

Table 2: Salinity control measure of crops grown under different medium by using K_2SO_4

SN	Crop	Medium	Treatment	Best	Reference
1.	Wheat (*Triticum aestivum*)	Sand culture	150 mM NaCl	200 mM	Kausar and Gull (2014)
2.	Lettuce (*Lactuca sativa*)	Nutrient film technique (NFT)	40mM NaCl	10 mM	Tzortzakis (2009).
3.	Tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and pepper (*Capsicum annum*)	Sand culture	60 mM NaCl and pH 8.5	3 mM	Kaya *et al.,* 2002
4.	Bean (*Phaseolus vulgaris*)	Control condition of temperature and light	20 mM of NaCl	1000 and 2000 $mg^{-1}kg$	Erdinç *et al.,* 2018
5.	Mustard (*Brassica juncea*)	Hydrophonic	150 mM of NaCl	6 mM	Yousuf *et al.,* 2015
6.	Rice (*Oryza sativa*)	Hydrophonic	12-24 mM L^{-1}	1.2 mM L^{-1}	Munir *et al.,* 2019
7.	Olive (*Olea europaea*)	Medium-textured soil	0, 50, 100 and 150 mM of NaCl	100 mM of K	Chartzoulakis *et al.,* 2006
8.	Sugarcane (*Saccharum officinarum*)	Hydroponic	100 mM L^{-1} of NaCl	3 mM L^{-1}	Ashraf *et al.,* 2010

derived from the weathering of different primary salt minerals. Presence of salts in an optimum condition is vital for plant growth as all of it (Ca, Mg, Na, Cl) are either essential or beneficial nutrients. Soil salinization is all about higher concentration of dissolve salts in soil near to the root zone which incite osmotic stress and ions toxicity to the plant. The extent of toxicity differs from salts to salts and also from plant to plant (Zhang *et al.,* 2018). Optimum ratios of different ions are essential in plant cells for the proper functioning and yield development. So, the antagonistic relation of K with other ions can be used as a strategy to maintain the ionic level in the plant cell that will reduce the toxicity of salts to plants. K is an essential elements required by the plant which primarily involve in water movement and stomatal functioning. Plants can absorb K in an amount far exceed from the requirement when readily available in the soil, and is called as luxury consumption of K (Jungers *et al.,* 2019). Luxury consumption of K does not influence toxicity and crop yield. Its availability in turn will reduce the ionic imbalance/toxicity impart by dissolved salts in saline soil. The antagonistic effect of K with different ions in saline soil can reduce the uptake and toxicity of ions such as Na, Ca and Mg.

Different researchers have worked under different crops to see the effect of K for reducing the salinity stress for better productivity (Table 2). A study conducted by Kausar and Gull (2014) on wheat crop initiate salt stress in sand culture at a concentration of 150 mM. Under this salinity stress different concentrations of KSO_4 (50, 100, 150, 200 mM) were applied to see its ameliorating nature on growth and yield of the crop. It has been observed that the application of 200 mM of KSO_4 proved to be the best for ameliorating the saline stress. There is also evidence from the finding of Tzortzakis (2009) in lettuce grown with the nutrient film technique (NFT) under greenhouse conditions that inclusion of K_2SO_4 reversed the negative impact of salinity on plant growth. Another worked done by Kaya et al., 2002 under different vegetable crops (tomato, cucumber and pepper) shows the effect of salts concentration (60 mM of NaCl) and pH (8.5) of the medium affecting chlorophyll content, plant growth, water absorption and membrane permeability. The adverse effect on plant is primarily due to the high salinity condition than that of high pH. But inclusion of K in the form of K_2SO_4 improved the plant health and maintained the proper ratio of the ions inside the plant. K_2SO_4 application under constant exposer of plant (beans) with 20 mM of NaCl shows positive results on nutritional element content, root and shoot dry matter content (Erdinç *et al.,* 2018). A study conducted by Taha *et al.* (2020) comparing different methods of K application for controlling salinity stress under soybean (*Glycine max*) shows the exogenous application of K_2SO_4 were more

effective than seed soaking (SS) and foliar spray (FS) at improving the yield, seed quality and physio-biochemical attributes of soybean. Under saline condition maintaining higher concentration of K in any medium for plant growth serve as an effective measure for regulating the growth and productivity of mustard crop (Yousuf *et al.,* 2015). In saline-sodic stress condition, higher concentration of Na^+ results in lower uptake of K^+ in plants. Application of higher doses of K^+ results in maintaining/improving K^+:Na^+ in plant tissue enhancing rice (*Oryza sativa*) growth by improving the concentration of enzymes, physiological activities and biochemical properties (Munir *et al.,* 2019). Yield increments of wheat (*Triticum aestivum*) with the inclusion of K^+ along with phosphorus (P) in saline-sodic soils of Pakistan were observed. Application of K and P together at the rate of 150 kg K_2O ha^{-1} and 120 kg P_2O_5 ha^{-1} increased the yield of wheat by improving the nutrients dynamic of soil (Hussain et al., 2016). An experiment conducted in salt affected soils of Egypt under rice-wheat cropping system shows that inclusion of K in the form of Mono potassium phosphate along with compost treatment increases the grain and straw yield of both rice and wheat. A study conducted on a tree species of olive in medium textured soil of subtropical region of Greece shows a significant correlation of salinity with the concentration of Na^+ and Cl^- in the leaves and fruits. But with increase in K^+ concentration salinity stress decreases, which shows a negative correlation (Chartzoulakis *et al.,* 2006). A hydroponic experiment conducted to see the effect of salt stress under different genotypes of sugarcane shows enhancement of Na^+ concentration in plant tissue and significantly reduced the root and shoot growth (Ashraf *et al.,* 2010). It has been showed that application of K^+ alone or combination with silicon (Si) significantly prevent the uptake and transport of Na^+ in plant and can improve its yield even under salt stress condition. Activities of different antioxidant enzymes decreases under salt stress condition nevertheless inclusion of K^+ along with zinc significantly increase the enzymes activities. Furthermore, significant decrement of oxidative stress and increment of root, shoot and spike of wheat crop were reported with increase in concentration K^+ (Jan *et al.,* 2017).

Conclusion

Soil salinization has a significant impact on the physio-biochemical aspects, growth and yield of crops/plants, which ultimately affect the future food security. Most of the factors responsible for soil salinization directly or indirectly related with the climate. So, chances of increasing the salinity problem in the near future is expected with increasing global climate change. The continual increase of global population has a direct relation with the present global climate change. Certain unwanted activities taking so far (deforestation, landuse change, overexploitation of underground water, etc) that increased the soil salinization process is directly or indirectly related with the growing population. Complete halt of the growing population globally is not possible as so with the soil salinity problem. So, developing management practices that can reduce the salinity effect is vital for an agricultural sustainability. However finding the practice that suits the problem most is also a great concern since some management practices has certain negative effect to both soil and water. So, use of the essential nutrients (K^+ and SO_4^{-2}) that has an antagonistic relationship with the salts (especially Na^+ and Cl^-) present in soil is a new approach. Due to similar charge and physico chemical properties of K^+ and SO_4^{-2} respectively with toxic ions Na^+ and Cl^- of saline soil, there lies an antagonistic relationship. Furthermore, SO_4^{-2} of its less toxicity to plants and improve soil pH condition especially in arid and semi-arid region, the combination of K^+ and SO_4^{-2} salt is a good combination to ameliorate the Na^+ and Cl^- toxicity under saline soil for sustaining both soil and plant production.

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Conflict of interest

The authors declare that they have no conflict of interest.

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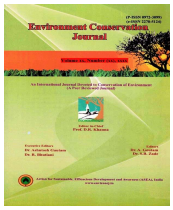

Possible remediation of hexavalent chromium by native fungi of Sukinda mining area: a review

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ABSTRACT

The expeditious industrialization is helping the world to give a new modern era with all sorts of amenities. But the consequences are following great risks that might result in a terrifying future. Heavy metal pollution and its hazardous effects are one of them. Though India is the 3rd largest chromium producing country and the Sukinda valley of Odisha, is the chief source for chromium, hence here the threat of chromium pollution is at a high point. Countermeasures to this problem have become of prime importance. Among several remedial measures, bioremediation is an approaching process to control the accelerated growth of heavy metal contamination including chromium. In the world of microorganisms, the congenital characteristics of fungi have great importance as they can grow easily in polluted habitats. Again, there is evidence of native fungi having the potential to bind with heavy metals and remove toxic agents from natural environments. The pathway of chromium toxicity and its possible remediation potential by fungi have been studied extensively in the Sukinda area. This study signifies some positive aspects that can be practised in the future as a convenient option for bioremediation. Fungal bioremediation improved with biotechnology tools will be suitable output for rapid remediation which is vital for this moment.

Introduction

After the industrial revolution, the anthropogenic exploitation of chromium increased rapidly in India as well as in Odisha.Chromium is an essential metal that dominates the domestic market as it is exported to other countries for ferroalloys. Other than ferroalloys, it is also used as a chief ingredient in refactories, ceramics and the preparation of chromium-containing chemicals. As Chromium is the most demanding mineral reserve of our country hence it stands as the economical, sociological, and financial backbone of our territory.Among all the forms of chromium, Cr (VI) is reffered as the most toxic form as is detrimental for all the ecosystems.Overdose of Cr (VI) results in depletion of seed germination, plant growth and yield quality by disturbing the enzymatic activity, nutrients and oxidative balance. Whereas in case of animals it leads to mutagenesis and several genetic disorders. Odisha contributes almost 97% of India's reserve of chromium (US EPA data 2004) and Sukinda is the chief source of chromites. Several mining industry of Sukinda and their methodologies are having a great impact for contaminating the nearby natural resources and making it inappropriate for the surrounding biological system. The open cast mines raised near Sukinda escalated the concentration of hexavalent chromium which is far above the permissible limits. This leads the environment toxic for the local biotic community. It captured everyone's attention when designated as the 4th most polluted area by Blacksmith report 2007(BI university 2007). The upcoming threat indicates that proper propaganda was essential to establish

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Figure 1: Mining sites of sukinda (Source from google map)

a non-toxic or less toxic environment for the people of Sukinda balancing the ecological, economical and ethical status.

Detoxification of chromium should include a procedure that is inexpensive, eco-friendly and could apply on an extended version. The remedial proposal forwarded for chromium reduction includes physical, chemical and biological cleaning procedures. Almost all methodsincluding some combinations are either having less impact on remediation or produces secondary pollutants which result in no action. In order to prepare an optimal remedial strategy, an understanding of the characteristics of chromium and its interaction with the environment focusing on its mechanism of contamination needs to be explained.

Remedial measures using living biomass or bioremediation raised is an emerging technology that has been used extensively. After analyzing the pathways of chromium contamination, conversion of chromium from a toxic form to a nontoxic form was an approachable option for remediation in mines area (Bhutiani *et al.,* 2019; Chuanhan *et al.,* 2019; Irfan *et al.,* 2022). Again, the traits of adsorption were traced in plants and in microorganisms which symbolizes utilization of these living organisms for detoxification of contaminating mining areas. The remediation using biological agents are cost effective, valuable and effortless option to be applied in the Sukinda region.Bioremediation is a cleaning process that includes a pathof investigation for molecular biology and ecological balance (Kumar *et al.,* 2011). It's a cleaning process in which microbes are used to transform harmful substances to achieve a contamination-free nontoxic environment. The whole phenomenon of bioremediation is accompanied by several sub-processes like biosorption, bioabsorption, bioaugumentation, bioaccumulation, biosolubilization, bioreduction, bio precipitation, mineralization and methylation. For the removal of heavy metals methods like biosorption, bioaccumulation, bio leaching, biomineralizations are applicable. Here the emphasis has been given to adsorption and accumulation. Presently bioremediation incorporation with nano-technology in a voyage for remediation of heavy metals (Karmacharya *et al.,* 2016; Bhutiani *et al.,* 2021; Bhutiani and Ahamad, 2018). The results showing positive symptoms in the field of heavy metals (Tyagi *et al.,* 2017). Evidencesshow that Heavy metals like arsenic ,aluminium and nickel remediated from the respective contaminated system with this technology (Dehghani *et al.,* 2015).Even nanoparticles have certain impact on chromium also (Gupta *et al.,* 2016). So more exploration in this field can be proved as beneficiary forboth bioremediation and nano technology field. Fungi are the dominating microorganism of the biotic community. Fungal biotechnology is now on a voyage to explore the absorbance capacity of metallic ions from contaminated soils in order to give a solution to the leading pollution problem.

Table 1: Mines of Sukinda and their overburden generation in Sukinda valley

Name of the mines	Overburden generation in million /year	Over burden dump area in ha	Reference
Saruabil,M.L. Mines Pvt. Ltd.	10.37	62.02	Mishra and Sahoo, 2013
TISCO Sukinda	5.4	79.8	
Kaliapani , OMC	3.0	48.1	
Sukinda , IMFA	0.60	45.0	
South Tailangi , IDCOL	0.54	9.995	
Kaliapani, Balasore Alloys	0.48	22.41	
Ostapal FACOR	0.47	17.18	
Mahagir , IMFA	0.20	4.49	
Kamarda, B.C. Mohanty & Sons	0.2	17.74	
Kaliapani ,OMC	0.1	-	
Sukrangi , OMC	0.03	-	
Kathpal , FACOR	0.03	27.25	
Chingudipal , IMFA	-	4.38	

Table 2: Role of microorganisms in remediation of heavy metals

Micro organisms	Compound	Reference
Saccharomyces cerevisiae	Heavy metals,Pb, Hg and Ni	[chen *etal.,*2007,kilar *et al.,* 2009 ,infanate 2014]
Cunninghamella elegans	Heavy metals	[tiginiv *et al* 2010]
Pseudomonas fluorescens and Pseudomonas Aeruginosa	Fe 2+, Zn2+, Pb2+,Mn2+ and Cu2	[paranthaman *et al* 2015]
Lysinibacillussphaericus CBAM5	Co, Cu, Cr and Pb	[Montengro *et al* 2015]
Microbacteriumprofungi strainShh49T	Fe	[Wu *et al* 2015]
Geobacterspp	. Fe (III), U (VI)	[Mirlahiji *et al* 2014]
Bacillus safensis (JX126862) strain (PB-5 and RSA-4)	Cd	[Rajesh *et al* 2014]
Pseudomonas aeruginosa, Aeromonas sp.	U, Cu, Ni, Cr	[Sinha *et al* 2011
Microorganisms Compound Reference Aerococcus sp., Rhodopseudomonas palustris	Pb, Cr, Cd	[Sinha *et al* 2014, Sinha *et al* 2014]

Table 3 : Microbial Contribution To Chromium Remediation

Organisims	Mode of Action	Reference
Pseudomonas fluorescens	LB300 Uptake of Cr2O4- by the strain with plasmid	[Ohtake*et al.,* 1987]
Schizosaccharomyces pombe	Lysine and leucine auxotrophic and heterothallic strains of this microbe were used to obtain Cr-sensitive and tolerant mutants by UV radiation-induced and nitrosoguanidine induced mutagenesis	[czako-ver*et al.,* 1999]
Pseudomonas ambigua G-1	Bioreduction of the Cr-concentration from 150-35mgL-1 in 36hr in liquid media	[losi *et al.,* 1994]
Bacillus firmus	Capable of absorbing Cr6+ efficiently into their biomass	[Bennett *et., al.,*2013]
Klebsiella pneumoniae	Capable of absorbing Cr6+ efficiently into their biomass	[Bennett *et al.,* 2013]
Mycobacterium sp.	Capable of absorbing Cr6+ efficiently into their biomass	[Bennett *et al.,* 2013]
Bacillus cereus IST105	Absorption of chromate on the bacterial cell wall takes place through surface functional groups like carboxyl, amide, phosphoryl and hydroxyl	[Naik *et al.,* 2012]
Bacillus megatarium TKW3	Hexavalent chromium reduction associated with membrane cell fraction	[Cheung *et al*., 2006]
Bacillus circulans	Removal of chromium by bioabsorption	[Khanafari *et al.,* 2008]
Bacillus subtilis	Able to reduce chromate at concentrations ranging from 0.1 to 1 mM K2CrO4	[Garbisu *et al.,* 1998]
Bacillus methylotrophicus	Chromate reduction activity was found to be 91.3% at 48hrs	[Mala *et al.,* 2015]

Fungi, the dominating organisms found in sukinda area are variably capable to survive and retrieve in the highly concentrated heavy metal fields, so recovery of precious metallic ions using fungal based cleaning approaches is one of the best solutions that can be applied for detoxification.

The study gives an idea that bioremediation using fungal isolates present in the Sukinda soil are possibly able to start a new methodology in the world of remediation.

Sukinda and its environmental scenario:

In India, Odisha state is blessed with vast deposits of mineral reserves like coal, iron, manganese and bauxite but chromium is the principal ore element that stabilizes its economy.Major share of chromite deposits (98.6%) associated with ultramafic complexes are in Sukinda and BaulaNuasahi region. Sukinda chromium valley is the largest chromium deposits of Odisha (Pattnaik*et al*., 2016) that counts almost 195 million tons of reserve which is 98% of the total chromium in India (Mishra and Sahu*.,* 2013). The valley encloses 200 sq. Km area bounded by latitudes 20°53' and 21°05' and longitudes 85°40' and 85°53' surrounded from Tomka-Daitari Range, North to Mahagiri Range in the South with a general slope of 18-20° towards South-West (Figure 1.)

Status of chromite mines

Chromite is chiefly used and exported in the form of ferroalloys, which accounts for about 85% of the total chromites demand of Odisha state. Some chromites are also utilized for refractory, ceramics and chromium containing chemicals. Due to demands as key industrial raw materials 17 mining leases granted for chromites mining in jajpur district from which 12 are operating smoothly while others have some statutory clearance problem (www.orissaminerals.gov.in). Among these mines most are open cast mining except two, engaging anthropogenic activities which causes negligence in environmental controls posing major hazards to the flora and fauna in and around

Toxicity of Sukinda area

Blacksmith Institute USA has declared Sukinda as the fourth most polluted place (BI report 2007) of the world. The reason of pollution is the exceeded level of hexavalent chromium as particulate matter in the airwater and soils affecting severely the nearby population (Das *et al*.,2011.). Due to open cast mining, overburden material generating soild waste results in damage of abiotic and biotic community (Viti *et al.,* 2014). In rainy days leaching occurs which may lead to the detoriation in quality of ground and surface water (Mohanty and Patro 2011). The water also washed out with chromium from mining sites and reaches to nearby water reservoir. This causes harm to the aquatic organisms and to human society both directly and indirectly (Kumari *et al.,* 2017). Due to exploitation high amountof chromium dust generates and fuses in the air and inhalation of this polluted air may have carcinogenic effector can causes cardiac arrest (Das *et al.,* 2010). The state pollution board conducted tests for Sukinda area in October 2018 and the report reveals the presence of hexavalent chromium far from permissible limit . According to another prevailing report 70% of water and 28% of soil are inappropriate for irrigation due to high concentration of Cr(VI) (EPA 1998a). This data signifies a red alert to nearby 75 villages and 40 perennial streams.

Sukinda environmental situation creates a major health hazard to the residents and workers of the Sukinda valley (Pattnaik *et al.,* 2012) as well as to the floral population. Increased number of open cast mining is the prime reason for promoting the contamination of the nature (Mishra *et al*., 2010). The solid waste (Around 7.6 tons) deposited in the boundaries of mining areas facilitates the contact of hexavalent chromium with the soil and air (Das *et al.,* 2011). Again because of drilling, blasting and transportation a large amount of dust is produced and the dust is nothing but particulate matter of hexavalent chromium that has a lethal effect to the biomes. Also, the duping of the overburden in the nearby area interrupts the natural balance of that ecosystem causing disturbance in the plant and animal diversity.

Utilizing better mining technologies for ore exploitation is a progressive step for the development of mankind but simultaneously destroying our own environment is the major drawback. Time already alarmed us to investigate on more sustainable methods for a better future for all living beings for a better future because all creatures have their rights to live and evolve in future.

Chromium chemistry and its toxicity:

Chromium can easily locate in rock, soil, volcanic dust and in living organisms but in trace amount.Chromium is the first element in group 6 with atomic number 24.This element derives from only ore complex, chromites, discovered first from United States nearly about 1808. It is the chief and most indispensible industrial metal because of its significance characteristics like resistance to corrosion, hardness and high melting point. Chromium is a lustrous steel grey metal used in plating on steel and other nonferrous alloys and also owned as raw materials by the chemicals and leather industries.These properties of chromium are responsible for its huge demand in the market

Valance of chromium vary from –II to VI, whereas the only possible stable forms are III and VIavailable as ores, such as ferrochromite. Hexavalent chromium is produced due to anthropogenic activity (EPA 1984a) (ATSDR 2017). Because of various utilizations in different commercial field its demand in this present world has achieved in its peak. Today's modernized world demands chromium in several industries for electroplating, timber preservation and in leather tanning etc (Madhavi *et al*., 2013). Chromium also has a significant role in living bodies but its increased concentration may lead to toxic, mutagenic, carcinogenic and teratogenic (Kilic *et al*., 2011).

Chromium and its utilization

Chromium and its derivative compounds are important raw material for industries like metallurgical, chemical, and refractory. Metallurgical industries utilizes for preparation of steel and other non alloys, chemical industries for preparation of different chemicals and Refractory uses for metallurgical furnace linings and granular chromites for production of heat resistant appliances. It is also a vital agent for the living world as it stimulates fatty acid and cholesterol synthesis essential for brain and nerve systems and other metabolic reactions (Kumari *et al*., 2017). Organ meats, mushrooms, wheat germ, and broccoli are examples of rich sources of chromium. Chromium also involves in several metabolic processes and can acts as a catalyst when taken as supplement.

Negative impact of chromium

The valence of chromium determines the intensity of its toxic nature (Tchounwou *et al.,* 2012). Chromium with valance VI more harmful than chromium valence with III for its high oxidizing potential, greater solubility and smaller size as compare to another valance state (Liang *et al*., 2017). It easily enters in to the cells causing mutation or apoptosis. Hexavalent chromium is so harmful that inhalation, ingestion or even dermal contact can cause severe damage to the living body. Hence US EPA has set a limitation value i.e., beyond 0.1 milligram per litre or 100 ppb of chromium forms will beconsidered toxic to all form. The toxicity of chromium for many agronomical fields varies from five to one hundred mg/kg in soil (EPA 1984) (Bakshi *et al* 2022). The issued limit for chromium or hexavalent chromium for potable drinking water is up to 0.05 mg/L and drinking water near industries ranges from 2-5 g/L in the effluents (Indian standard specification for drinking water). Excess of chromium leads to yellow impacts on water and unfit for drinking (Dhal *et al*., 2013). So hexavalent chromium above its permissible limits originates some incurable health hazards in plants and animals.

Mechanisms of chromium toxicity

Reduction is a normal phenomenon of chromium but not necessarily to less toxic form (Kawanshi*et al*., 1986). Basically, this is the most supported mechanism of chromium involvement in biological process. It destroys the cell by producing free radicals (Fenti *et al*., 2020). Overdose of hexavalent chromium inside a cell may mislead some important pathways like transcription, translation and DNA replication causing mutagenesis (Su *et al.,* 2014). DNA damage show miserable condition to cell as it concludes to genotoxicity . Hexavalent chromium affects the male reproductive system as well as the development of fetus (Kim *et al*., 2012). Chromium elements are highly toxic to plants.Excess of chromium deposition affect germination and limits the growth of plant which results in reduced dry matter production and decreased yield. It also interrupts several physiological and metabolical processes causing oxidative stress to the plant. Earlier symptoms can

be identified by chlorosis and necrosis effects (Oliveira *et al.,* 2012).

Hexavalant chromium is unable to act directly with the DNA. It enters into the cells through various transport systems. As it has similarities with sulphate oxyanions (SO_4),it can affect the cells either by creating oxidative stress or by attaching to the DNA in its reduced form. When it enters into the cells it immediately reduces to an intermediate form i.e Cr (V)/(IV) due to the presence of biologicalascorbate and thiol group.The intermediate stage forms hydrogen peroxide (H2O2) and free radicals that causes oxidative stress and leads to cell proliferation and mutation. The intermediate forms are unstable so it further converts to trivalent chromium (Cr (III)) which inserts into the structural DNA forming chromium DNA adduct which interrupts the central dogma of life (Shahid *et al*., 2017).

Effect of chromium on Animal cells

Carcinogenic effects of the chromium have been studied from years with sufficient evidence which clarifies the toxicity and mutagencity in animal and plant cells (Narayani *et al*., 2013). Animal cells encountered by chromium through dermal contact, inhalation and ingestion and each of these aspects create a threat to different part of animal cells. Chromium exposure to epidermis of animal cell can cause dermatitis and dermatosis while inhalation can cause irritation, itchiness and nose bleed in nasal septum (Alvarez et al., 2021) and exceeded contact may cause respiratory disorder. Normally ingestion of chromium after a certain limit can result into cancer in gastro intestinal tract, oesophagus or may be in stomach. Studies also represent the cytogenetic impact of Cr in different biological systems (Mayotte *et al.,* 2018) which leads to point mutation, alternation of physico-chemical properties of nucleic acid and DNA damage (Mayotte *et al.,* 2018), but mechanism responsible for Cr oxidation specially with genetic material is still doubtful (Masinire *et al*., 2021). Depending on the proportion of exposure the toxicity can show minimum to lethal effect in animal body.

A survey on the workers releted to chromium Industriesconfirms that Chromium causes Carcinogenic effect on human (den Braver-Sewradj *et al.,* 2021). An experiment on workers of chromite mines in the United States reports that lung cancer was initiated with 1,445 workers, those who are directly involved in extraction from chromite mines from 1930 to 1947 (Clementino *et al*., 2018). Further study shows that hexavalent chromium can cause skin ulceration, lesion and other allergies through dermal contact. It can result into asthama or perforation in nasal septum by extreme inhalation of hexavalent form. (Halasova *et al*.2009).Ingesting Cr (VI) causes abdomen and viscous injury which will cause cancer (Langård *et al*., 2019)

Chromium toxicity on Plant cells

Plant Physiology and metabolisms gets affected by Chromium. Although the chromium absorption in still uncertain but it was assumed that depending on the valence of Chromium it get absorbed by various methods. Active absorption of chromium is found in case of hexavalent chromium as it attached with a carrier ion like sulphate for its translocation (Singh *et al.,* 2013). Hexavalent chromium also has affinity towards Fe and P ion for carrier binding. In case of trivalent chromium passive absorption takes place with requires no energy for its translocation in plants. Again there is a lack of reports that justify the enzymes for reduction or gaining of electrons to thevalence of chromium within a plant. Thus metal speciation is the only responsible factor to exert its path for accumulation or translocation as well as intensity of virulence.

Effect of chromium toxicity has been studied in different stages of plants. Hexavalent chromium creates some serious problem in plant tissues at higher concentration. With increase concentration of chromium symptoms of chlorosis and necrosis is progressively visible with a sharp decline in protein production and nitrogenase activity. (Paiva *et al.,*2014) Also reduced shoot and root growth with wilted leaves observed at early stage(Rai *et al.,* 2014). The morphological parameters severely affected by the application of chromium correspondingly the yield and production is also affected that may lead to no harvest condition. Again, the biochemical and physiological parameters are also disturbed by the increasing chromium concentration (Pattnaik *et al*., 2022). Again Rosko & Raclin (1977) showed hexavalent chromium concentration affect growth, photosynthesis, morphology and enzyme activities

in algae and is toxic in concentrations ranging from 20 -10,000 ppb . Thus, the effect of chromium toxicity has a direct impact on plant growth and yield.

Remedial measures and bioremediation:

Contamination of chromium toxicity at the manufacturing sites is due to hexavalent chromium that is stable by nature. So, it is our prime importance to dive into the detailed information of chromium and its chemistry of conversion to its stable state. Excess disposal of waste products from mining industries decreases the capacity of self-cleaning, for which soil, water, air and crops get affected. Consequent contamination in these biotic elements with hazardous metals and toxic chemical led this area into jeopardy. Hence development of new technology is essential which should emphasize on conventional approach for disposal of pollutants without producing any secondary pollutant and without disturbing the ecological food chain. (Asha *et al*., 2013).

Remedition proposals and their incapacities

While a lot of environmental investigation and remediation work involves chemical and physical protocols, it is important to remember that this type of methods is just one of the hundreds that can have impact on particular or limited areas (Bahi *et al*., 2012).Physical procedures like excavation solidification/stabilization, filtration, reverse osmosis, membrane technology, evaporation and electrochemical treatment were introduced earlier for complete removal of pollutants. But it is unable to give a persistent solution to this problem. These proposals are not acceptable due to the complicated application procedure which is difficult when it comes to large quantity remediation and second is its high cost.

Chemical detoxification includes chemical precipitation, oxidation or reduction ion-exchange and other sulphur or iron-based compounds, such as Fe (II) [Jagupilla*et al.,* 2009], amorphous FeS2 [Li Y et al., 2016], calcium polysulphide (CaSx) [Chrysochoou et al., 2015] and sodium thiosulphate (Na2S2O3) [Li et al., 2011].Again, as hexavalent chromium is water soluble; it can never be separated by means of physical separation (Pradhan *et al*., 2020). These detoxification procedure produces huge secondary pollutants so application of this procedure may be possible for industries but not for mining sites as Sukinda.

Now the bioremediation process has become a prime importance to deal with chromium pollution issue using microorganisms to detoxify the hazardous component from a particular area (Vargas *et al*, 2019). The microbes may be indigenous or may be exported to the contamination site (Kumar *et al*., 2011). Some varieties of bioremediations are phytoremediation, bioventing, bioleaching, land farming and biostimulation etc. (Verma *et al*., 2008). As living organisms are involved to reduce pollutant concentration and a maintain biodiversity balance hence bioremediation can be used as a better clean-up programmed for metal contaminated and polluted ecosystem (Park *et al*., 2011).

Bioremediation

Microbes prove to be the best remedial agents as they are able to degrade the contaminants with less energy as well as less costly ways. Again, aerobic microbes shows better results than the anaerobe (Arshi *et al*., 2021).Biological agents like yeast, bacteria and fungi take part in the cleaning programme called, Bioremediation(Kumar *et al*., 2011). Usually, microorganisms use the contaminants as their nutrients and utilizes in their metabolisms (Asha *et al*., 2013). Initially the interest was on anaerobes like *aeromonas, micrococcus* and *aerococcus* (Sharma *et al.,* 2021). There was a success found in *Thermus scoductus* and in certain *achromobacter sp*. In case of fungi the experiment starts with *actinomycetes*. Now several bacteria and fungi have been reported to reduce or adsorb, transform or bioaccumulate heavy metals from different contaminated soils. Bioremediation can be natural or intervention processes (Asha *et al*., 2013). Metals has a significant role in microbial metabolisim and bioremediation is perfect approach to utilize it as a treatment facility.

Mechanisim of bioremediation

Absorption of metals by microorganisms can take place actively through bioaccumulation and passively through biosorption. Several bioremediation cases were witnessed of having

impressive landmark on the field of heavy metal detoxification.

Biosorption is a type of bioremediation where that aim is to attach the toxic metals or contaminants to the surface of living organisms. The surface of the Cell walls are composed of different complexes including various catatonic and anionic properties that helps the heavy metal compound to latch on the surfaces (Fernández *et al*., 2018). Biological complex like Polysaccrides, lipids, amino acids and other functional groups are responsible for biosorption. Again, functional group like carboxylate, hydroxyl, amino and phosphate groups are actively present in microorganisms that may shows biosorption (Rathi *et al*., 2021). So, microbes mediated biosorption process is an affordable and large scale applicable process that can go commercial. There is also some draw backs as these are microorganisms they also have certain metabolism that requires oxygen or other gases that increase the COD and decreases the BOD in water bodies. In soil again they can release some gases that may produce secondary pollutants. The major factor is applying microorganisms may bring risk on the healthy environment of living biomass and other environmental factors.

Bioaccumulation is a process where the living entity completely engulfs the toxic materials and utilizes in its own metabolism. The mechanism of bioaccumulation is ambiguous but studies may conclude that the metabolically active uptake leads to the intercellular space and allow attaching with the protein and peptide ligands (Mishra and Malik, 2013). But it also implies that the microorganismsneed to be alive for metabolic action that may imposes unique challenges like necessity of nutrition, environment for maximum propagation and most important heath risk to the nearby biotic community. So, using native micro organisims could be an effective option for bioaccumulation.

Biosorption and bioaccumulation can be proved as best remedy for various polluted sites as they have the ability of regeneration. Other advantages include low cost, removal substantial quantities of metals and recovery of metals.

Bioremediation of Chromium

Trivalent chromium is less harmful because of its impermiability larger size lack of oxidation capacity, so conversion of Cr (VI) to Cr(III) can be a applicable process for the treatment of chromium contaminated wastes and industrial effluents. Cr (VI) at normal environmental state, get reduced in the presence of ascorbate and glutathione to form pentavalent, tetravalent free radicals and finally trivalent form. The conversion of pentavalent to hexavalent process are reversiable process that under go redox reaction inside the cell membrane leading to the formation of ROS complex that can combine affect DNA directly Cr. The common physiological mechanisms inside a cell can be directly affected by Cr (IV) through mutation (Mishra *et al*., 2019). But there are sulphate utilizing microbes absorbs hexavalent chromium through the membrane sulphate transport channels present in the cells.capacity So microbes have a capacity to intact or intake the chromium through their body again the self-replicating and cost effectiveness makes bioremediation an effective biological tool for chromium detoxification.

Fungi in the Field of Bioremediation

The kingdom fungi comprise a vast and diversified group of organisms that are found in almost every ecosystem which makes it ubiquitous in nature. The ability of producing spore makes the fungi survive the stress conditions. Extended mycelia growth may help fungi to grow in a large effected area with low nutrient requirement. Again, these organisms don't require a special condition for their dispersal, like other microorganisms. Fungi can produce several extracellular oxido-reductase that can degrade lingo-cellulose which can be used as pollutant degrading agents without utilization of carbon and energy sources and hence are called as the cleaning agents or the decomposers of the environment. Unique properties of Fungi and the mutualistic relationship with other organisms make it an excellent experimental organism for bioremediation. However, fungi of heavy metal polluted Indian habitat aren't that exploited for bioremediation

Native Fungi in heavy metal reduction:

The extremophilic nature of fungi makes mycoremediation an emerging subject and attracts attentions in recent years. Among the diversified group filamentous fungi are the significant group that used due to its low-cost values and easy

growing mycelia. Binding properties of microbes are already evident, again in fungi involving gene i.e.,hydrohobin has also been described for metal tolerance. Again, microbes belong to area of extreme metal condition develops metal resistance ability by their own. There may be involvement of more than one mechanism in case of fungi Sukinda is a well-known chromium contaminated site and hence it is the ideal source for chromium tolerant fungi. There are notable indigenous fungal strain that can show tolerance to varieties of heavy metals. Filamentous fungi like *Asperigillus, Penicillium, Rhizopus, Tricoderma and Fusarium* have been reported showing tolerance to heavy metals.

Fungal bioremediation Mechanism

Fungi can interact with different metals depending on the metal type, environmental condition and type of organisms. Possible mechanism of fungal bioremediation may include extracellular remediation or intracellular remediation. Extracellular remediation otherwise called biosorption and the intracellular remediation is called bioaccumulation. In biosorption the outer layer of cells acts as a chealating agent to bind the hexavalent chromium where as in bioaccumulation in is uptake inside to the cell allowing reduction to the trivalent form. The aim of biosorption to prevent the chromium inside the cell whereas bioaccumulation aims to reduce the hexavalant chromium concentration inside the cell.Fungal cell wall may be the responsible bioremediating organ that chleate Cr (VI). The proteins and the peptides present on cell wall acts as chelating agent to bind chromium In yeast it was evident that gulothine binds the hexavalent chromium, whereas it was suggested that presence of other pigment like melanin and other polymorphic materials are helping in binding the hexavalent chromium. Some FTIR report confirms that biosorption occur in the presence of functional group like Carboxyl, Amine and Hydroxyl that presumes to help in biosorption (Kumar *et al*., 2021). Biosorption can also be performed in dead fungal biomass (Akhtar *et al*., 2020). There are also other External environmental factors can affect the biosorption capacity. Factors like Biomass , Initial concentration (Kavita et al., 2011), contact of time and Temperature (Sarkar *et al*., 2013) may disturb the biosorption capacity.The Bioaccumulation reductions have not been studied in brief. There are a number of channels through which the hexavalant chromium can enter into the cell. It can actively be transported into the body through sulphate ion channel as it has structural similarity with sulphate ion (Zhio *et al*., 2009). As bioaccumulation can successfully observed in fungi like *Asperigillus* and *Fusarium*, its mechanisim and potential need to be discovered.

Conclusion

- Antagonistic activities of hexavalent chromium are accelerating in speed since 1990 in sukinda area, has a serious implication on the flora and fauna. So our first priority is to regulate the environment in such a manner that its outbreak should be limited in that particular area for that a frequent surveys and continuous monitoring is essential.
- Scientists should encourage the bioremediation protocols and removal of hexavalent chromium without producing any secondary pollutants. Current situation of sukinda reviews explains that the bioremediation is capable to fulfill the needs for detoxification. It is an attractive option to clean, manage and remediate the hexavalent chromium contamination through microbial activity.
- Recent bioremediation research activity mainly focuses on the bacterial and plant based remediation processes whereas fungi are the natural pollution cleaning agents. So scientists need to change their vision other than bacteria and plants.
- Again, the over qualifying properties of fungi makes it a perfect bioremediation tool for Sukinda. Novel species of fungi family should be explored by which the speed of remediation can be elevated with great potential. Presently an increase in fungal research has been noticed, but only a few are towards their destination.
- Again now a days, the use of biotechnology is at its peak, so exploration and editing in fungal genes using biotechnological tools is essential to constitute a better promising candidate for removal of chromium toxicity.
- Although the speed of remediation depends on the environmental condition that may not favor to the

organisms to Sukinda again while working with microorganisms there is a risk of infection or mutation whose results can't be ignored. Bioremediation has been accepted and used in different corner of world.

- The diversified characteristics of fungal species incorporated with biotechnological techniques can be applied as an improved tool for bioremediation against chromium toxicity after further investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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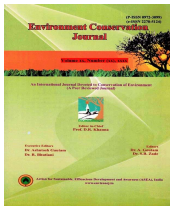

Growth, yield and economics of amla (*Emblica officinalis* L.) based agri-horticultural system in Alfisols of semi-arid tropic

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ABSTRACT

An intercropping trial conducted during 2011 to 2017 using five year old amla (*Emblica officinalis* L.) orchard planted at 4 X 4 m spacing and grown under rainfed condition to identify the suitable and profitable intercrops. The intercrops *viz,* finger millet, fodder maize, field bean, grain amaranth, cowpea, horsegram were considered in the study besides their pure stand. Growth parameter of amla such as plant height (369 cm), number of branches/tree (2.73), stem diameter (35.31 cm), canopy spread (279 cm) and biomass yield (296 kg/ha) was found to be statistically significant with Amla intercropped with field bean compared with sole amla. The higher amla equivalent yield was recorded in intercropping with finger millet (1517 kg/ha) and was at par with cowpea (1298 kg/ha). Finger millet proved to be better intercrop in amla and registered 57.11 per cent higher net returns and Benefit cost ratio than sole amla. Overall, Amla + finger millet cropping system was found to be more sustainable both interms of benefit cost ratio (2.43) and improving system productivity (104.44 %) followed by pulse crop such as cowpea and field bean. The higher sustainable yield index (0.83) was with amla + finger millet intercropping system while Land Equivalent Ratio and Area Time Equivalent Ratio were higher with amla + field bean intercropping system.

Introduction

Climate change induced an unsustainable production system under rainfed situation, demands climate smart crops (Ramachandrappa *et al*., 2016a; Bhutiani and Ahamad, 2018) combating climate change demands enhancing forest ecosystem, which is difficult to increase under populated India. Alternate land use involving Agri- horti systems seems to the long term operation for sustainability. Amla or Indian goose berry (*Emblica officinalis* L.) based agri-horticultural system has enormous potential to use and conserve rainfall particularly under dryland condition for betterment of poor farmers (Thimmegowda *el al.,* 2019). Amla is an

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deep rooted deciduous tree species, which has a wide adaptability in wider range of soil. It is potential fruit species suitable for growing under dryland condition. Sole amla orchards provides gives higher gross returns with lesser investments for planting and its management, but taking agricultural crops as intercrops along with amla provides an opportunity for better land utilization also reduction in the risk due to aberrant climate condition.

Agri horticulture systems in rainfed conditions are the ideal systems for controlling runoff, soil erosion and land degration. The major problem in rainfed area is increased competition between trees and crops for water. But the varied nature of crops in agri-horti system, utilize the water as well as other resource efficiently with added seasonal revenue. Intercropping has been proved as potential crop production systems and it will provide substantial yield advantage over sole cropping system (Willey, 1979).

Intercropping systems have ability to cover land surface very efficiently, which check soil erosion and helps to check soil erosion through sufficient ground coverage and also improve the soil physico-chemical condition. Traditionally, intercropping in the interspaces of fruit orchards is practiced (Adiveppa Mallappa Asangi *et al*, 2019), but only a few results are available for amla based horticultural system. Hence, the present study was undertaken to know the suitability and profitability aspects of different intercrops under *rain-fed* conditions in *Alfisols* of semiarid tropic.

Material and Methods

Study area: The field study was carried out at the AICRP for Dryland Agriculture, University of Agricultural Sciences, Bengaluru. The centre is situated in Eastern Dry Zone of Karnataka at 12^0 58' North latitude and 77^0 35' East longitude with altitude of 930 m above mean sea level. The site experiences climate with bimodal distribution of rainfall *i.e.* the rainfall during 2014 and 2015 was positive normal with 8.6 and 15.9 per cent excess higher values. The deviation during *kharif* 2013 and 2016 was -7.4 and -24.5 %, respectively compared to normal and the impact on crop production activities under conventional farming practices was more pronounced during 2016 (Table 1).

Plate1: Geotagged image of experimental site

The intercropping trial was carried out in well-established five year old amla orchards for seven years from 2011-12 to 2017-18.

Treatment details: The intercrops considered in the study are finger millet, cowpea, horsegram, field bean, fodder maize and grain amaranth besides their sole crop and compared with amla. The intercrops were sown one meter away from the trunk. The experiment was laid out in a Randomized Block design with three replications. Well decomposed compost 15 t ha^{-1} was applied well before 15 days prior to sowing of the intercrops. Nitrogen, phosphorus and potassium were applied as basal doses @ 50:40:25 kg ha^{-1} for finger millet, 25:50:25 kg ha^{-1} for cowpea and field bean, 25:38:25 kg ha^{-1} for horsegram, 100:50:25 kg ha^{-1} for fodder maize and 40:20:20 kg ha^{-1} for grain amaranth. In case of finger millet, fodder maize and grain amaranth. Nitrogen was applied in two equal splits one as basal dose and at 30 DAS. The soil of the experimental site was acidic in reaction [pH(1:2.50): 5.4], deficient in organic carbon (0.32 %), medium in available N (372.8 kg ha^{-1}), P_2O_5 (49 kg ha^{-1}) and K_2O (169.9 kg ha^{-1}).Observations on growth parameters of amla in terms of plant

height, number of branches per plant and crown diameter were recorded. The data on fruit yield per plant were recorded at harvest during all four years and were statistically analyzed, similarly the intercrops yield was also recorded. The yield of intercrop was converted into amla equivalent yield considering the yield and prevailing price of the produce (Thimmegowda *et al.,* 2016).

$$\text{Crop equivalent yield (kg/h)} = \text{Yield of main crop (kg/ha)} + \left\{ \frac{\text{Yield of inter crop (kg/ha)} \times \text{Price of inter crop (Rs./kg)}}{\text{Price of main crop (Rs/kg)}} \right\}$$

Land Equivalent Ratio (LER) along with Area Time Equivalent Ratio (ATER) were calculated as intercropping efficiency with below given formula (Willey, 1979):

$$\text{Land equivalent ratio} = \frac{(Y_{ab})}{Y_{aa}} + \frac{(Y_{ba})}{Y_{bb}}$$

Where,
Y_{aa}: Sole yield of crop a
Y_{bb}: Sole yield of crop b
Y_{ab}: Intercropping yield of crop a
Y_{ba}: Intercropping yield of crop b

Area Time Equivalent Ratio of different cropping systems are calculated by formula given by Hiebsch and Mc Collum (1987).

$$\text{Area time equivalent ratio} = \frac{(R_{ya} X_{ta}) + (R_{yb} X_{tb})}{T}$$

Where,
Rya: Relative yield of the crop 'a'
Ryb: Relative yield of the crop 'b'
ta: Duration (days) for crop 'a'
tb: Duration (days) for crop 'b'
T: is the total duration (days) of the intercropping system.

The Sustainable yield index of amla based intercropping systems was calculated with the formula given by Ramachandrappa *et al.* (2016b).

$$\text{Sustainability yield index (SYI)} = \frac{A - SD}{Y_{max}}$$

Where,
A = Average yield over the years for a particular treatment;
SD = Standard deviation for the treatment;
Y_{max} = Maximum yield obtained in any of the treatments over the years.

The economics was calculated for individual treatments for all the years by respective price of inputs and produce. The net return received during study was worked out by subtracting cost of cultivation (Rs/ha) from the gross return (Rs/ha) of respective years.

Statistical analysis: The data from 7 years were analyzed to check the significant difference between the treatments and to draw valid conclusions with Analysis of Variance technique (Gomez and Gomez, 1984. The level of significance used in 'F' and 't' test was p=0.05. Critical difference (CD) values were calculated, wherever 'F' test was found significant.

Results and Discussion

Growth Parameters of amla

Plant height, Number of branches and Collar diameter: Inter crops grown in association with amla varied significantly for different parameters. The plant height, branches and collar growth are the important attributes, which greatly influenced by supply of water and nutrient. The increased plant population per unit area due to addition of intercrops resulted in higher competition for soil moisture, nutrients and light, which influenced the vertical/ horizontal growth and intern growth parameters. Amla + field bean recorded significantly higher plant height (369 cm), number of branches (2.66) and collar diameter (35.31 cm) followed by amla + cowpea, amla+ horsegram compared to amla sole (309, 2.26, 31.03 cm, respectively) (Table 2). The higher growth parameters are mainly attributed due to enhanced availability of nitrogen through symbiotic nitrogen fixation and increased organic matter addition in the form of leaf litter by the legume crops. Due to higher biomass production, incorporation and further decomposition has led to higher availability of nutrients for uptake (Adiveppa Mallappa Asangi *et al.,* 2019). The increase in stem collar diameter could also be due to increase in leaf canopy spread, number of leaves and number of branches. These results are in conformity with the findings of Chauhan *et al.* (2013), Ramulu *et al.* (2015) and Swain *et al.* (2014).

Table 1: Meteorological data of the experimental area during 2011-2017 at UAS, GKVK, Bengaluru

	2011	2012	2013	2014	2015	2016	2017
Normal rainfall (mm)	923.1	925.2	915.4	913.8	917.2	920.4	915.7
Actual rainfall (mm)	804.5	571.9	847.5	992.3	1061.2	694.9	1115.8
Number of rainy days	61	34	58	54	71	43	64
Number of dry spells	3	6	6	3	2	4	2
Excess / deficit rainfall (%)	-12.8	-38.2	-7.4	8.6	15.9	-24.5	17.93

*** Normal rainfall was calculated taking average annual rainfall from 1978 to previous year**

Canopy spread/plant and biomass (kg/tree): At harvest, the canopy spread of amla differed significantly due to intercropping. Amla + field bean recorded higher canopy spread/plant (279 cm) followed by amla + cowpea (250 cm). Significantly lower canopy was recorded by amla +fodder maize (197.2cm) (Table 3). Higher biomass (kg/tree) was noticed in amla + field bean (296 kg/tree) followed by amla +horse gram (286 kg/tree) as compared to other intercrops (Table 3). Enhanced growth of amla plants in with intercrops might have attributed to the improved soil porosity and aeration from frequent soil management practices and also due to the better response for applied inputs by intercrops than in sole plantation. Interspaces of sole crops were left uncultivated and not received additional inputs like manure, fertilizer *etc.,* Awasthi and Saroj (2004) reported positive effect of intercrops on growth and vigour of amla and mango. The finding also supports the views of Saroj *et al.* (2003) in ber.

Yield of Amla as influenced by intercrops

Amla yield: Among the different intercrops, higher amla fruit yield (749 kg/ha) was recorded from the amla trees inter cropped with field bean, while it was minimum in fodder yield (535 kg/ha) followed by 721 kg/ha in amla + cowpea, 655 kg/ha in Amla + horse gram and 604 kg/ha in amla + finger millet than amla + fodder maize (535 kg/ha) and sole amla (655 kg/ha) (Table 5). Growing of pulse crop helped in building up of soil fertility and better utilization of applied nutrients which resulted in improved growth and yield of main crop (Meena *et al.,* 2011). Maize being an exhaustive crop removed much nutrients for its growth and yield and there by resulted in reduced yield of amla (Chaturvedi and Jha, 1998). The other reason for increase in fruit production under agri-horticultural system may be also due to application of fertilizers and manure to intercrops and its utilization by amla trees as there was no physical barrier between root systems of intercrops and trees (Korwar *et al.,* 2006).

Amla equivalent yield: Significantly higher amla equivalent yield was observed in intercropping with finger millet (1517 kg/ha) followed by cowpea (1298 kg/ha) and field bean (1235 kg/ha) compared to other intercrops in amla based agri-horti system (Table 6). Better performance of small millet even under drought and erratic rainfall both as sole and intercrop during the different growing period over the years was due to their drought tolerance (Shashidhar *et al.,* 2000). With respect to legume as intercrops which act as good cover crop and helps in better moisture conservation helped in yield enhancement

Intercropping efficiency

On the basis of mean data among different intercrops, maximum land equivalent ratio was recorded with amla+ field bean (1.69) followed by amla + finger millet (1.61) intercropping system, indicating more efficient use of land than sole amla and among the intercrop less land equivalent ratio was recorded in amla + fodder maize (1.46) (Figure 1). Intercropping efficiency analysis using the ATER approach has also shown differences among different associations (Figure 1). The higher mean values of ATER were recorded by the Amla + field bean (1.67) intercropping system. While, the lowest ATER value was recorded by the alma + finger millet (1.06). The higher yield were recorded in intercrops was mainly due to complementary effects among component crops and also due to efficient use of resources when compared to sole cropping systems (Mudalagiriyappa *et al.*, 2011). The inherent capacity of crops will efficiently utilize natural resources and complementary interaction plays vital role in resource utilization (Maitra *et al*., 2019). Further, higher yield of both the crops in maize-cowpea intercroppingombination was noted than pure stands (Kimou *et al*., 2017).

Table 2: Plant height (cm), No of branches and stem diameter (cm) of amla as influenced by amla based Agri-horti system

Treatment	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Pooled
Plant height (cm)								
Amla+ Finger millet	163	236	240	304	357	350	345	285
Amla+Fodder maize	174	254	248	334	355	365	359	299
Amla+Field bean	204	289	341	357	388	506	500	369
Amla+Grain amaranth	188	255	239	301	320	328	329	280
Amla+Cowpea	164	253	313	342	381	444	439	334
Amla+Horsegram	190	263	341	349	376	482	476	354
Amla sole	151	172	207	342	380	457	452	309
S. Em. ±	5.89	15.76	15.14	20.24	22.60	34.47	34.07	29.59
C. D. (p=0.05)	18.14	48.56	46.64	NS	NS	106.23	104.99	83.07
No. branches								
Amla+Finger millet	1.77	2.20	2.11	2.83	2.83	2.33	2.67	2.39
Amla+Fodder maize	2.10	2.30	2.58	3.00	3.00	3.00	2.67	2.66
Amla+Field bean	3.30	2.77	2.83	3.10	3.10	2.00	2.00	2.73
Amla+Grain amaranth	2.80	2.90	2.33	3.00	3.00	2.33	2.33	2.67
Amla+Cowpea	1.90	2.20	2.57	3.07	3.07	2.67	3.33	2.69
Amla+Horsegram	2.40	2.77	2.58	3.00	3.00	1.67	2.33	2.54
Amla sole	1.60	1.43	1.98	3.07	3.07	2.33	2.33	2.26
S. Em. ±	0.12	0.17	0.13	0.08	0.08	0.56	0.36	0.28
C. D. (p=0.05)	0.37	0.52	0.41	NS	NS	NS	NS	0.77
Stem diameter (cm)								
Amla + Finger millet	12.6	21.0	21.8	31.5	32.3	43.8	44.7	29.66
Amla + Fodder maize	12.8	24.2	25.9	37.8	38.2	43.7	42.7	32.19
Amla +Field bean	15.2	26.9	32.3	38.5	42.1	45.5	46.7	35.31
Amla + Grain amaranth	15.0	25.4	22.6	32.0	35.1	43.3	42.0	30.79
Amla + Cowpea	12.8	24.2	28.9	38.3	42.0	41.8	43.0	33.02
Amla + Horsegram	14.8	24.4	28.2	38.2	40.2	46.3	47.3	34.22
Amla sole	10.7	19.5	20.9	38.8	40.8	43.7	43.0	31.03
S. Em. ±	0.62	1.18	1.26	2.09	4.08	2.73	2.67	2.34
C. D. (p=0.05)	1.92	3.64	3.87	NS	NS	NS	NS	6.58

***NS: Non-significant at p=0.05**

Table 3: Canopy spread and biomass of amla as influenced by amla based Agri-horti system

Treatment	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Pooled
Canopy spread (cm)								
Amla + Finger millet	90	153	146	179	302	346	349	223
Amla + Fodder maize	106	152	156	207	282	307	307	217
Amla + Field bean	118	192	199	302	338	402	404	279
Amla + Grain amaranth	109	189	152	185	290	318	318	223
Amla + Cowpea	101	153	190	265	313	365	362	250
Amla + Horsegram	108	155	182	243	306	343	346	240
Amla sole	78	131	106	304	308	404	396	247
SEm±	3.50	15.86	6.62	9.57	22.20	14.98	12.56	13.43
CD (0.05)	10.79	NS	20.40	29.50	NS	46.15	38.69	37.70
Amla biomass (kg/ha)								
Amla+Finger millet	78	150	158	386	264	386	395	259
Amla+Fodder maize	80	180	197	384	323	384	367	273
Amla+Field bean	99	206	261	326	366	405	409	296
Amla+Grain amaranth	97	192	165	258	291	380	362	249
Amla+Cowpea	80	180	226	325	365	363	373	273
Amla+Horsegram	96	182	219	323	348	415	419	286
Amla sole	63	136	149	329	353	385	380	256
S. Em. ±	5.1	11.1	12.4	22.0	43.5	30.8	29.8	25.2
C. D. (p=0.05)	15.6	34.3	38.1	67.7	NS	NS	NS	70.8

***NS: Non-significant at p=0.05**

Table 4: Intercrop yield as influenced by amla based Agri-horti system

Treatment	Intercrop yield (kg/ha)						
	2011	2012	2013	2014	2015	2017	Mean
Amla + Finger millet	2610	1843	2187	2296	1746	1324	1620
Amla+Fodder maize	17989	12332	9840	7691	18057	13902	7825
Amla + Field bean	887	725	953	595	334	308	490
Amla + Grain amaranth	1287	1106	948	783	267	261	555
Amla+Cowpea	810	737	856	498	473	398	450
Amla + Horsegram	653	587	831	526	247	221	421
Finger millet	2576	1872	2424	2679	2167	2033	2292
Fodder maize	27683	13846	10758	17727	23070	18974	18676
Field bean	947	769	970	776	587	557	768
Grain amaranthus	1413	1295	1152	958	412	349	930
Cowpea	877	795	924	727	935	808	844
Horsegram	703	615	1030	697	424	405	646

***In 2016 due to scanty rainfall intercrop was not recorded.**

Table 5: Amla yield as influenced by amla based Agri-horti system

Treatment	Amla yield (kg/ha)					
	2013	2014	2015	2016	2017	Pooled
Amla+Finger millet	407	411	776	728	699	604
Amla+Fodder maize	379	399	577	591	730	535
Amla+Field bean	449	470	1045	914	867	749
Amla+Grain amaranth	338	386	711	699	716	570
Amla+Cowpea	422	453	1012	861	858	721
Amla+Horsegram	458	425	816	838	739	655
Amla sole	476	509	999	846	879	742
S. Em. ±	16.90	25.39	60.22	78.92	51.49	49.16
C. D. (p=0.05)	52.07	NS	185.55	NS	NS	138.72

***NS: Non-significant at p=0.05**

Table 6: Amla equivalent yield as influenced by amla based Agri-horti system

Treatment	Amla Equivalent yield (kg/ha)						SYI
	2013	2014	2015	2016	2017	Pooled	
Amla+Finger millet	1849	1845	1736	728	1427	1517	0.83
Amla+Fodder maize	858	591	1254	591	1251	912	0.43
Amla+Field bean	1255	1214	1504	914	1290	1235	0.64
Amla+Grain amaranth	825	1561	1112	699	1108	1061	0.53
Amla+Cowpea	1903	1076	1604	861	1355	1298	0.68
Amla+Horsegram	1264	846	1032	838	932	982	0.48
Amla sole	476	509	999	846	879	742	0.32
S. Em. ±	51.88	97.68	57.81	56.34	60.35	67.22	
C. D. (p=0.05)	151.42	285.10	168.73	164.43	176.16	188.00	

***NS: Non-significant at p=0.05**

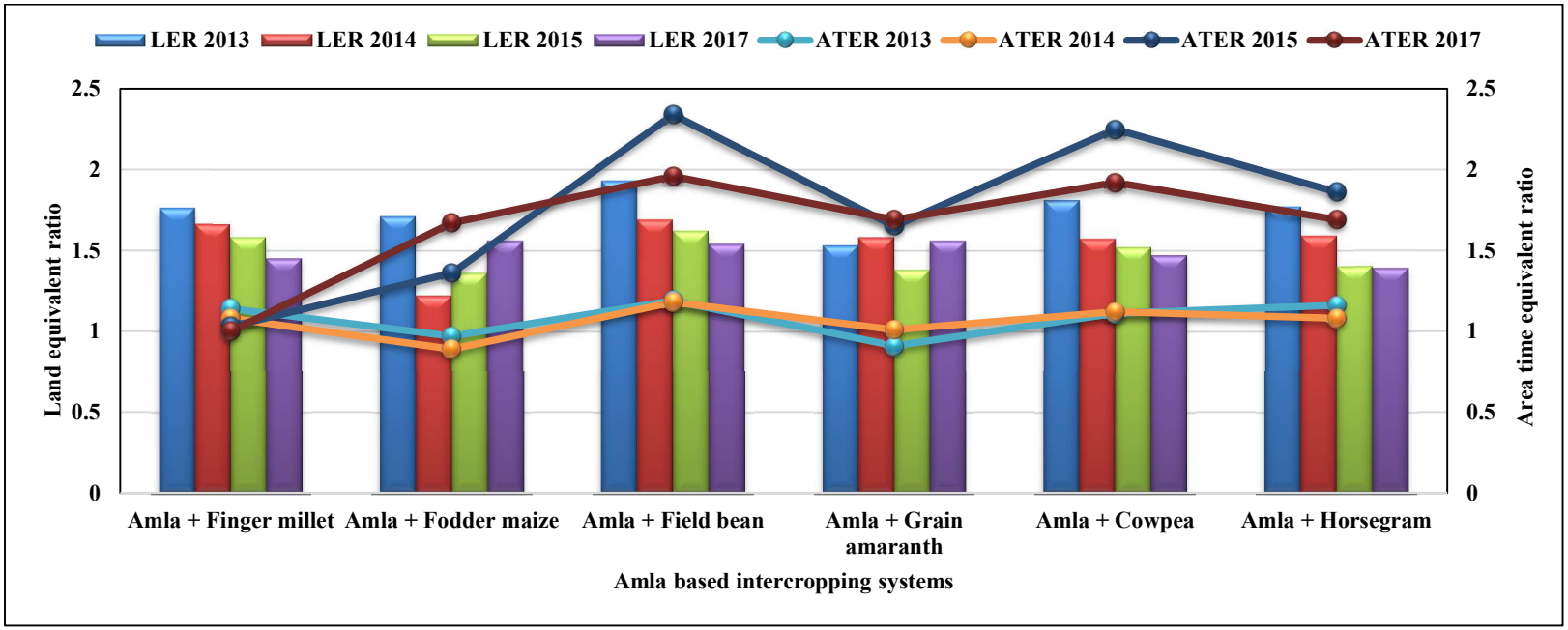

Figure 1. Land equivalent ratio and Area time equivalent ratio of amla as influenced by amla based Agri-horti system.

Table 7: Net returns and B: C ratio of amla as influenced by amla based Agri-horti system

Treatment	Net returns (Rs./ha)								B:C ratio							
	2011	2012	2013	2014	2015	2016	2017	Mean	2011	2012	2013	2014	2015	2016	2017	Mean
Amla + Finger millet	18400	20618	29831	51632	43110	10824	29446	29123	2.38	2.27	2.74	3.33	2.64	1.59	2.07	2.43
Amla + Fodder maize	13300	9321	-2726	2573	28499	5591	28737	12185	2.21	1.73	0.90	1.12	2.31	1.31	2.13	1.67
Amla + Field bean	3300	1310	9210	23570	34548	19037	24332	16472	1.51	1.17	1.32	1.94	2.35	2.09	2.88	1.89
Amla + Grain amaranth	14300	8939	-2687	38665	18736	13635	25212	16686	2.16	1.70	0.90	2.63	1.73	1.95	1.96	1.86
Amla + Cowpea	1628	1197	29548	18867	39917	16794	27824	19396	1.10	1.07	2.07	1.78	2.65	1.95	2.25	1.84
Amla + Horsegram	5650	2590	11994	11513	28876	17914	18501	13863	1.41	1.19	1.46	1.52	3.33	2.15	1.72	1.83
Amla sole	-	-	6537	9958	29094	23039	24053	18536	-	-	1.79	3.68	2.82	3.14	3.16	2.91

Sustainable yield index (SYI)

The data given in Table 6 revealed that amla + finger millet intercropping system recorded the higher sustainable yield index (0.83) followed by amla + cowpea (0.68) and amla + amla + field bean (0.64) as compared to sole amla (0.32) which indicated that at least 159 per cent of the maximum observed yield over years is assured with high probability in intercropping system as against 159 per cent in sole cropping system. Hence, higher sustainable yield index shows that the intercropping helps in providing yield stability (Henry and Kumar, 2005). Similar findings were reported by Koli *et al*. (2004). Finger millet was found to be a compatible intercrop with amla for efficient use of resources and sustainability under dryland situations.

Economics

Economic analysis of different inter cropping system showed that higher returns were obtained when the intercrops were grown in association with amla compared to sole cropping. Finger millet intercropping in amla earned maximum net returns (Rs. 29,123/ha) followed by amla + cowpea (Rs. 19,396/ha). These two intercrops estimated an additional income of Rs. 10,587/ha and 860/ha, respectively over sole amla. Lower returns obtained from other intercropping system was due to lower prevailing market price and increased cost on amla in all other treatments. Similar was the trend in B:C ratio with 2.13 in amla + finger millet with 104.44 % improvement in system productivity compared to other inter crops (Table 7). The increased returns from tree- crop combination have been reported by Nath *et al*. (2007) in perennial fruit based multi storied production system

Conclusion

Agri-horticulture system is an essential approach to have higher farm income and for maintaining better soil fertility. Even though the yield of individual crops including amla was higher under sole crops but the additional yield from component crops is an added advantage under intercropping system. Amla trees inter cropped with finger millet was better cropping system, since it has recorded 104.47 per cent higher finger equivalent yield, higher net returns, inter cropping efficiency and sustainable yield index when compared to sole amla. Besides finger millet, pulses like cowpea and field bean are also best intercrops in amla to get higher yield, profit and sustainability.

Conflict of interest

The authors declare that they have no conflict of interest.

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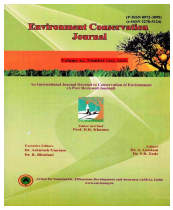

Varietal and moisture effect on physical properties of various pearl millet (*Pennisetum glaucum*) cultivars

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ABSTRACT

Engineering properties of pearl millet varieties (Pusa composite 443, Pusa composite 701, Pusa1201 and Pusa1801) were evaluated at varying moisture content (10-25% wb). A significant varietal difference was found on studied properties. GMD, Surface area, thousand grain mass, the angle of repose, porosity, internal coefficient of friction, static coefficient of friction (Poly, GI, MS and Al) increased linearly with increase in moisture content within the range of 10 to 25% (w.b.) while the bulk density, true density and hardness decreased linearly with increase in moisture content within the same range. But the value of sphericity showed that direct and indirect relation with moisture content depending variety. The mean value of different cultivars observed and found extreme high and low value of bulk density, true density and porosity for PC701 and Pusa1201, geometric mean diameter and surface area for pusa1801 to Pusa1201, sphericity and internal coefficient of friction for Pusa1201 and PC443, grain mass for Pusa1801 and PC 701, angle of repose for Pusa1201 and PC701, hardness for PC701 and PC443 respectively at moisture ranges from 10 to 25% (wb).

Introduction

Pearl Millets (Pennisetum glaucum) are getting more attention for its gluten free nature and It belongs to the family of poaceae (Sharma & Niranjan, 2018). India is one of the largest producers of pearl millet with 8.61 MT production and 1243 kg/ha yield from the 6.93 million ha area during the period 2018-19 (Project Coordinator Review, 2020). Its nutritional composition and health benefits attracted today's market focused present health segment highlighting the commercial viability of the crop (Satankar *et al.,*2020). Pearl millet accounts high proportion of germ and it is twice that of sorghum, so it plays a main factor in containing the higher nutritive value than other cereal crops (Andrew and Kumar 1991). (Meera *et al.,* 2019) studied about physical and mechanical properties of brown rice and paddy which is helpful in designing instruments for various agricultural practices such as processing, milling, drying (Singh *et al.,* 2021), heating, cooling, handling, extraction (Patil *et al.,* 2020), transfer and storage of grains, thereby reducing post-harvest losses. Before designing a model for viscoelastic materials, it is important to understand the mechanical properties of the material (Satankar *et al.,*2020). (Mwithiga & Sifuna, 2006) reported that the sorghum seed properties vary from variety to variety and these properties are also affected by moisture content. Many researchers have studied the properties of various agricultural produces like millets (Singh *et al.,* 2010)(Baryeh, 2002)(Jain & Bal, 1997), Pomegranate dried seeds (Kingsly *et al.,* 2006), grass seeds (Singh *et al.,* 2021). Although some information on the properties of pearl millet is

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available (Jain & Bal, 1997) (Baryeh, 2002) (Ojediran *et al.,* 2010), data on engineering properties of pearl millet grain cultivars of India is still lacking. Thus, the objective of this study was to collect data on the effect of varietal and moisture on engineering properties of different cultivars of pearl millet grains i.e. GMD, ϕ, A, W, ρb, ρt, P, ɵ, μs on various surfaces {i.e. poly, GI, MS and Al}, μi, and H of the grain as a function of moisture content.

Material and Methods

The pearl millet cultivars (Pusa1201, Pusa1801, PC43 and PC701) were obtained from the farm of Genetics and Plant Breeding Division, IARI, Pusa, New Delhi for carrying out the experiments. The moisture content of the grains was determined by standard oven-dry method (AOAC, 2002). The initial moisture contents of Pusa1201, Pusa1801, PC443 and PC701 were 8.77, 9.32, 8.37 and 8.72% (w.b.) respectively. Each variety sample was divided into four equal amounts for performing different experiment. The four desired moisture contents (10, 15, 20 and 25%, w.b.) were obtained accordance with recommended procedure (Subramanian & Viswanathan, 2003). The geometric, gravimetric, mechanical and frictional properties of each pearl millet variety were determined or four moisture levels (10%, 15%, 20% and 25%). Three replications at each treatment were taken for accuracy of results. The mean of each engineering property determined at four moisture levels for each variety and standard deviation values of each engineering property were calculated, analyzed and presented (Table1).

Measurement of Geometric properties

GMD (mm) was calculated using the measured sizes of the pearl millet grain L (length), W (width), and T (thickness). Digital vernier caliper (least count 0.01 mm) was used for measuring dimensions of hundred randomly selected grains for each variety. GMD was calculated by using the following equation

$GMD = (LWT)^{1/3}$ (Baryeh, 2002)

The sphericity (decimal) of the pearl millet grain was calculated by following equation

$\phi = (LWT)^{1/3}/L$ (Ramashia *et al.,* 2018)

Where, L=length (mm), W=width (mm) and T=thickness (mm)

The surface area (mm^2) of pearl millet cultivars were calculated using following equation

$A = \pi (D_g)^2$ (Sologubik *et al.,* 2013)(Altuntaş & Yildiz, 2007)

Measurement of Gravimetric properties

Determination of the value of thousand grain mass (W) expressed in gram, was done by random selection of pearl millet sample containing hundred seeds from each variety and measured using electronic weighing balance (least count 0.01mm). Then measured values were multiplied by ten to give the value of W (Figueiredo *et al.,* 2011).

Bulk density, ρ_b (kg/m^3) was determined by using 100 ml measuring cylinder filled with grains. The weight of the sample without any compaction was recorded for known volume. The bulk density was determined by using formulae (Ramashia *et al.,* 2018)(Nwabueze *et al.,* 2020)

True density, ρ_t (kg/m^3) of the pearl millet grains was determined using the toluene displacement method. The true volume of the grain was the final volume displaced by toluene. The following equation was used to calculate the true density of grain.(Konak *et al.,* 2002)).

$\rho_t = W/ V_d$

where, ρ_t= True density (kg/m3),

W= weight of the sample (kg)

V_d= Displaced volume (m^3)

Porosity, P (decimal) of pearl millet grains was determined from bulk and true density using the equation given by (Figueiredo *et al.,* 2011) (Nwabueze *et al.,* 2020).

$P = (1- \rho_b/ \rho_t)\times 100$

Where P = porosity (%),

ρ_b = Bulk density, kg/m^3

ρ_t= True density kg/m^3.

Frictional properties

The angle of repose (θ) was calculated using a cylindrical container with both ends open. Grain was placed in a cylindrical container, which was slowly raised until the grain formed a cone on a platform. The value of was then calculated using the height and diameter of a naturally formed cone. The angle of repose was calculated using the following relationship.

$\theta = \tan^{-1}\frac{2h}{d}$ (Kaleemullah & Gunasekar, 2002)

where, θ = Angle of repose (degree),

h= height of cone (cm)

d= diameter of the cone (cm)

The experimental set up was fabricated for determining internal and static coefficient of friction in the workshop of Division of Agricultural Engineering, IARI, Pusa, New Delhi. Subramanian & Viswanathan (2007) studied coefficient of internal friction was calculated using the following equation

$$\mu_i = F_i/N_i$$

where μ= coefficient of internal friction

N_i= normal force in internal friction (kg)

Fi= frictional force in internal friction (kg)

The coefficient of static friction was determined on four surfaces viz. polythene (poly), galvanized iron (GI), mild steel (MS) and aluminium (Al) sheet. It was performed according to the method of Subramanian & Viswanathan (2007).

$$\mu_s = F/N$$

where μ= coefficient of static friction

N= normal force in static friction (kg), and

F= frictional force in static friction (kg)

Mechanical properties

The rapture force of pearl millet grain was measured to determine grain hardness (H) using a texture analyzer (Stable micro system, U.K.) and a load cell weighing 50 kg. The test speeds during the analysis were 2 mm/s with 50% strain. The individual grain along with its thickness loaded between horizontal plate and load cell, compressed until rupture occurred. The peak value of the force in curve was recorded as the hardness of the grain (Altuntaş & Yildiz, 2007).

Results and Discussion

The cultivars of pearl millet collected and maintained at four different equilibrium moisture content (MC) were subjected to different physical and mechanical tests. Mean values and their standard deviations of selected engineering properties measured at four different equilibrium moisture contents of 10%, 15%, 20%, 25% (wb) were presented in Table 1. Significant varietal effect was observed on all studied engineering properties except coefficient of internal friction and hardness. Figures show the mean values of engineering properties of various pearl millet cultivars at various moisture content levels. All the studied engineering properties were varying significantly with moisture content for all the pearl millet cultivars.

Geometrical properties of pearl millet cultivars

Geometric Mean Diameter (GMD)

Dimensions play an important role in typically designing the different processing equipment. The results for the geometric mean diameter of pearl millet varieties shown in Tables1. Significant difference in GMD values was observed for all of the pearl millet varieties. GMD for pearl millet varieties varied from 2.69±0.07 mm in PC 443 or Pusa 1201 to 2.75±0.08 mm in Pusa 1801. (Ramashia *et al.,* 2018) reported geometric mean diameter for finger millet and ranged from 2.81 ± 0.71 mm to 1.35 ± 0.06 mm. Similar results were also found for pearl millet (Nwabueze *et al.,* 2020) (Jain & Bal, 1997) (Asoiro *et al.,* 2020) (Koocheki *et al.,* 2007). The GMD value for all varieties increased linearly as the moisture level increased (Fig. 1).

The increase in GMD could be due to the moisture absorption that results in the expansion of the grain dimensions (Solomon and Zewdu, 2009). The values of GMD for MC and variety interaction were found non-significant and there was no difference in cultural values of GMD at each moisture level.

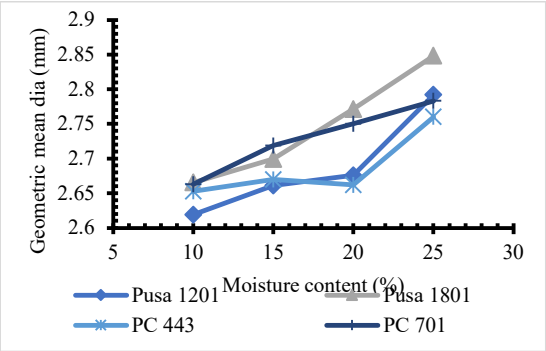
Sphericity (ϕ)

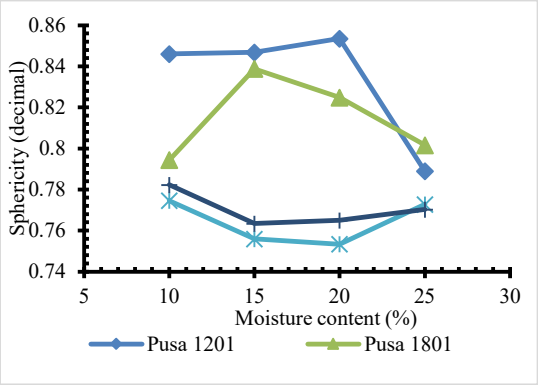
The sphericity of pearl millet showed a variation from 0.76± 0.01 (PC 443) to 0.83± 0.03 (Pusa 1201). The sphericity range for pearl millets was reported between 0.937-0.942 (Jain and Bal, 1997). (Kaleemullah & Gunasekar, 2002)(Meera *et al.,* 2019) showed the variation in sphericity from 45% to 56% and 0.787 to 0.723 for rice and arecanut respectively. The sphericity of PC 443 and PC 701 varieties was inversely related to moisture for 10-15% moisture but had little effect in the moisture range of 15-20% moisture; however, after that, the sphericity increased with increase in moisture. Whereas the sphericity of other varieties i.e. Pusa1201 and Pusa1801, was showing direct relation with moisture up to certain moisture level then showed inverse (Fig1). (Sologubik *et al.,* 2013) found the similar results that initial increase in sphericity followed by decrease in sphericity of barley seed.

Surface area (A)

The surface area of the various pearl varieties studied differed significantly. Surface area ranged

from 22.73± 1.22 mm^2 in Pusa1201 to 23.87± 1.72 mm^2 in Pusa1801. The surface area increased as the moisture content increased. This shows the hygroscopic nature of pearl millet. This increase in surface area was caused by an increase in grain dimensions as the moisture content of the grains increased. Similar trend was also observed for soyabean (Deshpande *et al.,* 1993). It was found that not much difference in values of surface area at each moisture level (fig 1).





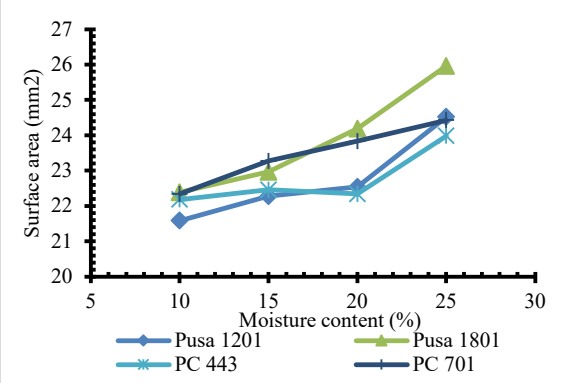


Figure 1. Varietal moisture effect on geometric characteristics of various pearl millet cultivars of India.

Gravimetric properties of pearl millet cultivars

Bulk density (ρb)

The bulk density for selected pearl millet varieties significantly ($p < 0.05$) varied from 737.42± 34.51 kg/m3 in Pusa1201 to 765.83± 43.91 kg/m3 in PC 701 (Table 1). For all varieties, the bulk density was found to decrease linearly with moisture content. (Fig.2). This decrease may be due to the increase in mass caused by moisture absorption, which is less than the volumetric expansion of the bulk. Similar patterns were found for different seeds i.e. chickpea seeds, bambara groundnut, faba bean, sorghum seed, (Konak *et al.,* 2002)(Altuntaş & Yildiz, 2007)(Mwithiga & Sifuna, 2006).

True density (ρt)

True density values for various pearl millet varieties ranged from 1208.98± 31.92 kg/m3 in Pusa 1201 to 1286.48± 20.77 kg/m3 in PC 701 (Table 1). True density significantly ($p<0.05$) decreased for all the verities when the moisture content increased (Fig.2). This decrease indicates that there was a smaller increase in grain mass compared to an increase in grain volume as moisture content increased. Similar results were found for minor millet and caper seed (Balasubramanian & Viswanathan, 2010) (Dursun & Dursun, 2005).

Porosity (P)

Porosity varied significantly ($p < 0.05$) among the different pearl millet cultivars. The values ranges from 39.03± 1.35% in Pusa 1201 to 40.51± 2.47% in PC 701 (Table.1). (Konak *et al.,* 2002) found that because porosity is primarily determined by bulk and true density, the magnitude of the increase in porosity can be attributed to changes in true and bulk density as moisture content increases. For all pearl millet varieties, the value of porosity was found to have a direct relationship with an increase in moisture content (Fig.2).

Thousand grain mass (W)

The thousand grain mass of pearl millet cultivars ranged from 10.19 ± 0.68 g in PC 701 to 12.95 ± 0.68g in Pusa1801 (Table 1). For all pearl millet varieties, the thousand grain weight increased as moisture increased (Figure 2). Same results was obserbed by (Nwabueze *et al.,* 2020; Kingsly *et al.,* 2006; Kaleemullah & Gunasekar, 2002; Baryeh, 2002). Thousand seed mass of pearl millet increased from 7.3 to 10.1g and 9.5 to 11.94g for Ex-Borno and SOSAT C88 varieties respectively, in the moisture range of 10-20% w.b. (Ojediran *et al.,* 2010).

Table 1: Varietal effect on engineering properties of various pearl millet cultivars of India.

Property		Pearl millet cultivars			
		Pusa 1201	**Pusa 1801**	**PC 443**	**PC 701**
GMD$^{\#}$ (mm)		2.69 ± 0.07^{bc}	2.75 ± 0.08^{a}	2.69 ± 0.07^{bc}	2.73 ± 0.07^{a}
ϕ (decimal)		0.83 ± 0.03^{a}	0.82 ± 0.02^{b}	0.76 ± 0.01^{c}	0.77 ± 0.01^{c}
A$^{\#}$ (mm^2)		22.73 ±1.22^{bc}	23.87 ± 1.72^{a}	22.74 ±1.10^{bc}	23.47 ±1.17^{ab}
W (g)		12.38 ± 0.52^{b}	12.95 ± 0.68^{a}	10.53 ± 0.90^{c}	10.19 ± 0.68^{d}
ρ_b (kg/m^3)		737.42 ± 34.51^{c}	747.08 ± 39.44^{b}	765.08 ± 41.78^{a}	765.83 ± 43.91^{a}
ρ_t(kg/m^3)		1208.98 ± 31.92^{d}	1227.60 ± 12.19^{c}	1285.43 ± 22.22^{a}	1286.48 ± 20.77^{a}
P (%)		39.03 ± 1.35^{b}	39.16 ± 2.73^{b}	40.51 ± 2.28^{a}	40.51 ± 2.47^{a}
ɵ (degree)		28.71 ± 2.71^{a}	28.62 ± 2.64^{a}	28.58 ± 3.07^{a}	25.55 ± 2.98^{b}
μ_i *$^{\#}$		0.66 ± 0.10^{a}	0.65 ± 0.08^{a}	0.65 ± 0.06^{a}	0.66 ± 0.07^{a}
μ_s	**Poly**	0.51 ± 0.07^{b}	0.48 ± 0.05^{c}	0.51 ± 0.08^{b}	0.54 ± 0.09^{ab}
	GI	0.56 ± 0.09^{ab}	0.53 ± 0.06^{bc}	0.56 ± 0.10^{abc}	0.57 ± 0.10^{a}
	MS	0.57 ± 0.06^{a}	0.56 ± 0.07^{a}	0.59 ± 0.10^{a}	0.58 ± 0.07^{a}
	Al	0.55 ± 0.10^{ab}	0.52 ± 0.08^{b}	0.53 ± 0.11^{ab}	0.55 ± 0.12^{a}
H*$^{\#}$ (N)		2.87 ± 1.16^{ab}	3.21 ± 1.01^{ab}	2.86 ± 0.58^{ab}	3.37 ± 1.23^{a}

All data were means of triplicates. Values with the same superscripts in a row did not differ significatly (p≤ 0.05) by DMRT
*** Non significant w.r.t. varieties at p=0.05, # Non significant w.r.t. varieties and MC interection at p=0.05**

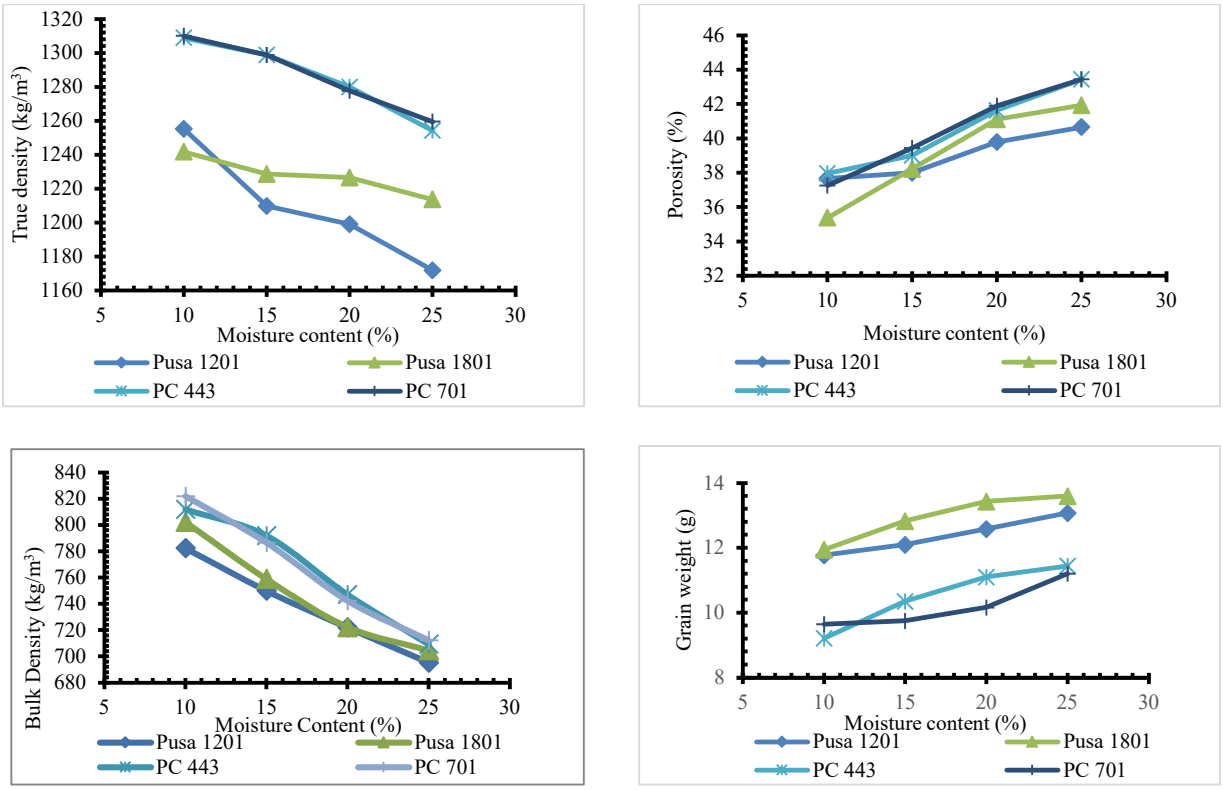


Figure 2: Varietal moisture effect on gravimetric properties of various pearl millet cultivars of India.

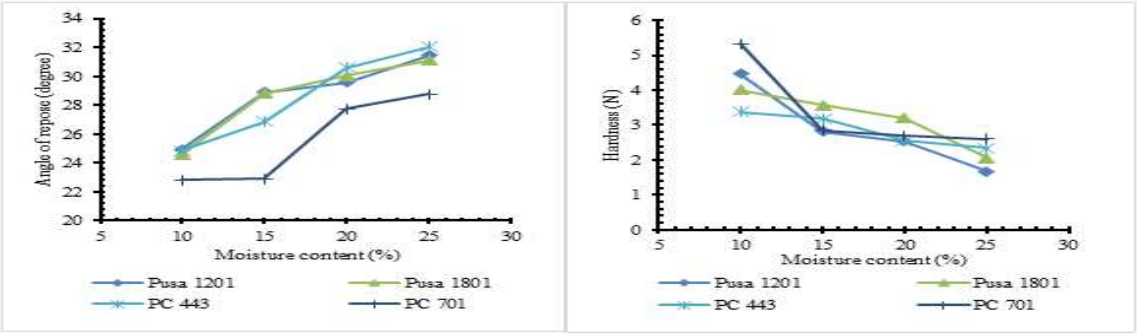


Figure 3: Varietal moisture effect on mechanical properties of various pearl millet cultivars of India.

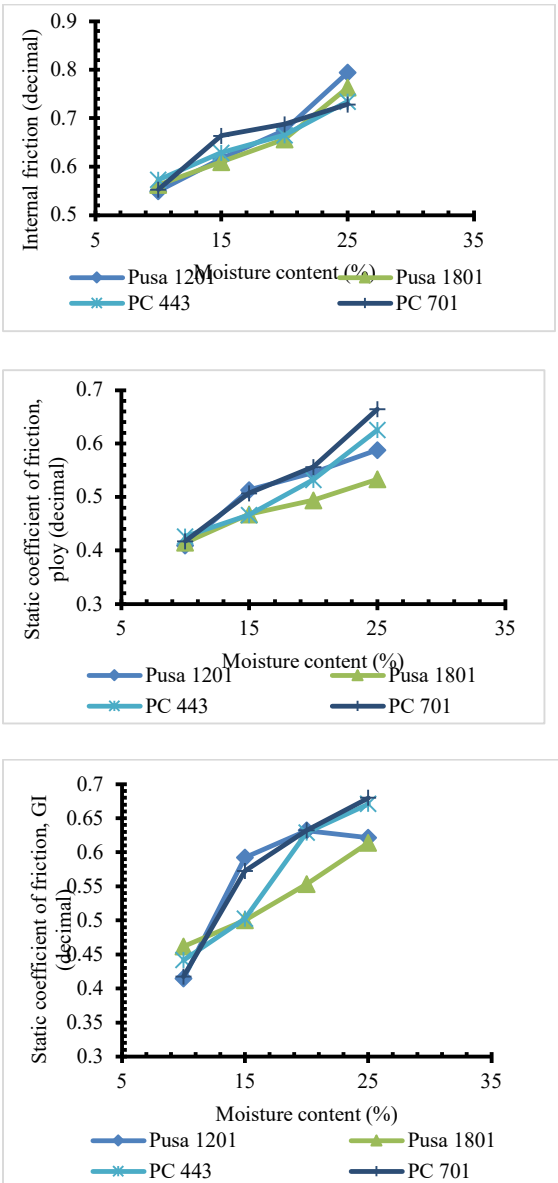


Figure 4: Varietal moisture effect on frictional properties of various pearl millet cultivars of India.

Mechanical properties of pearl millet cultivars

Angle of repose (ɵ)

The angle of repose for various pearl millet varieties differed significantly (Table 1) and it varied from 25.55± 2.98º in PC 701 to 28.71± 2.71º in Pusa1201. The values of angle of repose were increasing with increase in moisture for all varieties (Fig.3). The greater the moisture content of the seed, the greater the angle of repose, which may increase the internal friction of the seeds. (Dursun & Dursun, 2005) reported in the moisture range of 6.03–16.35%, the angle of repose of caper seed increases from 21º to 32º.

Hardness (H)

The Hardness of pearl millet varied from 2.86 ± 0.58 N in PC443 to 3.37± 1.23 N in PC 701 (Table 1). With increasing moisture, the hardness of all pearl millet varieties decreased (Fig.3). The hardness of barnyard millet decreased linearly from 45.67 to 36 N, while the moisture content increased from 0.065 to 0.265 kg kg-1 dry matter (Singh *et al.,* 2010). The results showed that the higher the moisture content, the softer all cultivars of pearl millet. At higher moisture content, the seed became soft, requiring low rupturing forces, making it more susceptible to cracking. A similar pattern was observed for minor millet and pomegranate seed (Balasubramanian & Viswanathan, 2010; Kingsly *et al.,* 2006).

Frictional properties

Internal friction (μi)

Non-significant difference was observed for internal friction of all the varieties of pearl millet. The value of internal friction was found between 0.64 to 0.66 (Table 1). The coefficient of internal friction ranged from 0.59 to 1.25 in the moisture content ranges of 11.11–42.86% d.b., with kodo and barnyard millet having the highest value compared to other minor millets (Balasubramanian & Viswanathan, 2010). But significant difference was found for the moisture level and direct relation was found between internal friction and moisture for pearl millet (Fig.4). This could be due to grain cohesion increasing with moisture content.

Static friction (μs)

Significant difference was observed for pearl millet cultivars for plastic surface, galvanized iron sheet (GI), MS and AL frictional surface. The value of static friction varied 0.48 ± 0.05 in Pusa 1801 for poly to 0.59 ± 0.10 in PC 443 for MS (Table 1).

The highest friction coefficient was found in MS, followed by galvanized iron sheet, solid plastic surface, and finally Al. This could be due to the surface roughness which is greatest in the case of MS and probably the least for Al. For all of the surfaces tested, the static coefficient of friction increased as the moisture content increased. (Fig.4). The same trend observed for lentil seed, water melon seed, arecanut, barnyard millet, minor millet (Amin *et al.,* 2004) (Koocheki *et al.,* 2007) (Kaleemullah & Gunasekar, 2002)(Singh *et al.,* 2010) (Balasubramanian & Viswanathan, 2010).

Conclusion

The GMD, sphericity, surface area, thousand grain mass, bulk density, true density, porosity, angle of repose and static coefficient of friction (Poly, GI, MS and Al) of pearl millet cultivars vary significantly from variety to variety measured at different moisture content (10%-25% w.b.) of grains. It was found that non-significant difference was obtained for internal coefficient of friction and hardness. Analysis of variance was performed for all the pearl millet cultivars showed that moisture content had a significant effect on all the engineering properties studied. The mean value of different cultivars at varying moisture content must be consider during design of different milling machinery of pearl millet. Moreover, the maximum or minimum extreme value of different cultivar at moisture range helps for the selection of cultivar on the basis of specific requirement of operation.

Conflict of interest

The authors declare that they have no conflict of interest.

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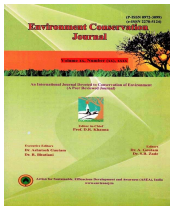

Yield attributing traits of high zinc rice (*Oryza sativa* L.) genotypes with special reference to principal component analysis

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ABSTRACT

Total 21 high zinc rice genotypes were evaluated under five different locations for 14 different yield attributing traits, including grain yield/plant (gm) to determine the pattern of variation, the relationship among the individuals and their characteristics through Principal Component Analysis (PCA) during the *Kharif*-2017. PCA was done for all the locations individually as well as pooled analysis for all locations using R software. Out of the 14 PCs, the initial four PCs contributed more to the total variability. The highest cumulative variability of the first four PCs found at Bhikaripur (81.11%) followed by BHU Agriculture research farm-II (79.23%) etc. and Pooled variability was 76.61%. Pooled data analysis indicates PCA biplot or loading plot of first two principal components revealed that days to maturity, days to 1st flowering date and days to 50% flowering loaded more on the first component and number of spikelets per panicles, number of grains/panicles, grain weight per panicle, grain yield/plant accounted more variation in the second component compared to the other parameters. Thus, the pooled analysis of principal component analysis revealed the characters contributing to the variation and genetic variability that exists in these rice genotypes. This is because the genotypes BRRIdhan 72, Sambamahsuri and Swarna were identified in different principle components related to grain yield and grain quality, and were also located farthest away from biplot origin in individual PCA based biplot. So they may be employed to improve yield attributing factors like total effective tiller number. PC1, PC2 and PC3 have days to first flowering and days to 50% flowering, hence their genotypes may be valuable in producing early maturing cultivars. Thus, the results revealed that wide range of variability was shown by different traits of the genotypes which can be utilized in rice improvement programmes.

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop that belongs to the family Gramineae. It delivers the daily intake of two-thirds of the world's population and about 20% of a human's calories intake (Wogu *et al*., 2010). It is cultivated in more than 120 countries, largely in tropical and subtropical Asia (Mulyaningsih *et al*., 2021). In Asia, rice accounts for 60-70% of energy consumption for over two

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billion people (Tiruneh *et al.,* 2019). Rice grains hold 75-80% starch, 12% water, and 7% protein (Hossain *et al*., 2015). Rice grain with good nutritional value contains vitamins like thiamine, riboflavin, niacin, and minerals like calcium, phosphorus, and magnesium (Negussie and Alemu, 2011). Rice plays a pivotal role in the food security of several countries. By 2050, the world population will reach 10 billion so the demand for rice will grow faster than other crops. To meet the demand for rice in the future development of high-yielding rice varieties is necessary. The successes of crop breeding programs are greatly dependent on the effective manipulation of the genetic variability and genotype selection utilizing all available desirable yields and quality contributing characteristics (Mignouna *et al*., 1996). Zinc is one of the essential micronutrients, which serves as a co-factor for more than 300 enzymes involved in metabolism. Zinc deficiency is a serious health concern, especially in developing countries where many people rely on cereal-based foods for their daily diet and cannot afford to diversify their meals (Shahzad *et al*., 2014). Moreover half of the world's population consumes rice as a staple diet and source of energy. Popular rice varieties are deficient in important minerals like zinc. Rice biofortification with higher amounts of zinc in its polished state could be a cost-effective and long-term option (Sharma *et al*., 2013). In rice breeding programs identification of usable variability is possible through one of the multivariate techniques like Principal Component Analysis (PCA).

Rice breeders observe a number of different characters. Some of which may not be helpful in germplasm discrimination. PCA can be used in these situations to discover patterns and minimize redundancy in data sets (Maji and Shaibu, 2012). PCA is a classical statistical method for simplifying a multidimensional dataset to lower dimensions for analysis, visualization, or data compression. It involves the calculation of the Eigenvalue decomposition of a data covariance matrix or singular value decomposition of a data matrix, usually after mean centering the data for each attribute. It is the simplest of the true eigenvector-based multivariate analyses (Kambo and Yerpude, 2014).The crucial benefit of PCA arises from quantifying the significance of each dimension for describing the variability of the data set. PCA can identify the minimal number of components that may represent the maximum amount of variation out of the overall variability (Morrison, 1978), as well as rank genotypes based on PC scores. Attributes with higher variability are more likely to provide high levels of gene transfer in breeding practices (Aliyu, 2000; Gana, 2006). The present experiment was carried out on rice germplasm to dissect yield-related traits at the individual and pooled environment to classify higher variability into total variability by taking into account many characters and relationships between the traits.

Material and Methods

Plant materials

A set of twenty-one genotypes and germplasm lines were used for the present study and are mentioned in (Table 1). The materials were collected from International Rice Research Institute (IRRI) South Asia Hub, Hyderabad, India. The experiment was conducted at five different locations (Table 2, Figure.1) in Uttar Pradesh, India during the *Kharif*-2017.

Experimental Design

The experiment was laid out in a completely randomized block design with three replications. The weather conditions during the evaluations period from June 2017 to November 2017 were almost normal and favourable for crop growth. All the experiments of five different locations were carried out at irrigated ecosystem and medium upland with transplanted nursery establishment.

Cultural Practices

The single seedling was transplanted at a 15 cm × 20 cm distance. All the standard recommended cultural practices were followed. Fertilizers were applied as 120 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare.

Quantitative Data observations

Five competitive plants were selected randomly from each row of each genotype in each replication and observations were noted for all yield and yield attributing characters except for days to first flowering, days to 50% flowering, and days to maturity. The performance of the cultivars was judged by recording observations on the following attributes mentioned in Table.3. The depiction and

evaluation stage of the quantitative traits were as stated by the descriptor of Biodiversity International (IRRI and WARDA, 2007).

Statistical analysis

Multivariate Analysis was carried out in using R (4.0.5) software packages. Principal components analysis, eigenvectors, Eigen values, and 2D PCA gg-biplots were obtained using FactoMineR, factoextra, and psych packages. Multi-trait multi-environment analysis for PCA was analyzed using metan packages (Olivoto and Lúcio, 2020).

Results and Discussion

Pooled analysis of variance (ANOVA) of rice germplasm among morphological characters of five environments

Pooled analysis of variance among morphological characters *viz*., days to 1st flowering date, days to 50% flowering, days to maturity, total effective tiller number, plant height (cm), panicle length (cm), number of spikelets per panicles, number of grains/panicles, spikelets fertility percentage, grain weight per panicle, weight of 1000 seed (gm), grain length/breadth ratio, grain zinc content (ppm) and grain yield/plant (gm) were observed in the study was presented in the Table-3a and Table-3b. There were significant difference was present in the germplasm for all the traits at 5% level of probability, hence suitable for further genetic analysis. This significant difference among the genotypes explains the presence of dissimilarity in their genetic composition. However, this significant difference might be due to the influence of environment on the genotypes. High significant difference was recorded among the genotypes for all the observed traits which disclosed the presence of considerable variability among tested germplasm (Bharadwaj *et al*., 2001; Maji and Shaibu, 2012; Tuhina-Khatun *et al*., 2015; Pachauri *et al*., 2017; Tiruneh *et al*., 2019 and Kumari *et al*., 2021).

Principal Component Analysis (PCA)

Variable Principal Component Analysis (PCA) in the multi environment with special reference to the pooled environment

The PCA described the proportion of the relative contribution of the different characters to the total variance of the rice genotypes under study. The study of several morphological traits is important for the assessment of the variances between the genotypes and their breeding potential. PCA pools the capacity to provide a synthetic summary of the most relevant traits and assessment of the relative contribution of various characters to the total variability of the population. Data were measured in each component with Eigenvalues more than one as per the recommendations given by Brejda (2000). The genetic variation present in the genotypes was divided into principal components (PCs). Table 4a. and Table 4b. represent the principal components, Eigenvalues, and percentage contribution of each component to the total variation in the rice genotypes tested in the five different environments and pooled data of the same five environments.

The scree plot displayed in Figure 2.described the percentage of variation related to each principal component obtained by drawing a graph between Eigenvalues and principal component numbers. The PCA at five different environments and pooled data of five different environments indicated that among 14 principal components (PCs) primary four PCs contributed more to the total variability *viz*., BHU Agriculture research farm-I (76.23%), BHU Agriculture research farm-II (79.23%), Bhikaripur (81.11 %), Karsada (72.55%), Rampur (76.23%) and Pooled data (76.61%).

In pooled data, the first principle component (PC1) added the highest proportion of the total morphological variation (31.90%) in the rice germplasm. Days to maturity (0.119) followed by days to 1st flowering date (0.115), days to 50% flowering (0.110), total effective tiller number (0.064), grain length/breadth ratio (0.054) contributed positively to the PC1 variation while other characters *viz*., spikelets fertility percentage (-0.066),grain zinc content (-0.100),weight of 1000 seed (-0.268), panicle length (-0.278), plant height (-0.368), number of spikelets per panicles (-0.368), number of grains/panicles (-0.378), grain yield/plant (-0.421) and grain weight per panicle (-0.449) contributed negatively to the PC1 variation. Tuhina-Khatun *et al.* (2015) were not in support of this result as they reported that, plant height and panicle length were contributing more for PC1 in explaining total variability.

Table 1: List of high zinc rice genotypes used for the experiment (Source: IRRI South Asia Hub, Hyderabad, India).

Entry No.	Entry Name	Grain Zinc Content (ppm)	ENTRY No.	Entry Name	Grain Zinc Content (ppm)
1	IR 95044:8-B-5-22-19-GBS	20.6	12	BRRIdhan 64	24.97
2	IR 84847-RIL 195-1-1-1-1	21.8	13	BRRIdhan 72	20.7
3	IR 99704-24-2-1	14.67	14	DRR Dhan 45	18.13
4	IR 99647-109-1-1	23.7	15	DRR Dhan 48	19.2
5	IR 97443-11-2-1-1-1-1 -B	14.45	16	DRR Dhan 49	17.63
6	IR 97443-11-2-1-1-1-3 -B	23.47	17	IR 64	23.57
7	IR 82475-110-2-2-1-2	24.73	18	MTU1010	21.70
8	IR 96248-16-3-3-2-B	27.18	19	Sambamahsuri	24.47
9	R-RHZ-7	26.61	20	Swarna	18.89
10	CGZR-1	24.43	21	Local check(HUR3022)	16.9
11	BRRIdhan 62	23.33			

Table 2:Five different locations used for the experiment

Location Code	Location Name	Latitude	Longitude	Altitude
E1	BHU Agriculture Research farm –I	25.18° N	80.30° E	81M
E2	BHU Agriculture Research farm –II	25.18° N	80.30° E	81M
E3	Bhikaripur	25.26° N	82.83° E	87M
E4	Karsada	25.22° N	82.90° E	85M
E5	Rampur	25.23°N	82.89°E	80M

Table 3a: Pooled mean square result of the five environments of morphological characters of rice

Source	D.F	Fst DF	FDF	DM	ENT	PH	PL	NSP
Env	4	1364.82	1278.54	1851.51	17.83	7931.79	163.00	39182.85
Rep (Env)	10	2.52	1.11	1.89	1.42	65.31	5.30	160.21
Gen	20	1428.20	1160.65	1607.58	12.46	602.60	35.00	13053.30
Gen:Env	80	12.92	35.88	21.63	1.80	117.21	4.97	1258.23
Residuals	200	1.64	1.07	1.41	0.90	29.71	2.48	472.26
CV (%)	-	1.36	1.06	0.94	12.04	5.11	6.04	19.89
Overall mean	-	93.74	97.96	126.81	7.88	106.72	26.01	109.27

Table 3b: Pooled mean square result of the five environments of morphological characters of rice

Source	D.F	NGP	SFP	GWP	STW	GLBR	GZC	GYP
Env	4	25731.94	371.42	15.69	264.42	0.15	1362.36	1242.51
Rep (Env)	10	190.18	35.44	0.07	1.56	0.01	2.97	8.16
Gen	20	7084.18	155.48	1.81	107.38	1.07	143.28	36.30
Gen:Env	80	616.34	51.41	0.24	6.56	0.21	14.45	7.91
Residuals	200	236.92	21.66	0.08	1.13	0.02	3.92	2.40
CV (%)	-	18.52	6.09	18.76	5.82	3.37	8.93	13.34
Overall mean	-	83.12	76.37	1.51	18.26	4.00	22.16	11.62

Where, DF-Degree of freedom; Env-Environments; Rep-Replications; Gen-Genotypes; CV-Coefficient of variation; Fst DF-Days to 1st flowering date; FDF-Days to 50% flowering; DM-Days to maturity; ENT-Total effective tiller number; PH-Plant height (cm); PL-Panicle length (cm); NSP-Number of spikelets per panicles; NGP-Number of grains/panicles; SFP-Spikelets fertility Percentage; GWP-Grain weight per panicle; STW-Weight of 1000 Seed (gm); GLBR-Grain Length/Breadth ratio; GZC- Grain Zinc content (ppm); GYP-Grain yield/plant (gm)

About 25.12% of the morphological variation in the rice genotypes was reported by the second principle component (PC2) in the pooled data. Days to maturity (0.484) followed by days to 1st flowering date (0.483), days to 50% flowering (0.480),number of spikelets per panicles (0.250), number of grains/panicles (0.249), grain weight per panicle (0.120) and grain yield/plant (0.105)contributed positively to the PC2 variation while other characters *viz*., grain length/breadth ratio (-0.020), spikelets fertility percentage (-0.027) , plant height (-0.043), panicle length (-0.044), total effective tiller number (-0.054), weight of 1000 seed (-0.233) and grain zinc content (-0.299) contributed negatively to the PC2 variation. But Kumar *et al*. (2014) reported that yield related traits *viz*., total spikelets/panicle and grain yield/plant were contributed more for PC1 rather than to PC2.

The third principle component (PC3) contributed about 11.86% of the total variation in the rice genotypes at the pooled data. The variation was due to traits *viz*., total effective tiller number (0.618), grain length/breadth ratio (0.434), spikelets fertility percentage (0.389), grain yield/plant (0.215), plant height (0.205), grain zinc content (0.199), days to 1st flowering date (0.164), days to 50% flowering (0.146), days to maturity (0.133), panicle length (0.127), weight of 1000 seed (0.090) having higher positive values to the PC3 variation. While other characters *viz*., grain weight per panicle (-0.088), number of grains/panicles (-0.115) and number of spikelets per panicles (-0.199) added negatively to the PC3 variation.

About 7.72% of the variability was contributed by the fourth principal component (PC4) in the rice genotypes at the pooled data with high positive values grain length/breadth ratio (0.415), number of grains/panicles (0.266), spikelets fertility percentage (0.228),number of spikelets per panicles (0.215),total effective tiller number (0.156), grain yield/plant (0.102), grain weight per panicle (0.032) and high negative values were found for other characters *viz*., panicle length (-0.213), days to 1st flowering date (-0.231), days to maturity (-0.243), days to 50% flowering (-0.251), plant height (-0.276), weight of 1000 seed (-0.371) and grain zinc content (-0.435). The maximum collective variability of the initial four PCs was reported at Bhikaripur (81.11 %) followed by BHU Agriculture research farm-II (79.23%), BHU Agriculture research farm-I (76.23%), Rampur (76.23 %), Karsada (72.55 %), and Pooled data was 76.61%. Tuhina-Khatun (2015) and Pachauri (2017) identified four principal components with Eigen value more than one (>1) and that explained 72.1% and 72.48% respectively of the total collective variance within the axes in an environment further strengthening current result.

The loading plot of pooled data component showed that the days to maturity, days to 1st flowering date, days to 50% flowering loaded more on the first component and accounted for more variation compared to the other parameters. Number of spikelets per panicles, number of grains/panicles, grain weight per panicle, grain yield/plant loaded more on the second component (Figure 3). The overall relationship among Eigen value with percentage variation with respect to PCA was presented in Figure-4. Kumari *et al*. (2021) in an environment identified days to 50% flowering, days to maturity, ear bearing tillers per plant, total grains and panicle length are important principal components that are in agreement with the present study except for panicle length.

Individual Principal Component Analysis (PCA) in the pooled environment

Principal components namely PC1, PC2, PC3 and PC4 of 21 genotypes contain positive PC scores (table 5a and 5b). Here in this pooled environment, genotypes with high PC scores (>1) have been taken under study. The range of positive PC scores for PC1 was from 4.38 (BRRIdhan 72) to 1.35 (Sambamahsuri). In PC2 the positive PC scores ranged from 3.65 (Swarna) to 1.64 (DRR Dhan 49). In PC3, the range of positive PC scores was from 1.96 (BRRIdhan 72) to 1.39 (BRRIdhan 64). In PC4 the positive PC scores ranged from 3.2 (IR 82475-110-2-2-1-2) to 1.02 (BRRIdhan 72). All the principal components contain the variable number of yield attributing traits and quality traits except PC2, because it has only yield and yield attributing traits. The highest number of traits were found in PC3 (10) followed by PC4 (7), PC2 (6) and least in PC1 (5). It indicates that the maximum variability of 21 genotypes was explained by the five traits which were present in PC1 (days to 1st flowering date, days to 50% flowering, days to maturity, Total effective tiller number and grain length/breadth ratio).

Table 4a: Eigen value, percent of the total variation, and component matrix for the principal component axes in rice at different environments

Parameters	BHU Agriculture research farm-I				BHU Agriculture research farm-II				Bhikaripur			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Days to 1st flowering date	0.339	0.328	-0.006	0.177	0.287	0.381	-0.195	0.139	0.316	0.299	0.242	-0.091
Days to 50% flowering	0.337	0.328	-0.005	0.186	0.290	0.376	-0.199	0.153	0.319	0.292	0.254	-0.094
Days to maturity	0.337	0.327	0.004	0.184	0.294	0.359	-0.235	0.156	0.322	0.291	0.252	-0.087
Total effective tiller number	-0.124	0.444	0.014	0.164	-0.223	0.386	0.078	0.061	-0.150	0.385	-0.091	0.173
Plant height	0.099	0.035	0.599	-0.139	0.092	-0.230	-0.336	0.472	0.093	-0.337	0.261	-0.134
Panicle length	0.076	-0.265	0.109	0.527	0.178	-0.075	0.420	0.014	0.051	-0.243	-0.336	0.102
Number of spikelets per panicles	0.355	-0.258	-0.200	0.098	0.391	-0.195	0.022	-0.206	0.391	-0.161	-0.143	0.021
Number of grains/panicles	0.395	-0.168	-0.164	-0.109	0.400	-0.115	0.154	-0.218	0.376	-0.170	-0.240	-0.158
Spikelets fertility Percentage	0.046	0.296	0.159	-0.576	-0.123	0.193	0.459	0.014	-0.130	0.023	-0.377	-0.742
Grain weight per panicle	0.321	-0.345	0.208	-0.077	0.341	-0.317	0.126	0.162	0.281	-0.387	0.032	-0.041
Weight of 1000 Seed	-0.171	-0.203	0.559	0.116	-0.175	-0.311	-0.035	0.570	-0.192	-0.334	0.277	0.277
Grain Length/Breadth ratio	-0.141	0.255	0.270	0.289	-0.120	0.270	0.419	0.362	0.094	0.182	-0.510	0.469
Grain Zinc content	-0.291	-0.012	-0.104	0.336	-0.286	-0.135	-0.141	0.041	-0.360	-0.135	0.124	-0.120
Grain yield/plant	0.325	-0.024	0.311	0.084	0.293	-0.034	0.353	0.363	0.310	-0.230	-0.031	0.167
Eigen value	2.250	1.719	1.279	1.008	2.239	1.779	1.305	1.102	2.300	1.931	1.112	1.047
Percentage of variance	36.16	21.12	11.69	7.26	35.80	22.60	12.16	8.68	37.80	26.64	8.83	7.84
Cumulative (%)	36.16	57.28	68.97	76.23	35.80	58.40	70.55	79.23	37.80	64.44	73.27	81.11

PC-1: First principle component; PC-2: Second principle component; PC-3: Third principle component; PC-4: Fourth principle component

Table 4b: Eigen value, percent of the total variation, and component matrix for the principal component axes in rice at different environments.

Parameters	Karsada				Rampur				Pooled data			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Days to 1st flowering date	0.239	-0.489	0.103	-0.056	0.249	0.455	-0.129	-0.121	0.115	0.483	0.164	-0.231
Days to 50% flowering	0.246	-0.485	0.101	-0.053	0.227	0.373	-0.283	0.030	0.110	0.480	0.146	-0.251
Days to maturity	0.233	-0.483	0.153	0.036	0.254	0.448	-0.135	-0.103	0.119	0.484	0.133	-0.243
Total effective tiller number	0.205	0.143	-0.373	-0.376	-0.189	0.345	-0.111	0.294	0.064	-0.054	0.618	0.156
Plant height	-0.108	0.036	0.390	-0.159	0.090	-0.223	-0.485	-0.178	-0.364	-0.043	0.205	-0.276
Panicle length	-0.031	-0.100	0.365	-0.540	0.180	-0.106	-0.367	-0.394	-0.278	-0.044	0.127	-0.213
Number of spikelets per panicles	-0.384	-0.277	-0.243	0.026	0.401	-0.140	0.276	-0.097	-0.368	0.250	-0.199	0.215
Number of grains/panicles	-0.414	-0.244	-0.194	-0.032	0.401	-0.135	0.298	-0.058	-0.378	0.249	-0.115	0.266
Spikelets fertility Percentage	-0.164	0.142	0.203	-0.354	0.150	0.057	0.112	0.561	-0.066	-0.027	0.389	0.228
Grain weight per panicle	-0.453	-0.193	0.022	0.003	0.412	-0.240	0.049	0.027	-0.449	0.120	-0.088	0.032
Weight of 1000 Seed	-0.231	0.087	0.481	0.101	0.075	-0.242	-0.498	0.236	-0.268	-0.233	0.090	-0.371
Grain Length/Breadth ratio	0.066	0.007	-0.250	-0.607	-0.067	0.184	0.243	-0.515	0.054	-0.020	0.434	0.415
Grain Zinc content	-0.027	0.165	0.291	-0.077	-0.249	-0.265	-0.112	-0.123	-0.100	-0.299	0.199	-0.435
Grain yield/plant	-0.404	-0.180	-0.123	-0.156	0.401	-0.105	-0.055	0.189	-0.421	0.105	0.215	0.102
Eigen value	2.054	1.712	1.246	1.205	2.250	1.719	1.279	1.008	2.113	1..8755	1.289	1.040
Percentage of variance	30.14	20.94	11.09	10.37	36.16	21.12	11.69	7.26	31.90	25.12	11.86	7.72
Cumulative (%)	30.14	51.09	62.18	72.55	36.16	57.28	68.97	76.23	31.90	57.02	68.88	76.61

Where, PC-1: First principle component; PC-2: Second principle component; PC-3: Third principle component; PC-4: Fourth principle component

Table 5a: Scoring of 21 high zinc rice genotypes in different PC's in pooled environment.

Sl. No.	Name of Genotypes	Code of genotypes	PC1	PC2	PC3	PC4
1	IR 95044:8-B-5-22-19-GBS	V1	-2.90	-0.17	-0.80	-0.62
2	IR 84847-RIL 195-1-1-1-1	V2	-1.77	-0.41	-0.14	0.74
3	IR 99704-24-2-1	V3	-0.52	-0.96	0.27	0.71
4	IR 99647-109-1-1	V4	-2.79	-0.64	0.83	-0.01
5	IR 97443-11-2-1-1-1-1 -B	V5	3.16	-0.96	-2.83	-0.18
6	IR 97443-11-2-1-1-1-3 -B	V6	3.84	-2.40	-2.50	-0.93
7	IR 82475-110-2-2-1-2	V7	-0.15	-1.45	-0.06	3.20
8	IR 96248-16-3-3-2-B	V8	-1.94	-0.56	0.26	0.15
9	R-RHZ-7	V9	-1.21	2.88	-0.45	0.45
10	CGZR-1	V10	-2.64	-1.74	-0.68	-0.25
11	BRRIdhan 62	V11	-3.83	0.53	-0.61	-0.52
12	BRRIdhan 64	V12	1.78	-3.56	1.39	-0.52
13	BRRIdhan 72	V13	4.38	-1.64	1.96	1.02
14	DRR Dhan 45	V14	0.36	-1.14	1.95	-1.19
15	DRR Dhan 48	V15	0.28	2.42	-1.25	-0.13
16	DRR Dhan 49	V16	1.96	1.64	-0.03	-1.36
17	IR 64	V17	-2.56	-0.35	0.31	-0.44
18	MTU1010	V18	-0.68	-0.52	-0.15	-0.03
19	Sambamahsuri	V19	1.35	3.62	0.17	1.08
20	Swarna	V20	2.87	3.65	0.55	0.44
21	Local check(HUR3022)	V21	0.99	1.77	1.83	-1.63

Table 5b: Rice genotypes are selected on the basis of PC scores in each component having positive values & more than >1.0 in each PCs in pooled environment

	PC1	PC2	PC3	PC4
Variable	DM, Fst DF, FDF,ENT, GLBR	DM, Fst DF, FDF, NSP, NGP, GWP, GYP	ENT, GLBR, SFP, GYP,PH,DM, Fst DF, FDF, PL, STW	GLBR, STW, ENT, GYP, PL, NSP
Genotype	BRRIdhan 72 (4.38)	Swarna (3.65)	BRRIdhan 72 (1.96)	IR 82475-110-2-2-1-2 (3.2)
	IR 97443-11-2-1-1-1-3 -B (3.84)	Sambamahsuri (3.62)	DRR Dhan 45 (1.95)	Sambamahsuri (1.08)
	IR 97443-11-2-1-1-1-1 -B (3.16)	R-RHZ-7 (2.88)	Local check(HUR3022)(1.83)	BRRIdhan-72 (1.02)
	Swarna (2.87)	DRR Dhan 48 (2.42)	BRRIdhan 64 (1.39)	
	DRR Dhan 49 (1.96)	Local check(HUR3022) (1.77)		
	BRRIdhan 64 (1.78)	DRR Dhan 49 (1.64)		
	Sambamahsuri (1.35)			

Where [DF-Days to 1st flowering date; FDF-Days to 50% flowering; DM-Days to maturity; ENT-Total effective tiller number; PH-Plant height (cm); PL-Panicle length (cm); NSP-Number of spikelets per panicles; NGP-Number of grains/panicles; SFP-Spikelets fertility Percentage; GWP-Grain weight per panicle; STW-Weight of 1000 Seed (gm); GLBR-Grain Length/Breadth ratio; GZC-Grain Zinc content (ppm); GYP-Grain yield/plant (gm)]

Figure 1.The map showing all the five different experimental locations.

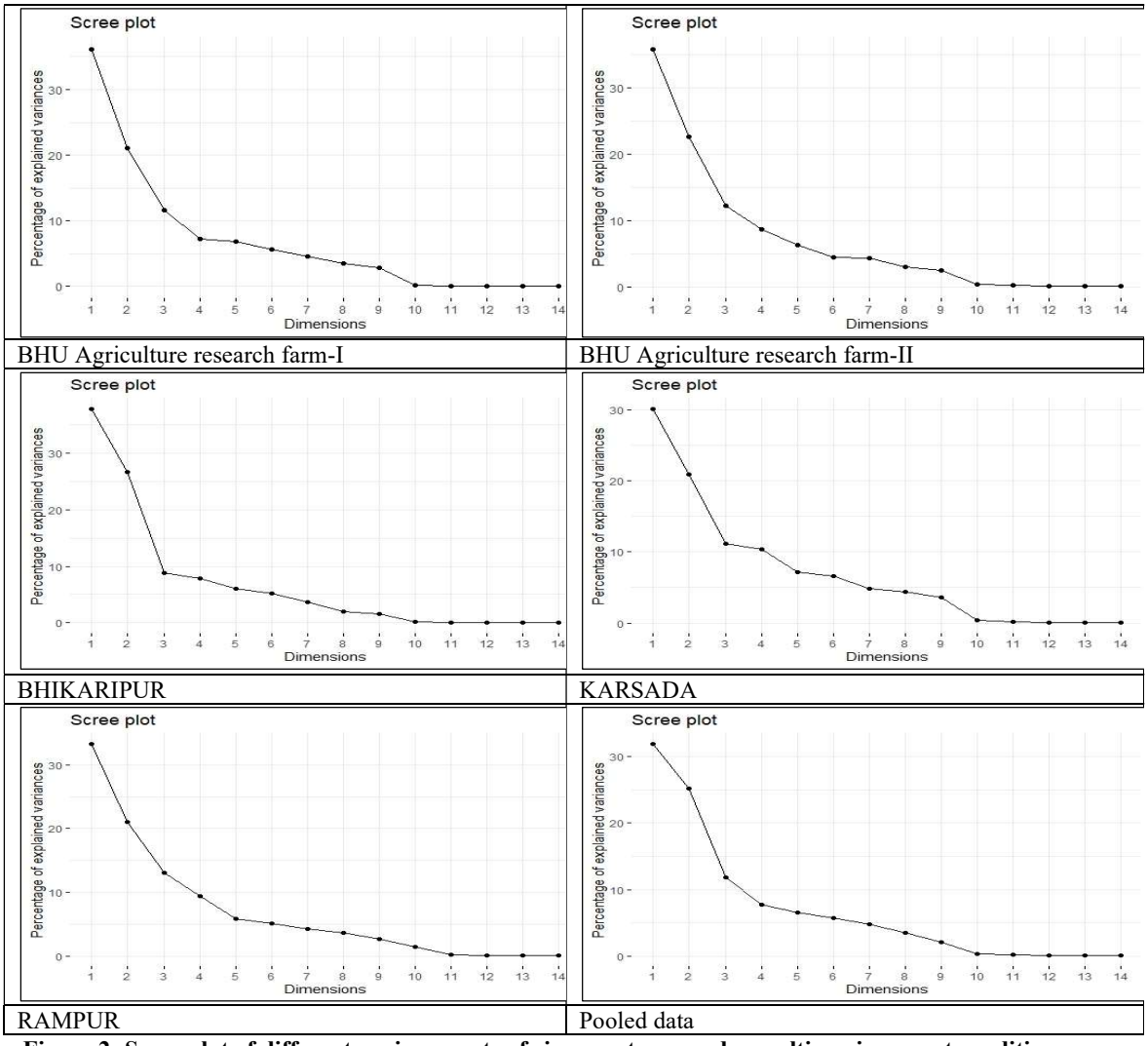

Figure 2: Scree plot of different environments of rice genotypes under multi-environment condition

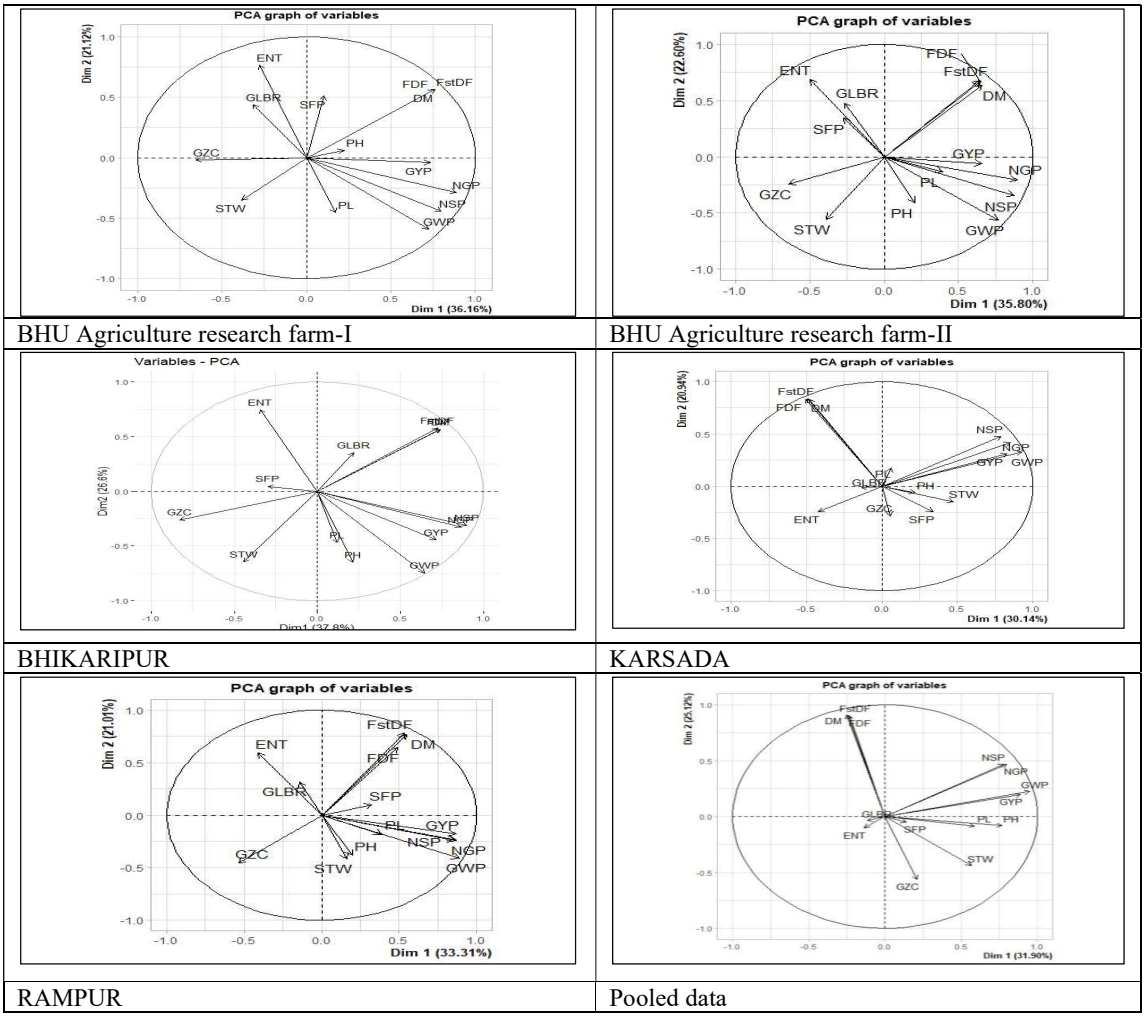

Figure 3: Loading plot of yield-related traits of rice genotypes at different environments

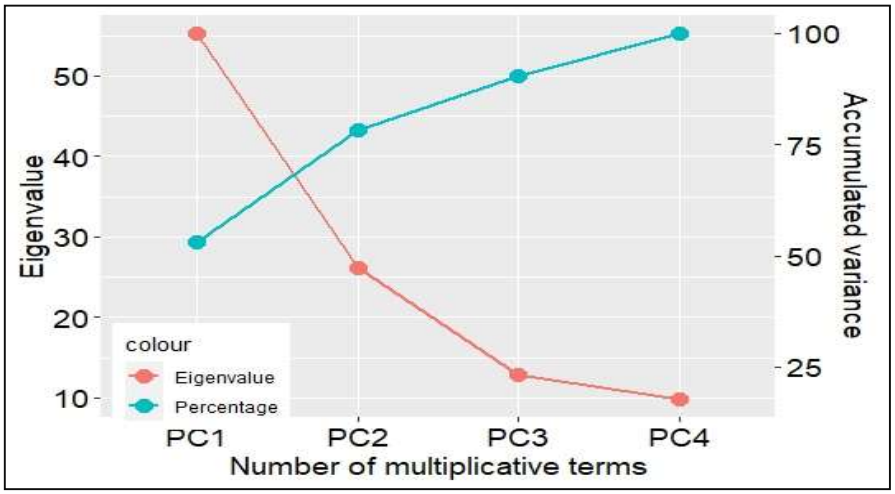

Figure 4: Overall relationship among Eigen value with percentage variation with respect to PCA.

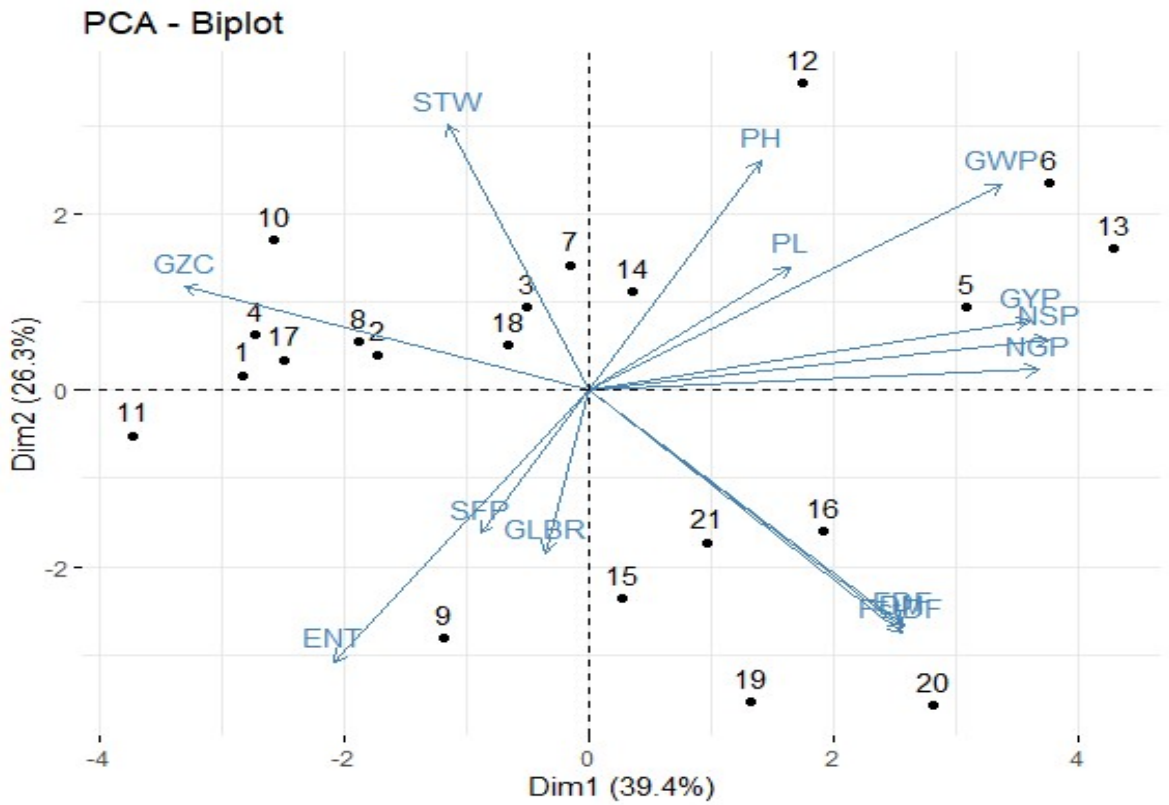

Figure 5: The bi-plot of 21 high zinc rice genotypes for PC1 and PC2 in pooled environment

The highest number of genotypes were fallen under PC1 (7) followed by PC2 (6), PC3 (5) and PC4 (4). Some genotypes are found in more than one principal component. The genotype Sambamahsuri has fallen under 3 different PCs (PC1, PC2 and PC4) and the genotype BRRI dhan 72 also fallen under 3 different PCs (PC1, PC3 and PC4), whereas the Swarna genotype was found in PC1 and PC2. This explains the relationship of genotypes with different yield attributing traits and quality traits to express their variability. Therefore such genotypes may be recommended directly for cultivation (Goure*t al*., 2017). R-RHZ-7, DRR Dhan 48, Samba mahsuri and Swarna exhibited the highest score in PC2. Genotypes DRR Dhan 45 and Local check (HUR 3022) had the highest PC score in PC3 and IR 82475-110-2-2-1-2 had in PC4 (table 5a).

BRRI dhan 72, DRR Dhan 45, Local check (HUR 3022) and BRRI dhan 64 exhibited high scores in PC3. This means that selection of genotypes with high PC scores in PC3 can be improved with good grain yield and grain quality traits. But Nachimuthu *et al*., (2014) were not in agreement with this result as they reported that variability of PC3 was explained by some yield attributing traits only, viz., spikelet fertility, single plant yield, and number of productive tillers.

With the help of two main PCs (PC1 and PC2), bi-plot analysis was done. For better understanding through visualization both germplasm and traits studied were merged in a single bi-plot. PCA bi-plot for PC1 and PC2 explained the 65.7% of total variability and showed that number of spikelets per panicles, number of grains/panicles, grain weight per panicle, grain Zinc content (ppm) and grain yield/plant (gm) had shown high variability among all studied traits (Fig. 5). The genotypes; Swarna, BRRI dhan 62, BRRI dhan 64, BRRI dhan 72, IR 97443-11-2-1-1-1-3-B, Sambamahsuri and IR 97443-11-2-1-1-1-1-B were located far away from biplot origin indicating better performance in comparison with other genotypes. Aslam *et al*., (2017) and Maqbool *et al*. (2015a, b), exploited bi-plot technique for evaluating crop genotypes in

diverse environments using first two principle components as they represent the most of data variability.

Conclusion

The PCA analysis revealed that the rice genotypes used in this experiment do not have close genetic relationships and could serve as a good genetic source for improvement of several traits. Therefore, to exploit the tested genotypes in crop improvement, selection for the traits that show high variability has to be done. Characters with greater variability are likely to contribute more to the diversity and provide a high level of gene transfer in breeding programmes. The prime goal of crop improvement is the improvement of yield, so the morphological traits related to yield are used as the primary evaluation tool. Therefore, the results from our study will be of greater benefit to select parents for improving various yield attributing traits analysed in this experiment for multi-environments.

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Conflict of interest

The authors declare that they have no conflict of interest.

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State of the science of environment, spirituality and health: An overview

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ABSTRACT

Indian culture is the oldest culture known in the world. It is enriched with the well-organized system of life and large number of literature, which enlighten cultural wealth of ancient India. Veda, Upanishads, Samhitas etc. provide sufficient knowledge about the cultural heritage and spiritualties and science. Health of human being is related to the state of mind, which is governed by various factors like anxiety, workload, mental tension etc. The objective of the present study was to elaborate the knowledge regarding vedic science, spirituality, environment, and health. To fulfill the objectives of the present study, searches were performed on the various research platforms (Web of Science, google scholar, research gate, science direct, and Scopus database). It was observed that there is a continuous debate on the definition of spirituality from its origin to till date. Spirituality and human health are related in positive and negative ways. People's health improved after practicing the spirituality and in certain cases, a downfall in health of spiritual people was noticed. Vedas considered as the base of modern science in literature. Spirituality was also found closely attached with the environment, as it not only revives the human soul but also to the environment. Spirituality and religious persons called environment as Mother Nature therefore the never damage its components. A clean and refreshing environment is the basis of good health of the persons living on the earth. Therefore, to revive the degrading human society, environment and health, there is a need of huge number of people with high spiritual mind.

Introduction

In the past century due to industrialization and urbanization, life style has changed, which imposed the impacts on the health as well as on the environment (Lagziel *et al.,* 2022). Literature shows the relationship between environment, spirituality and health (Puchalski, 2001; Williams and Sternthal, 2007; Reinert and Koenig, 2013; Oman, 2018). Sometimes the spirituality affects the health in a positive way and sometimes in a negative way, depending on the choices of people (Isaac *et al.,* 2016). Veda is the ultimate source of knowledge. Exact age when Vedas were written is not known. As per assumptions, Vedas were composed between 2000BC and 500BC. They were the literature of the Brahmans and the oldest literature in the world. Veda consists of entire knowledge of nature. The Word Veda was originated from the word 'Vid' means knowledge. It starts right from the origin of the creation. India is the land of Veda and spiritualism, culture and religion. Vedic knowledge is so deep and large that it is impossible to interpret and spread it in short time. The four Vedas, which include entire knowledge of nature, are as following-

Rigveda: It includes 10552 mantras, which are scientific and pragmatic. It teaches us how to live,

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how to let live, devotion spirit divinity. According to Indian mythology, it is the oldest Veda.

Yajurveda: It includes 1975 mantras in which rules of sacrifice has been given. It explains way of worship. It is also gives scientific knowledge to the world.

Sam Veda: It explains all about Taal, Chhand, Music, Poetry, Literature and Human beauty. It has 1875 mantras. It gives a complete system of living, culture and a systematic development of society.

Atharva Veda: It includes materialistic science like commerce, economy, civics, physics, chemistry, biology, geology, astrology, astronomy etc. Atharva Veda also enlightens the human health and the complete remedy by using medicines. Entire knowledge of modern sciences and their practical applications are included in it.

Scientific Knowledge in Vedas:

Vedic science is the final science, which fulfills the objectives of modern science with emerging learner and process of learning in the field of investigations. It gives the full knowledge about all laws of nature. Vedic science is the final science it covers knowledge of probable of natural laws and bring human awareness with creative impulses and are engaged in converting in field of intelligence into field of matter. Satpath Brahmana consists of all scientific discoveries.The origin of modern science is found in vedic literature like Samhita, Brahmana, Aranyaka, Upnishada. Maharishi Kanad was the first to propose that every matter is composed of small fundamental particle called 'Kana' Vaisesika Darshan of Maharishi Kanad played important role in growth of physical idea in India. Kanika sidhanta 'Paramanuvad' based on natural scientific views of the ancient Indians. According to which Anu (atom) does not possess any property, but it combines with another anu (atom) to form dvainuka (Molecule) which in turns form a transrenu (trimolecule). Only the transrenu possess properties of the matter. Vaisesika objectively defines size of an atom as it does not have any property, the only transrenu can be seen by naked eye.

Definition of spirituality, environment and health

The word 'spirituality' was originally derived from the Latin word "spiritualitas" which corresponds to the Greek pneuma, 'spirit', and its adjective pneumatikos, as they appear in the Pauline Epistles (Sheldrake, 2013; Holt, 2022). The base of division of the whole world was spiritual belief, the western countries follow Christianity belief and eastern countries follow the belief of Hinduism and Buddhism (Hasa, 2016). The concept of spirituality and religion are different from each other (Harvey, 1989). The modern concept of spirituality was come into light in late nineties, when the large number of people follows the concept of spirituality rather than religion and move away from this consumer world to obtain solace (Hay and Hunt, 2000; Roof, 2001). Based on the definitions available in the literature, spirituality can be defined as "the personal search of the objectives and meaning of life, which may or may not be related to religion. It encompasses the connections from self-chosen and religious belief, practices and values, which provide meaning to this life. These connections bring faith, hope, peace, and empowerment and motivate the human beings to obtain the optimum joy of life. The results of spirituality are joy, forgiveness, awareness and acceptance of hardship and mortality" (Tanyi, 2002; Puchalski *et al.,* 2014; McSherry and Cash, 2004). The meaning of spirituality changed over time and is understood different by different people (Zinnbauer and Pargament, 2005; Fisher, 2011; Huss, 2014; Jones, 2016; Oman, 2018). The word religion was derived from Latin word ""religo" which means "good faith," or it may have come from the Latin "religãre" which means "to tie fast" (Steinhauser *et al.,* 2017). Religion is a system of beliefs, including the belief in the existence of a supreme power (NHS, 2021). Finding of satisfactory and accurate definitions of both spirituality and religion is a difficult task. Health can be defined as the state of the body without any disease, illness, injury, and other physical and mental deficiencies in individuals (NHS, 2021). The word environment was derived from the French word "environ" which means total encircle, therefore, we can say that everything which envelops us may be referred to as the environment.

Spiritualism & Science:

"Science without spirituality is blind and
Spirituality without Science is lame"

Elbert Einstein

Spiritualism & Science are appearing to be unrelated, but when analyzed they are found to be much related to one and another like corners of a

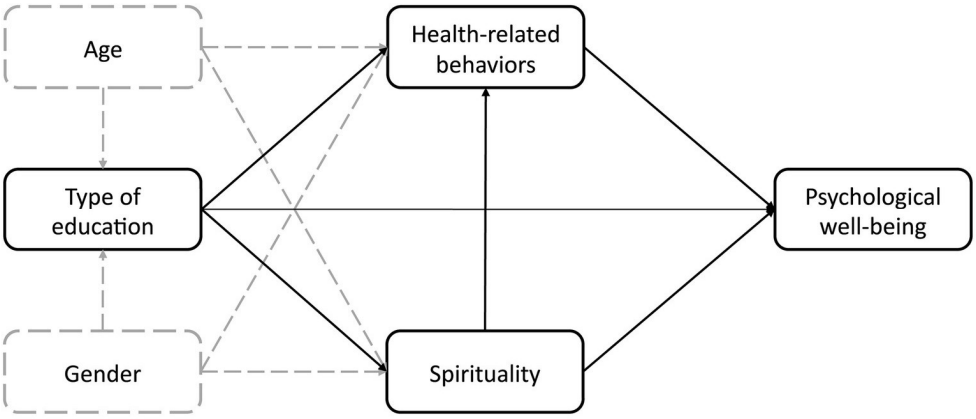

Figure 1: Showing the relation of spirituality and health (Source: Bożek *et al.*, 2020).

triangles, in triangle each side is a simple line but when these meets in a system makes a triangle. In the same way if scientific discovery do not include human views it will not provide any benefit to society and will be just a science for no use. In the same way, spiritualism without science will not provide any benefit to society. Without any one of them, the identity of the Humanity should not be there. Scientific increment without spirituality is such as body without soul. Science we look today it move to society with attributes of Selfishness, self-destruction, Greed, Bloated Egos and Intolerance. Today we become slaves of scientific infrastructure and gadgets we have put together for service. We totally depend on scientific tools. Humanity required in human and progressive culture on world, where scientific and spiritualism development goes all together. Culture based on goodwill, tolerance, and principles of love, desire to grow, to move ahead we should have come before for well-cultured society, which includes the human values.

To achieve goal of spiritual-scientific culture on Earth, spiritual level of society humanity has to increase. There must be huge number of people with high spiritual mind to bring about change at very high rate. This may be tough, but things are really traveling in positive way. In spite of greed, materialistic and hatred hullabaloo, lot of us are feeling required for an inner quest. One should think for what are we here, what are we doing, why we are doing so? Why so much misery and pain in life? How to make our lives more meaningful? What can be done to make our society better? Current inner quest will really help us to rise above normal. In addition, so long queues at doorsteps of religious preachers and Gurus. Only desire for an inside quest might not help enough. To achieve diversity, some more effort is needed dedicated and honest persons, working steadfastly and specifically to bring about change. Group should work honestly for the welfare of mankind; they should have humanity not to show but from inner corner of heart. In addition, one of way for them is to find for scientific-spiritual development of past.

Spirituality and health

Health of human being is directly relates to different beliefs and practices of spirituality and religion (Thoresen and Harris, 2002). Some aspect of spirituality and religion are overlapping means some values are included in both but both also have non-overlapping area means values (Thoresen, 1999). In a report of National Institute of Healthcare Research (NIHR), relationships between various religious activities and certain disease have shown (Larson *et al.,* 1997). The report says that it reduces the severity of pain (Yates *et al.,* 1981), blood pressure (Larson *et al.,* 1989; Koenig, 2013), cirrhosis (Comstock and Partridge, 1972; Koenig and Vaillant, 2009), and myocardial infarction (Medalie *et al.,* 1973). Most of the researchers pointed out positive effects of spirituality on health (McCullough *et al.,* 2000; Koenig *et al.,* 2001) but there are some researchers who found negative impacts on health (Bergin, 1983; Gartner *et al.,* 1991; Asser and Swan, 1998). Anxiety and

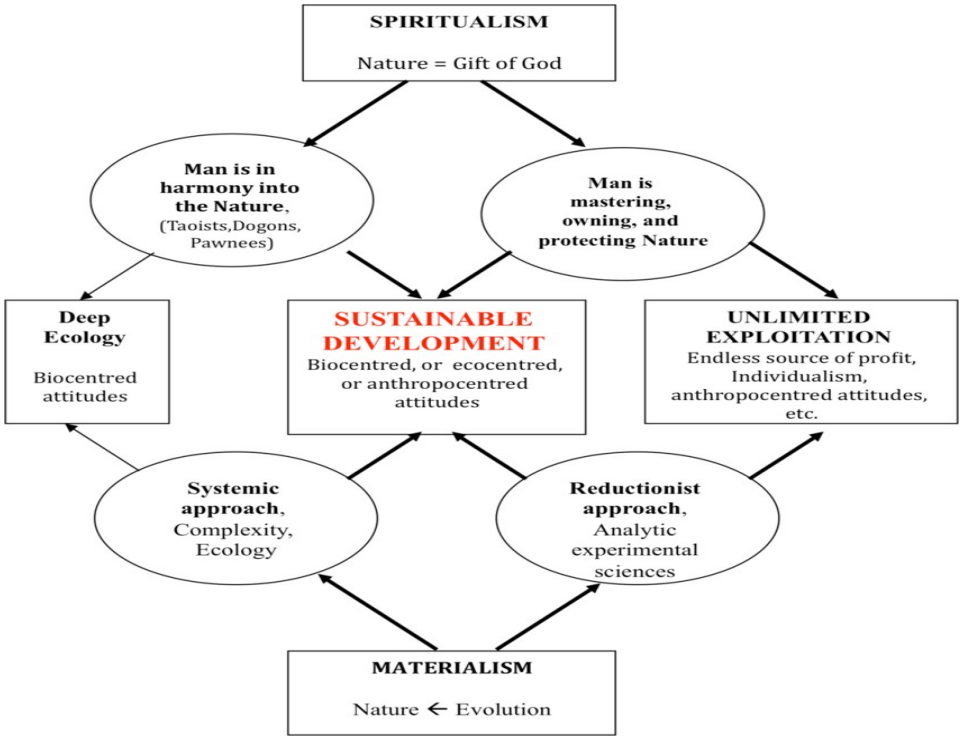

Figure 2: Situation of Sustainable Development and Environment (Source: Clément 2012).

depressions, mental health problems and suicidality were observed in persons that are more spiritual. The above said problems were observed in the persons, which intermixed religion with spirituality or they believe in old beliefs of spirituality. Although the spirituality reduces the anxiety among the peoples but due to the misconceptions, negative impacts were reported more in literature (Pargament *et al.,* 1998). An increasing interest for health and nutrition was reported around 1960. Dietary guidelines were first developed in USA in mid 1970s, which now has been developed by all industrialized countries around the globe giving the preference to the health of individuals (Trichopoulou and Vassilakou, 1990; Coveney, 1999). Faulty dietary habits disturb the functioning of the body, which made it difficult in achieving the spiritual purity (Foucault, 1982). A proper diet contains the nutrients in balanced proportions, which are important for the growth and development of the body as well as peace of mind, which helps in attaining the spiritual purity.

Spirituality and Environment

Industrialization and urbanization puts a stress on the weather and climate of this planet earth, which results into bad effects on the health of organisms. Spirituality not only revive the human soul but it also change the vision of human beings towards the nature, which results into the revival of this mother earth (Nurnazar, 2022). Spirituality provides the insights about the nature to human beings. Spirituality teaches the importance of every sphere of the environment (lithosphere, hydrosphere, atmosphere and biosphere). Spirituality inculcates the moral values in the human beings and due to all these values human beings become sensible towards the environment. Spiritual persons take responsibilities itself rather making the other person liable for any degradation of environment and with this feeling human being try to conserve every bit of the nature which results into harmony between human being and environment.

Environment and health

There is a need of detailed multidimensional assessment to analyze the environmental problems globally and the center point of all those assessments should be human being. Existence of human being without environment is next to impossible. All the anthropogenic activities are responsible for the

disturbance in all the sphere of the environment i.e. lithosphere, hydrosphere, atmosphere, and biosphere. Various researchers performed the assessment and reported the changes in the physical, chemical and microbiological characteristics of these spheres (Bhardwaj *et al.,* 2020; Bhutiani *et al.,* 2021; Ruhela *et al.,* 2022a&b; Ahamad *et al.,* 2022a&b). Changes in physicochemical and microbiological characteristics of these spheres pose threats to human health in various ways. Long-term exposure to various agricultural chemical such as pesticides, insecticides, herbicides, fungicides and fertilizers affects the human health badly as presented in figure 2 (Sharma *et al.,* 2017; Rani *et al.,* 2021). Holloway *et al.* (2021) performed the satellite monitoring of air pollutants and assess their impacts on human health. The atmosphere near harbors and ports and especially in coastal areas, shipping is the main cause of air pollution, which results into respiratory diseases, inflammation, asthmatic disease, lung cancer, and cardiovascular diseases (Merico *et al.,* 2019; Yang *et al.,* 2021; Contini and Merico, 2021; Kalivitis *et al.,* 2022) and in some cases death (Verma *et al.,* 2021; Ryan *et al.,* 2021). Polycyclic aromatic hydrocarbons (PAH) released from electronic waste are also responsible for environmental pollution, which in turn affects the life of organism on this earth in a negative way (Ma *et al.,* 2022). Water is an essential element for the survival of human being on this planet earth. The entire water requirement is fulfilled by groundwater, surface water and some by rainwater. Industrialization and urbanization results into increased amount of wastewater generation. In India, due to lack of treatment facility, a large amount of wastewater is discharged into drains and on open ground either in treated or in untreated form, which causes water pollution of surface and groundwater pollution. Increased value of different physicochemical, microbiological and heavy metal parameters of water quality are responsible for various disease as predicted by several researchers after calculating the both carcinogenic and non-carcinogenic health risk index (HRI) of water quality and the long term exposure results into some other complex disease and sometimes death also (Adeloju *et al.,* 2021; Bhutiani *et al.,* 2021; Senoro *et al.,* 2022; Amuah *et al.,* 2022; Rahman *et al.,* 2022). Similar to air and water quality, soil quality is also important for the human health as we get various nutrients from soil. Pollution due to hazardous elements has raised the attention of common men and scientific community throughout the world (Haghnazar *et al.,* 2021). Dumping of solid waste, disposal of untreated and partially treated domestic and industrial wastewater and agrochemicals are the main causes, which disturbs the physicochemical, microbiological, micro and macronutrients, and heavy metal characteristics of soil (Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022b). Besides this, various industrial, mining, and agriculture processes, waste treatment, extraction and processing of fossil fuels are some other sources of soil pollution (Raimi *et al.,* 2022). Due to their toxic and persistence nature, these pollutants accumulate in the soil as well as in plant parts and in turn into human body via food chain and pose threats to human life (Khan *et al.,* 2021; Abbaszade *et al.,* 2022; Boente *et al.,* 2022). All the toxins ultimately last in the soil and pollute all the spheres of the environment including water, air and food (Raimi *et al.,* 2022).

Conclusion

The present study was to elaborate the knowledge regarding vedic science, spirituality, environment, and health. To fulfill the objectives of the present study, searches were performed on the various research platforms for the literature study using different keywords. From above discussion, one can conclude that science without spirituality is human body without soul. Veda is the richest source of scientific advanced knowledge, but Veda is written in ancient Indian language Dev-Vani Sanskrit, therefore to have deep research on Veda deep knowledge of Sanskrit is required. Large no. of research dimensions on Vedas are in progress, in abroad and India. Some more positive in this direction are still awaited. Aim of current words is to put together all such similar-minded researchers on one stage with their views, to have sincere effort in this field. Modern science has made our life comfortable with so many investigations, like Radio, Television, Cars, Trains, Aero planes, Mobile phone, Internet etc. but it could not remove violence, cruelty, depression, anxiety and disharmony. All such problems can be solved by making science and spirituality hands together. From its origin to till date, none of the scholar is able to define spirituality

in a perfect way. Each scholar defined it in their own way, therefore till date there is a lot of confusion among peoples. In certain cases, spirituality shows positive impacts on human health and in some cases negative. Spirituality, human health and environment were found closely related to each other. Spirituality declared environment as Mother Nature therefore spiritual and religious persons never damage its components. Therefore, to revive the degrading human society, environment and health, there is a need of huge number of people with high spiritual mind.

Conflict of interest

The authors declare that they have no conflict of interest.

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Lactose intolerance: A review for facts and fictions

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ABSTRACT

Milk is considered a complete food due to its high nutritional status. Regular consumption of milk reduces the problems of nutrition deficiency. Milk contains sugar, mainly lactose, in significant amounts with other ingredients of milk, which also contributes to maintaining the body's energy level. But, sometimes, a problem arises due to the intake of lactose owing to a deficiency of β-galactosidase (lactase). A lack or shortage of lactase enzymes in the human body results in the body's inefficiency in degrading lactose into minor constituents. Un-degraded/ undigested lactose is consumed by the bacteria and converted into several acids and gases, which results in the rise of several types of intestinal disorders. In this article, the focus is given to lactose intolerance, its types, and its remedies.

Introduction

Milk, as a complete food, contains several components like water, fat, protein, lactose, vitamins, minerals, etc. that help to grow and provide nutrition to the body. Among these components, lactose is one of the important components which provide energy and other health benefits to the body. Lactose is the primary source of sugar which is only present in mammalian milk. Human and bovine milk contains 7.2g/100 ml and 4.7g/100 ml, respectively, of lactose, whereas the milk from marine mammals' origin contains much less lactose (Solomons, 2002). Lactose is a rich source of energy for the infant during the early growth stage. To get the nutritional value and energy from milk and lactose, a person should be able to digest the lactose after its assimilation. Lack of ability to digest lactose falls under the category of lactose intolerance, and it depends on several factors, which are discussed in detail in this article. Additionally, lactose intolerance, the inability to digest lactose, is not a new term; it has existed for many years and is well known in foreign countries. People with lactose intolerance show sensitivity to products containing lactose (especially milk and milk products). Enough scientific knowledge of lactose and the importance of lactose in milk are vital in dealing with lactose intolerance. Many physiological factors affect lactose breakdown and its absorption, like passing

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through the digestive system, lactase activity, working of the visceral organs, various intestine-related disorders, and composition of the gut microflora. Considering these complex factors, there are variations in the symptoms of lactose intolerance. Simultaneously, the industry and academia are working on the causes of lactose intolerance and their remedies and exploring the dairy products' digestion regulation and their implementation in the product formulation to provide alternative products for lactose-intolerant people (Vesa *et al.,* 2000). Products derived from milk have excellent nutritional status and can be used as a functional ingredient to provide therapeutic benefits to the body. Milk is a good source of minerals (especially calcium), and vitamin D. Lack of consumption of milk causes the deficiency of these nutrients and results in several health-related issues. On the other side, most of the consumers are not that aware of lactose intolerance and the complications linked with it but being the most important part of the daily diet (milk and milk products). Thus, the facts and fiction for lactose intolerance must be explored, and consumers should also have enough scientific knowledge about lactose and its intolerance. Therefore, keeping these facts in mind, this manuscript is reviewed to enlighten the serious and important issue of lactose intolerance.

Role of Lactose as Nutrient

Lactose is the primary energy source for infants, as human milk contains higher lactose than bovine milk. Lactose also acts as a prebiotic for gut microflora, providing superior health benefits to the body. Generally, the human body has lactase to break the lactose (Venema, 2012), but its deficiency results in the passage of lactose in the large intestine, which acts as an excellent substrate for fermenting microflora (lactic acid bacteria) and results in the formation of undesirable acid and gas. Due to acid and gas production, several problems arise in the body (Hill, 1983; Gibson *et al.,* 2004). Most of the microbiota from the colon can split the lactose into glucose and galactose with β-galactosidases which also produce metabolites such as short-chain fatty acids (SCFA) and other gaseous compounds like hydrogen, carbon dioxide and methane. SCFA works as a substrate and provides energy to gut microflora and colonocytes. Oligosaccharides are well-known compounds that have bifidogenic activity, thus provide excellent health benefits to the body (Vandenplas, 2015). The activity and population of Bifidobacterium in the intestine decrease with increasing human age. So, milk consumption provides milk saccharides having a bifidogenic activity to maintain the intestinal health and population of gut microbiota (Vulevic *et al.,* 2015). It is proposed that galactose is produced due to the hydrolysis of lactose. It acts as a substrate for the gangliosides, cerebrocides and mucoproteins, which have various neural and immunological roles. Due to its probable bifidogenic activity, lactose may itself be involved in promoting innate immunity. If one cannot get these benefits due to lactose intolerance, there is a need to replace it in dairy products and even in breast milk with oligosaccharides which are like those in milk and can serve as prebiotics in place of the lactose (Zivkovic *et al.,* 2011)

Lactase and Importance of Lactase Activity

Lactose-phlorizin hydrolase, mostly known as lactase, is β-galactosidase which breaks down the lactose to its subunit's glucose and galactose. This activity of lactase enzyme in adulthood is termed lactase persistence. Intestinal enterocytes absorb glucose and galactose in the bloodstream. Glucose and galactose mainly provide energy as part of glycoproteins and glycolipids (Campbell *et al.,* 2005). Lactase is primarily present in the mid jejunum of the small intestine and some parts on the apical surface and attached to the molecules of the gastrointestinal lumen through the end of C terminal (as shown in Fig. 1). Enzyme is released with 220 kDa precursor peptide. But due to post-transcriptional change, the size of the peptide obtained was about 150 kDa. Various factors play a significant role in the production of active enzymes by alteration in the protein network. The pancreatin trypsin achieves the process of cleavage of peptide bonds (Zecca *et al.,* 1998). During pregnancy, there is a progressive increase in the activity of lactase enzyme occurring from 8th week to 37th week of pregnancy, and at birth, lactase activity is at its peak. After a few months of life, lactose non-persistence is observed, which decreases lactase activity. Lactase activity was observed to fall at variable rates with a progressive increase in the age of mammals (Vesa *et al.,* 2000; Matthews *et al.,* 2005).

Lactase persistence which is a continuation of the lactase activity is observed in 30% of the human population after weaning and in adulthood (Savaiano and Levitt, 1987). Lactose deficiency, also known as hypolactasia is classified under three distinct forms first one is a congenital deficiency, which is associated with the inferior activity of lactase. Primary lactose deficiency is mainly observed in most of the population, which is non the persistence of lactase activity. Secondary lactase deficiency in people with lactase persistence is the progressive loss in the activity of lactase enzyme due to gastrointestinal disorders, pathologies and surgery (Leis *et al.,* 2020). For efficient lactose utilization, only 50% of the lactose activity is required (Swallow, 2003). Non-persistence of lactose does not mean the people with it are all unable to digest lactose. They can utilize lactose up to 12g if spread throughout the day.

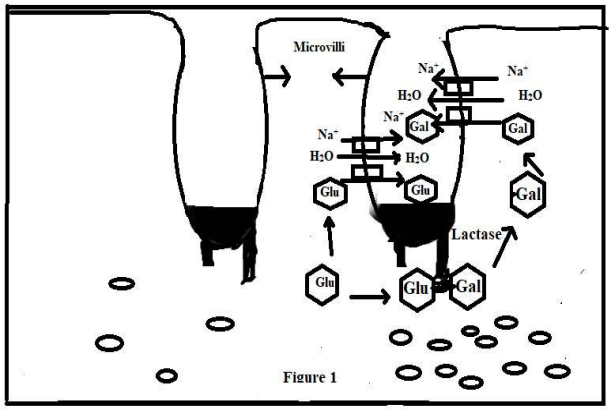

Figure 1: The mechanism of lactase activity (Reproduced from Lomer *et al.,* 2008)

Prevalence of Lactose Intolerance

The loss in lactose expression is generally completed in childhood, but some studies also revealed its decline with a progressive increase in age (Sahi *et al.,* 1994). The rate of loss of lactase activity varies concerning ethnicity. After 3-4 years of stoppage of consumption of mother milk, 80-90% decrease in the activity of lactase was observed in Chinese and Japanese, while 60-70% loss was observed in Jews and Asians over several years of stopping mother's milk. Lactase activity reached its minimal level after 18-20 years in the case of white Northern Europeans.

Congenital Deficiency

Deficient activity of lactase results in congenital deficiency and is characterized by the inability to break down lactose in the first-ever breast milk, resulting in diarrhea. It is very rare, with only 40 cases till now. It is known to be a single autosomal recessive disorder, and the exact reason at the molecular level is yet to be explored (Swallow, 2003). To overcome this type of problem, avoid the consumption of lactose altogether from birth. No treatment was available for congenital lactase deficiency before the 20th century due to the lack of availability of lactose-free human milk substitutes (Heyman, 2006).

Primary Lactase Deficiency/ Non-Persistence of Lactase

Primary lactase deficiency is a significant problem diagnosed in 70% of the world's population. Reports based on the clinical symptoms of lactase deficiency show variations according to the test subject and give confusing results during diagnosis. This means when a person showing lactose intolerance is fed with two glasses of milk or lactose hydrolyzed milk daily in a double-blind, crossover study. No statistical difference was observed in symptoms of lactose intolerance in both cases, even though the test subject said they were lactose intolerant (Suarez *et al.,* 1997). Even in the case of lactose-impatient people, some people tolerate one glass of milk and one scoop of ice cream without any symptoms, but when the same person consumes one more glass of milk or other dairy product can show symptoms.

Secondary Lactase Deficiency

In this case, various infections can cause damage to epithelial cells which contain lactase. The cells which replace these injured cells are immature and lactase deficient thus, cause secondary lactase deficiency (Sandhu *et al.,* 1997). Several studies showed that children with rotaviral diarrheal illness with the absence of or only mild dehydration do not offer any symptoms of lactose intolerance, including the status of dehydration, nutritional outcome or success of the therapy. Parasites like giardiasis and cryptosporidiosis infect the small intestine and cause damage to the epithelia cells, leading to lactose malabsorption. Diseases like celiac, Crohn's and other immune-related diseases are significant factors for secondary lactase intolerance in the case of children (Heyman, 2006).

Severe malnutrition disorders result in small intestinal atrophy, leading to secondary lactase deficiency (Nichols *et al.,* 1997). Children with

persistent post-infestation diarrhea (more than 14 days) are recommended to avoid lactose-containing milk according to the recommendations of the WHO when they fail in the dietary trial of milk or yoghurt (World Health Organization, 1996). In the case of secondary lactose deficiency, there is no need to exclude lactose from the diet; lactose consumption can be started after resolving the primary problem.

Detection of the Lactose Intolerance

In early studies, lactose digestion is detected by measuring the blood glucose level after injection of 50g of lactose, a significant rise in the blood glucose after the time interval of 30 minutes is an indication of high lactase activity (Swallow, 2003; Gugatschka *et al.,* 2005). The lactose hydrogen breath test is also one of the best-known methods for detecting the digestion of lactose. In this test, 50g gram of hydrogen is introduced into the human body orally, and the hydrogen content of the breath is measured in 3-6 hours. If it is more significant than 20 ppm above the baseline, it will indicate lactose intolerance. In case of the results of the 6 hours are taken, then the sensitivity increases from 40% to 60% (Matthews *et al.,* 2005). Real-time polymerase chain reaction (PCR) test for genotyping provides information about the specific lactase gene quickly and easily and is used to distinguish primary and secondary lactose intolerance (Gugatschka *et al.,* 2005).

Signs of Lactose Intolerance

In the case of lactose intolerance, as discussed above, the lactose ingested orally in the body remains unabsorbed in the intestine. The undigested lactose in its complex form, while passing through the digestive system, causes various health-related problems, considered as the symptoms of lactose intolerance. Symptoms include stomachache, gas in the stomach, and intestine, bloating, diarrhea, and rumbling and gurgling noise made by the movement of the gas and the water inside the abdomen. Occasionally, nausea and omitting are also observed (Vesa *et al.,* 2000; Gugatschka *et al.,* 2005; Matthews *et al.,* 2005). Abdominal pain and bloating result from the fermentation of unabsorbed lactose, which produces byproducts like SCFA and gases like hydrogen, methane and carbon dioxide. It further increases the transit time in the gut and intracolonic pressure. Due to the acidification of the colonic content and increased osmotic load resulting from the unabsorbed lactose, greater secretion of the fluid and the electrolytes occurs, which results in rapid transit time and causes loose stools and diarrhea (Swagerty *et al.,* 2002).

Gut Microflora and Lactose Intolerance

The human digestive system consists of about 17 bacterial families with over 500 species (Suau *et al.,* 1999). Colon has the highest gut microflora concentration, about $10^{12\text{-}14}$ ml^{-1}. It is observed that the malabsorbed lactose is fermented by the lactic acid bacteria present in the ilea and colon followed by the production of SCFA and gases (Hove *et al.,* 1999). In this process, the malabsorbed lactose is broken down by the lactase enzyme present in the lactic acid bacteria (Swallow, 2003) into glucose and galactose which is then absorbed (Fig. 2) in the small intestine. The lactase enzyme shows optimum activity at the pH 6-8 as in the small intestine. In the case of the colon where the pH decreases to 4, lactose remains unfermented due to lowering the bacterial lactase activity.

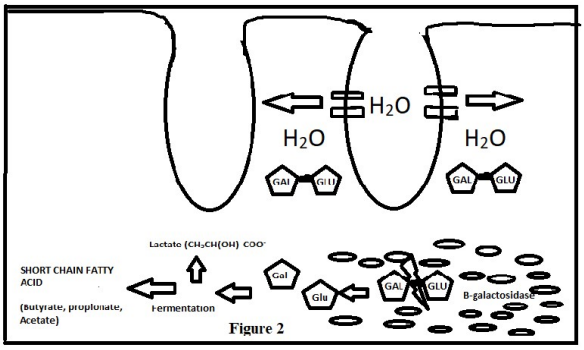

Figure 2: Mechanism of breakdown and absorption of lactose by lactase enzyme (Reproduced from Lomer *et al.,* 2008)

Dairy Foods and the Health

After weaning foods, milk and milk products are considered important, viable, convenient to feed and nutritious food materials for the children (Widodo *et al.,* 2016). Calcium is one of the major mineral constituents of milk required for bone health. During the first 50 years, the calcium requirement will be the same for males and females (Hodges *et al.,* 2019). From the previous research, it can be concluded that calcium absorption in healthy adults is independent of dietary lactose and concentration of lactase enzyme. The presence of lactose may enhance the absorption of calcium in animals (Weaver *et*

al., 2011) and infants (Abrams *et al.,* 2002) but has no stimulating effect in the case of humans (Zittermann *et al.,* 2000). Consumption of a variety of dairy products like milk, cheese, yoghurt etc., having different levels of lactose (Table 1) does not affect calcium absorption in adult women (Nickel *et al.,* 1996).

Table 1: Lactose content of milk and milk product

Particular	Type	g/100ml
Milk	Semi skimmed milk	4.7
	Whole milk	4.6
	Condensed, whole, sweetened	12.3
	Dried skimmed milk	52.9
	Evaporated whole milk	8.5
	Human	7.2
	Ship	5.1
	Single cream	2.2
	Double cream	1.7
	Sour cream	2.7
	Imitation cream	2.3-6.8
Cheese	Brie/camembert	Trace
	Cheddar	0.1
	Cheese spread	4.4
	Cheese spread, reduced fat	7.3
	Cottage cheese	3.1
	Cottage cheese, reduced fat	3.3
	Cream cheese	-
	Danish blue	Trace
	Stilton	0.1
	Edam	Trace
	Feta	1.4
	Goat cheese	0.9
	Mozzarella	Trace
	Parmesan	0.9
	Processed cheese slice	5.0
Yoghurt	Plain	4.7
	Fruit	4.0
	Drinking yoghurt	4.0
Puddings	Milkshake average	4.0
	Ice-cream non- dairy vanilla	4.8
	Ice-cream dairy vanilla	5.2
	Rice pudding	3.9

Calcium homeostasis in Chinese adults is achieved at a low calcium intake of ≤500 mg daily (Fang *et al.,* 2016), which depends on other factors such as lactose and calcium absorption. Horowitz *et al.,* 1987 conducted a study using a calcium isotope and observed that the calcium absorption is independent of lactase enzyme efficacy in postmenopausal women. However, calcium absorption largely depends on the lactose concentration and activity of lactase in the case of the elderly. Still, there is a lack of supportive data about this statement (Hodges *et al.,* 2019). Schuette *et al.* (1991) found that calcium absorption enhanced in postmenopausal women when 12 gm of lactose was added to the non-carbohydrate milk formula. Calcium absorption in postmenopausal women largely depends upon the food source and concentration of lactose present in that food (Obermayer-Pietsch *et al.,* 2007). Cederlund *et al.* (2013) also highlighted the possible immune-protective roles of lactose. Lactose malabsorption causes the problems like loose stools or diarrhoea because of osmosis exerted by the undigested lactose. Lactase deficiency provides an excellent substrate for colonic bacteria. As a result of bacterial fermentation and gas production, problems like intestinal flatulence and swelling are raised, as well as concerns like carcinogenicity (Gibson and Macfarlane, 1995; Aimutis, 2012). Malabsorption of lactose causes the problems like osteoporosis due to impaired calcium absorption (Casellas *et al.,* 2016).

Conclusion

Lactose digestion and its assimilation is vital for the human body to get the nutritional benefits of milk. It also helps in assimilating calcium and vitamin D and its prebiotic attributes. But the lack of ability to digest lactose due to unavailability, deficiency, and rendered functioning of lactase enzyme falls under lactose intolerance and sensitivity to lactose. Lactase (lactose-phlorizin hydrolase) is a β-galactosidase that breaks down the lactose to its subunit's glucose and galactose. It presents in the mid jejunum of the small intestine and some parts on the apical surface. It is attached to the molecules of the gastrointestinal lumen through the end of C terminal. Lactase deficiency results in the passage of lactose in the large intestine, which acts as an excellent substrate for fermenting microflora (lactic acid bacteria) and results in undesirable acids and gases. The symptoms of lactose intolerance include stomachache, gas in the stomach, and intestine, bloating, diarrhea, and rumbling and gurgling noise made by the movement of the gas and the water

inside the abdomen. Occasionally, nausea and omitting are also observed. To avoid the complications linked with lactose intolerance, the sensitive person should adopt the plant of diet with lactose exclusion in daily diets and should avoid food containing milk and milk ingredients like milk from any mammalian source; milk solids; lactose; whey powder; caseinate; condensed milk; cream; SMP; evaporated milk; buttermilk; feta; quark; curd; ricotta; butter; margarine; etc. Though the caseinate does not contain lactose, all the milk ingredients must be avoided initially. Lactose deficiency, also known as hypolactasia is classified under three distinct forms (1) congenital deficiency (inferior activity of lactase); (2) primary lactose deficiency (lactase amount declines with age); (3) secondary lactase deficiency (due to gastrointestinal disorders, pathologies, and surgery). The good thing is that for efficient lactose utilization, only 50% of the lactose activity is required, and the non-persistence of lactose does not mean that the people with it are all unable to digest lactose.

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Conflict of interest

The authors declare that they have no conflict of interest.

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