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

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Bhutiani, R., (2004). Limnological status of river Suswa with reference to its mathematical modeling, Ph.D. Thesis submitted to Gurukul Kangri University, Haridwar, Uttaranchal, India.

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Centers for Disease Control and Prevention (2021). Water treatment. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Retrieved March 3, 2021, https://www.cdc.gov/healthywater/drinking/public/water_treatment.html

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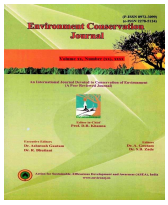

Assessment of environmental sustainability using ecological footprint in urban ecosystems of North Western Himalayas

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ABSTRACT

Rapid urbanization in cities is crafting major environmental problems, leading to degradation of urban ecosystems and is responsible for creating an imbalance between demand and supply of resources. Ecological Footprint Analysis (EFA) is a tool that can be used to assess this imbalance scientifically and determine the sustainability of a particular area. Our study aims to determine the urban sustainability of Kangra district in Himachal Pradesh, a hilly state in North Western Himalayas, India situated in North western Himalayas by using one of the Ecological Footprint Analysis (EFA) components, the built-up land footprint, as a pragmatic tool for analysis and planning of the urban region. The total built-up land footprint, total biocapacity and total ecological deficit are 18146.095 g ha,15968.564 g ha and 2177.531 g ha respectively whereas built-up land footprint per capita, built-up land biocapacity per capita and ecological deficit per capita are 1.371 g ha, 1.206 g ha, and 0.164 g ha respectively in different urban areas. Consequently, it is concluded that the built-up land results in an ecological deficit, and the system is considered unsustainable because its ecological footprint exceeds its bio capacity. It is suggested that urban sustainability should move and work on ecological principles so that the vision encompassing global goals and agenda 2030 for sustainable development can be achieved.

Introduction

Urban dwellers are expected to make up 68% of the world's population by 2050, according to the United Nations (2018). The global population is projected to reach approximately 9.6 billion by 2050, with 2.5 billion people living in urban areas, indicating a high concentration of growth in these areas (Turok, 2014). The 2011 census revealed that there were 1.21 billion people living in India, of which 31.1% were urban dwellers (Shaban *et al.,* 2020). Urbanization in India is accelerating at an alarming rate, which is supported by the statistics (Sudhira and Gururaja, 2012). Various issues related to urbanization exist on a local, national and international scale (Taipale *et al.,* 2012). The rapid pace of urbanization in recent decades has hastened the demand for urban land, leading to major challenging issues (Seto *et al.,* 2012; Uttara *et al.,* 2012) such as overpopulation, overconsumption, shortage of housing, infrastructure depreciation, overcrowding, water scarcity, poor air and water quality, increased pollution levels, expanded energy utilization, increased impervious surfaces and alteration in the functions of natural ecosystems such as biogeochemical processes and circulation patterns (Geng, 2012; Newman, 2006), collectively leading to ecological imbalance (Ren *et al.,* 2012; Karkazis and Boffey, 1997; Rees, 1996). Sustainable development gained popularity in the late 1980s and early 1990s from the Brundtland Report (Brundtland, 1987), which defined sustainable development as a form of development that meets the needs of the present without

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compromising the ability of future generations to meet their own needs (Wackernagel, 2014). However, sustainable development is interrelated with urbanization in three dimensions: economic, social, and environmental (United Nations, 2018).
Several methods for exploring and assessing sustainability exist across the globe, including emergy analysis, material flow analysis, data envelopment analysis (Geng *et al.,* 2013; Zhao *et al.,* 2006), analytical hierarchy process (Yue *et al.,* 2011), and ecological footprint analysis (EFA) (Wackernagel and Rees, 1996; Wackernagel *et al.,* 2005; Wackernagel, 2014; Swaider *et al.,* 2020). Of these, the EFA technique (Wackernagel and Rees, 1996) will be the most useful and will be employed to have a comprehensive view of the urban ecosystems existing in the Himalayas. In an ecological footprint analysis, six components are taken into consideration: carbon lands, built-up areas, forests, fishing grounds, cropland, and pastures.Ecological footprint is a function of these components together (Wilson and Anielski, 2004) (Figure 1). EFA is an approach to trace human impacts on the regenerative capacity of an ecosystem by calculating the amount of bio-productive land needed to sustain the average annual consumption and waste output of a given entity based on technology at the time (Wackernagel *et al.,* 2005; Monfreda *et al.,* 2004; Wackernagel *et al.,* 2002; GFN, 2017).

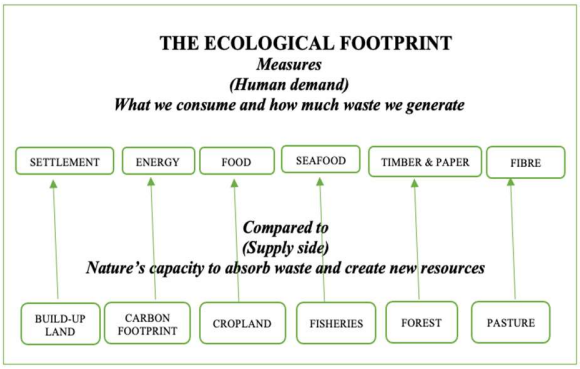

Figure 1: Illustration of the demand on and supply of nature

Cities are not only engines of economic and social development but also transportation corridors that pose significant challenges and risks to the environment, making planning a difficult task. However, sustainable and smart development can be achieved through urban planning, (Corrigan, 2004; Livingston, 2017) , which requires a comprehensive approach that includes new knowledge, inclusivity, integration, management, and sustainable ecosystems for urban habitats (Minea, 2008; Soltani and Sharifi, 2012).
To evaluate the environmental sustainability of one of the urban ecosystems in the North-western Himalayas, this study focuses on the *built-up land component*, such as buildings and infrastructure for housing, transportation, and industrial production. Kangra, which has 3,559,422 working people, is the most populous district in Himachal Pradesh, with a population of about 15.10 lakh and a density of 263 people per square kilometre. Therefore, it holds the first position among the 12 districts in terms of population, literacy rates, and working population (Census of India, 2011).
As no similar study has been conducted in Kangra district, in order to develop a utilitarian model to address ecological imbalance caused by the fast pace of urbanization, and to generate strategies for urban resilience that incorporate adaptive capacity and sustainability to face the challenges of the 21st century. (Dadashpoor and Ahani, 2019a; Diamond, 2012).

Material and Methods

Study area

The study was conducted in the Kangra district of Himachal Pradesh, located in the North western Himalayas. Kangra was selected for its diverse population, and it is the most populous district in Himachal Pradesh with a total population of approximately 1,510,075 and a geographical area of 5,739 square kilometres, which accounts for 10.31% of the state's total area. The district's coordinates lie between 31° 21′ to 32° 59′ North latitude and 75° 47′ 55″ to 77° 45′ East longitude (see Figure 2). The study included three types of urban areas based on their degree of urbanization: Municipal Corporation, Municipal Council, and Nagar Panchayat (refer to Table 1). These urban areas account for approximately 5.7% of the state's population.

Methods and measurements of EFA tool

This study was carried out during 2019-2020 in the urban areas of Kangra district. The necessary data, including the built-up area, the equivalence factor, and the yield factor, were gathered from field

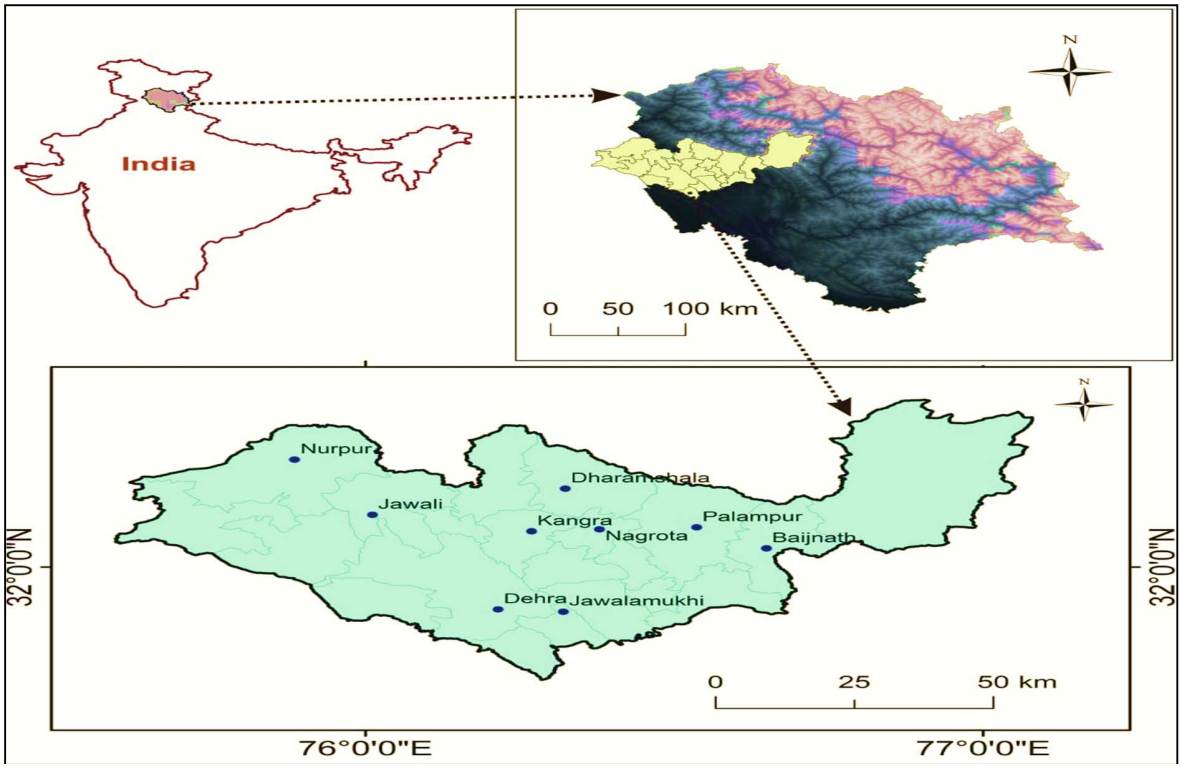

Figure 2: Map showing study area

Table 1: Description of different urban areas of Kangra district

Urban Areas	No. of wards	No. of Households	Total population
	Municipal Corporation		
Dharamshala	17	12500	53543
Municipal Council			
Kangra	9	2250	9528
Nurpur	9	2160	9807
Palampur	7	766	3543
Nagrota	7	1779	5900
Dehra	7	1221	4816
Jawalamukhi	7	1428	5361
Nagar Panchayat			
Baijnath-Paprola	11	2661	16124
Jawali	9	2966	10564
Total	**83**	**27731**	**119186**

surveys, official records from the Census of India, and global footprint account data.

Built-up land Footprint (EF $_{\text{Built-up land}}$)

"The footprint of built-up land is calculated based on the area covered by man-made infrastructure, such as transport, housing, industry, and reservoirs for hydropower generation." According to the National Footprint Accounts (NFA, 2011), which assumes that built-up land is located in fertile areas, it can lead to irreversible losses of biocapacity (Kandil *et al.,* 2019; Geng *et al.,* 2014). The ecological footprint (EF) of built-up land was derived using the following equation and methodology, based on the assumption that the built-up area was largely converted from prime agricultural land (NFA, 2011; Wackernagel *et al.,* 2005).

$$\mathbf{EF_{Built-up\ land}(gha)} = \frac{\mathbf{A(ha)} \times \mathbf{EQF}\left(\frac{gha}{ha}\right) \times \mathbf{YF}}{\mathbf{N}}$$

Where;

- **EF built-up land is the ecological Footprint of built-up area per capita in global hectares**
- **A stands for the area in hectares of the built-up land**
- **EQF is the global equivalence factor per hectare of built-up land**
- **YF is the yield factor of built-up which is equal to the yield factor of the cropland**
- **YF stands for yield factor of the cropland which equals yield factor of built-up land**
- **N is the population of the area under study**

Built-up land Biocapacity (BC $_{\text{Built-up land}}$)

"As a lens, an important aspect of biocapacity is its ability to regenerate and provide the necessary natural resources and services to meet the competing needs of humans, such as producing energy, eliminating waste, recycling water, and making urban areas productive and liveable". Overall, biocapacity gives us a sense of how sizable the material metabolism of human economies is in comparison with what nature can replenish. It is standard practice to allocate 12% of available land for the preservation of domestic biodiversity when determining the biocapacity (BC) of a given land use type. The biocapacity was calculated using the methodology described by Kandil *et al.* (2020), Zhao *et al*. (2006), and Borucke *et al*. (2013).

$$\mathbf{BC_{Built-up\ land}(gha)} = \frac{\mathbf{A(ha)} \times \mathbf{EQF}\left(\frac{gha}{ha}\right) \times \mathbf{YF}(\mathbf{100-12\%})}{\mathbf{N}}$$

Where;

- **BC stands for total built-up land bio-capacity**
- **A stands for the total available supply in a given year**
- **EQF stands for the Equivalence Factor**
- **YF stands for the Yield Factor**
- **N stands for the population of the area under study**

Equivalence Factor (EQF)

It is used to convert actual areas (hectares) of different land types into global hectares. Equivalence factors represent the relative productivity of world average hectares of different land use types that apply to all countries and change slightly from year to year. For the year 2017, the equivalence factor for built-up areas was 2.49 global hectares per hectare, while for 2019 it was 2.51 global hectares per hectare (Lin *et al.,* 2019; NFA, 2021)

Yield Factor (YF)

According to the yield factors, each country, for each land use type, has its own yield factor. The yield factor represents the average productivity of both national and global hectares of a given land use type. These yield factors are included in biocapacity calculations when the biocapacity statistics are expressed in global hectares. Similarly, there are similar yield factors for all countries, which change slightly from year to year. For the year 2017, the yield factors for built-up areas were 1.05 global hectares per hectare, while for 2019 they were 1.08 global hectares per capita (Lin *et al.,* 2019; NFA, 2021).

Ecological deficit (EF_D)

Ecological deficit or an ecological balance issue can be determined by deducting ecological footprint from biocapacity. If the ecological footprint is greater than the biocapacity, it results in an ecological imbalance problem; then the system is considered to be unsustainable and there is a presence of Ecological Deficit (ED). On the other hand, if biocapacity exceeds ecological footprint, then the system is considered sustainable and Ecological Reserves (ER) are present (Geng *et al.,* 2014; Cucek *et al.,* 2012; Galli *et al.,* 2019).

$$\mathrm{EF_D} = \mathrm{EF} - \mathrm{BC}$$

Where

- **EF_D stands for ecological deficit of the study area**
- **EF stands for ecological footprint by various categories of consumption**
- **BC stands for biocapacity given by bio productive area**

Results and Discussion

Built-up land footprint

The Ecological Footprint (EF), Biocapacity (BC), and Ecological Deficit (ED) vary largely as separate variables. These can be used to determine how much the local environment is capable of supporting the study system when the first two variables are larger, or when they are present in pools. The built-up land footprint is an important factor that gives insight to planners and researchers to ensure the balance ratio of EF and BC. The data presented in Figure 3 illustrates that the total built-up land footprint and built-up land footprint per capita are 18,146.095 and 1.371 g ha, respectively, in different urban areas. The Kangra district of the hilly state has different total built-up land footprints ranging from 181.624 to 7,988.728 g ha. Urban areas exhibit the following trends: Dharamshala (7,988.728 g ha) > Jawali (2,434.298 g ha) > Baijnath-Paprola (2,339.420 g ha) > Nurpur (1,287.630 g ha) > Jawalamukhi (1,225.282 g ha) > Nagrota (959.623 g ha) > Dehra (883.721 g ha) > Kangra (845.770 g ha) > Palampur (181.624 g ha). Dharamshala has the highest built-up land footprint (7,988.528 g ha), likely due to the rapid development of the area.

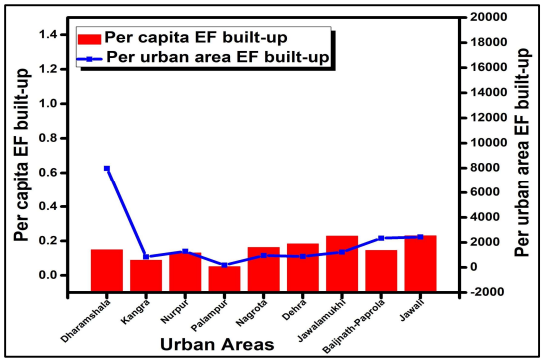

Figure 3: Built-up land footprint EF $_{built-up}$ (g ha) in different urban areas of district Kangra

These results are in line with Kassouri (2021), Pandit et al. (2021), and Jain et al. (2021), who have reported a positive and significant effect of urbanization on built-up land footprints. The results clearly depict that increased rates of urbanization will result in elevated levels of built-up land footprint as the growth of urbanization increases the amount of space needed for infrastructure, including buildings, bridges, roads, and industrial structures. High population pressure, tourism, waste generation, educational hubs, as well as material accumulations, including an abundance of many private and governmental offices, may also contribute to the high footprint. While Palampur has the smallest footprint of built-up land (181.624 g ha), which might be due to reasonably lower anthropogenic and developmental activities and lesser levels of population in the region compared to other urban areas.Per capita built-up land footprints varied among different areas, ranging from 0.051 to 0.230 g ha/capita and resulted in trend: Jawali (0.230 g ha/capita) > Jawalamukhi (0.229 g ha/capita) > Dehra (0.183 g ha/capita) > Nagrota (0.163 g ha/capita) > Dharamshala (0.149g ha/capita) > Baijnath-Paprola (0.145 g ha/capita) > Nurpur (0.131 g ha/capita) > Kangra (0.089 g ha/capita) > Palampur (0.051 g ha/capita). Similar results has been found by Kandil *et al.* (2020) and Pandit *et al.* (2021)*;* based on different population sizes, diverse geographic areas, and the different populations under study not proportionate to the current area available, no significant trends in per capita built-up land footprint were observed.

Built-up land biocapacity

The biocapacity of built-up land provides insight into the demands of humans for renewable resources in the study area. Calculating these values can help to clearly identify the balance between supply and demand over time. With respect to urban areas, Figure 4 shows a total biocapacity of 15968.564 g ha and a per capita built-up land biocapacity of 1.206 g ha. Different urban areas had a total built-up land biocapacity between 7030.081 and 159.829 g ha. The urban area-wise trend was: Dharamshala (7030.080 g ha) > Jawali (2142.183 g ha) > Baijnath-Paprola (2058.690 g ha) > Nurpur (1133.114 g ha) > Jawalamukhi (1078.248 g ha) > Nagrota (844.468 g ha) > Dehra (777.674 g ha) > Kangra (744.277 g ha) > Palampur (159.829 g ha). It is likely that Dharamshala has the highest built-up biocapacity due to its maximum built-up land area and Palampur has the lowest built-up biocapacity due to the smallest built-up land area.

Different urban areas exhibited varying levels of built-up biocapacity per capita from 0.045 to 0.203 g ha/capita and the order was : Jawali (0.203 g ha/capita) > Jawalamukhi (0.201 g ha/capita) > Dehra (0.161 g ha/capita) > Nagrota (0.143 g ha/capita) > Dharamshala (0.131 g ha/capita) > Baijnath-Paprola (0.128 g ha/capita) > Nurpur (0.116 g ha/capita) > Kangra (0.078 g ha/capita) > Palampur (0.045 g ha/capita). The results are in line with findings of Kandil *et al.* (2020) and Pandit *et al.* (2021) who reported that varying degrees of population density and lack of proportionate geographic area might result in no particular pattern in built-up land biocapacity.

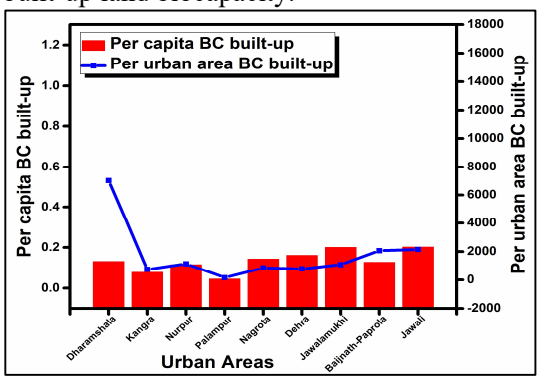

Figure 4: Built-up land Biocapacity BC $_{built-up}$ (g ha) in different urban areas of district Kangra

Ecological deficit

Ecological deficits highlight the need for more bio productive built-up land than is currently available in order to promote sustainability in urban

ecosystems. When an ecological deficit occurs, it indicates that the demands of the particular area have exceeded the critical capacity of the ecosystem. This can result in the scarcity of ecological reserves due to faster consumption of resources, which leads to ecological overshoot. According to Figure 5 , the total ecological deficit in different urban areas is 2177.531 g ha and the per capita ecological deficit is 0.164 g ha. The total ecological deficit in different urban areas ranges from 958.647 to 21.795 g ha, and it is arranged in the order : Dharamshala (958.647 g ha) > Jawali (292.116 g ha) > Baijnath-Paprola (280.730 g ha) > Nurpur (154.516 g ha) > Jawalamukhi (147.034 g ha) > Nagrota (115.155 g ha) > Dehra (106.046 g ha) > Kangra (101.492 g ha) > Palampur (21.795 g ha). These findings are in line with the results obtained from Kassouri (2021) and Zhang *et al.* (2019). Different consequences of expanding urbanization can be seen on ecological resources across different spatial locations occurring on a multidimensional scale including environment and social processes which are shaping sustainability as a whole.The greatest ecological deficit was recorded in Dharamshala (958.647 g/ha). This suggests that due to fancy lifestyles and rapid urbanization with a massive population, resources are being consumed at a faster rate than they can be regenerated or renewed. This could be attributed to the high clustering of various hubs of private, semi-government, and public institutions, companies, and a high flux of tourists to the region. Consequently, the natural capital of this region is under increased pressure. The lowest ecological deficit of 21.795 g/ha in Palampur may be explained by the low levels of anthropocentricity in the region, as well as the relatively low levels of development in the area.The ecological deficit per capita in different urban areas ranged from 0.004 to 0.027 g ha/capita and followed the following pattern: Jawali (0.028 g ha/capita) > Jawalamukhi (0.027 g ha/capita) > Dehra (0.022 g ha/capita) > Nagrota (0.020 g ha/capita) > Dharamshala (0.018 g ha/capita) > Baijnath-Paprola (0.017 g ha/capita) > Nurpur (0.016 g ha/capita) > Kangra (0.011 g ha/capita) > Palampur (0.006 g ha/capita). The results are in congruence with the findings of Kandil *et al.* (2020) and Pandit *et al.* (2021);the per capita ecological deficit has no characteristic pattern because of varying geographical areas and capricious amount of population which has not relatively magnified in these areas. According to the data, the expanded built-up land footprint of different urban areas exceeds the biocapacity of the environment, rendering the system unsustainable and pointing out the existence of an Ecological Deficit (ED). This implies that various urban areas must have vast built-up land in order to provide back up to urban sprawl in the hilly region and to sustain its associated activities which in turn also highlights the major losses to urban ecosystems and degradation of natural capital and its resources followed by elevated levels of accumulation of waste in the study area.

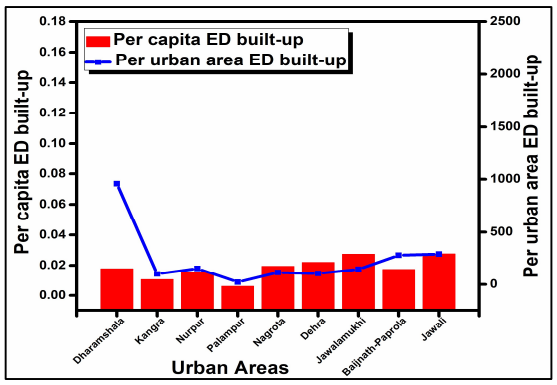

Figure 5: Ecological deficit EF_D (g ha) in different urban areas of district Kangra

Conclusion

The study revealed that there is a high total $EF_{built\text{-}up}$ in comparison to $BC_{built\text{-}up}$ in Kangra district, HP, which indicates unsustainability and a need for more bio-productive built-up land to support urban activities. To achieve sustainability in the urban ecosystems of the North Western Himalayas, it is important to rely on ecological principles and promote urban renewal through the development of smart cities/eco-cities. Additionally, more focus should be placed on advocating for regular updates and revisions to address significant shifts in the environment, society, and economy. To ensure ecological balance and resource sustainability for future generations, development should be carefully planned with similar studies followed by regular monitoring and assessments. Mitigation measures should be adopted through green practices such as improving production systems, altering consumption patterns, managing overpopulation,

and recovering, improving, protecting, preserving, managing, and recovering natural ecosystems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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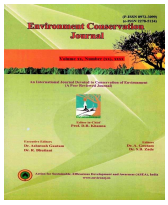

Detection of fumonisin among different strains of *Fusarium* spp. associated with bakanae disease of rice (*Oryza sativa* L.) using molecular markers

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ABSTRACT

Bakanae disease caused by *Fusarium fujikuroi* of basmati rice causes huge economic losses varying with varieties produced, with a frequency of 3.0-95.4%. The *Fusarium* spp. associated with bakanae disease produce fumonisins, a group of structurally similar sphingosine analogue mycotoxins, among which Fumonisin B1 is the most prevalent and active (FB1). The worst harm to both people and animal wellbeing is created by fumonisins, which infect feed and food sources. IARC, a global organization dedicated to cancer research, classified FB1 as a potential causing human cancer (Group 2B). Altogether 26 strains of *Fusarium* spp. from bakanae infected samples of various popular basmati rice varieties collected from Hisar, Jind, Fatehabad, Bhiwani, Sirsa, Panipat, Sonipat, Karnal, Yamunanagar, Kaithal and Kurukshetra (eleven) districts of Haryana state. Two specific primers namely VERTF and polyketide synthase (PKS) (involved in fumonisin biosynthesis) FUM (rp 32 and rp 33) were utilized in this investigation to differentiation between fumonisin-producing and non-producing strains employing PCR technique. Twenty-two strains were significant for the VERTF primer and showed the capacity to generate fumonisin, while four isolates evaluated negative for both primers. The FUM specific primer displayed positive respose only in nine strains and rest were negative. The present study provides a rapid and specific method that helped in accurate differentiation between fumonisin-producing and non-producing strains.

Introduction

In Asian nations where rice is grown, a disease known as bakanae induced by *Fusarium fujikuroi* has gained substantial importance (Asmaul *et al.,* 2021). Hori (1898) was the first to recognise *F. heterosporium* Nees as the disease's cause. Ito and Kimura renamed the imperfect stage *F. moniliforme* and revised Sawada's (1917) description of the fungal sexual stage from *Lisea fujikuroi* to *Gibberella fujikuroi* in 1931 (Sun and Snyder, 1981). In addition to damaging plant tissues, *Fusarium* spp. forms fumonisins, a group of mycotoxins which cause a variety of health problems in animals and humans. Genus *Fusarium*, which includes the major fumonisin producers, is primarily responsible for producing these chemicals (Tyska *et al.,* 2021). Mycotoxins being dangerous natural compounds created by various pathogenic fungus that occur in nature in a variety of products from all over the globe. Food and feed frequently include deadly mycotoxins called fumonisins (FBs), (Li *et al.,* 2022). The potential impacts on the wellbeing of the animals are considered in the

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threat analysis of mycotoxins in animal feed, together with the potential dangers of their compounds transferring to animal - based foods intended for human consumption (Mackay *et al.,* 2022). Fumonisin causes severe species- and organ-specific fatalities, such as neurological problems in horses, pulmonary edoema in pigs, gastrointestinal cancer in people, renal and liver related adverse reactions in rats (Wangia and Kizito, 2020).

In general, the morphological characteristics of fumonisin, which is produced by fungi, are insufficient (Jurado *et al.,* 2010). Using DNA-based molecular methods, precise and specific methodologies for detecting strains that can generate fumonisins have been created. A PCR test for fumonisin detection using the FUM1 primer has been developed in earlier research investigations. According to Moretti *et al.* (2004) the internal transcribed spacer section (IGS) is a non-coding region with a widely varying nucleotide. Two sets of primers (VERTF1/2) were found in the IGS (Patino *et al.,* 2004). Nayak *et al.* (2018) studied genetic variability of fumonisin generating *Fusarium* strains of bakanae employing PCR-RFLP of IGS-rDNA section. Current research intends to provide scientific data for upcoming initiatives to undertake corrective and preventive measures for the management of mycotoxins in the nation. So keeping in view the danger of exposure to carcinogenic compounds due to the large consumption of rice-based diets and a fairly quick precise method that aids in the distinction between fumonisin producing and non-producing strains with accuracy, present study was planned.

Material and Methods

Diseased samples collection

Bakanae infected diseased samples (64) from commonally grown aromatic rice cultivars viz., PB 1121, PB 1401, PB 1718, PB 1509 and Basmati 521, were collected from Hisar, Jind, Fatehabad, Bhiwani, Sirsa, Panipat, Sonipat, Karnal, Yamunanagar, Kaithal and Kurukshetra districts of Haryana during *kharif* season. From these samples, finally twenty six different isolates of *Fusarium* spp. were selected and maintained for this study.

Isolation, purification and maintenance

The infected samples were cut into pieces of three to four mm and surface disinfected using $MgCl_2$ (0.1%). Under completely sterile and aseptic conditions, the cut pieces were distributed evenly over potato dextrose agar media in petri plates. A BOD incubator was used to incubate the inoculated plates at a temperature 25±2°C. Purified and the related fungus from the culture plates, *Fusarium* spp. were identified through microscopic examination (Leslie and Summerell,2006) subcultured using the single spore culture technique (Hansen, 1926) and maintained using potato dextrose agar for future research.

DNA extraction

Twenty six purified strains of *F. moniliforme* were used to isolate DNA using CTAB technique. (Murray and Thompon, 1980). Using a sterile pestle and mortar, the fungus mat was crushed into a powder and then immersed in liquid nitrogen. The powder that resulted was collected in 2 ml centrifuge tubes. The powder was combined with 800 µl of 1% mercaptoethanol-containing CTAB buffer before being placed in the tubes for an hour in a water bath at 65°C. After each 15 minutes, the tubes were carefully turned over to combine the contents, eighty hundred µl of chloroform : isoamyl alcohol (24:1) were supplied after incubating. In order to ensure proper mixing, the materials were chilled to outside temperature and the materials were shaken at seventy rpm for thirty to forty five minutes. After shaken, the samples were placed in a micro-centrifuge at ten thousands rpm for fifteen min. Using a pipette, the liquid was extracted from the tissue waste and placed in new 1.5 ml spin tubes. The supernatant was then given the RNase treatment by having 10 µl of RNase to every tube, which was then keeping them at 37°C for 30 min. Following the addition of 800 µl of cold isopropyl alcohol, moderate inversions were performed. After being kept at four degree celsius for fifteen minutes, materials spun once more for ten minutes at 10,000 rpm. The pellet was rinsed with 70% ethanol after the supernatant was disposed. After being wind drying, the pellet was combined with 50 µl of Tris-EDTA buffer. The isolates' entire DNA was kept in storage at -20°C until usage.

PCR amplification

In this investigation, a PCR test was utilized to differentiation between fumonisin-producing and non-producing strains using two specific primers called VERTF (Vertf 1 and Vertf 2 forward and reverse primers, respectively) and FUM (rp 32 and rp 33 forward and reverse primers), which are both

involved in fumonisin biosynthesis. PCR was conducted in a total amount of 25 µL with every tube holding 12.5 µL of the master mix (Promega corporation, USA) 7.5 µL water, 1.5 µL each primers (Vertf 1-5'-GCGGGAATTCAAAAGTGG CC-3') and (Vertf 2-5'-GAGGGCGCGAAA CGGATCGG-3') as studied by (Patino *et al*., 2004) and FUM (rp 32-5- ACAAGTGTCCTTGGGGTC CAGG-3') and (rp 33-5'-GATGCTCTTGGAAGT GGCCTACG-3') as reported by (Jeon *et al.,* 2013) and 2 µl DNA of each isolates. The primers were synthesized by Integrated DNA Technologies (USA). The PCR conditions used included pre-incubation at 94^0C for 4 min, followed by multiplication for 35 rounds, including denaturation at 94^0C for 1 min, annealing at 60^0C for one min, extension at 72^0C for 1 min and a last extension stage of seven min at 72^0C. Amplicons were observed by electrophoresis on 2% agarose gels utilizing the EtBR dye and documented with a (Bio-Rad, Philadelphia, PA, USA) gel documentation system.

Results and Discussion

A total of twenty six *Fusarium* isolates were analyzed in the present investigation which obtained from common aromatic paddy cultivars. The infected samples were collected from different locations of Haryana state.

A PCR product of size approximately 400bp was observed for primers (Verf 1 and Vertf 2) in Fig. (1a) lane 2 M 100 bp lanes (3-14) isolates numbers as FM 3, FM 7, FM 10, FM 12, FM 16, FM 18, FM 20, FM 25, FM 28, FM 31, FM 34 and FM 36 (Table 1) and in Fig. (1b) lane 1 M 100bp lanes (2-15) isolates FM 37, FM 40, FM 44, FM 50, FM 51, FM 52, FM 53, FM 56, FM 59, FM 60, FM 62, FM 63, FM 64 and FM 66 (Table 1) in sequence. Twenty two isolates were showing fumonisin producing ability and four isolates (FM 52, FM 56, FM 59 and FM 62) were negative.

Similarly in Fig. (1c) lane 2 M 100 bp lanes (3-14) isolates viz., FM 3, FM 7, FM 10, FM 12, FM 16, FM 18, FM 20, FM 25, FM 28, FM 31, FM 34 and FM 36 (Table 1) and in Fig. (1d) lane 1 M 100bp lanes (2-15) isolates numbers as FM 37, FM 40, FM 44, FM 50, FM 51, FM 52, FM 53, FM 56, FM 59, FM 60, FM 62, FM 63, FM 64 and FM 66 (Table 1) in sequence for FUM gene a product of size approximately 680bp was observed. Nine isolates viz., FM 3, FM 7, FM 10, FM 12, FM 16, FM 18, FM 40, FM 52 and FM 56showed amplification of FUM (rp 32 and rp 33 primers) therefore regarded as producer of fumonisin while the remaining strains were non-fumonisin producers.

This study further demonstrates that PCR analysis is an effective and fast way to detect fumonisin producing *Fusarium* strains. Similar to our study, as we detect almost 85% fumonisin producing strains, Nayak *et al.* (2014) detected 85% fumonisin producers among 28 *Fusarium* isolates from Indian rice cultivars with a rapid molecular method using primer Fum5 F and Fum6 R.

Elsharnouby *et al.* (2015) studied twelve *Fusarium* isolates for PCR assay to distinguish between isolates that produce fumonisin and those that do not. Single strain (*F. verticillioides*) from damaged maize, 10 samples (*F. moniliforme*) from infested paddy with bakanae disease, and one strain (*F. solani*) from damaged wheat. In that investigation, two distinct primers by the names of VERTF-1 and FUM1 were employed. The polyketide synthase (PKS) gene FUM1 and the intergenic spacer region (IGS) of rDNA. Only sample (*Fusarium solani*) was evaluated negatively for both primers while 11 strains were positive for the VERTF-1 primer and had the capability to generate fumonisin. Five strains of *Fusarium moniliforme* while one of *Fusarium verticillioides*, respectively, responded negatively to the primer FUM1. In this study, Vertf primer also showed good results as in our investigation in comparison to primer FUM. *Fusarium* spp. isolates infecting wheat and maize were also used in this investigation while we studied only rice isolates.

Deepa *et al.* (2015) collected a total of 135 cereal samples from different districts of Karnataka, India in which 69 samples were infected with *Fusarium* species. Among these 51 samples were having *Fusarium verticillioides* infection and among them 42 samples were positive for fumonisin production. Similar to our study, Vertf primer also showed good results for fumonisin detection among different strains of *Fusarium* species.

Hinojo *et al.* (2006) showed deviation in methods as used in present study for detection of fumonisins among the isolates, optimized analytical method for

Table 1: Details of *Fusarium* isolates collected from different locations of Haryana for fumonisin producing and non-producing ability

SN	Isolates ID	Locations (Districts)	Isolates positive/ negative (Vertf)	Isolates positive/ negative (FUM)	Variety grown
1	FM 3	Dhad (Hisar)	+	++	PB 1121
2	FM 7	Gurana (Hisar)	+	++	PB 1121
3	FM 10	Kheri Jalab (Hisar)	+	++	PB 1121
4	FM 12	Intal Khurd (Jind)	+	++	PB 1121
5	FM 16	Ikkas (Jind)	+	++	PB 1121
6	FM 18	Saniana 1 (Fatehabad)	+	++	PB 1121
7	FM 20	Saniana 2 (Fatehabad)	+	--	PB 1401
8	FM 25	Pirthala1 (Fatehabad)	+	--	PB 1121
9	FM 28	Pirthala 2 (Fatehabad)	+	--	PB 1121
10	FM 31	Kungar (Bhiwani)	+	--	PB 1509
11	FM 34	Alakhpura (Bhiwani)	+	--	PB 1121
12	FM 36	Barsi (Bhiwani)	+	--	PB 1121
13	FM 37	Patli Dabar (Sirsa)	+	--	PB 1401
14	FM 40	Mochiwali (Sirsa)	+	++	PB 1121
15	FM 44	Bajekan (Sirsa)	+	--	PB 1121
16	FM 50	Naiwala(Sirsa)	+	--	PB 1718
17	FM 51	Bapoli (Panipat)	+	--	PB 1509
18	FM 52	Panipat 1	-	++	PB 1718
19	FM 53	Panipat 2	+	--	PB 1718
20	FM 56	Sonipat	-	++	PB 1121
21	FM 59	Taraori (Karnal)	-	--	PB 1121
22	FM 60	Sikri (Karnal)	+	--	PB 1718
23	FM 62	Kartarpur (Yamunanagar)	-	--	PB 1509
24	FM 63	Sandhala (Yamunanagar)	+	--	PB 1121
25	FM 64	Kaithal	+	--	Basmati 521
26	FM 66	Babain (Kurukshetra)	+	--	PB 1509

Note: Isolates positive for primer Vertf shown by symbol (+) and negative (-) and isolates positive for primer (FUM) shown by symbol (++) and negative (--)

determination of fumonisins in rice was applied to the study of FB_1 and FB_2 production by four isolates of the *G. fujikuroi* species complex in rice cultures carried out at different temperatures and water activities to establish the influence of strain and environmental conditions on fumonisin production in this cereal. In general, fumonisin production was the highest at 20°C and lowest at 37°C. Four of the five assayed water activity (a_w) values (0.97, 0.98, 0.99, and 1.0) did not affect significantly fumonisin accumulation but fumonisins were not detected in cultures when a_w was 0.96.Similar to our study, Maheshwar *et al.* (2009) studied the occurrence of fumonisin producing *Fusarium verticillioides* in 90 samples of stored paddy (*Oryza sativa* L.) collected from different geographical regions of Karnataka, India. Fumonisin producing *F. verticillioides* was identified based on micro-morphological characteristics and PCR using two sets of primers. One set of primers was *F. verticillioides* species specific, which selectively amplified the intergenic space region of rDNA. The other set of primers was specific to fumonisin producing *F. verticillioides*. Eight paddy samples were positive for *F. verticillioides*. Eleven isolates obtained from these samples were capable of producing fumonisin. Less no. of isolates were taken for the detection of fumonisins as comparisons to our investigation.

Sreenivasa *et al*. (2008) reported that out 83 *Fusarium verticillioides* strains 64 were positive for Fumonisin production. Choi *et al.* (2018) assessed the genetic potential for fumonisin production among different isolates using a PCR assay designed to detect the presence of the FUM1 gene.The results of this assay showed that about 98% of the FFSC isolates tested were positive for FUM1

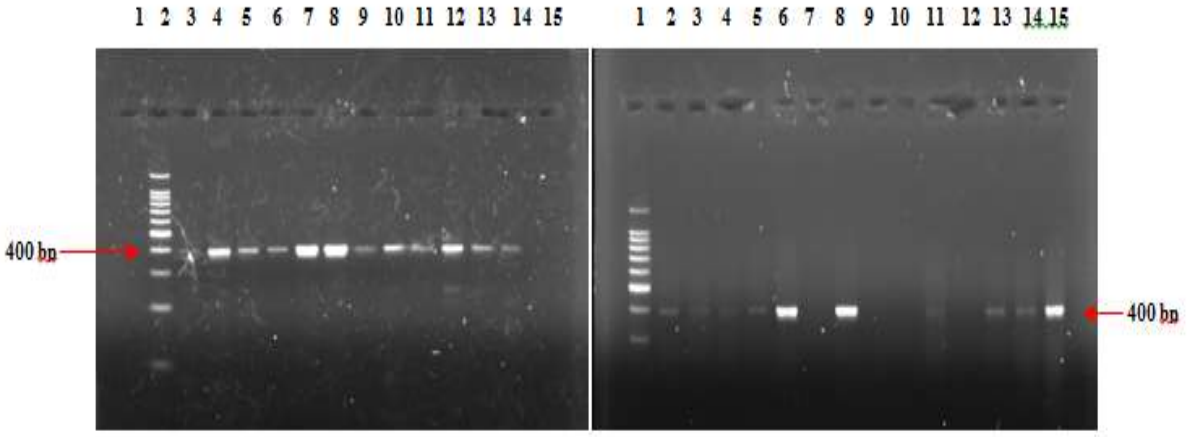

Fig. (1a) lane 2 M 100 bp lanes (3-14) isolates FM 3 FM 7, FM 10, FM 12, FM 16, FM 18, FM 20, FM 25, FM 28, FM 31, FM 34 and FM 36 size 400 bp

Fig. (1b) lane 1 M 100 bp lanes (2-15) isolates FM 37, FM 40, FM 44, FM 50, FM 51, FM 52, FM 53, FM 56, FM 59, FM 60, FM 62, FM 63, FM 64 and FM 66 size 400 bp

Figure (1a) and (1b): Details of *Fusarium* isolates for fumonisin producing and non-producing ability by using primers (Verf 1 and Vertf 2)

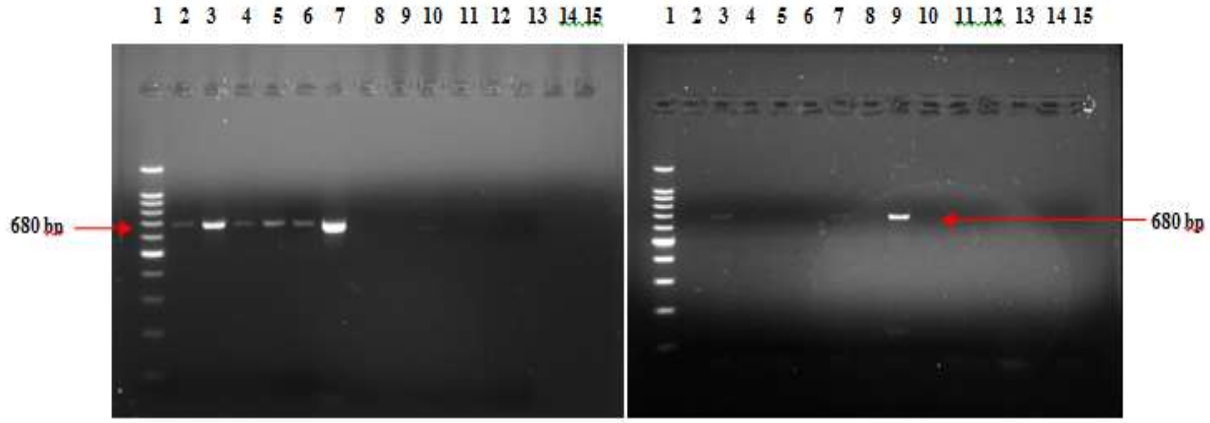

Fig. (1c) lane 1 M 100 bp lanes (2-13) isolates FM 3 FM 7, FM 10, FM 12, FM 16, FM 18, FM 20, FM 25, FM 28, FM 31, FM 34 and FM 36 size 680 bp

Fig. (1d) lane 1 M 100 bp lanes (2-15) isolates FM 37, FM 40, FM 44, FM 50, FM 51, FM 52, FM 53, FM 56, FM 59, FM 60, FM 62, FM 63, FM 64 and FM 66 size 680 bp

Figure (1c) and (1d): Details of *Fusarium* isolates for fumonisin producing and non-producing ability by using primers FUM (rp 32 and rp 33)

including all of the *F. fujikuroi*, *F. proliferatum*, *F. verticillioides* and *F. thapsinum* isolates. Actual production ability was assessed in rice medium and 76.0% of *F. fujikuroi*, 96.3% of *F. proliferatum*, and 94.1% of *F. verticillioides* isolates produced both FB1 and FB2. There was a precise detection of fumonisins producing strains using rapid method similar to our investigation. Similar to our study, as we detects the fumonisins among *Fusarium* spp. which causing bakanae, Jeon *et al*. (2013) reported that three isolates of *F. fujikuroi*, *F. proliferatum* and *F. verticillioides* were found to have FUM1 (the fumonisin biosynthetic gene); however, FUM1 was not found in isolates of *F. concentricum*.

Conclusion

Four strains were evaluated negatively for both primers, while 22 strains tested positive for VERTF primer and showed the capability to generate fumonisin. The primer FUM (rp32 and rp 33) showed positive signal in nine strains and rest of all were negative. The approach presented in this investigation enables quick and precise differentiation between fumonisin-producing and non-producing strains. According to the study, sensitive methods are required for the quantification of fumonisins in rice meant for human consumption. Through this research, it will be feasible to protect both humans and animals from hazardous compounds like fumonisins.

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Conflict of interest

The authors declare that they have no conflict of interest.

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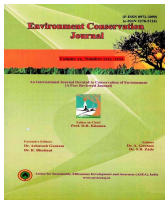

Assessment of carbon loss related to Soil loss in the tropical watershed of Maharashtra, India

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ABSTRACT

Soil carbon pools have a significant impact on the global carbon cycle and soil erosion caused by natural or human activities is one of the main drivers of changes in soil carbon sequestration. The present study aimed to estimate the carbon loss associated with soil loss in the watershed using remote sensing and GIS techniques. The study was carried out at the Central MPKV Campus Watershed, Rahuri, located in the rain shadow region of the Maharashtra state, India. The soil loss from the watershed was estimated using USLE model. The soil loss and carbon loss from the watershed were estimated before the implementation of conservation measures and after the implementation of conservation measures. It was found that the average annual soil loss from the watershed before and after conservation measures was 18.68 t/ha/yr and 9.41 t/ha/yr, respectively. Carbon loss was determined by soil loss rate, organic carbon content and the carbon enrichment ratio. The carbon loss from the watershed before and after conservation measures was 348.71 kgC/ha/yr and 205.52 kgC/ha/yr. The findings revealed that soil and carbon erosion was very severe on steep slopes without conservation measures and with limited vegetation cover. It was found that by reducing the carbon loss associated with soil loss, soil conservation measures not only aid in the conservation of natural resources but also serve as a climate change mitigation measure.

Introduction

Soil carbon pool, which is the dominant terrestrial carbon pool, is roughly 3.3 times bigger than atmospheric carbon pool and 4.5 times bigger than biotic carbon pool (Lal, 2004a). Soil erosion and subsequent sediment transport through runoff are important pathways for lateral soil carbon movement at the land surface and have a significant impact on the carbon flux of terrestrial ecosystems (Kuhn *et al.,* 2012; Li *et al.,* 2018; Wang *et al.,* 2019). Soil erosion induced from water and wind has a significant impact on both the lateral SOC dispersion within a landscape and vertical CO_2 fluxes into the atmosphere (Lal, 2003; Yue *et al.,* 2016). Key mechanisms governing the net carbon transfer between the soil and the atmosphere were enumerated by Van Oost *et al.,* 2005 as follows: 1) SOC replacement at eroding sites 2) deep burial of carbon-rich topsoils towards depositional sites 3) increased SOC degradation through physico chemical soil breakdown during detachment and

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transport process. In particular, last two mechanisms are vulnerable to changes in the precipitation pattern (Wang *et al.,* 2014).

Many studies have found that topsoil erosion caused by intense rainfalls and strong winds degrades soil quality and lowers SOC (Lal, 1990, 2013). However, following widely accepted land management practices (RMPs) can help to reduce soil erosion below tolerable limit and create an environment conducive to carbon sequestration. Soils that are degraded and depleted by soil erosion have a large carbon (C) sink capacity to replenish atmospheric CO_2 into SOC stocks when converted to regenerative land use and the use of effective soil conservation practices (Stallard, 1998; Jacinthe *et al.,* 2002) Due to anticipated changes in the Earth's climate, soil loss rate is likely to accelerate in the future (Berc *et al.,* 2003; Yang *et al.,* 2003). Accelerated soil erosion is one of four prime global pressures threatening human survival, the others being changing climate, increasingly rapid population explosion and biodiversity extinction (Ontl and Schulte, 2012). In recent years, a large number of research studies have been conducted in different regions of the world in order to better understand the dynamics and redistribution of carbon (C-erosion) associated with soil erosion (Bajracharya *et al.,* 2000; Mabit *et al.,* 2008; Wang *et al.,* 2014; Karmakar *et al.,* 2016; Wang *et al.,* 2019).

SOC loss can have a significant impact on soil quality by lowering soil stability, water holding capacity and productivity (Lal, 2015). Furthermore, soil organic carbon loss through soil erosion depleted carbon uptake by terrestrial ecosystems, lowering soil carbon sequestration capacities (Lal, 2004b). Carbon flux from soils as recently reported by Kindler *et al.,* (2011), is an important component of the ecosystem's net carbon balance. Despite its significance, it has gone unnoticed in tropical and subtropical regions, where episodic but intense rainfall storms can significantly damage soil productivity through soil erosion and carbon erosion (Li and Fang, 2016).

Erosion-induced carbon fate in India is poorly studied at the state and national levels, and even less at the watershed level. As a result, the purpose of this research is to estimate the lateral transport of carbon by erosion at the watershed level by multiplying amount of soil loss by SOC content and carbon enrichment ratio (CER). The present research was conducted at the Central Mahatma Phule Krishi Vidyapeeth (MPKV) Campus Watershed located in the rain shadow region of Maharashtra, India. The watershed receives moderate rainfall and is prone to water erosion. Nearly half of the watershed is treated with diverse soil and water conservation (SWC) measures. Therefore, carbon loss induced from the soil erosion was estimated before and after conservation measures. The impact of conservation measures on soil loss and subsequently on carbon loss was evaluated.

Material and Methods

Specifics about the study area

The study was carried out at "Central MPKV Campus Watershed" located in Rahuri Taluka in Ahmednagar District of Maharashtra State, India. The study area lies between latitudes $19^0$21.77' N and $19^0$18.73' N and longitudes $74^0$37.79' E and $74^0$36.49' E. The study area is 1260 ha in size, with an altitude of 441 to 542 m above mean sea level (Figure 1).

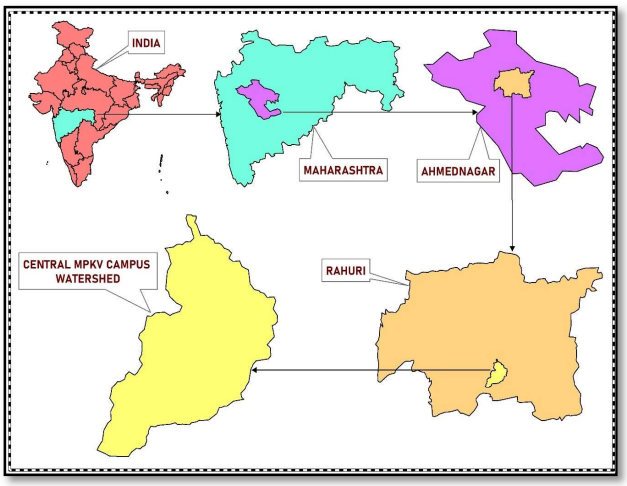

Figure 1: Location map of study area

Climate

The study area exhibits a unimodal precipitation pattern. The study receives rainfall from the monsoon, with the main rainy season extending from delayed June to early September. It receives 592 mm of average annual rainfall and the normal lowest and highest annual temperatures are 19°C

and 31°C, respectively. The study area is located in hot and dry climate zone.

Soil and water conservation measures in the study area

The Central MPKV Campus Watershed is treated with various soil and water conservation measures. In the year 2019 nearly half of the watershed was treated with both land area and drainage line treatments. The land area treatments in the watershed include Deep continuous contour trench (DCCT) and compartment bunding, while drainage line treatments include earthen nala bund, loose boulder structure and percolation tank. The details of SWC measures are given in the (Table 1) and (Figure 2, 3)

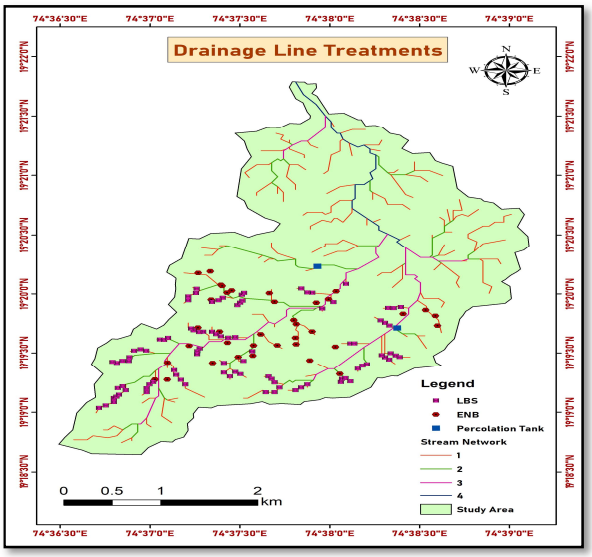

Figure 2: Drainage line treatments in the watershed

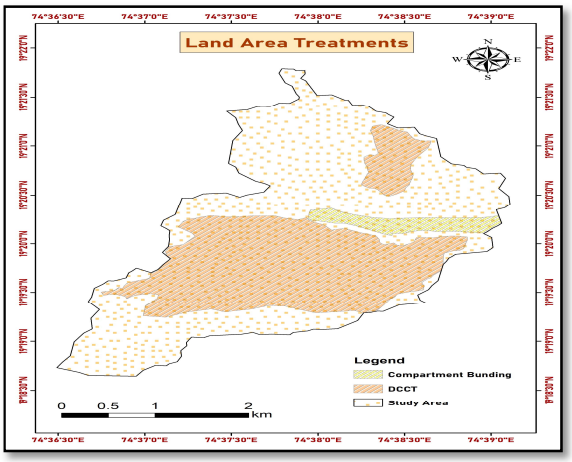

Figure 3: Land area treatments in the watershed

Table 1: Soil and water conservation measures in the watershed

Total Study Area	1260 ha
Total Treated Area	545 ha
Perimeter of treated area	12.77 km
Area Under DCCT	495 ha
Length of DCCT	99,600 running m
Area Under Compartment Bunding	50 ha
Earthen Nala Bunds	38 nos.
Percolation Tanks	2 nos.
Loose Boulder Structures	97 nos.

Land use pattern and crops grown in the study area

The watershed has six primary land-use types: barren land (37.95%), natural vegetation land (24.20%), agriculture land (21.48%), horticulture land (7.30%), settlement (5.78%) and waterbody (3.29%). The majority of cultivated land is concentrated in the lower reaches of the watershed, while natural vegetation land is found in the middle part. Farmers' landholding in the watershed is described as small and dispersed, with less than 0.5 ha per household. Cultivation is the primary source of income in the watershed and main crops cultivated are sugarcane (*Saccharum officinarum L.*), sorghum (*Sorghum bicolor L.*), Maize (*Zea mays L.*) and Onion (*Allium cepa L.*).

Estimation of soil loss risk in the watershed

The soil loss from the Central MPKV campus watershed was estimated using the universal soil loss equation (USLE) model coupled with GIS software. The Arc GIS 10.8 software was used for the estimation of soil loss. The soil loss in the watershed was estimated under two conditions: one without any SWC measures and another after implementation of SWC measures in the watershed. The construction of SWC measures started in the year 2017 and completed in the year 2019. Therefore, before conservation measures data was taken for the year 2016 and after conservation measures readings were taken in year 2021.

Description of data sources used

The data sets required for USLE model parameters were acquired from a variety of sources. The annual rainfall data for watershed was obtained from the Department of Agro-meteorology, Rahuri. Rainfall erosivity factor (R-value) of study area was calculated using annual rainfall data. Soil erodibility factor (K value) was calculated using

organic matter, structure, texture and permeability of the study area's soil. Slope length and gradient factor (LS value) were calculated using Shuttle Radar Topography Mission (SRTM) digital elevation model (DEM) with a resolution of 30m. The crop management factor (C) and conservation practise factor (P) were calculated using Sentinel-2A imagery and DEM data. The DEM and satellite images were acquired from Earth Explore web portal maintained by United States Geological Survey (USGS) (https://earthexplorer.usgs.gov/).

Estimation of USLE parameters

Rainfall erosivity factors (R)

The R factor of study area was calculated using an equation developed specifically for the hot and dry region of Rahuri tehsil and derived from spatial regression analysis. The average rainfall over the last 30 years (1991 to 2021) was used to calculate the R factor. The R factor value was kept constant for estimating soil loss before and after conservation measures.

The Eq. 1 was used to calculate R factor:

$$\mathbf{R} = \mathbf{0.0022}X^{\mathbf{2}} + \mathbf{0.7526X} + \mathbf{152.35} \quad \ldots(1)$$

Where, R= Annual Erosivity, MJ-mm/ha-hr-yr
X= Annual Rainfall, mm

Soil erodibility factor (K)

Total 50 soil samples were collected from the watershed using a 500×500 m grid, with samples collected from the centre of each grid for analysis. Soil samples were collected under different land use patterns from top 15 cm depth using soil auger in order to determine the physicochemical properties of soil. Soil samples were collected in November 2021 when most of the soil in the watershed had dried up. The soil samples were analysed according to standard laboratory procedures. The different soil properties such as organic matter content, soil texture, soil structural and permeability were estimated in the soil testing laboratory.

The K factor of different soil types was calculated using different soil properties such as texture, organic matter, permeability and structure (Foster *et al.,* 1981; Panagos *et. al.*, 2015). The K factor was calculated using Eq. 2 and mapped in this study (Tamene and Vlek, 2007; Addis and Klik, 2015; Wolka *et. al.*, 2015).

$$\mathbf{K(factor)} = \mathbf{2.77 \times 10^{-7}(12 - OM)\ M^{1.14} + 4.28 \times 10^{-3}(s - 2) + 3.29 \times 10^{-3}(p - 3)} \quad \ldots(2)$$

Where,

$$M = [(100 - C)(L + A_{rmf})] \quad \ldots(3)$$

C is % of clay (< 0.002 mm), L is % of silt (0.002–0.05 mm) and A_{rmf} is % of very fine sand (0.05–0.1 mm), OM is the organic matter content (%), p is a code denoting the class of permeability and s is a code for the structure size. It was found that soil physical and chemical properties did not change significantly over a 5-year period, therefore the K-factor is considered constant when estimating soil loss before and after conservation measures. Soil erodibility map of the watershed was prepared in Arc GIS software using interpolation techniques. The Inverse Distance Weighting (IDW) interpolation technique was used to transform soil sampling location points of erodibility factors (K) to surface raster data.

Slope length and gradient factor (LS)

Slope length and gradient factors i.e topographic factor was estimated in ArcGIS 10.8. The SRTM DEM with a spatial resolution of 30m was used to prepare slope map of study area. The DEM was pre-processed in ArcGIS environment to remove discontinuation in data set then different thematic layers such as flow direction, flow accumulation, slope steepness and slope gradient were prepared. The Eq. 4 developed by Wischmeier and Smith (1978) was used to generate LS factor map of study area. Similar approach also followed by other researchers (Shiferaw, 2011; Gerawork and Awdenegest, 2014).

$$\mathbf{LS} = (\mathbf{X/22.1})^{m}\ (\mathbf{0.065 + 0.045S + 0.0065S^2}), \quad \ldots(4)$$

$$\boldsymbol{X = (FLow\ Accumulation \times Cell\ size\ value)} \quad \ldots(5)$$

Where, LS = slope length–steepness factor/Topographic factor, S = slope gradient (%), X = length of slope (m) and m = exponent (slope-length exponent).

Since the slope pattern of the watershed did not significantly change before or after conservation measures, the LS factor was also held constant for both scenarios.

Crop management factor (C)

Land use land cover mapping of study area was performed to prepare crop management factor map of study area. The ratio of soil loss from areas with a particular vegetation cover to soil loss from areas that are fallow under the same rainfall conditions is represented by a C factor. (Wischmeier and Smith, 1978). The Sentinel-2A satellite imagery was used to generate the land-use and land-cover (LU/LC) map of the watershed. Image classification was performed using supervised digital image classification technique in ArcGIS 10.8 software. To create LU/LC maps before and after conservation measures, satellite images from December 15, 2016 and December 16, 2021 were used.The validation of the land cover classification was performed using Google Earth. A total of 105 reference points were generated in Google Earth and these points compared to the obtained land cover classification. Finally, seven LU/LC classes were identified as agriculture, horticulture, barren, natural vegetation, current fallow, settlement and waterbody (Table 1). The standard C-factor values of various LU/LC classes were assigned to the appropriate landcover class using the Reclassify tool in the ArcGIS 10.8 environment to obtain the watershed C-factor raster layer.

Conservation practice factor (P)

The conservation practice factor (P) is defined as the ratio of soil loss expected for a given soil conservation practice to that expected for uphill and hillside plowing (Wischmeier and Smith, 1978). The area under different conservation practices in the watershed was mapped by conducting field survey. The GPS device was used to map the area of different conservation measures. The P factor value of one was given for the entire watershed before conservation measures. However, after the implementation of recommended SWC measures on half of the watershed area, corresponding P factor values were assigned to the conservation measures in the Arc GIS 10.8 environment. Finally, the watershed's P factors raster layer was created by allocating adapted P factor values for conservation measures.

Estimation of soil loss from the watershed

The average annual soil loss from the Central MPKV campus watershed before and after conservation measures was calculated by interactively multiplying (Eq. 6) the USLE factor values (R, K, LS, C, and P) in the Arc GIS 10.8 environment using the Raster Calculator tool.

$$\boldsymbol{A = R \times K \times LS \times C \times P} \quad \text{.....(6)}$$

Where A = Average annual soil loss (t/ha/yr); R = Rainfall erosivity factor (MJ-mm/ha-hr-yr); K =Soil erodibility factor (t-ha-hr/ha-MJ-mm); LS = Slope length factor (dimensionless); C = Crop management factor (dimensionless); and P = Conservation practice factor (dimensionless).

Estimation of carbon loss from the watershed

The C-loss due to soil loss depends on soil erosion rate, SOC concentration and carbon enrichment ratio values. The C-loss from the watershed before and after conservation measures was estimated using (Eq. 6) developed by Mandal *et al.,* (2020)

$$\boldsymbol{C - loss}\left(\frac{t}{ha}{yr}\right) = \frac{\boldsymbol{Soil\ loss}\left(\frac{t}{ha}{yr}\right) \times \mathbf{SOC}\ (\%) \times \mathbf{CER}}{\mathbf{100}} \quad \text{...(6)}$$

Soil organic carbon content in the watershed

A total of 50 soil samples were collected from the watershed to determine SOC content of the watershed. The GPS locations of the sampling points were recorded to map the SOC content. The SOC content was determined for different land use classes by taking soil samples from different land cover classes using the grid sampling method. Soil samples were analysed in the laboratory to estimate after conservation measures SOC content. The SOC data prior to conservation measures was obtained from the Department of Soil and Water Conservation Engineering, Mahatma Phule Krishi Vidyapeeth, Rahuri. The SOC layer for the watershed was generated in Arc GIS environment by providing corresponding SOC values to the soil sample locations. The raster layer of SOC for before and after conservation measures was prepared using interpolation techniques.

Carbon enrichment ratio for the watershed

The CER is defined as the ratio of SOC content in the eroded sediment sample to that of the original soil (Sharpley, 1985). Mandal *et al.,* (2020) calculated CER values for various erosion classes for Maharashtra state (Table 2). In the Arc GIS environment, these values were assigned to the various erosion classes of the watershed, and CER layers for the watershed before and after conservation measures were created.

Table 2: Erosion class wise CER values

SN	Erosion class	Erosion range (t/ha/yr)	CER value
1	**Very low**	< 5	3.62
2	**Low**	5 to 10	3.28
3	**Moderate**	10 to 20	2.3
4	**Severe**	20 to 40	2.3
5	**Extremely severe**	>40	2.04

Carbon loss from the watershed

The average annual carbon loss from the watershed was estimated using raster calculator tool in the Arc GIS 10.8 environment. Soil loss rate, SOC concentration and CER ratio layers generated in the Arc GIS software were used for the estimation of C-loss from the watershed. The Eq. 6 was used in Raster Calculator to generate C-loss layer.

Results and Discussion

Rainfall erosivity (*R*) factor

Rainfall erosivity factor is directly influenced by amount of rainfall and intensity of rainfall. Average annual precipitation in the study area is 592.19 mm, resulting in rainfall erosivity of 478.19 MJ-mm/ha-hr-yr. The lower the R-value, the lower the erosivity of rainfall to erode the soil (Asmamaw and Mohammed, 2019) and lower the rainfall intensity in the study area (Devatha *et al.,* 2015). The estimated moderately low rainfall erosivity index for the study area signifies further risk of soil erosion hazards, especially under conditions of increasing rainfall. The rainfall erosivity is highly dependent on the frequency and intensity of precipitation. Additionally, variations in climatic conditions and weather patterns can also affect the rainfall erosivity by modifying precipitation patterns and intensities. Consequently, fluctuations in rainfall erosivity can greatly impact soil erosion rates in the watershed. In estimating soil loss before and after conservation measures, the erosivity factor was held constant. Bagwan, 2020 found similar rainfall erosivity values, ranging from 392 to 1014 MJ-mm/ha-hr-yr, in the rainfed region of the Urmodi river watershed, Maharashtra.

Soil erodibility (*K*) factor

The soil erodibility value indicated the susceptibility of soil to erosion. Soil erodibility is mainly affected by the kinetic power of rain drop and surface runoff (Khairunnisa *et al.,* 2020). The structural stability and water infiltration capacity of the soil influence the value of the K factor (Devatha *et al.,* 2015). The soil structure in the watershed is coarse granular, with moderate to rapid permeability. The greater the soil erodibility, the higher will be the soil erosion, and vice versa. Soil erodibility in this watershed ranged from 0.0310 to 0.0599 t-ha-hr/ha-MJ-mm (Fig. 4). Low soil erodibility was observed in regions with low levels of organic matter and high soil bulk density. The watershed has three major types of soil: sandy clay loam, sandy loam and clay loam. Among the different soil types found within the watershed, sandy loam soil has the highest erodibility and clay loam soil has the lowest. The areas with clay loam soil type were found in the lower reaches of the watershed, where agriculture land is the dominant land cover. Therefore, the majority of agricultural land cover in the watershed was found to have lower soil erodibility values. Similarly, areas with sandy clay loam and sandy loam soil types were found in the upper reaches of the watershed, where barren land is the predominant land cover. Consequently, values of soil erodibility were found to be greater in the majority of barren land covers than in other types land covers. It indicates that barren lands with high soil erodibility values in the watershed are more vulnerable to soil erosion hazards and require immediate soil conservation measures. The soil type wise average K factor values are given in (Table 3).

Topography factor (*LS*) factor

The LS factor varied from 1.02 in the plains to 5.92 in the highlands (Fig. 5). The watershed's slope ranges from 0 to 30.23%, with a mean slope of 4.17%. Around 90% of the watershed had a slope of 0-9%, with the remaining 10% having a slope greater than 9%. The majority of the watershed, 90%, has a moderate slope range, indicating moderate soil erosion potential, while the remaining 10% has a high erosion potential.

Table 3: Soil type wise soil erodibility (K) factor values (t-ha-hr/ha-MJ-mm)

Soil Type	Minimum	Maximum	Mean	Coefficient of Variation
Sandy Clay Loam	0.031	0.052	0.044	15.64
Sandy loam	0.052	0.060	0.056	4.48
Clay Loam	0.029	0.033	0.031	6.08

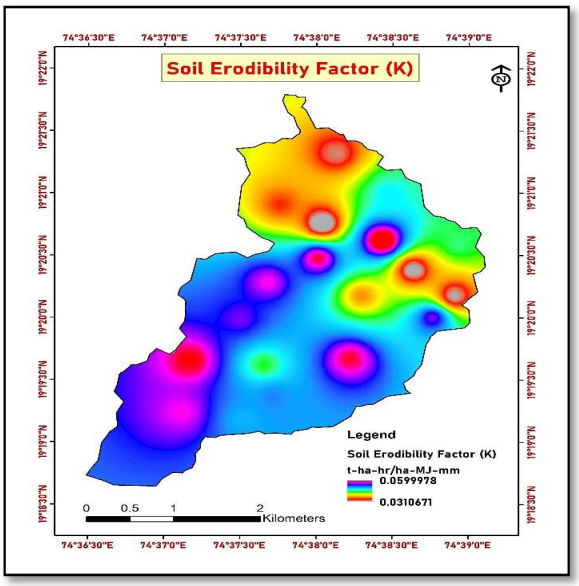

Figure 4: Soil erodibility (K) factor map of watershed.

This 10% area of the watershed with hilly terrain is located in the watershed's middle reaches and requires soil conservation measures that intercept long slopes into several short ones in order to keep runoff water at less than a critical velocity. Karhade and Vangujare (2018) found the LS factor in the range of 0 to 11 in the Kham River Basin, Aurangabad, Maharashtra.

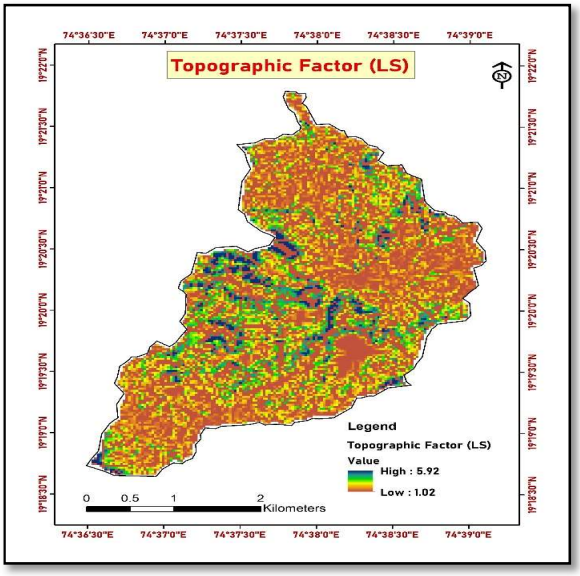

Figure 5: Topographic factor (LS) map of watershed

Crop management factor (C)

Two land cover maps were created, one before conservation measures and one after the implementation of conservation measures in the watershed (Fig. 6, 7). The conservation measures implemented in the watershed affected the land cover within the watershed. Through satellite image classification seven land classes in the watershed were identified as agriculture, horticulture, barren, natural vegetation, current fallow, settlement and waterbody. The overall accuracy of image classification and Kappa coefficient for watershed were 88% and 0.78, respectively, for before conservation measures image and 89% and 0.80, respectively for after conservation measures image. The land cover classification before and after conservation measures is given in the Table 4. It was observed that barren land was dominant land cover class in the watershed followed by natural vegetation. After implementation of conservation measures in the watershed area under barren land and current fallow land was decreased while area under all other land cover classes were increased. Before conservation measures nearly 37.5% of the watershed area was under vegetation cover but after implementation of conservation measures in the watershed vegetation cover area increased upto 50%.

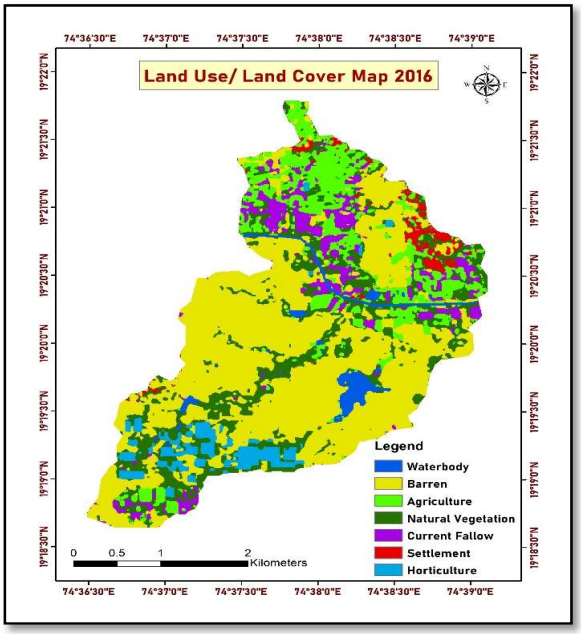

Figure 6: Before conservation measures land use/ land cover map of watershed

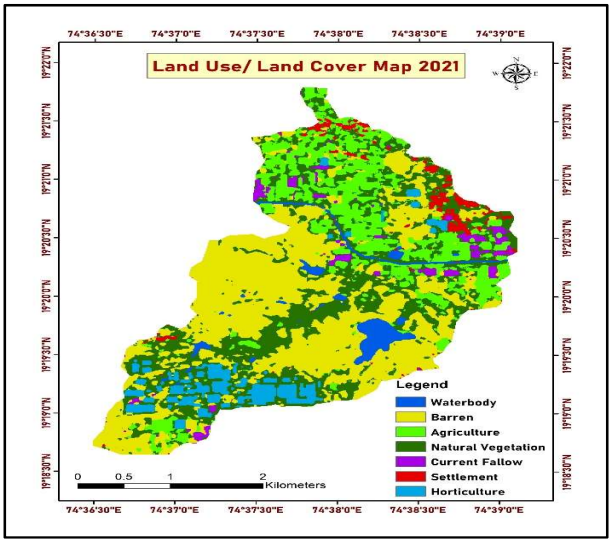

Figure 7: After conservation measures land use/land cover map of watershed

Table 4: Area coverage by different land use/ land cover classes before and after conservation measures

Land Cover Class	Year 2016 (Before Conservation Measures) Area (ha)	Year 2021 (After Conservation Measures) Area (ha)	Change in Area (ha)	Change in Area (%)
Waterbody	32.91	41.48	8.57	26.04
Barren Land	605.65	478.17	-127.48	-21.05
Agriculture	162.17	230.1	67.93	41.89
Natural Vegetation	231.95	304.97	73.02	31.48
Current Fallow	93.74	40.49	-53.25	-56.81
Settlement	58.39	72.82	14.43	24.71
Horticulture	75.19	91.97	16.78	22.32
(-) ve value indicates decrease in area.				

Table 5: Crop management (C) factor for different land cover classes

Land use/land cover	C value
Forest (Rasool *et al.*, 2014)	0.04
Barren land (Rasool *et al.*, 2014)	0.84
Settlement (Rasool *et al.*, 2014)	0
Horticultural crops (Pal and Samanta 2011)	0.1
Agriculture land (Pancholi *et al.*, 2015)	0.45
Waterbody (Pancholi *et al.*, 2015)	0
Current fallow (Pancholi *et al.*, 2015)	0.6

The C factor values of respective land cover class are given in Table 5. The mean value of the C factor in the watershed area was 0.27 and ranged from 0 to 0.84. The barren land comprises most of the land use in the watershed and has a maximum C-factor value, indicating that the area is at high risk of erosion. According to previous research, the value of crop management factors tends to decrease as vegetation cover increases, which is consistent with the findings of our study (Manik *et al.,* 2019). As the area covered by various land covers changed after the implementation of conservation measures, the different C factor layers were used to estimate soil loss before and after conservation measures from the watershed. The before conservation measure LU/LC image was used to generate C factor layer prior to conservation measures, and the after conservation measure LU/LC layer was used to generate C factor layer later conservation measures.

Conservation practice factor (P)

P factor value of one is considered for the entire watershed prior to any conservation measures in the watershed. Following the implementation of conservation measures in the watershed, the respective P factor value of the conservation measure was provided to the respective area, with one value considered for the untreated area. The P-factor value ranges from 0 to 1, with 0 indicating complete protection from soil erosion and 1 indicating no protection against soil loss. The P factor value for the watershed were ranged from 0.03 to 1. The conservation measures constructed in the watershed and their P factor value is given in the (Table 6). Other studies have also reported a wide range of P factor values for watersheds. For instance, López-Ballesteros *et al.,* 2019 found P factor values ranging from 0.02 to 0.8 in their study, while ElKadiri *et al.,* 2023 reported values ranging from 0.04 to 0.9. The P factor layers after conservation measures is given in Fig. 8.

Table 6: Conservation practice (P) factor

Conservation Measure	Area (ha)	P factor
Deep Continuous Contour trench	495	0.15
Compartment Bunding	50	0.03

Soil erosion in the watershed

The yearly average soil loss rate from study area was estimated by multiplying five USLE parameters (rainfall erosivity, soil erodibility,

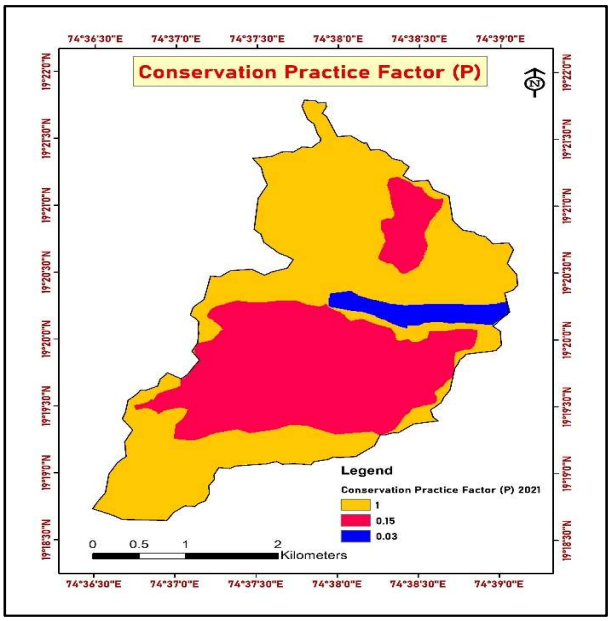

Figure 8: After conservation measures conservation practice (p) factor map of watershed

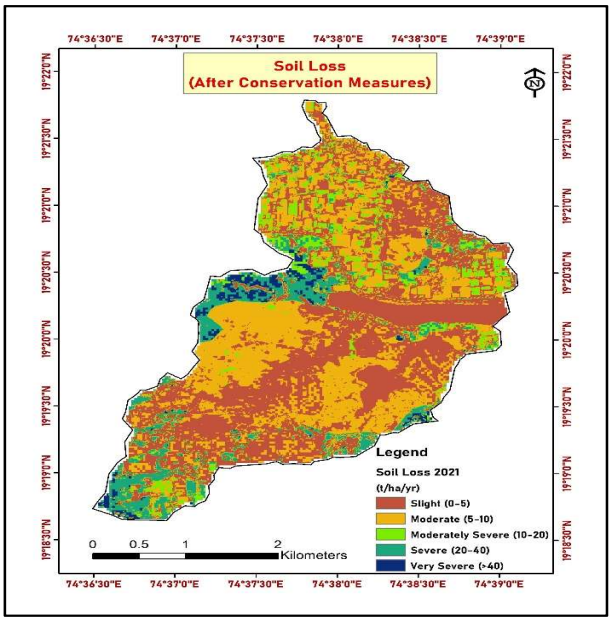

Figure 10: Soil loss from watershed after conservation measures.

topography, crop management and conservation practice factor) in Arc GIS software. The final USLE maps of before and after conservation measures display the yearly average soil loss potential (A) of the Central MPKV Campus watershed shown in Fig 9, 10.

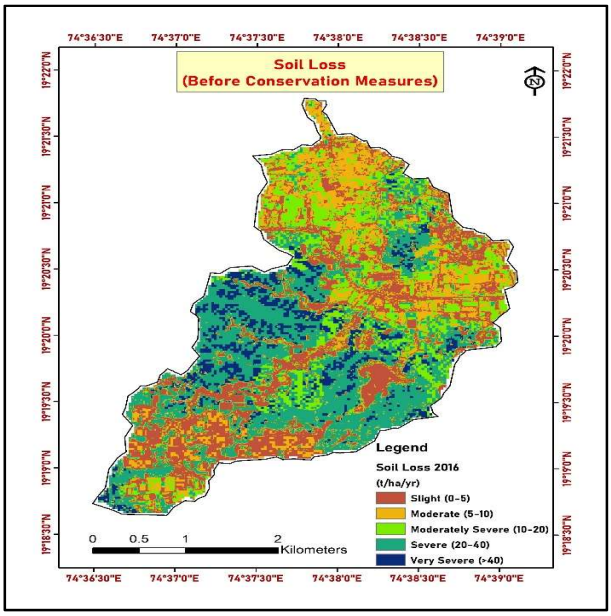

Figure 9: Soil loss from watershed before conservation measures

Soil loss before conservation measures

The yearly average soil loss rate before conservation measures was estimated at 18.68 t/ha/year. The soil loss rate in the watershed was ranged from 0 to 78.23 t/ha/year, with negligible soil loss in plains and severe soil loss in hilly areas. The soil loss rate before conservation measure was greater than tolerable limit of 11 t/ha/yr (Hudson, 1981). The yearly soil loss estimated from the watershed was found to be 23119.36 tonnes. The soil erosion rate was classified into five classes as shown in (Table 7).

Table 7: Area under different soil erosion classes before and after conservation measures

Soil Erosion Class	Soil loss (t/ha/yr)	Before Conservation Measures	After Conservation Measures
		Area (ha)	Area (ha)
Slight	< 5	365.27	574.75
Moderate	5 to 10	161.54	414.36
Moderately Severe	10 to 20	216.51	102.53
Severe	20 to 40	397.13	130.12
Very Severe	>40	119.54	38.224

The result indicated that 28.99% of the area has a slight erosion rate (0–5 t/ha/year) and such areas can be considered as areas with low erosion-risk. The slight erosion risk area was mainly found in

flat lands with vegetation cover. The remaining areas were classified as moderate (5–10 t/ha/year) erosion risk area (12.82%), which was mostly found in agricultural land; moderately severe (10–20 t/ha/year) erosion risk area (17.18%), which was found in barren land with spare vegetation cover; severe (20–40 t/ha/year) erosion risk area (31.52%), which was found in hilly area with spare vegetation and extremely severe (>40 t/ha/year) erosion risk area (9.49%), which was found in slopping areas without any vegetation cover. The severity of soil erosion was directly affected by the LU/LC, soil type, topography and rainfall intensity. Areas with dense vegetation cover, flat lands and cohesive soils were found to have less soil erosion. Whereas areas with no or sparse vegetation, steep and long slopes were found to have severe soil erosion. The results of soil loss before the adoption of conservation measures emphasise the importance of soil conservation within the watershed in order to maintain soil quality and fertility. Implementing site-specific conservation measures in the watershed can help to keep soil loss within a tolerable limit while also improving soil quality. Other studies have also reported higher soil loss rates without soil and water conservation measures, which is consistent with the findings of our study. A study conducted by Li *et al.* 2016 in semi-arid Yellow river basin of china found that soil loss rates without conservation measures ranged from 4.2 to 31.9 t/ha/yr in different watersheds.

Soil loss after conservation measures

The yearly soil loss rate after conservation measures was found at 9.41 t/ha/year. This post-conservation soil loss rate was 9.27 t/ha/year lower than the pre-conservation soil loss rate. The post-conservation measures soil loss rate was ranged from 0 to 53.24 t/ha/year in the watershed. The implementation of recommended SWC measures in the watershed reduced the soil loss rate below tolerable limit (11 t/ha/yr). The annual soil loss estimated from the watershed was found to be 11560.6 tonnes. Soil loss after conservation measures was reduced by half compared to soil loss before conservation measures. Similar to above, erosion rate risk was classified into five classes as shown in (Table 7). The result indicated that 45.62% of the watershed area has a slight erosion rate (0–5 t/ha/year), which was increased by 20%. The area under moderate (5–10 t/ha/year) erosion risk (32.89%) increased by 20%; area under moderately severe (10–20 t/ha/year) erosion risk (8.14%) decreased by 10%.; area under severe (20–40 t/ha/year) erosion risk (10.33%) decreased by 20% and area under extremely severe (>40 t/ha/year) erosion risk (3.03%) decreased by 6% post-conservation measures. The effectiveness of conservation measures in reducing soil loss rates has been confirmed by multiple studies. Lal (2015) and Wen and Zhen, (2020) both reported similar findings, demonstrating that implementation of conservation measures led to a significant reduction in soil loss rates.The average annual soil loss from the watershed before conservation measures was 40% and 50% higher compared to tolerable soil loss limit and the soil loss after conservation measures, respectively. The maximum soil loss before conservation measures occurred in hilly terrains and in the mainstreams, possibly due to high LS factor values and steep slope gradients greater than 25%. Areas with spare vegetation cover also have high rates of erosion as there is no any obstruction to the runoff. Implementation of conservation measures in the watershed increased water availability in the watershed. The water spread area in the watershed was increased by 25%. The increased water availability increased vegetation cover in the watershed, agricultural area increased by 40%, natural vegetation increased by 30% and horticulture plantation increased by 22%. This increased vegetation cover acted as a natural barrier to runoff, reducing the rate of water flow to a safe limit. Increased vegetation cover has a considerable impact on the rate of soil loss after conservation measures. Implementation of conservation measures in the watershed reduced the average annual soil loss rate below the tolerable limit and by 50% less than the soil loss rate before conservation measures. It was observed that after the implementation of conservation measures in the watershed, area under slight and moderate erosion risk class increased while area under moderately severe, severe and extremely severe erosion risk class decreased. Almost 75% of the watershed area is now classified as having a low to moderate risk of erosion. Only 13% of the watershed area is remained in the severe to extremely severe erosion risk class. The spatial distribution of soil loss

reveals that areas with high erosion risk have long slopes, sparse vegetation and fine soils with no conservation measures. This suggests that scientifically appropriate implementation of conservation measures in the watershed can drastically reduce soil loss from the watershed. This allows soil fertility and productivity to be maintained. It was suggested that additional conservation measures can be implemented in the watershed to further minimize the soil loss rate from the watershed.

SOC content before and after conservation measures

The SOC content before and after conservation measures under different land covers in the watershed is given in the Table 8. The average SOC content before and after conservation measures in the watershed was 0.74% and 0.77%, respectively. It was observed that SOC content was increased in each major land cover class but the rate of increase was varied depending on the land cover class. Natural vegetation land cover has the highest SOC increase rate while barren land has the lowest. There is ample evidence in the literature to suggest that the implementation of conservation measures can lead to significant increases in soil organic carbon (SOC) content. He *et al.,* (2022) demonstrated that the implementation of conservation practices resulted in a significant increase in SOC content in the topsoil and subsoil. Another study by Lai *et al.,* (2022) found that the application of conservation measures such as straw mulching and intercropping significantly increased SOC content in the topsoil. Thematic layers of SOC generated with Arc GIS software were used to estimate carbon loss from the watershed (Fig. 11, 12).

Table 8: Land Cover wise SOC content before and after conservation measures

Land cover	SOC content before conservation measures (%)	SOC content after conservation measures (%)	Total number of samples
Agriculture land	0.74	0.76	10
Barren land	0.67	0.69	15
Natural Vegetation land	0.78	0.82	15
Horticulture land	0.76	0.79	10
Average	**0.74**	**0.77**	

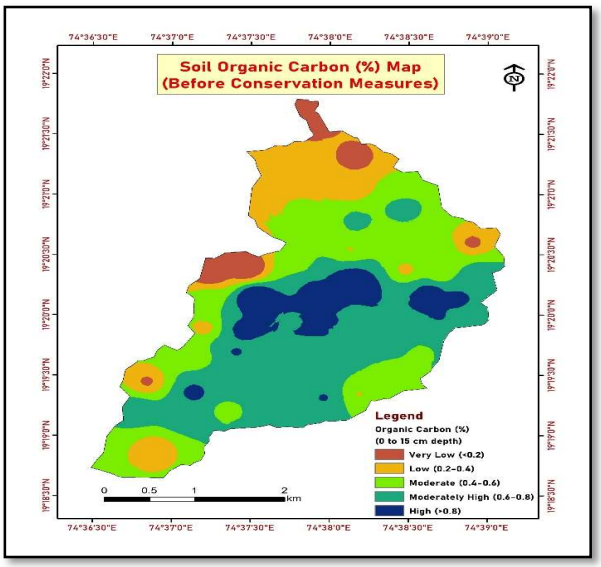

Figure 11: Soil organic carbon content before conservation measures

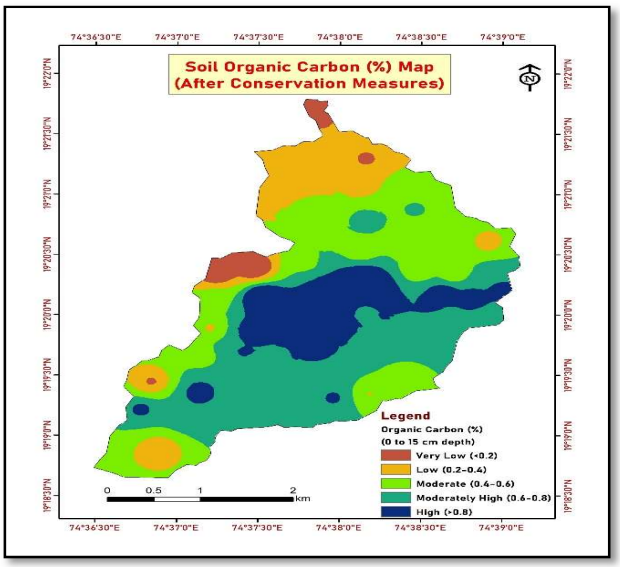

Figure 12: Soil organic carbon content after conservation measures

Carbon Enrichment Ratio

The CER layers generated using Arc GIS software were used in the estimation of carbon loss from the watershed. The CER value depends on the classes of soil erosion rate; higher soil erosion rates have lower CER values, while lower soil erosion rates have higher CER values. The CER values in the watershed were ranged from 2.04 for extremely severe erosion class to 3.62 for slight erosion class.

The 48% of watershed area was having the CER value of 2.3 before conservation measures and after conservation measures 45% area was having the CER value of 3.62. The change in the area under CER ration was observed due to significant difference in the rate of soil erosion before and after conservation measures. Similarly, a study by Wang *et al*. (2019) used a range of carbon enrichment ratio values, from 1.6 to 3.4, to estimate carbon loss in soil aggregates.

Carbon loss risk assessment

The yearly carbon loss from the watershed was estimated using soil loss rate, soil organic carbon content and carbon enrichment ratio layers in raster calculator tool in Arc GIS software. The C-loss layer depicts the average yearly carbon loss potential of the Central MPKV Campus Watershed before and after implementation of conservation measures (Fig 13, 14).

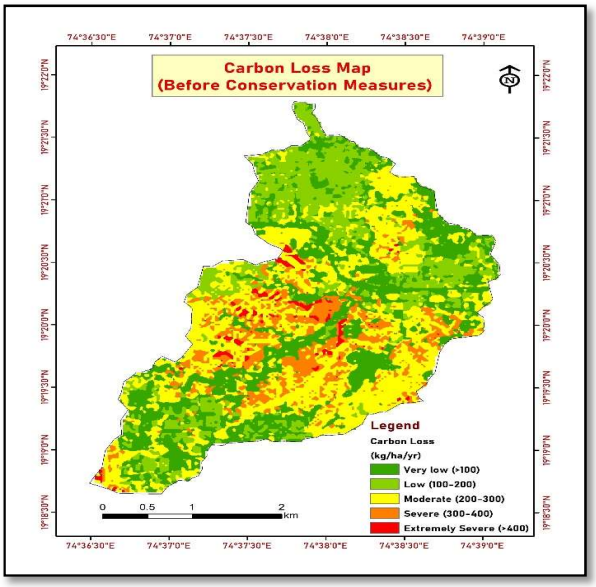

Figure 13: Carbon loss from the watershed before conservation measures

Carbon loss before conservation measures

The average annual carbon loss before conservation measures was 348.71 kgC/ha/yr, ranging from 0 to 618.42 kgC/ha/year. The total carbon loss from the watershed was 439.37 tonnes of C. The carbon loss was found higher on steep slopes and in baren land class without any vegetation cover. Similar to soil loss, carbon loss rate was categorized into five distinguished classes. The carbon loss rate categories varied from very low carbon loss rate (0-100 kgC/ha/year) to extremely severe carbon loss (>400 kgC/ha/year) rate (Table 9). It was found that area under very low carbon loss class (0-100 kgC/ha/year) was 25.63% this area is categorised as very low risk carbon erosion area. The remining area was classified as low (100–200 kgC/ha/year) carbon erosion area (19.84%); moderate (200–300 kgC/ha/year) carbon erosion area (34.52%); severe (300–400 kgC/ha/year) carbon erosion risk area (14.68%) and extremely severe (>400 kgC/ha/year)

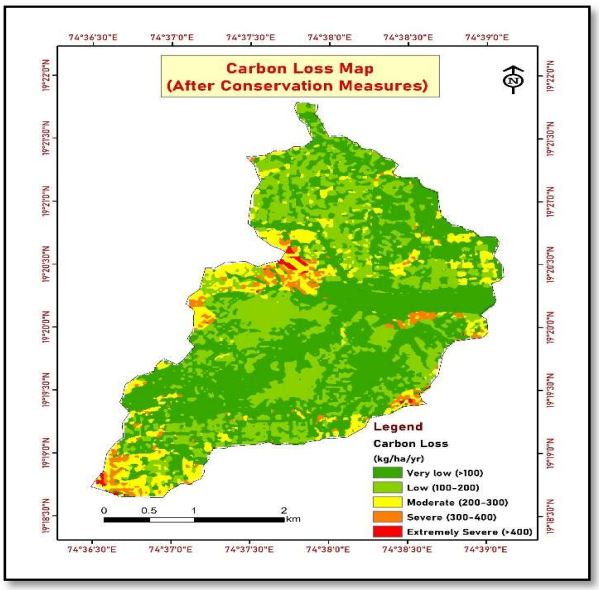

Figure 14: Carbon loss from the watershed after conservation measures

Table 9: Area under different carbon erosion classes before and after conservation measures

Carbon Loss Class	Carbon Loss Range (kgC/ha/yr)	Before Conservation Measures	After Conservation Measures
		Area (ha)	Area (ha)
Very low	<100	323	560
Low	100-200	250	415
Moderate	200-300	435	172
Severe	300-400	185	81
Extremely Severe	>400	67	32

bon erosion risk area (5.32%). Nearly 53% of the watershed area had a carbon loss rate above 200 kg C/ha/yr before the adoption of conservation

measures. It was observed that as the severity of soil erosion increased, so did the rate of carbon erosion. The rate of carbon loss in the watershed varied according to land cover, with the highest rate observed in barren land and the lowest rate observed in natural forests. Soil carbon loss was also significantly affected by the organic carbon content and carbon enrichment ratio in the top soil layer. Similarly, a study by Krauss *et al.,* (2017) found that the application of soil conservation measures significantly reduced carbon loss in a hilly area of southwestern China. The carbon loss rate in the untreated plots was approximately three times higher than in the treated plots.

Carbon loss after conservation measures

The average annual carbon loss after conservation measures was 205.52 kgC/ha/yr, ranging from 0 to 538.30 kgC/ha/year. The total carbon loss from the watershed was 258.95 tonnes of C. The carbon loss from the watershed after conservation measures was reduced by 40%. Similar to before conservation measures, carbon loss after conservation measures was also classified into five classes (Table 9). It was found that area under very low carbon loss class (0-100 kgC/ha/year) was 44.44%. The remining area was classified as low (100–200 kgC/ha/year) carbon erosion area (32.94%); moderate (200–300 kgC/ha/year) carbon erosion area (13.65%); severe (300–400 kgC/ha/year) carbon erosion risk area (6.43%) and extremely severe (>400 kgC/ha/year) carbon erosion risk area (2.54%). The carbon loss values from the watershed are comparable to those found by Lense *et al.,* (2021) in the tropical watershed, which ranged from 0.16 kgC/ha/yr to 209.50 kgC/ha/yr.

Similar to the soil loss rate, the yearly carbon loss rate in the watershed prior to the conservation measures was 40% higher than the after conservation measures carbon loss rate. It was found that nearly 75% of the watershed area is now classified as very low to low carbon erosion risk after the implementation of conservation measures. The area under severe to extremely severe carbon erosion risk also decreases from 20% to 8%. The severe carbon erosion risk area is associated where there is spare vegetation cover and no conservation measures. The significant reduction of 60% was observed in the moderate carbon erosion class. It is evident that SWC measures not only help to conserve natural resources but also reduces carbon emissions from soil. This can be validated through comparison of SOC content before and after conservation measures given in (Table 8). The SOC content increased under each major land cover class following implementation of conservation measures. This is due to increased vegetation cover and reduced soil degradation in the watershed, achieved through proper natural resource conservation planning. Implementation of conservation measures increases the carbon sequestration capacity of natural resources. Therefore, soil and water conservation measures can be regarded as climate change mitigation measures. The study is useful for sustainable watershed planning in order to counteract future climate change challenges.

Conclusion

The average soil loss before conservation measures ranged from 0 to 78.73 t/ha, with an average of 18.68 t/ha/year, which is above the tolerable limit. The soil loss after conservation measures ranged from 0 to 53.24 t/ha/yr, with an average of 9.41 t/ha/yr, which is below the tolerable limit. Implementation of recommended conservation practices reduced soil loss by half compared to soil loss before conservation measures. Soil loss rate, organic carbon content, and carbon enrichment ratio were used to calculate the carbon loss associated with soil loss. The carbon loss from the watershed before and after conservation measures was 378.71 kgC/ha/yr and 205.52 kgC/ha/yr, respectively. Carbon loss from the watershed was reduced by 40% after conservation measures. The study found that USLE model coupled with GIS technique makes soil loss estimation simple and credible. It was observed that implementing recommended conservation measures reduces soil loss rate as well as carbon loss rate from the watershed. The conservation measures serve the dual purposes of protecting natural resources and reducing climate change. The remaining portion of the watershed, where there are no existing conservation measures, can be used to implement additional conservation measures. That will further cut down on soil loss and carbon loss in the watershed.

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Conflict of interest

The authors declare that they have no conflict of interest.

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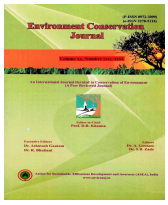

Hexavalent chromium bioreduction by chromium-resistant sporulating bacteria isolated from tannery effluent

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ABSTRACT

The main polluting source of heavy metal contamination of water is the leather tanning industry, which uses chrome powder and discharges it into the nearby ecosystem. In this investigation, chromium-resistant bacterial strains were isolated and characterized from tannery effluent. Based on morphological and biochemical characterization, the predominant sporulating *Bacillus sp.* was isolated and identified as *Bacillus subtilis* based on 16S rRNA gene sequencing. Chromium degradation by the bacterial strain was evaluated using the flask culture method at three different concentrations (300, 600, and 900 µg/ml) of Cr (VI), and the reduction potential of the isolated bacterium was analyzed by Atomic Absorption Spectrophotometry. A maximum reduction of approximately 78% was found at 24 hrs of incubation at pH 7 and at a constant temperature of 30°C. More than 50% of the Cr(VI) was decreased in 24 hours when the Cr(VI) concentration varied from 300 to 900 g/ml. FTIR analysis showed the involvement of hydroxyl and amine groups in chromium adsorption. As an outcome, this strain could be a promising bioagent for the environmentally friendly elimination of toxic Cr(VI) from polluted environments.

Introduction

Rapid urbanization has elevated the growth of industries at an exponential rate (Seragadam *et al.,* 2021 Bhutiani *et al.,* 2021a&b; Bojago *et al.,* 2023). There is a significant threat to the current environment because of the inappropriate discharge of industrial effluents with high concentrations of heavy metals into natural water resources and soil (Nagajyoti *et al.,* 2010; Bhutiani and Ahamad, 2018; Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022; Ahamad *et al.,* 2022;). A class of pollutants known as heavy metals accumulates in the food chain and in living things because they are not biodegradable (Kobya *et al.,* 2005; Ruhela *et al.,* 2019). This has jeopardized the environment's ability to support life. Toxic heavy metals such as chromium, cadmium, mercury, arsenic, and lead are major environmental pollutants due to their negative effects on both living ecosystems and public health (Ray and Ray, 2009; Bhutiani *et al.,* 2022). Cadmium, lead, mercury, nickel, copper, chromium, cobalt, and zinc are the heavy metals that the WHO (2010) study lists as being of the most concern (WHO, 2010). Chromium (Cr) and its derivatives cause many industrial processes to pollute the environment, including mining, chrome plating, pigment production, petroleum refining, leather tanning, wood preservation, textile production, pulp processing, and electroplating. (Wang & Xiao,1995). The primary source of chromium contamination in the immediate environment is the tannery industry. Due to the wet process used in tanning hides and skin, which generates 30-35 L of effluent for every kg of treated skin or hides, tanning factories need an enormous amount of water (Nandy *et al.,* 1999). There are approximately 3000 tanneries in India, with the major tanning clusters in Tamil Nadu being Ambur, Vaniyambadi, Pallavaram, Pernambut, Ranipet, Dindigul, and Trichy (Kavitha and Ganapathy, 2015). Tannery effluent contains poisonous solid and liquid heavy metal waste that is discharged after treatment (Muhammad *et al.,* 2015).

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Chromium (Cr) is a widespread contaminant that may be found in practically every aspect of the ecosystem, including the air, land, water, and biological systems (Rahman & Singh, 2019). Cr exists primarily in the ecosystem as trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Due to the emergence of hydroxide and oxide compounds, Cr(III) is relatively insoluble at environmentally relevant pH (James *et al.,* 1997), and it stands for a crucial nutrient needed for healthy fat and sugar metabolism. (Cefalu & Hu, 2004). Cr (VI) has much higher solubility, mobility, bioavailability, and toxicity than Cr(III) (Cervantes *et al.,* 2001), is structurally similar to sulfate (SO42-), is readily used up by bacterial and mammalian cells via the sulfate transport system and undergoes instantaneous mitigation reactions that result in the creation of various reactive intermediates (Cervantes *et al.,* 2001). Due to its great solubility in water and ability to be transported, Cr(VI) is 100 times more hazardous than Cr(III) (Katsayal *et al.,* 2022). Due to its rapid permeability and strong oxidizing nature, Cr(VI) is more harmful than Cr(III), which can cause severe damage to proteins and nucleic acids (Mishra *et al.,* 2012) and is extremely noxious to living organisms, causing allergies, eczema, irritations, and respiratory tract disorders. It has also been designated as a priority pollutant by the US Environmental Protection Agency (EPA,1998).

Over the past few years, conventional chemical or physicochemical treatment procedures such as adding lime, ion exchange, membrane separation, and adsorption followed by chemical precipitation and coagulation as Cr(OH)3 have been described. However Heavy metals such as Cr(VI) removed from industrial effluents using conventional metal removal techniques have significant drawbacks,, such as high energy requirements, incomplete metal removal, and the accumulation of a large amount of toxic waste sludge (Ahalya *et al.,* 2003). Bioremediation has become a viable substitute for conventional physicochemical techniques. By using biological agents, either in situ or ex situ, bioremediation is used for eliminating inorganic contaminants from contaminated environments such as water, soils, sludge, and waste streams. (Sundari, 2017).

Many bacteria have been found to convert hexavalent chromium to trivalent chromium. (Wang *et al.,* 1989) (Dong *et al.,* 2013) (Shen & Wang 1993) (Bopp *et al.,* 1983) (Camargo *et al.,* 2003. In recent studies, several more microorganisms, such as strains of *Pseudomonas* (Wani *et al.,* 2019), *Cellulosi microbium* (Bhargava & Mishra 2018), *Bacillus* (Li *et al.,* 2020), *Staphylococcus* (Ahmad *et al.,* 2022), and *Stenotrophomonas* (Sundari, 2017), have been shown to have significant alleviative effects on chromium. Compared to traditional physical and chemical approaches, the use of Cr(VI)-resistant bacteria for the detoxification of environmentally harmful chromium has been deemed to be more cost-effective, efficient, and secure. (Ganguli & Tripathi 2002) (Cheung & Gu 2007)(Polti *et al.,* 2009)(Piñón-Castillo *et al.,* 2010). Many species exploit hazardous Cr(VI) reduction to nontoxic Cr (III) form as one of their survival strategies in Cr (VI) polluted effluents. Acinetobacter and *Ochrobactrum arthrobacter*, *Serratia marcescens*, *Bacillus spp*., *Pseudomonas fluorescens* LB300, Intrasporangium sp. Q5-1, *Enterobacter cloacae*, *Bacillus sp.* ES29, and *E. coli* (Camargo *et al.,* 2003, Bopp *et al.,* 1983, Wang *et al.,* 1989, Sheng and Wang, 1993). Furthermore, a combination of microorganisms can lower the mobility and toxicity of Cr(VI) by converting it to a less mobile and less toxic form of Cr(III). Because of the involvement of carboxyl, hydroxyl, and amine functional groups in the complexation of chromium metals, microorganisms have a high metal binding capacity for chromium. (Chatterjee *et al.,* 2011)

The study's objective was to identify and characterize a Cr(VI)-resistant bacterial strain with the ability to reduce Cr(VI) in tannery effluent. They were efficiently tested for chromium (VI) bioreduction.

Material and Methods

Study area and collection of sample

The effluent sample from the Begumpur Leather processing industrial area of Dindigul district, Tamil Nadu (longitude 77.9695°E, latitude 10.3624°N), was collected in a labeled presterilized screw-capped bottle, placed in an icebox and immediately transferred to the laboratory. The sample was kept at 4°C, analyzed, and used within

6 hours of collection. The collected effluent was subjected to physicochemical parameter analysis, and the findings were tabulated.

Heavy metal evaluation in tannery wastewaters

The estimation of trace heavy metals such as cadmium, chromium, cobalt, copper, iron, lead, manganese, magnesium, nickel, and zinc in the industrial effluent was estimated by atomic absorption spectrophotometry (AAS) using the standard method.

Isolation of chromium-resistant microorganisms: To isolate chromium-resistant bacteria, the industrial wastewater sample was serially diluted up to 10-8 dilutions, and 100 µl of each dilution was inoculated into a Luria Bertani (LB) agar plate containing 50 µg/ml potassium dichromate (K2Cr2O7) using the spread plate method (Zahoor & Rehman 2009) and incubated at 37°C for 24 hours. Colonies with morphological differences were chosen and subcultured on a Luria Bertani agar plate containing 100 µg/ml potassium dichromate (K2Cr2O7) to isolate and enumerate the desired bacteria.

Characterization of bacterial isolates: The selected colonies were subjected to biochemical characterization using previously described standard methods (Holt *et al.,* 1994). The isolate was molecularly characterized using 16S rRNA sequence analysis. The bacterial genomic DNA was extracted using the phenol–chloroform method, which Sambrook *et al.* described in 1989. (Sambrook *et al.,* 1989). Its quality was assessed using a 1.0% agarose gel, which revealed a single band of high-molecular-weight DNA. The 16S rRNA gene fragment was amplified using the 16S rRNA-F and 16S rRNA-R primers. On an agarose gel, a discrete PCR amplicon band of 1500 bp was observed. To remove contaminants, the PCR amplicon was purified. The PCR amplicon was sequenced forward and reverse using BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer with 16S rRNA-F and 16S rRNA-R primers. Using aligner software, a consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data. The 16S ribosomal RNA sequences were used to search the NCBI blast search tool for similar sequences. Based on the maximum identity score, closely related sequences were chosen and aligned using the multiple alignment software program Clustal W. A distance matrix and a phylogenetic tree were generated using MEGA 10.

Determination of minimum inhibitory concentration (MIC)

MIC in agar plates: To determine the MIC, the isolates were grown on chromium-infused nutrient agar media**.** The MIC concentration was evaluated by gently inclining the chromium concentration until the isolates failed to form colonies in the Petri plate. The starting concentration of metal was 100 µg/ml from a 100 mg/100 ml stock solution. The streak plate method was used to transfer growing colonies from one concentration to the next higher concentration. After 48 hours of incubation at 37°C, the minimum inhibitory concentration was measured based on the analysis.

MIC in broth: Isolates that exhibited maximum tolerance in agar plates were further subjected to varying chromium concentrations from 100 µg/ml to 1000 µg/ml in broth to confirm their chromium susceptibility. Following incubation, the bacterial growth was estimated with a UV spectrophotometer. For further research, the isolate with the strongest tolerance was carefully selected.

Optimization of *Bacillus subtilis*

For effective chromium degradation, various physicochemical factors, such as pH, temperature, incubation time, inoculum concentration, and glucose percentage, were optimized. The proliferation of the bacterium was analyzed in a UV spectrophotometer, and chromium degradation was assessed by the 1,5 – diphenylcarbazide method (Lace *et al.,* 2019) at 540 nm by spectrophotometry**.**

Chromate bioreduction by free cells of *Bacillus subtilis*: The bacterial strain was cultured in LB broth overnight. Three varying concentrations of Cr(VI) were added to culture flasks containing LB broth medium. (300 µg/ml, 600 µg/ml, 900 µg/ml). Media without Cr(VI) served as a control. The microorganisms were inoculated into these flasks with 0.1 ml of cells under aseptic conditions. The flasks were incubated at 37°C and 120 rpm in a shaking incubator. Samples from each flask were taken at regular intervals (3, 6, 12, 24, and 48 hours), and the biomass of the isolated bacterium was assessed using UV spectrophotometry at OD 600 nm. The reduction of Cr (VI) by growing cells was investigated by taking a 1-ml culture from each flask at the same interval and centrifuging it at 8000 rpm for 10 min. AAS

was used to test the supernatant for Cr(VI) reduction. The pellet was analyzed by FTIR for any functional group changes.

Fourier transform - infrared spectroscopy (FTIR): Infrared spectra were obtained using an FT-IR spectrophotometer (Shimadzu) using KBr pellets. The bacterial pellet was formed by centrifuging the sample at 10,000 rpm for 10 minutes. The dried sample was powdered finely and thoroughly mixed with KBr. It was examined with a spectrophotometer in the 4000 - 400 cm -1 range, and FT - IR analysis of biomass in the availability and nonavailability of metal was carried out to recognize the functional moieties existing in the cell wall of the bacteria that are responsible for biosorption efficiency.

Results and Discussion

Physicochemical and heavy metal analysis of the tannery effluent sample

Tannery wastewater is alkaline (pH 8.1) in nature, light brown in color, and low in dissolved oxygen. In addition, the BOD (biological oxygen demand), TDS (total dissolved solids), and TSS (total suspended solids) were measured to be 550±40 mg/L, 3199 mg/L, and 1989.30 mg/L, respectively. (Table 1). The tannery effluent revealed high TSS and low dissolved oxygen. The suspended particles that settle on the soil can harm soil fauna and cause changes in the porosity of the soil, water-holding capacity, and soil texture (Chowdhury *et al.,* 2013) (Jeyasingh and Philip, 2005). Dissolved oxygen concentrations of 5–8 mg/L are suitable for aquatic environments, and dissolved oxygen concentrations of less than 4 mg/L are considered critical (Akan *et al.,* 2007) (Trivedy and Goel, 1984) (Alam, and Malik, 2008). According to Verma and his colleagues (Verma *et al.,* 2008), the low dissolved oxygen levels (4 mg/L) in the effluents were caused by an increased rate of organic pollution, indicating that the effluents had a high level of BOD. Ten different heavy metal concentrations of the tannery effluent were analyzed by a Shimadzu Atomic Absorption Spectrophotometer (AA-6300) at the Instrumentation Centre, Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi. The tannery effluent sample contained a greater concentration of chromium (0.60 mg/L) (Table 1). Chromium is highly lethal even in lower quantities, as it has biomagnification properties and accumulates in the food chain, causing toxicity at a cellular level (Barthwal *et al.,* 2008). Continuous discharge of low-concentration chromium is reported to be harmful to aquatic life and disrupts the aquatic food chain (Fent, 2004).

Table 1: Physicochemical characteristics and heavy metal analysis of the tannery effluent

Parameter	Standard Value	Effluent Sample
pH	6-9	8.1
TSS (mg/L)	150-500	1989.30
TDS (mg/L)	2100	3199
BOD (mg/L)	50-250	550±40
Chloride (mg/L)	600	793.3
D.O (mg/L)	4.5-8	1.38
Alkalinity	500	629.7
Temperature	27^0	31°
Heavy metals	**Permissible limit (WHO) mg/L**	**Conc. in mg/L**
Cadmium	0.01	0.0398
Copper	1.5	0.9438
Iron	0.30	0.4013
Chromium	0.05	0.6068
Cobalt	0.01	0.0362
Lead	0.10	0.1606
Magnesium	50	1.4682
Manganese	0.5	0.4497
Nickel	0.1	0.1584
Zinc	5.0	1.3694

Isolation and screening of chromium (Vi) resistant bacterial strains

Five morphologically distinct bacterial strains (C1-C5) were isolated from collected tannery industrial effluent using a serial dilution method, followed by an enrichment culture technique on LB agar plates containing 100 mg/L Cr(VI). Minimum inhibitory concentration screening was performed on all five isolates in both agar plates and broth to determine their resistance to high concentrations of Cr(VI) ranging from 100 to 1000 mg/L. Only one among the five bacterial strains, C1, was identified to tolerate 900 mg/L Cr(VI) in both agar plate and broth culture (Table 2) (Fig. 1). Previous research found that chromium-resistant microorganisms can tolerate concentrations ranging from 100 to 4000 mg/L Cr(VI) (Thacker *et al.,* 2007) (Zhu *et al.,* 2008). The bacterium C1 isolated falls well within the previously described tolerance range. High chromium tolerance bacterial strains were better at

Table 2: Minimum inhibitory concentration of the isolates on nutrient agar plates

S. No.	Test Isolates	Minimal Inhibitory Concentration (µg/ml)									
		100	200	300	400	500	600	700	800	900	1000
1	C1	+	+	+	+	+	+	+	+	+	-
2	C2	+	+	+	+	+	+	-	-	-	-
3	C3	+	+	+	-	-	-	-	-	-	-
4	C4	+	+	+	+	+	+	+	+	-	-
5	C5	+	+	+	+	+	+	+	-	-	-

+ = Growth, - + = No growth

converting Cr(VI) to Cr(III) and can be used to develop a more effective bioagent for chromium bioremediation (Ilias *et al.,* 2011). The C1 bacterial strain was chosen for further Cr(VI) reduction studies based on the results of the above screening.

Morphological, biochemical, and molecular characteristics of isolated chromium (Vi) resistant bacterium

Bacterial isolate C1 was identified as gram-positive, rod-shaped, motile, endospore-forming bacteria producing white colonies on nutrient agar medium. Biochemical tests were performed for the characterization of bacteria using Bergey's Manual of Determinative bacteriology (Holt *et al.,* 1994). Table 3 summarizes the morphological and biochemical analysis of the isolated bacterium (C1). This bacterium showed positive reactions for catalase, Voges Proskauer, citrate, gelatin hydrolysis, starch hydrolysis, and nitrate reductase tests but negative responses for the indole and

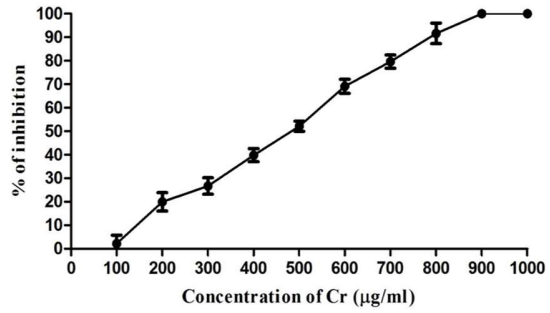

Figure 1: Minimum Inhibitory Concentration of Isolate C1 in Broth

methyl red tests. The TSI slants were alkaline, and they fermented only glucose but could not ferment lactose and sucrose. Furthermore, 16S rRNA gene sequence findings indicate that the isolated bacterium (C1) was 99.6% similar to that of the genus *Bacillus*, and thus, based on sequence similarity and blast analysis, the isolated bacterium (C1) was identified as *Bacillus subtilis* (Figure 2) with accession number MW737447. *Bacillus* has a widespread distribution and has adapted to harsh environments. (Arahal *et al.,* 2002). The spore-bearing distinctive feature most likely confers high Cr(VI) resistance to this Bacillus strain and allows it to remain in the stationary phase for an extended period of time. (Zouboulis *et al.,* 2004).

Optimization of chromium tolerant *Bacillus subtilis*

The proliferation of bacteria and simultaneous deduction of Cr(VI) by *Bacillus subtilis* C1 was assessed by subjecting it to various parameters, such as pH, temperature, incubation time, inoculum concentration, and glucose percentage, to optimize the environmental factors for better chromium degradation. Environmental variables such as pH, temperature, concentration, and the length of the bioreduction process all have a significant impact on the bioremediation process (Selvi *et al.,* 2014) (Sathishkumar *et al.,* 2017).According to the findings of this study, *Bacillus* subtilis C1 exhibited maximum cell growth and reduction efficiency of chromium at pH 7.0 (Fig. 3), a temperature of 30°C (Fig. 4), glucose 2% (Fig. 5), inoculum concentration of 6% (Fig. 7) and incubation time of approximately 24 hrs. (Fig. 6). Previous research has shown that weakly acidic conditions are more favorable for Cr6+ removal (Liu *et al.,* 2020). Similarly, when the pH was greater than 8 or slightly less than 5, the interaction of the Cr6+ removal enzyme synthesized by Bacillus subtilis was altered, which led to a substantial decrease in Cr6+ bioreduction. (Dhal *et al.,* 2010). Following a24-hour growth phase, the microbial community enters a death phase, and the microbial activity will decrease (Rolfe *et al.,* 2012). Temperature plays a

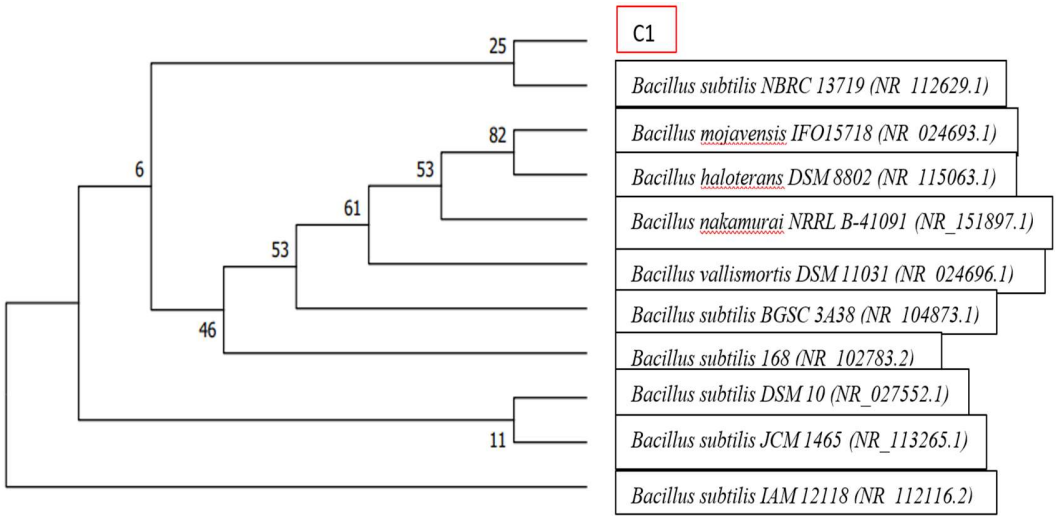

Figure 2. Molecular phylogenetic analysis of *Bacillus subtilis C1* by maximum likelihood. Accession numbers are indicated after the name of the isolate

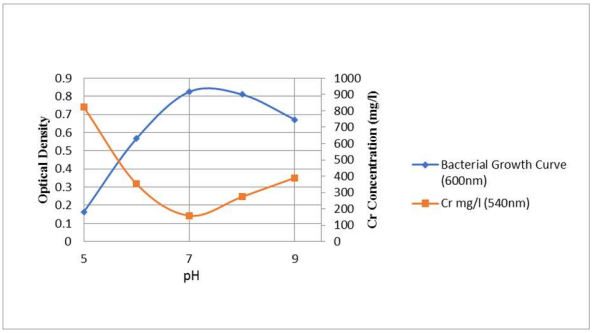

Figure 3: The effect of pH on chromium degradation by *Bacillus subtilis* C1

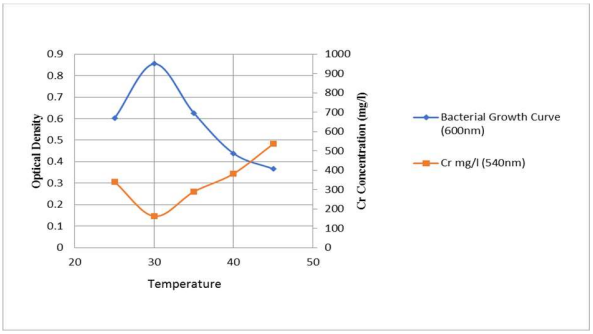

Figure 4: The effect of temperature on chromium degradation by *Bacillus subtilis* C1. Error bars indicate the standard deviations.

critical role in bacterial growth and enzyme activity, and the optimal temperature for both B. cereus D and B. cereus 322 growth is 30°C (Li *et al.,* 2020). Glucose, fructose, lactose, pyruvate, lactate, citrate, glycerol, acetate, formate, NADH/NADPH, and reduced glutathione are well-known electron donors for Cr(VI) reduction (Murugavelh,& Mohanty,2013) (Mala *et al.,* 2015). Because glucose is the easiest carbon source to metabolize, it is capable of offering most electrons for Cr(VI) reduction (Zheng *et al.,* 201).

Reduction of chromium (Vi) by free cell of *Bacillus subtilis*

The flask culture method of *Bacillus subtilis* C1 for Cr(VI) reducing capability was monitored in LB broth medium at 300, 600, and 900 µg/mL Cr(VI) at five different intervals of 3, 6, 12, 24, and 48 hours under aerobic conditions. In our experiment, a maximum Cr(VI) reduction of approximately 78% was discovered in 300 µg/mL Cr(VI) in 24 hours, confirming the exponential bacterial cell growth phase. At high concentrations of 600 µg/mL and 900 µg/mL, the chromium was reduced to 70% and 50%, respectively (Figure 8). The bacterium *Bacillus subtilis* C1 alleviated Cr(VI) in a concentration-dependent manner, which meant that the bacterium's removal efficiencies decreased as the Cr(VI) concentration increased. When the Cr(VI) concentration ranged from 300 to 900 g/ml, more than 50% of the Cr(VI) was reduced in 24 hours. However, after 48 hours of incubation, the reduction at 900 g/ml was only 40%. The bacterium's lower chromium reduction potential at

Table 3: Morphological and biochemical characteristics of the bacterial isolate *Bacillus subtilis* (*C1*)

S.No	Characteristics	*Bacillus subtilis (C1)*
1	Cell Shape	Rod
2	Color	white
3	Gram's staining	+
4	Motility	+
5	Spore formation	+
6	Indole	-
7	Methyl red	-
8	VP	+
9	Citrate utilization	+
10	Triple sugar agar test	K/A
11	H2S production	-
12	Starch hydrolysis	+
13	Gelatin hydrolysis	+
14	Catalase	+
15	Nitrate reductase	+

+ = Positive, - = Negative

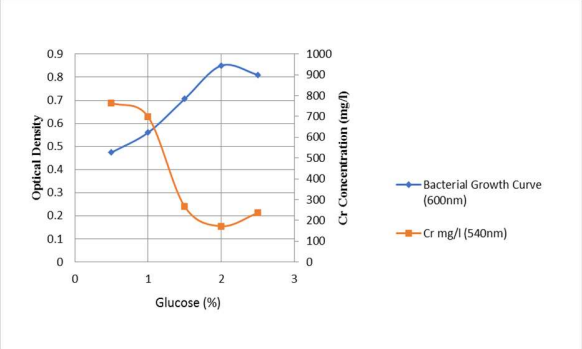

Figure 5: The effect of glucose (%) on chromium degradation by *Bacillus subtilis* C1. Error bars indicate the standard deviations

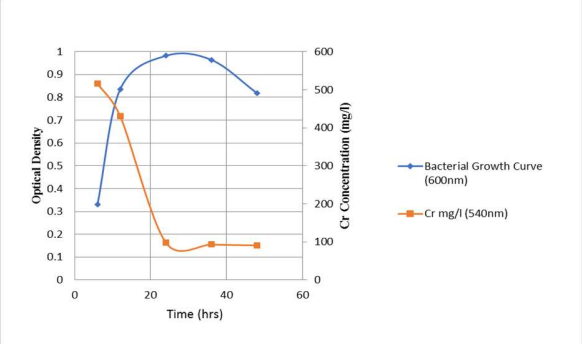

Figure 6: The effect of time on chromium degradation by *Bacillus subtilis C1*. Error bars indicate the standard deviations

elevated concentrations may indeed be related to Cr(VI) mutagenic and toxic effects on bacterial cell metabolism. (Thacker *et al.,* 2007). Because of hexavalent chromium's inhibitory action, bacterial cell density decreases slightly as concentration increases (Xiao *et al.,* 2017). In previous studies, *Bacillus sp.* is known to be resistant up to 40 mg/L and decreases thereafter. This indicates that as the concentration increases, the growth of *Bacillus sp.* is inhibited. *Bacillus.* isolated by Murugavelh and Mohanty removed 96.8% of Cr(VI) in 48 h with a 1.15 mM initial Cr(VI) concentration (Murugavelh & Mohanty, 2013). It has also been reported earlier that after 120 hours of incubation, Alcaligenes faecalis was reduced by approximately 70.0% and Bacillus sp. (accession number FM208185.1) was reduced by only 73.41% of 100 mg/L Cr(VI) (Sun *et al.,* 2018). When the bacterial growth curve reaches a certain point, it appears saturated, indicating adaptive mechanisms that enable the isolate to confer resistance to toxic Cr(VI) and grow in its presence (Cervantes & Campos-García, 2007)

FTIR analysis

FTIR spectroscopy was employed to determine the properties of the functional groups and chemical bonds that were essential in the biosorption of hexavalent chromium. The FTIR spectra of chromium-treated and untreated biomass revealed some absorption bands at different wavelengths, indicating the presence of a number of functional groups, such as amine, carboxyl, amide, aliphatic, aromatic groups, and bonded and unbonded hydroxyl groups. The biomass was treated with three concentrations of chromium (300, 600, and 900 µg/ml), and the IR spectra were compared. Absorption peaks differed slightly between chromium-treated and untreated biomass. The untreated bacterial biomass revealed peaks at 1067. 53 cm- 1, 1643.24 cm- 1, 2041.51 cm- 1, and 3524.67 cm- 1 correspond to C-N, -C = C-, N=C=S and (-COOH) bonds, respectively. (Figure 9). These bonds suggest the involvement of various functional moieties, such as amides and carbohydrates, on the outer surface of Bacillus sp. in the metal adsorption process (Bağcıoğlu *et al.,* 2019). The FT-IR spectra of metal-loaded bacteria showed a highly significant shift in frequency from 3524.67 cm-1 to 3489.95 cm-1 (300 µg/ml) (Figure 10), 3444.63 cm-1 (600 µg/ml) (Figure 11) and 3444.63 cm-1 (900 µg/ml) (Figure 12), suggesting the strong NH2 asymmetric stretching mode of

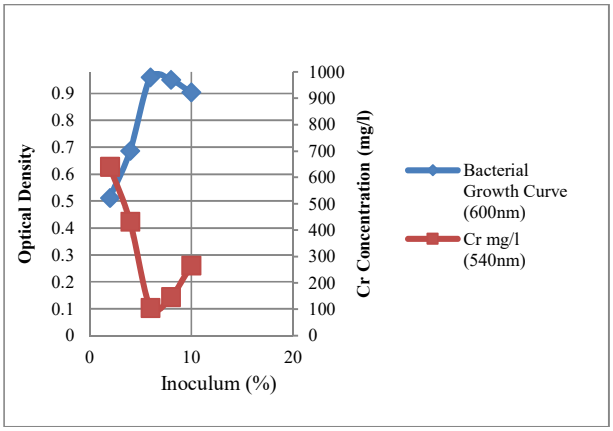

Figure 7: The effect of inoculum (%) on chromium degradation by *Bacillus subtilis C1*. Error bars indicate the standard deviations

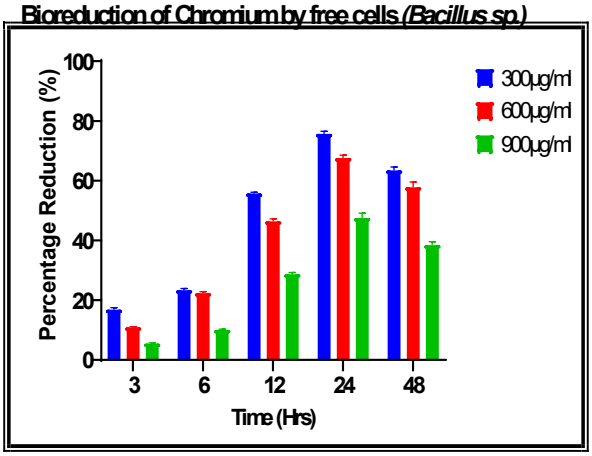

Figure 8: Bioreduction of chromium by free cells of *Bacillus subtilis C1*

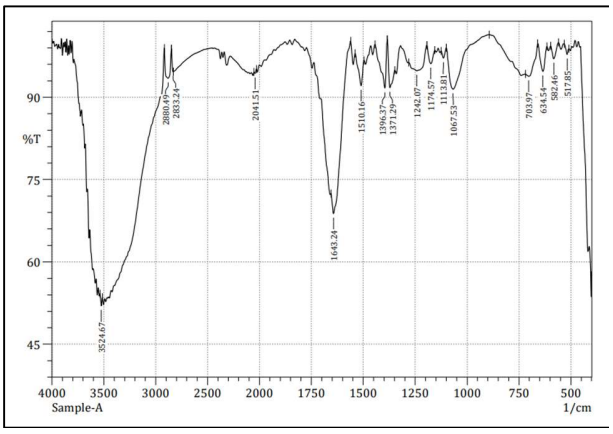

Figure 9: FTIR analysis of untreated cells of *Bacillus subtilis C1*

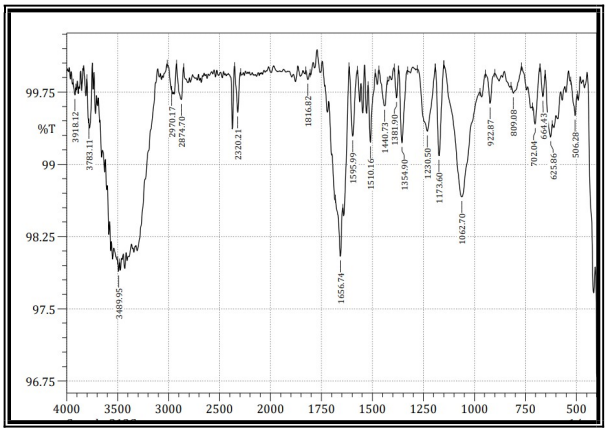

Figure 10: FTIR analysis of chromium (300 µg/ml)-treated cells of *Bacillus subtilis C1*

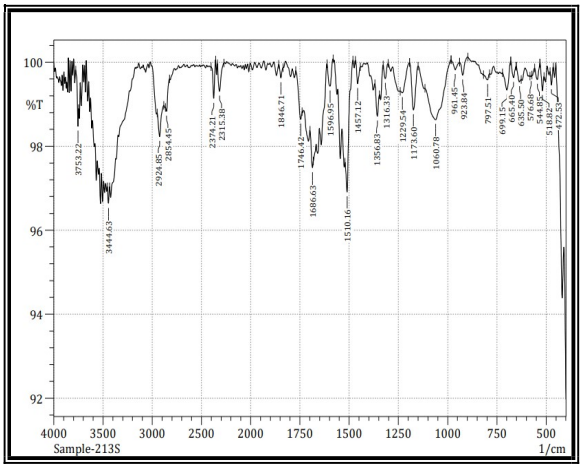

Figure 11: FTIR analysis of chromium (600 µg/ml)-treated cells of *Bacillus subtilis C1*

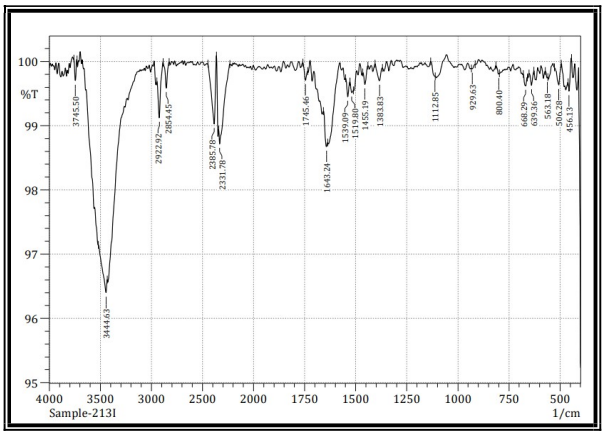

Figure 12: FTIR analysis of chromium (900 µg/ml)-treated cells of *Bacillus subtilis C1*

amines, which indicates the overlapping of amines and hydroxyl stretching on the surface of the bacterial cell (Mungasavalli *et al.,* 2007). Similarly, 1643.24 cm^{-1} (C=C stretching) of untreated biomass shifted to 1656.74 cm^{-1} (C=C stretching) in the 300 µg/ml chromium-loaded sample, 1686.63 cm^{-1} (C=N stretching) in the 600 µg/ml sample and 1643.24 cm^{-1} (C=C stretching) in the 900 µg/ml chromium-loaded sample. The slight shift from 1643 cm^{-1} to a higher frequency region of 1650-1680 cm^{-1} indicated the intervention of the C=O group of the amide I bond stretching (Doshi *et al.,* 2007). The peak at 1510 cm^{-1} remains constant in both treated and untreated biomass and is attributed to the aromatic ring's C-C stretching vibration, which indicates the presence of aromatic CH bending vibrations (Pham *et al.,* 2016). Similarly, the absorption peak that appeared at 2967 cm–1 remained unchanged in regard to both Cr(VI)-exposed and -unexposed cells. It might be said that the complexation of Cr(VI) with hydroxyl, carbonyl, or amide moieties causes reduction, more specifically between the wavelengths of 3400 and 3550 cm-1 and 1600 and 1750 cm-1. (Shahadat *et al.,* 2015).

Conclusion

The important findings of this paper were that the isolated chromium-tolerant bacterium *Bacillus subtilis* C1 had a higher Cr(VI) reducing power and could reduce up to 78% of Cr(VI)when compared to other isolated bacterial species. *Bacillus subtilis* C1 also exhibited the highest MIC value of 900 mg/L Cr(VI) and the spore-forming capability of *Bacillus* isolates gives an added advantage of lyophilizing the sporulating biomass and utilizing it in the biological clean-up of chromium-contaminated sites and the biological treatment of wastewater. These discoveries can be transcribed into technology through small-scale pilot testing and, sooner or later, on a massive scale to clean up chrome-polluted wastes, including tannery effluents, before they are discharged into the environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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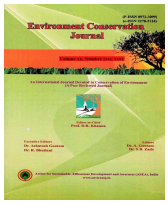

Yield attributes of Cassava (*Manihot esculenta* Crantz) and soil properties in Southern Laterites, Kerala as influenced by consortium biofertilizers

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ABSTRACT

A field experiment was undertaken at College of Agriculture, Vellayani during June to December 2019, to assess the efficacy of liquid consortium biofertilizer, Plant Growth Promoting Rhizobacteria (PGPR) Mix - I in cassava and to examine the changes in soil chemical and biological properties with the application. The treatment combinations included four levels of biofertilizer [PGPR Mix - I liquid (L) @ 2 %, PGPR Mix - I liquid (L) @ 5 %, PGPR Mix - I powder (P) formulation @ 10g of 2 % mixture per plant, without biofertilizer] and three levels of nutrients, with 50: 50: 100 kg NPK/ha as the standard dose of nutrients (SDN), [50 % SDN, 75 % SDN, 100 % SDN]. The 4 × 3 factorial experiment was laid out in randomized block design with three replications. The results of the study revealed that the liquid biofertilizer consortium at 5 per cent + 75 percent SDN (37.5: 37.5: 75 kg NPK /ha) recorded significantly superior yield attributes in cassava and improved the soil organic C, available K status and microbial count.

Introduction

The COVID 19 pandemic has invoked an element of uncertainty in all frontiers of life. Malnutrition has succumbed the weaker sections to fatality, food security is under threat and nutritional insecurity is sure to add to the catastrophe. This coupled with the unprecedented climate change events and challenges in agriculture production scenario have sparked the interest in energy efficient, high biomass producing resilient crops to sustain the food and dietary requirements of the in mass population worldwide. Carbohydrate rich nutritive tuber crops partly offer solution to the impasse on account of their low input and management requirements, adaptability to marginal lands and global warming and high yield potential. Cassava, the most important tuber crop of the tropics is climate resilient and with its high bioconversion efficiency fosters food and nutritional security in many countries. The production of cassava globally and in India is 311.5 mt and 4.98 mt respectively (Radhakrishnan *et al*., 2022). While, in Kerala, the production of cassava is 259.26 t (FIB, 2022). Being a concentrated source of carbohydrate, cassava can effectively bridge the likely demand - supply gap in major food grains. Extensive studies on the fertility status of the soils in Kerala (KSPB, 2013) have documented an acidic pH in majority of the soils of the state coupled with a high P status (> 25 kg/ha) and low to medium N and K contents in the Southern Laterites of the state. Amelioration of the soil acidity with lime materials and inclusion of biofertilizers in the nutrient management package can mobilize the fixed forms of the nutrients in soil (P and K) and the atmosphere (N) thus ensuring its availability over a longer period. The conventional practice of using microbial inoculants is in the form of carrier based formulations, especially talc. One of the major challenges encountered during the development of biofertilizers and commercialization of an effective strain is its shelf

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life and purity of inoculants (Basu *et al.,* 2021). Brar *et al*. (2012) opined that compared to the powder form of biofertilizers, liquid formulations are more advantageous on account of its ease in use, longer shelf life and compatibility in micro irrigation systems. The Department of Agricultural Microbiology, College of Agriculture, Vellayani has developed a liquid formulation of biofertilizer, PGPR Mix – I, which is a consortium of nitrogen (N) fixers (*Azospirillum lipoferum, Azotobacter chroococcum*), phosphorus (P) solubiliser (*Bacillus megaterium*) and potassium (K) solubiliser (*Bacillus sporothermodurans*).

Use of biofertlizers is of practical significance in cassava as the crop is of 6-10 months duration and depletes the nutrients in the soil even when the available nutrient status is high. Therefore a field experiment was undertaken in cassava (*Manihot esculenta* Crantz), to assess the efficacy of PGPR formulations on yield attributes of cassava and soil properties in Southern Laterites.

Material and Methods

Experimental site and conditions

The project work was undertaken at College of Agriculture, Vellayani, located at 8°30'N latitude, 76°54'E longitude and at an altitude of 29 m above mean sea level during June to December 2019. Soil was sandy clay loam in texture belonging to the order ultisol. Chemical properties assessed revealed the soil to be strongly acidic (pH, 5.23), high in organic carbon (1.25 %), medium in N (294.37 kg/ha) and K (138.32 kg/ha) and high in P (42.63 kg/ha) before the layout of experiment. The experiment was laid out in factorial Randomized Block Design with two factors, biofertilizers [PGPR Mix -I liquid (@ 2 % ; PGPR Mix -I liquid (@ 5 %; PGPR Mix -I powder @ 10 g of 2 % mixture per plant ; without biofertilizer] and nutrient levels [50 %; 75 % ; 100 % of standard dose of nutrients (SDN), 50:50:100 kg NPK /ha].Cassava setts (20 cm long cuttings of stem, 4-5 noded) of short duration variety Vellayani Hraswa, (5-6 months) were planted with two nodes of each sett below the soil and remaining nodes above, at a spacing of 90 cm x 90 cm. Full dose of P was applied basally, N and K were given in three equal splits (basal, 1 and 2 MAP) using the chemical fertilizers, urea, rajphos and muriate of potash. The biofertilizers were applied thrice, at planting (one week after fertilizer application), 2 and 4 months after planting (MAP). The 2 and 5 % concentrations of PGPR Mix -I liquid were prepared by mixing 20 mL and 50 mL of liquid consortium in 1000 mL water respectively. From the prepared solution 200 mL was applied in the root zone, on each mound according to the treatments. The mixture of the powder formulation was prepared by mixing 20 g talc based PGPR Mix-I with one kg of powdered cow dung and 10 g of the mixture was applied on each mound.

Yield attributes

The crop was ready for harvest, six months after planting (MAP). The percentage of productive roots per plant was calculated by dividing the number of tuberous roots by the total number of roots in each plant at harvest and multiplying by 100. Tubers were cut from the base of the stem and the length and girth of ten randomly selected tubers in each treatment were measured and recorded. Fresh weight of each part of the harvested plant (stem, leaves and tuber) was taken and sub samples were made for estimating the dry weight. The sub samples were dried in an oven at 70 ± 5^{o}C to constant dry weight and weight of each part was computed. To record the dry matter production (DMP).

Soil analysis

Samples were collected from the treatment plots at 1-15 cm depth at harvest, air dried and sieved through 2 mm sieve for the chemical analysis (soil pH, available N, P and K) and through 0.5 mm sieve for the estimation of soil organic carbon (C). Soil pH was determined in a 1: 2.5 w/v soil -water extract using pH meter and organic C by Walkley-Black chromic acid wet oxidation method and expressed in percentage. Available N was estimated by distillation with alkaline potassium permanganate, Bray extractable P by ammonium molybdo-blue colour method and K, by flame photometry with neutral normal ammonium acetate as extractant. Microbial counts, bacteria, fungi and actinomycetes were enumerated in the fresh soil samples by serial dilution method (Gopal, 2018).

Statistical analysis

The experimental data were statistically computed by applying the technique of analysis of variance (ANOVA) for 4 x 3 factorial RBD experiment and the significance was tested by F test (Cochran and

Cox, 1992) using OP Stat software, an online agriculture data analysis tool developed by O. P. Sheoran, Computer Programmer, Hisar.

Results and Discussion

Yield attributes in cassava

Biofertilizer application had significant influence on percentage of productive roots, length of tuber, girth of tuber and DMP in cassava (Table 1). The percentage of productive roots (56.96), tuber length (40.37 cm) and tuber girth (16.69 cm) were found to be superior in treatment with 5 % PGPR. (L). Formulation of PGPR (L) 2 % recorded the highest DMP (2.19 kg per plant) which was on par with 5 % PGPR (L) application.

Table 1: Effect of biofertilizer and nutrient levels on yield attributes

Treatments	Percentage of productive roots	Length of tuber (cm)	Girth of tuber (cm)	Dry matter production (kg per plant)
Biofertilizer (B)				
b_1 - PGPR (L) 2 %	50.96	38.85	15.39	2.19
b_2 - PGPR (L) 5 %	56.49	40.37	16.69	2.18
b_3 - PGPR (P)	53.28	34.30	13.78	1.81
b_0 - without biofertilizer	54.33	35.37	13.57	1.51
SEm±	0.25	0.18	0.04	0.04
CD (0.05)	0.764	0.554	0.125	0.105
Level of nutrients, NPK (N)				
n_1 - 50 % SDN	51.38	36.95	14.14	1.78
n_2 - 75 % SDN	54.06	38.47	15.42	2.08
n_3 - 100 % SDN	55.80	36.63	14.56	1.91
SEm±	0.22	0.16	0.01	0.03
CD (0.05)	0.661	0.480	0.029	0.091

***L- Liquid P- PowderSDN- 50: 50: 100 kg NPK/ha**

The nutrient application with 75 % SDN recorded the highest tuber length, girth and DMP, 38.47 cm, 15.42 cm and 2.08 kg per plant respectively. The percentage of productive roots were significantly superior in treatment with 100 % SDN. Among the treatment combinations (Table 2), the highest percentage of productive roots was observed in PGPR (L) 5 % + 100 % SDN. The length of tuber, girth of tuber and DMP were significantly superior for the treatment combination of PGPR (L) 5 % + 75 % SDN, 43.45 cm, 18.37 cm and 2.66 kg per plant respectively.

The positive influence of consortium biofertilizers on growth and yield of tuber crops have been illustrated by several workers (Dhanya, 2011; Jayapal, 2012; Radhakrishnan *et al*., 2013; Suja *et al*., 2014). The use of consortium biofertilizer in combination with chemical fertilizers significantly increased the yield attributes over the sole chemical fertilizer application. Further, it has been documented that the microorganisms in the consortium biofertilizers have the ability to synthezise phyto-hormones, decompose organic matter and recycle essential elements, augment the soil flora and improve the soil structure for root development (Singh, 2013). It is hence presumed that the enhanced root proliferation and absorption of water and nutrients, consequent to the PGPR application resulted in better plant yield parameters and DMP.

Table 2: Interaction effect of biofertilizer and nutrient levels on yield attributes

B × N Interaction	Percentage of productive roots	Length of tuber (cm)	Girth of tuber (cm)	Dry matter production (kg per plant)
b_1n_1	51.11	37.11	14.31	1.82
b_1n_2	58.30	39.89	16.56	2.31
b_1n_3	50.96	39.55	15.30	2.43
b_2n_1	48.95	40.78	16.28	2.07
b_2n_2	53.31	43.45	18.37	2.66
b_2n_3	61.96	36.89	15.39	1.82
b_3n_1	51.04	35.11	13.40	1.82
b_3n_2	52.23	34.00	13.15	1.80
b_3n_3	56.58	33.79	14.78	1.82
b_0n_1	54.41	32.00	12.56	1.43
b_0n_2	54.06	36.56	13.58	1.55
b_0n_3	54.54	37.56	14.56	1.56
SEm±	0.44	0.32	0.03	0.06
CD (0.05)	1.322	0.960	0.078	0.181

Vendan and Thangaraju (2006) documented the advantages of liquid formulations of biofertilizers over powder formulation *viz*., higher microbial counts, near zero contamination, greater protection against environmental stresses and increased field efficacy. According to Hoe and Rahim (2010), liquid biofertilizers have more viable cells than carrier based biofertilizers.In addition, as a higher cell count is realized in the liquid formulation, the dosage requirement for application can be reduced by 10 folds as compared to carrier based biofertilizers. Maheswari and Elakkiya (2014) reported the positive effects of foliar spray of liquid biofertilizer in black gram, on growth attributes and biochemical constituents such as chlorophyll, carbohydrate, protein and carotenoids content. It is deduced that cassava variety Vellayani Hraswa, being a crop of longer duration (180 days) required

a higher concentration (cell count) to realize the benefits of the formulation. This is justified by the significantly superior results on growth and yield parameters attained in the present study with the application of 5 per cent biofertlizer consortium. In addition to this, it is inferred that the cell protectants present in the liquid cultures helped to increase the survival of beneficial bacteria which play a vital role in nutrient uptake as enumerated by Krishan *et al.* (2005). The better performance of liquid biofertilizers over carrier based formulations has also been reported by other workers (Maheswari and Kalayarasi, 2015; Gopal, 2018; Lakshmi *et al*., 2019).

Soil properties

Soil organic carbon, available N, P and K content

The results on post experiment soil organic carbon, available N, P and K status as influenced by effect of biofertilizer, level of nutrients (NPK) and their interactions are presented in Tables 3 and 4.

Table 3: Effect of biofertilizer and nutrient levels on soil nutrient status after the experiment

Treatments	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
Biofertilizer (B)				
b_1- PGPR (L) 2 %	1.44	264.95	53.72	211.56
b_2 - PGPR (L) 5 %	1.47	244.03	52.97	191.16
b_3 - PGPR (P)	1.45	209.71	59.20	223.70
b_0 - without biofertilizer	1.29	204.17	46.09	186.60
SEm±	0.01	14.30	2.11	1.63
CD (0.05)	0.031	42.201	6.235	4.818
Level of nutrients, NPK (N)				
n_1 - 50 % SDN	1.14	219.63	51.47	206.76
n_2 - 75 % SDN	1.37	240.54	56.93	192.81
n_3 - 100 % SDN	1.40	235.31	54.26	196.56
SEm±	0.009	12.38	1.89	1.41
CD (0.05)	0.027	NS	NS	4.172

***L- Liquid P- Powder SDN- 50: 50: 100 kg NPK/ha**

Organic carbon in soil was found to vary significantly with PGPR Mix-I application and the treatment PGPR (L) 5 %, recorded the highest content (1.47 %) and was on par with PGPR (L) 2 % and PGPR (P), the values being 1.44 per cent and 1.45 per cent respectively. The lowest carbon (1.29 %) was observed in the treatment without biofertilizer. The mean data corresponding to effect of the levels of nutrients revealed that the organic carbon content was influenced by full dose SDN and was on par with 75 per cent of SDN with values 1.40 per cent and 1.37 per cent respectively. Among the interaction effect the highest organic carbon content (1.64 %) was observed in combination of PGPR (L) 5 % + 75 % SDN.

Table 4: Interaction effects on soil nutrient status after the experiment

B×N Interaction	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
b_1n_1	1.24	230.08	47.74	182.49
b_1n_2	1.52	251.00	58.63	178.64
b_1n_3	1.51	251.00	60.79	184.67
b_2n_1	1.35	231.48	57.20	175.64
b_2n_2	1.64	292.83	66.55	177.32
b_2n_3	1.41	271.92	55.84	185.00
b_3n_1	1.05	209.17	54.89	226.47
b_3n_2	1.09	271.91	54.70	217.87
b_3n_3	1.54	212.67	50.01	209.31
b_0n_1	0.90	209.17	46.05	196.83
b_0n_2	1.23	206.17	47.85	189.39
b_0n_3	1.16	217.16	50.38	194.26
SEm±	0.02	24.76	3.67	2.83
CD (0.05)	0.054	NS	NS	8.344

The variations in available N status of the soil with treatments were significant. Among the biofertilizer treatments, the use of liquid PGPR (L) 2 % (b_1) resulted in a significantly higher soil N status (264.95 kg/ha) which was on par with the 5 % treatment (244.03 kg/ha). The lowest N content was observed in b_0 (204.17 kg/ha) that was statistically comparable to powder formulation of PGPR (209.71 kg/ha). However, the different levels of nutrients and interaction failed to bring about marked variations in available N status post the experiment. The statistically analysed data revealed that biofertilizer application significantly influenced the P status and higher values were recorded in the treatments in which PGPR Mix-I was used. Among these, powder formulation PGPR recorded the highest soil P value (59.20 kg/ha). It

was on par with PGPR (L) 2 % and PGPR (L) 5 % with values 53.72 kg/ha and 52.97 kg/ha respectively. The different levels of NPK and interaction did not exert any significant influence on soil P status.

Potassium content in soil differed significantly with the two factors and their interaction. Significantly superior K status (223.70 kg/ha) was recorded in the treatment PGPR (P) and 50 % SDN, 206.76 kg/ha. The significant influence of the treatment combinations was revealed and the combination PGPR (P) + 50 % SDN, recorded the superior value of 226.47 kg K ha^{-1}. Soil available N status was lowered from the initial level and is ascribed to the crop uptake. Nevertheless, the P and K status were higher than the initial status. Meenakumari *et al*. (2008) reported the efficiency of P solubilizers in enhancing soil P pool by solubilizing P from insoluble sources. The PSB secretes the different organic acids which act on insoluble phosphate to convert them into soluble phosphate near the root of the plant and hence, availability of P is increased. Further, the mycotrophic nature of cassava makes it efficient in P extraction from the soil with the symbiotic association between its roots and AMF (Howeler, 2001). This also minimizes P depletion. A similar trend was noticed with K.

Potassium is considered as the most limiting factor in cassava system (Ezui, 2017) and the key nutrient for cassava cultivation. The nutrient is highly dynamic in soil. The results of *in vitro* assessment of K solubilization by *Bacillus sporothermodurans* present in liquid consortium indicated the high efficiency of the bacterium to solubilize insoluble inorganic (non exchangeable and fixed) K (Gopi, 2018), by its inherent ability to release organic acids that effectuates the solubilisation. This could be the plausible reason for higher available K in soil despite the higher uptake. The treatment with half the dose of SDN recorded the highest available K among the different nutrient levels. This can be related to the lower uptake of K in this treatment. The available N and P status of the soil did not show considerable variation with application of inorganic fertilizers. This was in conformity with the findings of Radhakrishnan *et al*. (2013). The interaction effects were significant with respect to organic carbon content and available K alone.

Microbial Count

Significant variations in the bacterial, fungal and actinomycete counts were observed with PGPR applications (Table 5 and 6) and the liquid formulations could enhance the microbial activities better than the powder formulation. Enumeration of the microbial counts in the rhizosphere revealed the population to be higher in the treatment with liquid consortium biofertilizer (PGPR Mix - I) indicating their proliferation and increased activity in the soil. Gopi *et al*. (2020) based on the results of analysis of rhizosphere population after application of PGPR Mix - I in amaranthus, reported successful colonization of organisms of PGPR Mix - I.

Table 5: Effect of biofertilizer and levels of nutrients on soil microbial count

Treatments	Microbial count (cfu/g soil)		
	Bacteria ($x 10^6$)	Fungi ($x 10^4$)	Actinomycetes ($x 10^4$)
Biofertilizer (B)			
b_1 - PGPR (L) 2 %	42.89	18.98	29.43
b_2 - PGPR (L) 5 %	44.11	20.44	28.11
b_3 - PGPR (P)	40.00	16.83	26.16
b_0-without biofertilizer	31.48	13.33	22.38
SEm±	0.57	0.49	0.63
CD (0.05)	1.686	1.466	1.887
Level of nutrients (N)			
n_1 - 50 % SDN	37.33	12.58	23.33
n_2 - 75 % SDN	37.91	15.83	24.74
n_3 - 100 % SDN	35.33	13.08	22.91
SEm±	0.50	0.43	0.55
CD (0.05)	1.460	1.270	NS

***L- Liquid P- Powder SDN- 50: 50: 100 kg NPK/ha**

Table 6: Interaction effects on soil microbial count

B ×N Interaction	Microbial count (cfu g^{-1} soil)		
	Bacteria ($x 10^6$)	Fungi ($x 10^4$)	Actinomycetes ($x 10^4$)
b_1n_1	38.27	16.13	24.67
b_1n_2	39.32	14.67	24.33
b_1n_3	42.12	15.95	23.00
b_2n_1	41.29	20.03	29.00
b_2n_2	43.33	21.67	26.53
b_2n_3	40.47	19.67	29.51
b_3n_1	38.23	15.00	25.67
b_3n_2	35.91	13.67	26.33
b_3n_3	36.67	13.52	23.33
b_0n_1	34.13	11.67	23.59
b_0n_2	31.21	12.75	22.67
b_0n_3	32.67	10.33	21.00
SEm(±)	0.989	0.53	1.10
CD (0.05)	2.021	1.372	NS

Among the microbes assessed, bacterial, fungal and actinomycetes counts were found to be significantly the highest in b_2 [PGPR (L) 5 %] followed by b_1 [PGPR (L) 2 %]. As expected, the lowest counts were observed in the treatment which did not receive any biofertilizer addition but was higher than the initial count. It is inferred that the rhizospheric deposits of root exudates, mucilage polysaccharides, phyto-hormones etc. produced by cassava would have promoted microbial activity, but, not to the extent of augmentation as evidenced with biofertilizer application. Microbial associations in cassava studied by Arotrupin and Akinyosoye (2008) revealed the array of microorganisms in cassava cultivated soils and Suja *et al.* (2014) reported the potential of the rhizobacteria for plant growth promotion and biocontrol. Dotaniya and Meena (2015) documented that plants can release carbohydrates, amino acids, lipids, and vitamins through their roots to stimulate the activities of microorganisms in the soil. Variations in the total microbial count due to application of different level of nutrients depicted in Table 5, reveal that the bacterial population was the lowest with application of full dose of SDN. The use of higher doses of fertilizers like urea increases ammonium toxicity in bacteria (Damodaran *et al*., 2016), whereas P and K fertilizers, reduce the substrate induced respiration in bacteria (Bolan *et al*., 1996). The growth of fungi decreases in N depleted conditions (Tuomela *et al.,* 2000) and this supports the results on fungal population with NPK application observed in this study. With respect to interaction, the bacterial and fungal counts varied significantly with the interaction of 5 % PGPR (L) and 75 % SDN. This indicates the positive interaction of consortium biofertilizer and nutrients. Khipla *et al*. (2017) documented the highest soil microbial population and enzyme activities with application of 100 % chemical fertilizers along with the consortium of *Azotobacter* and phosphate solubilizing bacteria in poplar. Variations in the actinomycete count due to the different level of fertilizers and interaction failed to reach the level of significance.

Conclusion

Optimisation of nutrient dose is important for improving the nutrient use efficiency and reducing losses. Cassava is a heavy feeder of nutrients and often leads to the depletion of the soil fertility unless replenished through efficient nutrient management practices. Inclusion of consortium biofertilizers in the nutrient management practice of short duration cassava has proven to be an effective strategy for enhancing the yield attributes and sustaining soil properties. Considering the impacts on yield parameters and soil fertility, application of PGPR Mix I liquid formulation (5 %) along with 37.5: 37.5: 75 kg NPK ha^{-1} can be recommended for short duration varieties of cassava.

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Conflict of interest

The authors declare that they have no conflict of interest.

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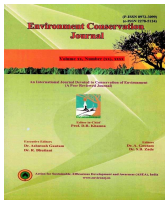

In-vitro selection of drought tolerant doubled haploid rice lines using polyethylene glycol (PEG)

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ABSTRACT

The present study was conducted to determine the response of 55 double haploid (DH) rice lines developed for drought tolerance from the cross Swarna × IR159B in polyethylene glycol (PEG) induced drought stress under *in-vitro* conditions (DH lines named as double haploid rice lines-DRL). Drought stress was created using PEG-6000 at different level of external water potential. Analyzed seedling traits of DRLs showed significant differences in response to different PEG concentrations. A decrement in plant growth at seedling stage with the increase in PEG concentration was observed as expected. Among 55DRLs, 14 DRLs were found to be drought tolerant sustaining the stress level till -7.5 bar as of the tolerant checks. Further, Drought linked SSRs were also evaluated in developed rice lines. Out of 8 SSRs, RM55 (R^2 value- 13.5%) and RM259 (R^2 value- 13.9%) found to be exhibiting significant association with the shoot/root ratio at - 7.5 bar stress level. Out of 14 DRLs, 9 DRLs were found to be showing drought tolerant in phenotypic and genotypic screening. Hence, PEG induced stress screening method used in this study will serve as the baseline for screening of rice lines for drought tolerance at very early stage without exploitation of much resource.

Introduction

Rice being a predominant food crop has devastating effect from drought stress limits the crop productivity by impeding plant growth and development, and thus reduces harvest size (Subba *et al.,* 2013). Approximately, 90% of the world's rice is produced and consumed in Asia. However, by 2025 production of 17 million hectares of traditionally grown irrigated rice will be affected by physical water scarcity and 22 million hectares will be hindered by "economic water scarcity" (Hibberd *et al.,* 2008; Prasad, 2011). Increase in water scarcity with the ongoing climatic changes will further worsen the scenario by posing a potential risk to productivity and food security in these rice growing areas (Li *et al.,* 2011). Studies of rice plants in response to identification of drought tolerance mechanism towards the development of promising drought tolerant lines under natural condition could be done by exploiting molecular, morphological, physiological and agronomic traits can make more crop per drop a reality (Degenkolbe *et al.,* 2009; Swamy and Kumar, 2013). Conventional breeding has led to the development of drought tolerant rice varieties still there is a cumbersome due to changing climatic regime accompanied with continuous nature of abiotic stresses. Thus, there is a need to fasten the breeding method. Double haploid (DH) technology *via in vitro* anther culture would accelerate the development of drought tolerant rice lines in short period of time. Looking to the success of this technology, since 2016, our lab is continuously working to develop DH lines in rice through *in vitro* anther culture for biotic and abiotic stresses

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which resulted into the release of variety CG Tejaswi Dhan high yielding and BLB resistance (Proceeding state seed sub-committee, Govt. of C.G. 2021). Many are in pipeline for high yielding, BLB, blast, drought tolerance, aroma (yet to be released). Field screening for drought tolerance is time consuming, labor intensive and requires suitable environmental conditions for the effective, repeatable phenotypic expression attributable to the genotype (Kacem *et al.,* 2017). It is therefore, necessary to use simple and effective early screening methods that relate to the field phenotypes. Selection of drought tolerant line *in vitro* is thought to be one of the ways to improve selection efficiency in addition to marker assisted selection.

Drought stress could be induced *in vitro* by using high molecular weight osmoticum *i.e*., PEG as it mimics in a way like soil drying resulting into selection of drought tolerant rice lines (Nepomuceno *et al.,* 1998; Widyastuti *et al.,* 2016).

To identify drought tolerant DH plants, molecular marker is also reliable tool which detect high degree of polymorphism in rice and hence are ideal for studies to identify tolerant genotype (Okoshi, 2004). In this study, attempt was made to identify the best promising drought tolerant line (DTL) derived from the cross Swarna and IR159B using varying level of external water potential given by PEG 6000 followed by genotyping of lines using random markers at seedling stage. The objective was to develop drought tolerant high yielding rice variety. Therefore, Swarna being a very popular yielder was selected and crossed with drought tolerant variety IR 159B. The above screening method together with the genotypic data can be employed to select superior drought tolerant lines varietal trials at very early stage, without exploitation of resources and yield loss due to stress.

Material and Methods

The experimental material consists of 55 DH rice lines developed through anther culture derived from the cross of Swarna × IR159B. Among 55 DH rice lines, 30 lines were mutagenized during its haploid callus stage using gamma irradiation (20 Grey) to increase variability for better selection. All the DH lines were named as DH rice lines no. 1-55 (DRL). Rice cultivars Dagaddeshi, RRF-127, IR-159B were used as tolerant check and Danteshwari, MTU1010, Swarna were taken as susceptible check. Mature rice seeds were harvested, manually dehusked using hand dehusker, and was treated with 0.2 % bavistin for two hours. Seeds were washed thrice with RO-system purified water followed by surface sterilization in 0.1% mercuric chloride for 8-10 minutes and again washed thrice with distilled water in laminar cabin. These seeds were placed in petri dish on to the moistened blotting paper for germination. After 3 days of germination, drought stress was induced *in vitro* using PEG (PEG6000) at 0.0 bar, -5.0, -7.5 and -10.0 bars of external water potential which was prepared by dissolving 196, 235 and 289 grams respectively in 1000 ml of distilled water (Hadas, 1976; Sabesan and Saravanan, 2016). After 10 days of PEG treatment, data were recorded at different level of external water potential on seedling traits such as root length, shoot length and root/shoot ratio. Shoot/root ratio (SL/RL) was calculated by measuring the ratio of shoot length over root length. The experiment was designed as factorial randomized design taking first factor as DTLs and second factor as different concentration of PEG treatments. Data analyzed with ANOVA using SPSS 16 software (SPSS *Inc*.). Further, least significant difference (LSD) among means was calculated at 0.05 level of significant.

For molecular characterization, genomic DNA was extracted from leaves of 10 days seedling from all DTLs and checks by modified CTAB method (Keb-Llanes *et al.,* 2002). The quality and quantity of DNA was checked in 0.8% agarose gel stained with ethidium bromide and UV spectroscopy using a Nano Drop Spectrophotometer. Eight SSR markers associated with drought tolerance QTLs were evaluated for screening of 55 DH lines derived from cross Swarna×IR159B (Table-1). Amplification reactions was carried out on thermal cycler (Applied Biosystems) by preparing 20µl final volume reaction containing 50 ng template DNA and EmeraldAmp GT PCR Master Mix (Takara Bio) (Table-1). This master mix includes an optimized buffer, PCR enzyme (2 U/µl), dNTP mixture (10 mM), gel loading dye (green), and a density reagent in a 2X premix format. The PCR condition were: denaturation at 94°C for 3 minutes,

Table 1: List of SSR markers associated with drought tolerance QTL

SN	Marker	Chr no.	Primers (5' →3')	Product size (bp)	Position (cM)
1	RM259	1	F: TGGAGTTTGAGAGGAGGG R: TGGAGTTTGAGAGGAGGG	162	54.2
2	RM472	1	F: CCATGGCCTGAGAGAGAGAG R: AGCTAAATGGCCATACGGTG	296	171.6
3	RM2634	2	F: GATTGAAAATTAGAGTTTGCAC R: TGCCGAGATTTAGTCAACTA	154	80.95
4	RM55	3	F: CCGTCGCCGTAGTAGAGAAG R: TCCCGGTTATTTTAAGGCG	226	168.2
5	RM451	4	F: GATCCCCTCCGTCAAACAC R: GATCCCCTCCGTCAAACAC	207	115.5
6	RM553	9	F: AACTCCACATGATTCCACCC R: GAGAAGGTGGTTGCAGAAGC	162	76.7
7	RM215	9	F: GAGAAGGTGGTTGCAGAAGC R: TGAGCACCTCCTTCTCTGTAG	148	99.4
8	RM271	10	F: TCAGATCTACAATTCCATCC R: TCGGTGAGACCTAGAGAGCC	101	59.4

35 cycles of denaturation for 45 sec at 94°C, annealing for 30 seconds at 50°C followed by 30 sec at 72°C and final elongation at 72°C for 10 minutes. The PCR products were detected using 1.5% agarose gel electrophoresis along with 100bp ladder and visualized by ethidium bromide staining under Gel documentation unit (Biorad). The genotypic data of DRL population were scored as "R" for parent 2 like bands *viz.*, IR-159B and "S" for parent 1 like band (Swarna). The scores were then used for analysis using SPSS 16.0 (*SPSS Inc.*) for associating markers (RM55, RM259) with the shoot/ root ratio of selected DRLs showing drought tolerance at 7.5 bar water potential treatment.

Results and Discussion

The phenotypic observations were recorded based on growth of root, shoot length in centimetres and root/shoot length ratio after 10days of PEG treatment by measuring it with scale in population along with parents and check (Fig-1). Data analyzed with ANOVA in factorial completely randomized design using SPSS 16.0 (*SPSS Inc.*) to test for significant difference among DRLs (G), PEG treatment (T) and their interactions (G×T). In the study, significant differences were observed for the seedling parameters (Table 2). Shoot length were found to be significant at 5% level of significance for DRLs, 1% level of significance for PEG treatments and for interaction between DRLs and PEG treatment. Similarly, for root length and shoot/root ration found to be significant at 1% level of significance for DRLs, PEG treatment and their interaction. Similarly, Akte *et al*., 2016 reported significant difference for seedling parameters, different PEG treatment and interaction between them in rice. Khakwani *et al*., 2011, Mansour and Elbagrmi, 2019 reported significant differences for wheat genotypes and PEG treatment.

Table 2: Analysis of variance for seedling trait of 55 DH rice lines during drought induced by polyethylene glycol (PEG)

Source	DF	Mean Square		
		Shoot length (cm)	Root length (cm)	Shoot/root ratio
DH rice line (G)	54	1.90*	17.58**	0.404**
Treatment (T)	3	858.94**	1580.039**	21.89**
Interaction (G × T)	162	1.38**	4.52**	0.16**
Error	440	0.043	0.073	0.004

** Significant at 1 % probability levels, *Significant at 5% level of significance

Shoot response under different PEG concentration

The shoot bears most economic part of the crop and is an important parameter while selecting the superior genotypes against drought. Shoot length of 55 DRLs along with parents and check varieties were measured from the root base to the tip of the shoot after 10 days of PEG treatment at different concentration. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced shoot length (Table-

3). With the increase in moisture stress level using different concentration of PEG (-5 bar, -7.5 bar, -10 bar), mean shoot length found to be decreased by 68 % at -5 bar, 95 % at -7.5 bar and 99 % at -10 bar of external water potential with respect to control (0 bar) in DRL (Fig-2). Out of the 55 DRLs, 14 DRLs *i.e*., DRL-4 (0.53cm), DRL-6 (1.06cm), DRL-14 (0.66cm), DRL-18 (0.58cm), DRL-22 (1.03cm), DRL-23 (0.35cm), DRL-31 (0.61), DRL-36 (0.28cm), DRL-48 (1.06cm), DRL-49 (0.5cm), DRL-50 (1cm), DRL-51 (0.56cm), DRL-53 (0.26cm), DRL-55 (0.53cm) along with drought tolerant check (Dagaddeshi:1.2cm, RRF-127:1.3cm) and parent (IR159B:1.1cm) shown average shoot length at -7.5 bar external water potential. Also, DRL-10 (0.22cm) and DRL-42 (0.55cm) shown average shoot length at -10 bar external water potential. The lines showing shoot response together with drought tolerant check (Dagaddeshi and RRF-27) and parent (IR-158) at -7.5 bar and -10 bar moisture stress induced by PEG indicate drought tolerance. Shoot length of 24 DRLs along with drought susceptible check (Danteshwari, MTU1010) and parent (Swarna) showed response in -5 bar external water potential. Rest of the lines didn't produce any kind of shoot growth indicate drought susceptibility. Previous studies on PEG treatment in different crops reported that the increase in external water potential leads to the decrease in shoot length when compared with control (Jajarmi, 2009; Khakwani *et al.,* 2011; Govindaraj *et al.,* 2010; Sabesan and Saravanan, 2016, Mansour and Elbagrmi, 2019). Masour and Elbagrmi, 2019 reported slight increase in shoot length at level of -3 bar in 3 wheat cultivars and then reduced with the increase in PEG concentration. Similarly, Akte *et al*., 2016 reported decrement in shoot length from 15.76 cm in control to 15.76 cm in 4% PEG concentration in rice varieties. Decrement in shoot length with the increase in PEG concentration is due to decrease in turgor pressure resulting in reduced cell division and cell elongation (Lagerwerff *et al.,* 1961; Chandra, 2011; Nurhayati *et al*., 2017; Akte *et al.,* 2016; Sabesan and Saravanan, 2016). The water scarcity can in induced by PEG because it may cause effect on metabolic processes of plant *via* preventing nutrients transfer (Chandra, 2011; Govindaraj *et al.,* 2010). In contrast the normal shoot growth is also reported in the presence of high concentration of PEG (Purbajanti *et al.,* 2019; Hellal *et al.,* 2018). Similarly, in the present study 14 DRLs found to sustaining upto -7.5 bar PEG treatment. This may be due to increase in proline content resulting into drought tolerant genotype (Nurhayati *et al*., 2017; Kadir, 2007).

Root response under different PEG concentration

Roots are also an important seedling trait responsible for perceiving and transducing of water deficit signals to shoot which further triggers an array of physiological, morphological and molecular responses in the whole plant (Moumeni *et al.,* 2011). This combination of rapid sensing and signaling on both cellular and organ level enable the plant to tolerate water loss and thus survive in drought stress condition (Robbins and Dinneny, 2015). Root length of 55 DRLs along with parents and check varieties were measured from the root base to the tip of the root after 10 days of PEG treatment at different concentration. It was observed that the root length also declined with increased external water potential and consequently, all PEG treatments caused a decrease in root elongation in all DRL compared to their controls (Table-3). With the increase in external water potential using different concentration of PEG (-5 bar, -7.5 bar, -10 bar), mean root length found to be decreased by 21% at -5 bar, 59% at -7.5 bar and 96% at -10 bar external water potential with respect to control (0 bar) in DH rice lines (Fig-2). Among 55 DTL, 34 DTL showed root growth ranging from 1.05 cm (DTL-19) to 7.17cm (DTL-50) followed by 6.56cm (DTL-43) at -7.5 bars of PEG treatment. At -10 bar of PEG treatments, 17 DTL showed root growth ranging from 0.22cm (DTL-22) and 1.99cm (DTL-6). The lines showing root response together with drought tolerant check (Dagaddeshi and RRF-27) and parent (IR-158) at -7.5 bar and -10 bar of external water potential induced by PEG indicate drought tolerance. Similar to present study, several studies reported that the increased in PEG concentration leads to the root length deployment when compared with control (Jajarmi, 2009; Khakwani *et al.,* 2011; Govindaraj *et al.,* 2010; Sabesan and Saravanan, 2016; Akte *et*

Table 3: Effect of moisture stress on seedling traits of rice DH lines during drought induced by PEG

SN	DH lines	Shoot length (cm)				Root length (cm)				Shoot/root ratio			
		External water potential (Bars)				External water potential (Bars)				External water potential (Bars)			
		Control	-5	-7.5	-10	Control	-5	-7.5	-10	Control	-5	-7.5	-10
C1	DAGADDESHI	8.56	5.10	1.2	0.00	4.16	3.21	1.6	0.93	2.06	1.59	0.75	0.00
C2	RRF-127	7.11	4.20	1.3	0.00	6.37	6.01	1.67	1.12	1.12	0.70	0.78	0.00
C3	DANTESHWARI	7.06	3.20	0.00	0.00	4.23	3.00	0.00	0.00	1.67	1.07	0.00	0.00
C4	MTU1010	7.02	4.18	0.00	0.00	4.77	3.37	0.49	0.00	1.47	1.24	0.00	0.00
P1	SWARNA (SW)	5.32	3.60	0.00	0.00	5.30	2.61	0.56	0.00	1.00	1.38	0.00	0.00
P2	IR-159B (IR)	8.55	4.90	1.10	0.00	7.81	5.23	1.04	0.44	1.09	0.94	1.06	0.00
1	DRL-1	5.33	1.20	0.00	0.00	6.38	3.34	2.23	0.00	0.84	0.36	0.00	0.00
2	DRL-2	5.20	2.17	0.00	0.00	6.37	4.12	2.04	0.00	0.82	0.53	0.00	0.00
3	DRL-3	5.48	1.78	0.00	0.00	6.78	8.24	1.45	0.00	0.81	0.22	0.00	0.00
4	DRL-4	4.54	1.27	0.53	0.00	6.31	7.62	5.5	1.23	0.72	0.17	0.10	0.00
5	DRL-5	4.44	0.47	0.00	0.00	7.13	4.50	3.25	0.00	0.62	0.10	0.00	0.00
6	DRL-6	4.20	1.56	1.06	0.00	6.58	6.45	4.56	1.99	0.64	0.24	0.23	0.00
7	DRL-7	4.53	2.10	0.00	0.00	7.51	6.51	3.14	0.00	0.60	0.32	0.00	0.00
8	DRL-8	6.04	1.42	0.00	0.00	8.05	5.26	2.3	0.55	0.75	0.27	0.00	0.00
9	DRL-9	6.61	1.62	0.00	0.00	9.07	7.13	3.21	0.00	0.73	0.23	0.00	0.00
10	DRL-10	5.70	2.08	1.09	0.22	8.49	6.3	5.23	1.32	0.67	0.33	0.21	0.17
11	DRL-11	5.48	5.08	0.00	0.00	7.00	5.22	3.43	0.00	0.78	0.97	0.00	0.00
12	DRL-12	6.66	3.01	0.00	0.00	7.56	8.13	6.15	0.00	0.88	0.37	0.00	0.00
13	DRL-13	4.40	2.03	0.00	0.00	4.16	3.54	2.93	0.23	1.06	0.57	0.00	0.00
14	DRL-14	4.68	2.06	0.66	0.00	6.21	5.23	2.28	1.20	0.75	0.39	0.29	0.00
15	DRL-15	5.11	2.96	0.00	0.00	11.15	8.56	2.96	0.00	0.46	0.35	0.00	0.00
16	DRL-16	4.22	0.53	0.00	0.00	7.45	5.06	3.34	0.00	0.57	0.10	0.00	0.00
17	DRL-17	4.06	0.00	0.00	0.00	5.22	4.26	1.11	0.00	0.78	0.00	0.00	0.00
18	DRL-18	4.78	2.63	0.58	0.00	3.16	1.54	0.00	0.00	1.51	1.71	0.00	0.00
19	DRL-19	5.10	1.02	0.00	0.00	5.96	2.41	1.05	0.00	0.86	0.42	0.00	0.00
20	DRL-20	3.96	0.00	0.00	0.00	5.20	3.56	3.02	0.00	0.76	0.00	0.00	0.00
21	DRL-21	6.24	1.17	0.00	0.00	11.66	6.94	1.52	0.00	0.54	0.17	0.00	0.00
22	DRL-22	5.33	2.81	1.03	0.00	10.28	5.7	1.51	0.22	0.52	0.49	0.68	0.00
23	DRL-23	4.92	1.07	0.35	0.00	5.86	3.26	0.00	0.00	0.84	0.33	0.00	0.00
24	DRL-24	4.93	1.07	0.00	0.00	3.87	7.46	3.02	0.00	1.27	0.14	0.00	0.00
25	DRL-25	3.72	0.00	0.00	0.00	3.69	2.72	0.83	0.00	1.01	0.00	0.00	0.00
26	DRL-26	3.49	0.00	0.00	0.00	7.74	7.79	1.92	0.00	0.45	0.00	0.00	0.00
27	DRL-27	3.36	2.86	0.00	0.00	6.68	3.45	0.00	0.00	0.50	0.83	0.00	0.00

28	DRL-28	4.98	0.00	0.00	0.00	8.84	5.56	2.6	0.00	0.56	0.00	0.00	0.00
29	DRL-29	5.15	0.00	0.00	0.00	7.56	5.36	4.15	1.20	0.68	0.00	0.00	0.00
30	DRL-30	5.26	2.76	0.00	0.00	7.64	3.62	3.00	0.00	0.69	0.76	0.00	0.00
31	DRL-31	4.44	2.13	0.61	0.00	6.96	4.23	2.56	0.56	0.64	0.50	0.24	0.00
32	DRL-32	3.88	0.74	0.00	0.00	5.95	4.23	2.69	0.44	0.65	0.17	0.00	0.00
33	DRL-33	5.99	0.00	0.00	0.00	6.39	6.51	2.32	0.00	0.94	0.00	0.00	0.00
34	DRL-34	6.02	1.33	0.00	0.00	8.86	5.23	2.45	0.00	0.68	0.25	0.00	0.00
35	DRL-35	5.62	0.00	0.00	0.00	8.72	5.63	2.15	0.00	0.64	0.00	0.00	0.00
36	DRL-36	5.08	3.38	0.28	0.00	10.37	6.25	4.26	1.34	0.49	0.54	0.07	0.00
37	DRL-37	4.92	0.00	0.00	0.00	9.07	5.94	5.73	0.00	0.54	0.00	0.00	0.00
38	DRL-38	4.19	0.00	0.00	0.00	7.22	5.52	4.90	0.00	0.58	0.00	0.00	0.00
39	DRL-39	4.54	0.50	0.00	0.00	5.57	7.86	4.88	0.00	0.82	0.06	0.00	0.00
40	DRL-40	5.26	1.54	0.00	0.00	10.66	9.42	3.70	0.56	0.49	0.16	0.00	0.00
41	DRL-41	3.84	2.46	0.00	0.00	8.88	6.83	3.75	0.00	0.43	0.36	0.00	0.00
42	DRL-42	4.68	2.48	1.28	0.55	9.85	6.42	5.60	1.56	0.48	0.39	0.23	0.35
43	DRL-43	4.53	2.25	0.00	0.00	8.02	7.08	6.56	0.00	0.56	0.32	0.00	0.00
44	DRL-44	5.32	1.20	0.00	0.00	4.86	6.37	4.16	0.00	1.09	0.19	0.00	0.00
45	DRL-45	5.57	0.00	0.00	0.00	6.98	5.23	2.52	0.00	0.80	0.00	0.00	0.00
46	DRL-46	5.06	2.59	0.00	0.00	7.42	5.33	0.00	0.00	0.68	0.49	0.00	0.00
47	DRL-47	5.15	2.03	0.00	0.00	7.23	7.59	3.44	0.00	0.71	0.27	0.00	0.00
48	DRL-48	5.24	2.60	1.06	0.00	9.04	7.28	6.65	0.92	0.58	0.36	0.16	0.00
49	DRL-49	5.54	2.15	0.50	0.00	9.16	8.2	7.17	1.00	0.60	0.26	0.07	0.00
50	DRL-50	6.03	1.56	1.00	0.00	9.16	7.95	6.14	1.00	0.66	0.20	0.16	0.00
51	DRL-51	5.24	1.36	0.56	0.00	5.18	4.23	2.14	0.00	1.01	0.32	0.22	0.00
52	DRL-52	4.51	1.88	0.00	0.00	7.99	6.23	2.23	0.00	0.56	0.30	0.00	0.00
53	DRL-53	4.36	1.38	0.26	0.00	7.99	7.47	5.10	0.50	0.55	0.18	0.05	0.00
54	DRL-54	3.47	2.96	0.00	0.00	8.06	7.41	4.15	0.00	0.43	0.40	0.00	0.00
55	DRL-55	4.35	3.03	0.53	0.00	7.98	7.12	5.06	0.00	0.55	0.43	0.10	0.00
Treatment mean (55 lines)		4.92	1..57	0.21	0.01	7.40	5.83	3.24	0.29	0.71	0.30	0.05	0.01
		Mean Standard Error											
	Rice DH lines(G)	0.059				0.077				0.059			
	PEG treatment (T)	0.016				0.021				0.0004			
	Interaction (G × T)	0.119				0.155				0.036			
		LSD (P > 0.05)											
	Rice DH lines(G)	0.169				0.221				0.025			
	PEG treatment (T)	0.072				0.094				0.022			
	Interaction (G × T)	0.334				0.435				0.101			

al., 2016; Ghosh *et al.,* 2020). For instance, Akte *et al*., 2016 observed the decrement in root length of rice genotypes from 5.022 cm in control to 3.898 cm in 4% PEG concentration. Similarly, Wickramasinghe and Seran, 2019 reported the declining of root length in tomato seedling with the increasing PEG concentration.

Effect of water stress on shoot/root ratio

In addition to root length and shoot length, shoot to root ratio plays a major role in selecting drought tolerant lines. The interdependence of shoot and root is required for the optimal growth and development of the crop. The shoot relies on the root for water, nutrients and mechanical support while the roots depend on the shoot for organic nutrients (Hoad *et al.,* 2001). The shoot to root ratio reflects shoot and root growth patterns of crop under drought stress. A high shoot to ratio means high shoot growth and lower shoot-root ratio mean comparatively high root growth. In the study, DRLs under control (0 bar) has highest shoot/root ratio in DRL-19 (1.51) and lowest shoot/root ratio in DRL-54 (0.43) (Table-3). With the increase in external water potential using PEG treatment (-5 bar, -7.5 bar and -10 bar), we observed decrement in shoot/root ratio by 57 % at -5 bar, 93% at -7.5 bar and 98% at -10 bar of PEG concentration compared to control (Fig-2). Among 55 DRLs, 12 lines DRL-4 (0.10), DRL-6 (0.23), DRL-14 (0.29), DRL-22 (0.68), DRL-31 (0.24), DRL-36 (0.07), DRL-48 (0.16), DRL-49 (0.07), DRL-50 (0.16), DRL-51 (0.22), DRL-53 (0.05), DRL-55 (0.10) shown average shoot/root ratio at -7.5 bar of external water potential induced by PEG. DH lines DRL-10 (0.17) and DRL-42 (0.35) shown average shoot/root ratio at -10 bar of external water potential induced by PEG. The lines showing response were together with the drought tolerant check and parent indicates drought tolerance. Similarly, Thabet *et al*., 2018 reported decrement by increasing the PEG concentration from 1.01 cm in control to 0.77 cm for shoot to root ratio in barley genotypes. A decrease in shoot/root ratio under PEG-induced external water potential indicates that PEG induced osmotic stress positively influence drought growth compared to shoot growth.

Molecular analysis of DTLs using SSR marker

Plant responses to stress factors can be considered on a variety of levels of their organization, beginning with the molecular background (through cells and organs) and ending at the whole plant. Selection for drought tolerant lines based on phenotypic traits may be accelerated by using molecular markers associated with trait. The recent identification of major QTLs governing grain yield under drought has made possible the use of marker assisted selection (MAS) for improving drought tolerance in rice. In the present study, genotyping of 55 DTLs of Swarna × IR-159 B were performed using 8 SSR marker linked to drought tolerance (Table-1). Out of the 8 SSR markers used, two of them namely, RM55 and RM259 were found to exhibit polymorphism among parents of DTL. Here, Swarna like alleles were designated as "P1" and IR-159 B like alleles were designated as "P2" (Fig-3). The lower band (226 bp) was observed in IR159B (drought tolerant) and upper band (290bp) was observed in Swarna (drought susceptible) for RM55. Among DTLs, banding pattern showing 61.8% alleles (34 lines) were like Swarna and 38.2% allele (21 lines) were like IR159-B. Similarly, RM259 marker showing lower band (162 bp) observed in IR159B and upper band (210 bp) was observed in Swarna. Here, banding pattern

Table 4: Genotypic data of DH rice lines sustaining at -7.5 bar PEG treatment

SN	DH lines	Shoot/root ratio	Genotyping	
		PEG treatment (-7.5 bar)	RM55	RM259
P1	Swarna	0.00	P1	P1
P2	IR-159 B	1.06	P2	P2
1	DRL-4	0.10	2	2
2	DRL-6	0.23	2	1
3	DRL-10	0.17	2	2
4	DRL-14	0.29	2	2
5	DRL-22	0.68	1	1
6	DRL-31	0.24	2	2
7	DRL-36	0.07	2	2
8	DRL-42	0.23	1	1
9	DRL-48	0.16	2	2
10	DRL-49	0.07	2	2
11	DRL-50	0.16	2	2
12	DRL-51	0.22	2	1
13	DRL-53	0.05	1	1
14	DRL-55	0.10	1	1

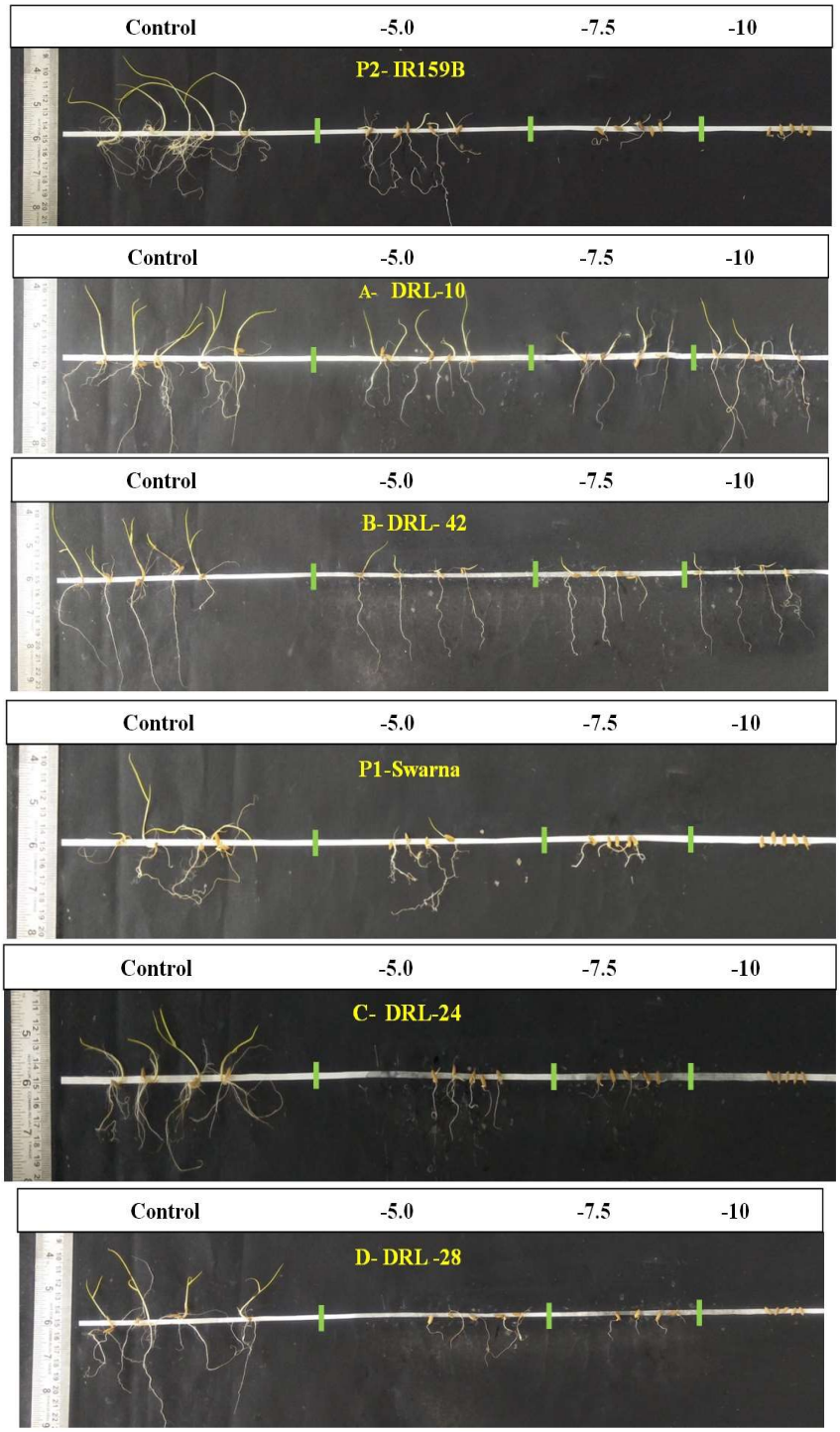

Figure 1: Response of seedling trait (Shoot and root length) for increased external water potential (0, -5.0, -7.5, -10 bar) using PEG.

Legends: P2- IR159B: Drought tolerant parent (showing shoot growth upto -7.5 bar and root growth upto -10 bar , A- DRL-10 indicating tolerance (showing shoot and root growth upto -10 bar), B- DRL-42 indicating tolerance (showing shoot and root growth upto -10 bar), P1- Swarna: Drought susceptible parent (showing shoot growth upto -5 bar and root growth upto -7.5 bar), C-DRL-24 indicating susceptibility (showing root growth upto -5 bar and root growth upto -7.5 bar), D-DRL-28 indicating susceptibility (showing shoot growth upto -5 bar and root growth upto 7.5 bar).

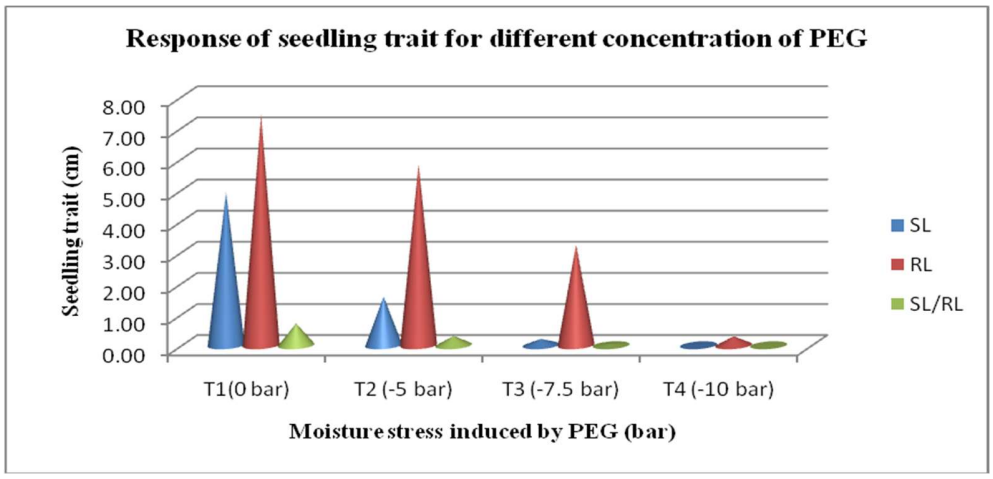

Figure 2: Effect of different levels of PEG induced water stress on 55 DH rice lines for seedling traits (SL-Shoot length, RL-Root length, SL/RL- Shoot-root ratio)

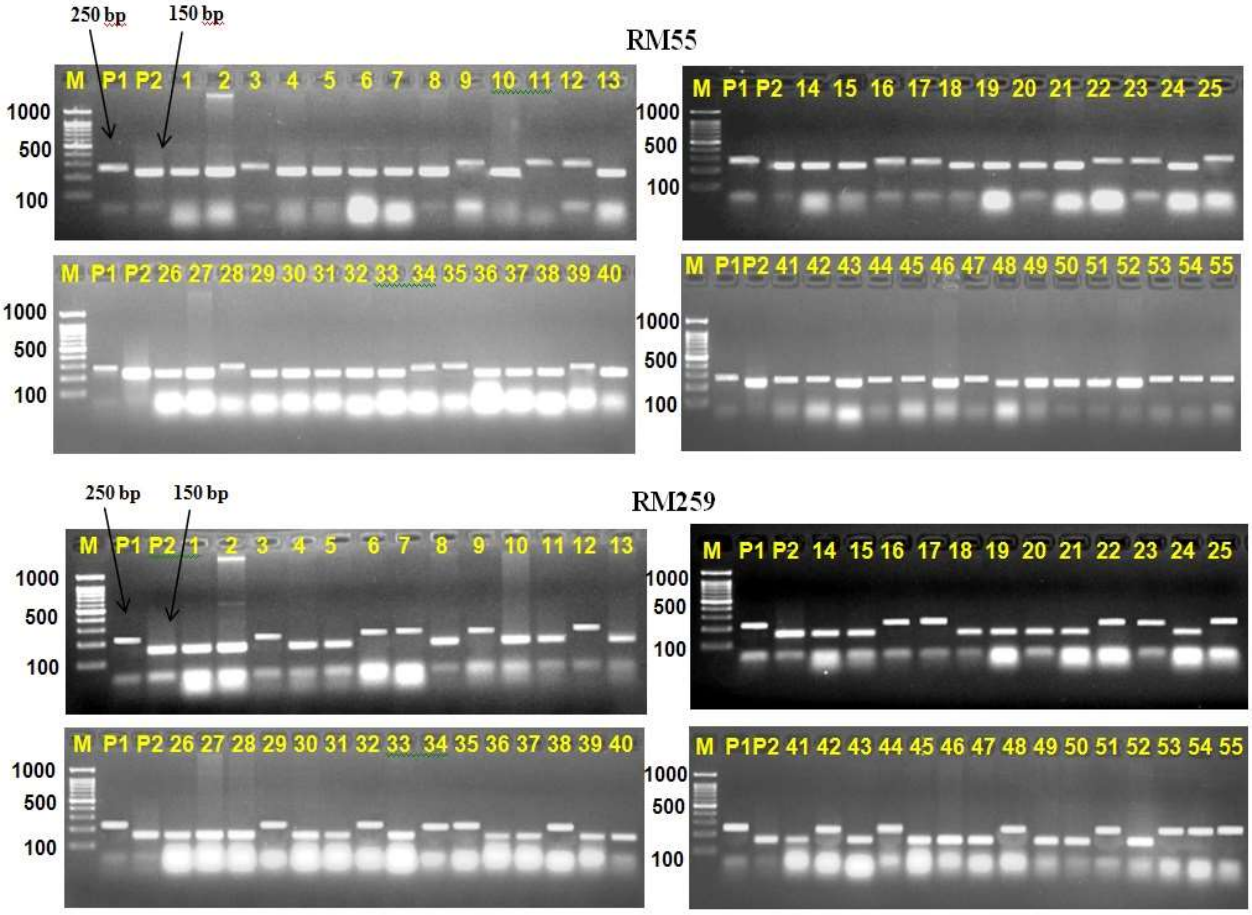

Figure 3: Molecular analysis of 55 DH rice lines derived from cross Swarna×IR159B

Legends: M: 100 bp ladder, P1: Swarna, P2: IR159B, 1-55: DH rice lines, SSR marker showing 290 bp (RM55) and 220bp (RM259) bands for Swarna like alleles indicating drought susceptible lines and 226bp (RM55) and 162bp (RM259) for IR159B like alleles indicating drought tolerant lines.

60% alleles (33 lines) were like Swarna and 40% alleles (22 lines) were like IR159B. Further, the scores generated in DRLs derived from cross Swarna and IR159B were used to find the association if any, with shoot/root ratio measured at -7.5 bar of external water potential induced using PEG. To study the association, 14 selected DRLs showing drought tolerance at -7.5 bar of external water potential were taken and analyzed using SPSS 16.0 software (SPSS *Inc*.) (Table-3). Phenotypic variance exhibited by RM55 is 13.5% (P value: 1.788E-10**, R^2: 0.135) and RM259 is 13.9% (P value: 1.267E-09**, R^2:0.138) for 7.5 bar stress level at 1% level of significance. Out of 14 selected DRLs, genotypic data of 9 DRLs (DRL-4, DRL-6, DRL-10, DRL-14, DRL-31, DRL-36, DRL-48, DRL-49, DRL-50) were found to be significant with the phenotypic data which could be further evaluated for drought tolerant trials in field. RM55 and RM259 marker was reported to be linked to drought tolerance in rice (Venuprasad *et al.,* 2009; Verma *et al.,* 2014, Sahoo *et al.,* 2019;). Wang *et al.* (2005) reported 16 candidate genes between markers RM212 and RM319 have potential role in drought tolerance and may be useful in marker assisted breeding for drought stress. Present investigation also shows the association of *in vitro* PEG screening with drought linked markers in DRLs developed for drought stress. It also indicates genetic stability in developed DRLs which could be utilized for new cultivar development in a short time span.

Conclusion

The study showed that application of PEG at different water potential has negative effect on *in vitro* rice growth due to negative osmotic pressure inside the cell. This inhibits plants to uptake available water to the seed and resulting in drought stress. Among 55 DRLs derived from cross Swarna and IR159B, 9 DRLs performed best by showing the significant seedling growth sustaining at - 7.5 bar external water potential. Drought linked markers *viz*., RM55 and RM259 found to be reliable based on data generated. The results of the PEG analysis in the study demonstrate the use of PEG at different concentration of external water potential as an effective method for studying the effect of water stress on seed germination and seedling growth characteristics, and adjudged it as a simple cost-effective, time saving method for screening large sets of DH lines/rice lines within a very short period and precision. However, validation under real field conditions is needed to further authenticate these results.

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Conflict of interest

The authors declare that they have no conflict of interest.

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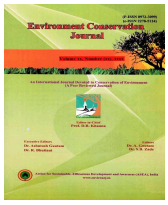

Mapping of supply chain and assessment of pre and postharvest losses of Alphonso mango in India

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ABSTRACT

One of the most popular commercial varieties of mango in India is the Alphonso variety. Due to its limited and specialised production zone, this cultivar attracts interest for its supply chain management research. Only a few studies on the management of the mango supply chain in India could be discovered in the literature. In order to better understand supply chain management and pre- and post-harvest losses for the Alphonso mango, a survey was conducted. To acquire the data, 123 observations from farmers, FPOs, retailers, exporters, and government officials were recorded. A socioeconomic study revealed that farmers who were young and educated (less than 50 years old and graduates) produced mangoes of higher quality and were more committed to exporting mangoes. The findings indicate that preharvest losses are primarily caused by variables including climate change, global warming, numerous illnesses and pests, spongy tissue, and fruit fly problem. During the harvest season, spongy tissue and abrupt, unseasonal rain have a negative impact on mango quality and supply. Mechanical damage, storage conditions, transportation, and mango handling all had a major impact on postharvest losses. According to the study, pre-harvest factors were responsible for 30 to 40% of mango loss, and post-harvest handling was found to be responsible for 15 to 20% of mango loss. The revenue of farmers is remains poor due to the current trading channels and lack of facilities for value addition. The results of this study provide insight on the current state of the supply chain and Alphonso mango losses. Researchers, governmental organisations, and policymakers can benefit from this study's findings by taking the appropriate actions to boost farmers' incomes, balance the market's supply and demand, and lower losses in other perishable fruits.

Introduction

Mango (*Magnifera indica* L.) is an important tropical fruit grown majorly in countries like India, China, Brazil, and Thailand. India is the world's largest producer of mango contributing 50% of the total mango production with an annual production of 21.38 million MT from an area of about 2.296 million ha (National Horticulture Board, 2018-19). Due to its delicious taste, pleasant aroma, excellent flavor, low calories, and high nutritional value, Indian mango has huge demand in the domestic as well as world market and is commonly called the king of fruits (Jha *et al.* 2012).India's mango is mostly exported to European and Arabian countries. In 2019-20 India exported about 49658 MT of mango and fetches about 400.21 cr rupees from the world market (APEDA 2021). In order to meet consumer's demands a consistent supply of fresh foods should have high quality, safefor consumption, and nutritious.In India, many mango varieties are cultivated, but varieties like Alphonso, Kesar, and Banganapalle have high demand in the local market and for export. Due to the favorablehot

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climate, volcanic soil conditions, coastal region, etc., Alphonso mango is typically grown in the Konkan region of Maharashtra, India. In the local language, it is called *Hapus Amba*. The Indian government's Ministry of Commerce awarded the Alphonso mango a Geographical Indication (GI) designation in 2003 to those mangoes coming from the 200 km coastal line of Ratnagiri and Sindhudurg districts of Maharashtra. Due to its thin and saffron-colored skin, thick, yellow, creamy, sweet pulp, and fibreless texture.Quality control plays important role in fruit export (Lawson *et al.* 2019). In India, harvested mango are subject to many unit operations like pretreatment, cooling, packaging, handling, etc. before reaching the consumer. From the production stage to the consumption stage many physiological changes occur in mango. Several factors such as time and season of harvest, the effect of mulching, location, high temperature, low fruit transpiration, and biological factors are known to influence the internal quality of mango fruit (Janave and Sharma, 2008; Oak *et al.* 2019;). Indian mangoes were prohibited by the United States (US) and the European Union (EU) due to the overuse of pesticides and the threat of spongy tissue, jelly seed, fruit flies, and stone weevil invasion from previous years.A supply chain of mango is described as the flow of activities, information, and resources connected to commodities from the producer to the consumer. Millions of individuals working in rural and urban areas, including farmers, wholesalers, logistics partners, and retailers involved in supply chains, the food supply chainsare essential in reducing waste and cutting down costs while maximizing overall values and generating money (Gebre*et al.* 2020).

So, the Konkan region in Maharashtra of India (shown in Fig. 1), which is regarded as the country's top producer of Alphonso mangoes, has received little attention in the literature. Additionally, no study has examined the challenges faced by the Alphonso mango industry, which plays a key role in the nation's export of mangoes and foreign exchange.This study's goals include identifying the difficulties that farmers, stakeholders, and policymakers face in producing, maintaining the quality, and authenticating Alphonso mangoes, as well as how to manage their supply chains at various levels (local markets, export, supermarkets, etc.), and study of losses occurred due to unavailability of various facilities for safeguarding the quality of Alphonso mangoes for enhancing marketing and identifying gaps through conducting the survey.

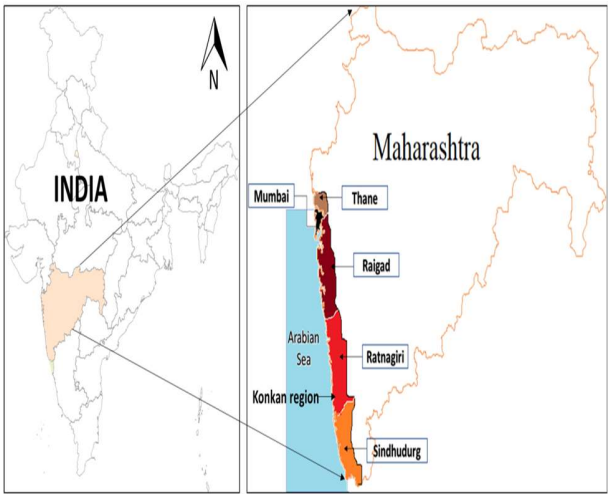

Figure 1: Map of study area

Material and Methods

Sampling methods and sample size

This study followed a stratified multistage sampling procedure for the collection of data. The purposive sampling was used because most of the farmers and growing area of Alphonso mango are concentrated in Ratnagiri and Sindhudurg districts from Maharashtra state, India shown in Fig. 2.From these two districts purposively two blocks from each district were selected, and from each block, three villages were selected on basis of high quality and authentic production, well-known, and famous for Alphonso mango for collection of information.So, primary data was collected from 123 stakeholders includes farmers, Farmer's Producers Organizations (FPO), contractual farmers, retailers, and logistics partners were collected from these districts. Information from retailers, wholesalers, cold storage agencies, APMC members, and export companies were collected fromAgriculture Produce Marketing Committee (APMC) Vashi, Mumbai.A survey was conducted in 2022 to gather data over the months of mid-April to June because the Alphonso harvesting season was at its peak at that time.

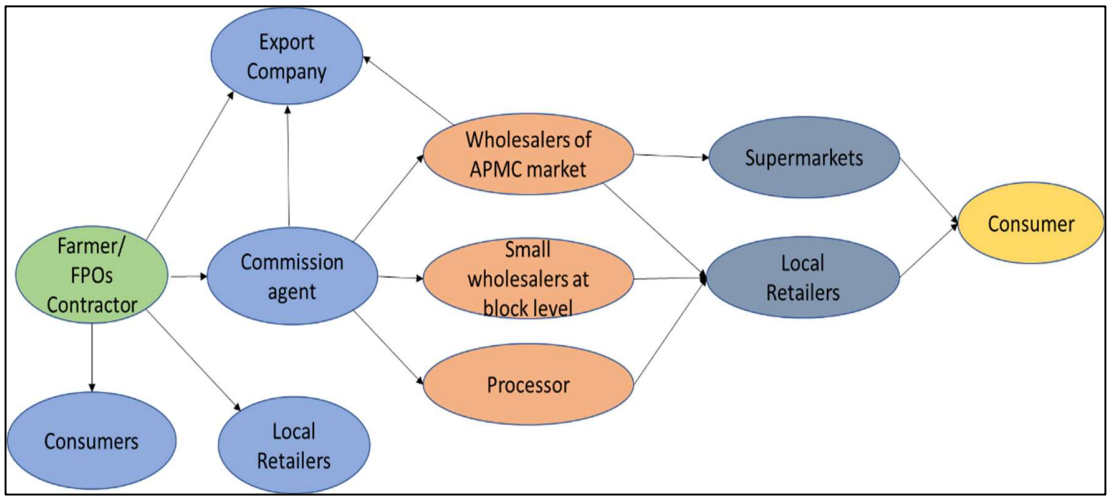

Figure 2: Stakeholders participated and marketing channels in supply chain of Alphonso mango in Konkan region

Table 1: Socio-economic characteristics of respondents

Stakeholder		Variable										
		Sex		Age (Year)			Experience (Year)		Education			
		Male	Female	<30	31-50	>51	<10	≥10	Illiterate	Primary	High. Sec	Graduate
Farmers	No.	49	6	8	38	9	25	30	4	5	16	30
	%	88.9	11.1	14.7	69.7	15.5	45.5	54.5	7.2	9	29	54.5
Wholesalers & retailers	No.	48	0	6	25	17	18	30	9	11	16	12
	%	100	0	12.5	52.0	35.5	37.5	62.5	18.7	22.9	33.3	25

Method of data collection

All stakeholders involved in the supply of mangoes were active in the state during the month of April, data collecting was initiated then. Different methods of gathering data were employed, including group conversations among farmers as well as observation, standardised questionnaires, checklists, and personal interviews. Table 1 lists the respondents' socioeconomic characteristics. Direct field visits and interactions with workers involved in the handling and processing of mangoes were conducted in addition to the aforementioned methods.Questionnaire created with the assistance of specialists in agricultural processing, fruit science, social sciences, and statisticians on the basis of the present issue related to the mango supply chain. Information gathering from farmers and neighbourhood merchants in the local tongue with the aid of an agent (a local educated individual well-known in English) and from other sources. Further data analysis is necessary because the stakeholder data were mixed in nature.Following that, data were divided into groups according to the type of information sources, such as farmers, dealers, exporters, logistic partners, agriculture officers, and APMC members, among others. Then, additional data analysis was performed using Excel and descriptive statistics including central tendency and frequency distribution. To visually depict the complete Alphonso mango chain in India, chain mapping was done.

Results and Discussion

Marketing and distribution system for Alphonso mango

Various stakeholders participate in the trading of mango shown in Fig. 2 during the operating supply chain, which is typically over long and fragmented and in which intermediaries take a substantial cost of what consumers pay for the fruit. There were primarily two supply chains that occurred during the distribution of Indian Alphonso mango: the first was the export supply chain, and the second was the domestic supply chain.

Export supply chain: Numerous parties are involved and given responsibilities for conforming to the formal specifications for quality export in compliance with the importing nations. Only grade 1 and 2 Alphonso mangoes are selected for export. Farmers that register their orchards with the Agriculture and Processed Food Products Export Development Authority (APEDA) in India, which is the supreme authority of the Indian government for overseeing and managing the import and export of agricultural commodities, sell their mangoes to export corporations. Following an assessment of the field, mango quality, maximum residue limit, pests, and diseases, export farms begin purchasing mangoes. In Fig. 3, the complete export supply chain is displayed.Send the sample for residue analysis, sanitary and phytosanitary certification, and Agmark quality assessment after obtaining it. Additionally, precautions are required to prevent fruit flies or spongy tissue disorders from infecting the farms or fruits. Young farmers (50 years old) were found to be more interested in exporting mangoes than older farmers during the survey. Nowadays, it was seen that more youthful farmers were working in the Alphonso mango industry. Table 1 shows that there were 84.4 and 15.6%, respectively, of younger and older farmers. The export of mangoes was found to be significantly impacted by the literacy rate.

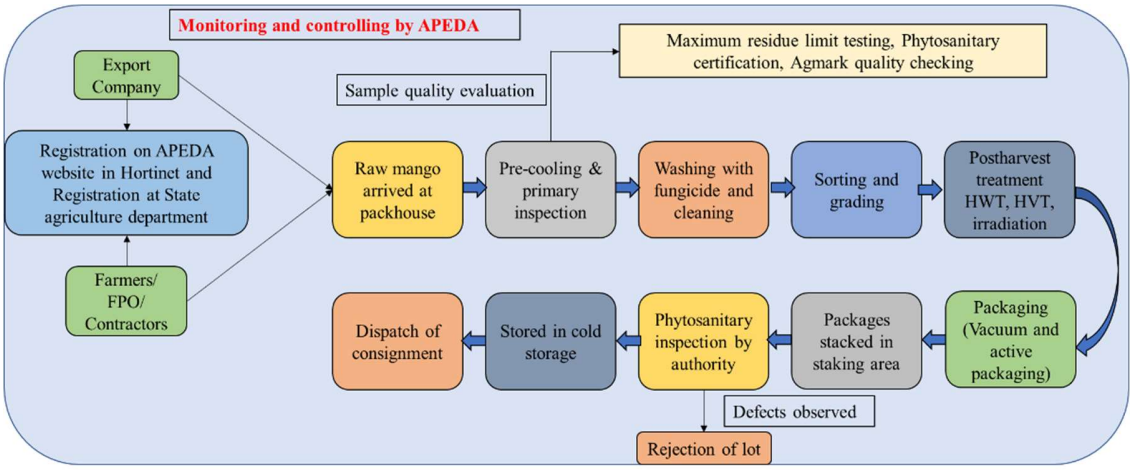

Figure 3: Export supply chain of Alphonso mango

In the study area, it was found that more literate farmers or business owners generate higher-quality product than illiterate individuals. More farmers and business owneri.e.,54.5 and 29%, respectively graduated or passed higher education. The biggest issue with older farmers is that they cannot adapt to changing circumstances or contemporary methods that are available on the market for high-quality produce. These findings more corroborate with results recorded by (Tadesse *et al.* 2018)they found that age, education, and experience significantly affect the quality of produce during value chain. Similarly,(Balyan*et al.*2015)examined dynamics of Indian mango export from 1990 to 2012. They noted that the Indian mango export business had a difficult time adopting the norms due to the high standards of sanitary and phytosanitary precautions from the importing countries.Truong and Sidique, (2022)investigated the specific cost structure, key supply chain players, and comparative advantage of Vietnam's Chu mango export supply chain.Similar study the impact of post-harvest practices on the quality of Ghana's mango export industry was studied by (Aboagye, 2009). They studied post-harvest practices, handling, value chain, and export chain in Ghana country.

Domestic supply chain: All of the leftover grades of mango were sold in the neighbourhood market. Farmers sorted and graded their products for the home market to get a better price from wholesalers. The APMC markets in Mumbai and Pune received Alphonso mangoes from farmers in the Sindhudurg and Ratnagiri districts. Each mango box was marked with the names of the farmer and the trader

who purchased the fruit from the farmer. Fig. 4 depicts the domestic supply chain and unit activities.Sending mangoes to wholesalers with government-issued licences was the initial and most popular method of distribution. After mangoes are delivered to the APMC market, wholesalers continue to compete for them with local retailers and others who bid higher prices to buy them. Mango prices were set through bidding, depending on the quantity and quality available. Wholesalers

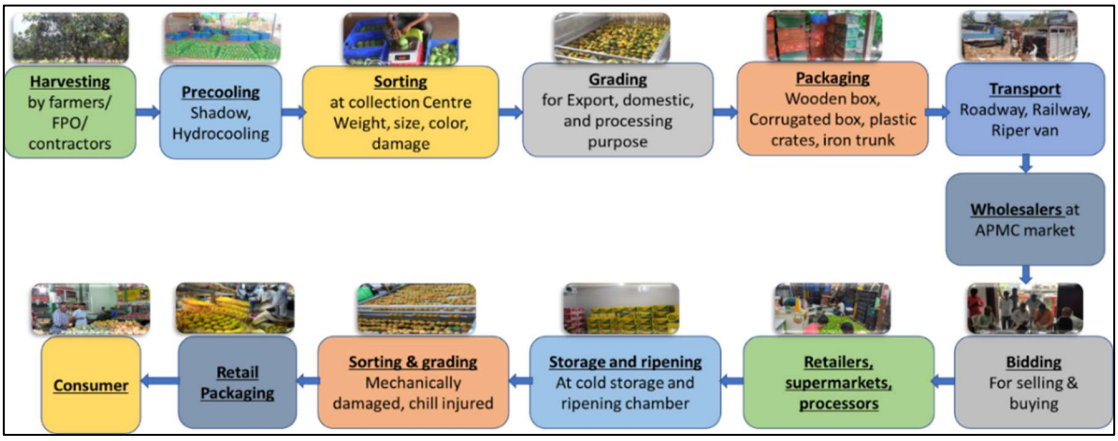

Figure 4: Domestic supply chain of Alphonso mango

occasionally offer farmers set pricing. Mango is sold by wholesalers to shops, supermarkets, restaurants, eateries, and even individual customers.After selling the mangoes, wholesalers reimburse the growers by reducing their own commissions by a certain proportion. The commissions range from 2 to 5% of the mango's overall cost. After purchasing the mangoes from the market, APMC market additionally charges the customers. Mango processors occasionally participate in the bidding process. Mangoes are a well-known fruit that is sold by seasonal small fruit vendors. There are a number of seasonal vendors who sell mangoes throughout the mango harvesting seasons. When selling the fruits to customers, they make a 10 to 15 percent profit.The second method of distribution involved certain local merchants contacting farmers or FPOs directly to purchase the mango in accordance with their needs. As a result, local shopkeepers benefited from higher pricing compared to those they received from wholesalers. then adhere to the same process as before. Farmers/FPOs selling mangoes directly to customers made up the third channel. Many people travelled to the tourist areas of Ratnagiri and Sindhudurg to visit these locations. As a result, farmers and FPOs have the chance to conduct business with consumers directly.Farmers that gather their own produce also keep some mango at their farmhouses before selling the fruits to the market. Traditionally, mangoes are stored for a week to ripen before being sold directly to clients. Customers receive authentic, fresh, and high-quality mangoes at a discount compared to market prices. With this kind of distribution strategy, farmers make more money. Social media allows them to share their contact information with clients and directly solicit orders from them. That claims to offer courier services for home deliveries. The fourth channel involved processors or the food industry purchasing mangoes directly from farmers or FPOs through commission agents. Mangoes that were rejected by the market or had mechanical damage, sap damage, or other defects were used for processing. The price of mango that processors acquire is determined by the weight of the mangoes. However, the first three distribution channels for mangoes are assessed on a count basis, or per dozen (12 pieces). Some public and private organizations offered the cold storage facilities. The cost for mango ripening is determined by the quantity of boxes and the number of days needed for ripening. They charge between 50 and 60 rupees a box for a box of four dozen, and between 20 and 30 rupees per box for a box of one or two dozen, according to the survey. Divide wholesale boxes into retail bundles of 1, 2, 3, or 4 dozen as well. Different sorts of mangoes are in high demand by

consumers. Some want completely ripe mangoes that are ready to use, others prefer mid-ripe mangoes that are ready for use in 3 to 5 days, while yet others prefer to purchase immature mangoes and use them once they have matured.Due to the process of handling and sale to customers, the retail sale of mangoes requires 7 to 10 days. Wooden or cardboard boxes with cushion made of paddy straw are chosen for retail packaging. Table 2 lists the packaging material along with its approximate losses and capacity. Found were wooden boxes with a capacity of 5–6 dozen and an iron trunk with a capacity of 7-8 dozen. About 42.5% of farmers in the Konkan region utilized wooden boxes to pack their mangoes, followed by 35.5% of farmers who used iron trunks, as shown in Fig. 5. Similar studies

Table 2: Packaging material used for Alphonso mango packing for marketing with its probable losses

Material	Capacity (dozen)		Losses (%)
	Wholesale	Retail	
Wooden box	5-6	3,4,5	5-6
Iron trunk	7-8	-	8-7
Plastic crate	5-6	2-3*	5-6
Cardboard box	3-4	1-2	10-12
Plastic bag	-	1-2	10-15

***Explains use & throw type crates**

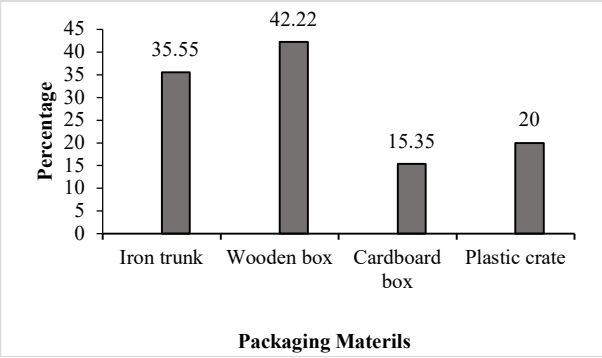

Figure 5: Packaging material used for Alphonso mango

were conducted for mango value chain in Ethiopia (Tarekegn and Kelem, 2022), for supply chain of grapes in Fiji (Morris *et al.* 2014). They found that two or more channels observed in domestic supply chain. Same experiment conducted by (Alam, 2018) for supply chain and value chain of different varieties of mangoes in Bangladesh by. They discovered that wholesalers and retailers dominated the mango supply chain and hold 54.6 and 23.29% share in total value, whereas producers holding the smallest part i.e., 22.35 only.

Factors affecting fruit losses at various levels

Preharvest losses (Fruit development and maturation)

One of the most fundamental and crucial elements that affects both the amount and quality of mango production is climate change. since both the timing and quality of flowering are entirely dependent on the temperature. Storms, rainfall, temperature, and other factors all had a significant impact on the number of fruits produced per tree, management of the harvesting season, and fruit quality. Farmers sustained significant losses over the past three years as a result of the unpredictable rainfall patterns, rapid global warming, and frequent hailstorms. Because of natural events, these losses cannot be prevented. Hailstorms cause mango to sustain mechanical damage and experience a heat shock that alters the fruit's physiology.The quality and financial losses are accelerated by the fruit fly infestation and the abnormally spongy tissue, which is specifically seen in Alphonso mangoes. Spongy tissue issues greatly increased when the local temperature rose above 35 to 36 C. In the survey, it was found that pre-harvest losses in Alphonso mangos were largely caused by spongy tissue and fruit flies, accounting for about 30 to 35% of losses, followed by infestations with insects, pests, and illnesses, accounting for about 12% of losses (Fig. 6).

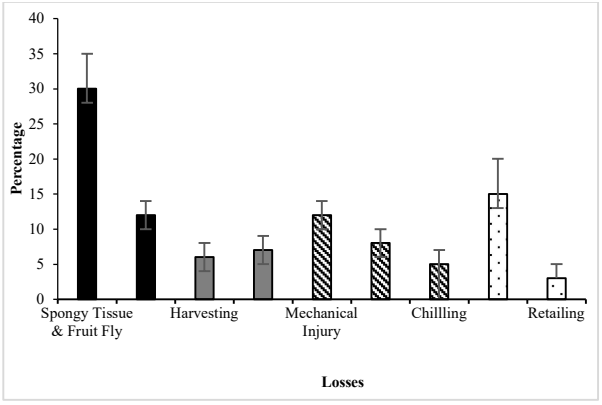

Figure 6: Share of each factor in pre and postharvest losses in Alphonso mango supply chain

Farmers overused fertilizers, pesticides, manures, and compost, which negatively impacted the nutritional value and internal quality of the crops. Mango growers now heavily rely on cultar to improve production, which has an adverse effect on the physiology of the tree and the fruit's quality. Farmers did not receive accurate and consistent weather forecasts, advice on fertilizer application rates, or market supply and demand data. Due to the aforementioned issues, the quality of Alphonso mangos declined, which decreased consumer demand and caused the export supply chain to collapse. Similar results recorded by (Bantayehu*et al.* 2019) in Ethiopia for tropical fruits and found similar reasons responsible for pre-harvest losses of agricultural commodities. Similar results were recorded by (Tarakegn and Kelem, 2022) for pre-harvest losses observed in mango supply chain of Ethiopia.

At the harvesting stage

Mango plantations were trained and pruned to achieve optimal yield that was simple to harvest and spray. However, some farmers do not follow these procedures, which causes difficulties when harvesting. In addition to scientific procedures like shaking trees, throw-and-catch methods were occasionally employed, which led to greater harvesting losses of 8 to 10% over modern techniques that only recorded 3 to 5% losses. Mangoes in the bottom part of the tree were picked by hand, and fruits in the top of the tree were harvested using cutting-edge equipment such blades coupled to nets and stick setups.The mango has a strong impact when it hits the ground immediately. As a result, the mango's physiology suffered a significant alteration, and the dropped mango rotted during ripening. The possibility of mangoes on the tree ripening rose due to the high temperatures, therefore growers kept constant surveillance on each tree to prevent losses. In order to minimize mechanical damage, Alphonso mangoes are typically harvested between 70 and 80% of their full ripeness before being sold to consumers. Similar observations were recorded by (Tian *et al.* 2010; Gianguzzi*et al.* 2021). Sometimes very immature mangoes were harvested to reach in good condition and with minimum damage. But harvesting at an early-stage flavor and taste of mango badly affects because there was no proper retention of calories and production of acids for imparting a good taste to mango. During harvesting, fruits were kept in open spaces so they were exposed to sunlight which significantly contribute to postharvest losses. Similar reasons found for losses due to the field heat. There were high chances of bruising, impact, and mechanical injury during carrying mango from the farm to the collection center/ packhouse due to rough handling by laborers. So, the harvesting losses mostly depend upon literacy and experience of farmers. These results were highly correlated with (Ullah *et al.* 2010; Siddiq *et al.* 2017; Troung*et al.* 2022) they showed that higher harvesting losses were estimated in developing countries due to the improper harvesting management and similar results observed in this study.

At the producers' stage (Handling)

The Konkan region had high relative humidity and temperatures reaching up to 39 to 42 °C during the Alphonso mango harvesting season. Because of these weather factors, precooling is essential for eliminating field heat. However, there are no pre-cooling facilities for farmers in Ratnagiri and Sindhudurg. They pre-cooled using hydro cooling or shadow cooling. However, these approaches lacked significant effectiveness. Farmers occasionally begin packaging right away after harvesting. It causes a rise in respiration rate and activity related to ripening. Fungicides were applied to mango to protect it from fungal infections, however some growers used unlicensed fungicides, endangering human health. Mechanical damage, cuts, and punctures happened when handling fruits for sorting, grading, and packaging. Worker observations of rough handling due to a rush to get the produce ready for marketing include bypassing cleaning and washing, packing without padding, etc. Table 3 displays the weight-based sorting of mangoes and their approximate market prices in India. Due to a lack of ripening chambers, producers may sell mangoes directly to nearby stores or consumers. Sometimes they employed the conventional method of ripening, which involved enclosing the mango in paddy straw for seven to nine days. because to poor ventilation and fungus and pathogen attacks. According to the conventional procedure depicted in Fig. 7, ripening losses of up to 15% were noted. 10 to 12% of the mangoes handled at the collecting centre were wasted, as indicated in Fig. 6. As a result,

substantial losses were seen at this time. Similar results were witnessed by (Yahia, 1999; Baltazari *et al.,* 2020) for postharvest losses of mango in Egypt and Tanzania.They showed that handling of mango after harvesting is very crucial stage and impacts the further postharvest life of mango fruit.Similar results regarding postharvest disease losses were recorded by (Prabakar *et al.* 2005).

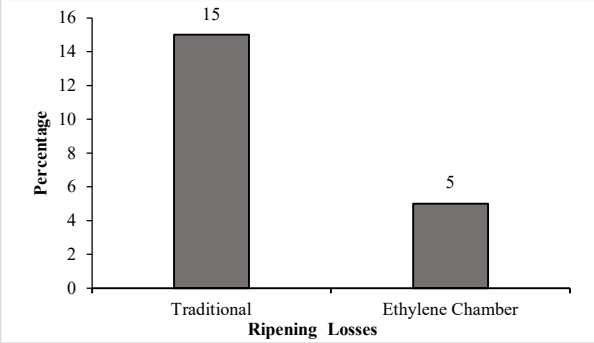

Figure 7: Losses observed in different ripening methods

Marketing stage losses

The transport department's job was to gather mango boxes from collector centres or packhouses and put them onto vehicles to be sent to the APMC market. Mountainous coastal districts with considerable rainfall include Ratnagiri and Sindhudurg. Due to these, there were winding roads across the highlands that were in poor shape and slowed down traffic. Due to traffic and poor roads, it took 10 to 12 hours to travel 300 to 400 kilometres to the Mumbai and Pune APMC markets to purchase mangoes.The hot, humid conditions during mango harvesting season also causes an increase in respiration rate and physiological weight loss (PLW) while handling and transit, both of which have a negative impact on the mango's quality. Trucks that were not ventilated were typically employed for transportation. According to Fig. 8, 75% of mangoes were transported by non-ventilated trucks, 15% by reefer vans, and 7% by railroads. For getting more profit transporters to put more loads out of capacity in each vehicle which increases injury and bruising to mango. Similar results were recorded by (Malik *et al.*2015). They found that due overloading of boxes in vehicles significant quality losses were occurred. During the marketing of mango highest losses were observed due to mechanical injury followed by transport losses followed chilling injury 12, 8 and 5% respectively shown in Fig 6. Similar study in Ethiopia was conducted by (Desalegn *et al.* 2016) and found that due limited infrastructure and improper postharvest handling had limits for processing.Alam, (2018) reported similar reasons for losses in mango on Bangladesh and recorded about 20 to 25% losses during marketing.

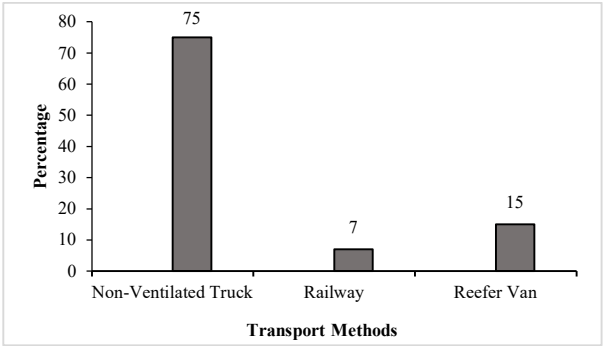

Figure 8: Transport methods used for marketing of mango.

At retail trading stage losses

Small business owners who buy mango directly from growers or occasionally through wholesalers during retailing. Retailers purchase unripe mango from farmers or wholesalers to lower the risk of bruising and mechanical damage.The primary challenge for retailers is the availability of transportation facilities to get goods from farms or marketplaces to cold storage or ripening chambers. Back to their businesses from the ripening room. As a result, produce is loaded and unloaded two to three times, resulting in higher postharvest losses of up to 12 to 15%, as shown in Fig. 6. There were no ripening chambers available for ripening or storing mango up to reach consumers during the peak season. This, given its perishable nature, raises the likelihood of losses and degradation. Fig. 9 depicts several ripening techniques applied throughout the supply chain. Mango ripening chambers were mostly utilized by retailers. There were no ripening chambers accessible at the busiest times for ripening or storing mangoes up to reach consumers. due to its perishable nature, it raises the risk of losses and degradation. In Fig. 9, various supply chain ripening techniques are depicted. Ripening chambers were mostly utilized by retailers for

mango ripening. Similar results were observed by (Yasunaga *et al.* 2012; Yasunaga *et al.* 2018) showed that elevated temperature during distribution accelerate the losses of mango. Retailers also suffered costs as customers rejected mangoes that were sometimes overripe due to consumer preferences. Mango losses owing to senescence and handling by consumers were up to 15% and 3%, respectively. Produce in the mango supply chain passes via a number of stakeholders before reaching consumers. Therefore, due to unclean conditions while handling, packaging, and selling, product suffers mechanical damage and is attacked by bacteria, which causes the quality of mangoes to rapidly decline. These findings are

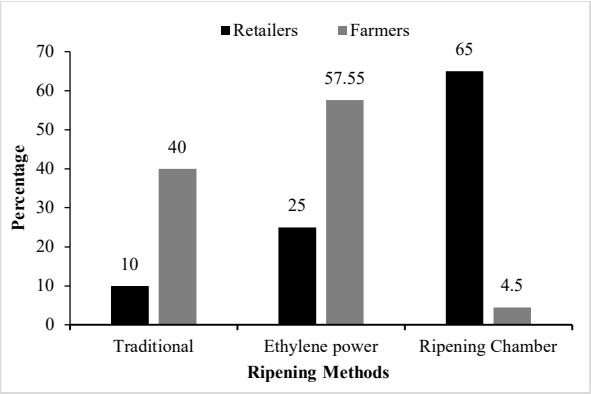

Figure 9: Ripening techniques used in supply chain of Alphonso mango

Table 3: Quality grades of Alphonso mango according to weight

Grade	A1	1	2	3	4	5	6
Weight	>300	250-300	225-250	200-225	175-200	150-175	<150
Average selling price (Rs/dozen) in the year 2022	>800	700-800	550-700	400-500	350-400	300-400	300-350

more in line with research by Siddiq *et al*. (2017). Challenges faced by mango farmers and stakeholders in Gamo zone of Ethiopia studied by (Tarekegn and Kelem, 2022). They evaluate the post-harvest losses along the mango value chain as well. Proper orchard management methods, harvesting techniques, packing techniques, postharvest treatments, temperature control, transportation and storage conditions, and destination ripening are just a few of the variables that affect mango fruit quality and shelf life along the supply chain. (Truong *et al.* 2022).

Conclusion

According to the findings of this study, if more efforts are made to reduce diseases and postharvest losses, all supply chain participants will profit more and farmers' income will also rise. Major actions including reduced production costs, integrated pest, nutrient, and water management, infrastructure building, and efficient marketing should be combined to develop FPOs in order to reap greater benefits. Because it was discovered during the survey that participating FPO farmers received higher benefits than independent farmers. Farmers lost their rights to fair pricing as a result of intense haggling between them and traders. Farmers should get training through field demonstrations, training, and seminars offered by exporters, APEDA, government departments, etc. in order to preserve the export quality of mangoes and raise awareness about GAP, phytosanitary requirements, and postharvest treatment. Spongy tissue and fruit flies were found to be the biggest impediments to a reliable export supply chain during the survey. To strengthen the supply chain for Alphonso mangoes, more study and effort are needed to identify and eradicate spongy tissue and fruit fly problems. Farmers had to contend with a variety of issues, including climate change (heavy and erratic rainfall and hail storms, high temperatures, etc.), adulterated or duplicate input materials (fertilisers, pesticides, fungicides, etc.), insufficient knowledge of marketing tactics, and a lack of infrastructure facilities, such as modern packhouses, ripening chambers, cold storage, logistic facilities, etc. Produce quality and hygiene are directly impacted by the absence of the aforementioned facilities. Postharvest losses were quite significant in developing nations like India. For farmers and FPOs to adopt new and highly recommended techniques and expand infrastructure, the government should offer subsidies and funding. For the domestic supply chain to run well, basic amenities like cleanliness, storage space, cold storage, and knowledge of daily rates for mangoes must be made available in the APMC market.

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Conflict of interest

The authors declare that they have no conflict of interest.

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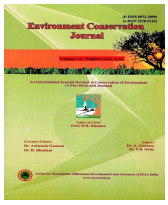

Evaluation of different management practices against yellow Sigatoka disease of banana (*Musa* spp.) caused by *Mycosphaerella musicola* Leach

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ABSTRACT

Leaf spot or yellow sigatoka disease (caused by *Mycosphaerella musicola*) of banana causes significant yield loss as well as in quality of fruits in every year and reported up to 65% or even more under favorable epidemic conditions. In this perspective, an experiment was conducted at ZBNF project Research field, college of horticulture, Sirsi (Uttara Kannada district of Karnataka) for consecutive two seasons (2020-21 & 2021-22). Experiment accompanied with RBD statistical design with five replications and four treatments. Four different management practices involving *viz.* Propiconazole 25EC @ 0.1% (Recommended package of practices-UHS, Bagalkot), *Trichoderma harzianum* 10g/lit. (Organic farming), sour butter milk 5 lit. per 200 liter of water (Natural farming) and Tebuconazole 50% + Trifloxystrobin 25% WG @0.5gm/lit. (Chemical farming) were evaluated against sigatoka leaf spot disease. Among the management practices, chemical farming comprises tebuconazole 50%+ trifloxystrobin 25%WG@1gm/lit was found effective in managing the disease (12.38% PDI) followed by recommended package of practice comprises propiconazole 25%EC @1ml/l (16.33% PDI), organic farming comprises of talk-based *Trichoderma harzianum* 10g/lit (17.33% PDI). Natural farming showed least effective to combat disease recorded maximum disease severity (19.66% PDI) after 210 days after planting. Although chemical farming can effectively control the disease but results in the serious risk on human health and environmental hazards. Therefore, organic and natural farming are an alternative approach that are eco-friendly and economically viable against sigatoka leaf spot disease management.

Introduction

Banana is cashcrop for small land holding farmers which belong to the family *Musaceae*, native to the Malaysia-Indonesian region of South-East Asia. It is widely cultivated in tropical and subtropical countries in the world.Among several diseases affecting banana plants, yellow Sigatoka leaf spot is a seriousfungal disease that involves ascomycetes fungi *viz*. *Mycosphaerella musicola* (anamorph:

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Pseudocercospora musae)leads to 11-80% yield losses in banana by reducing the photosynthetic tissues through necrotic leaf lesions (Shanthiyaa *et al.,* 2013).Initial symptom of the disease comprises appearance of pale yellow or dark brown indistinct linear streaks parallel to the veins on the lower leaves. In advanced stages,these streaks enlarge to form necrotic lesions with yellow halo andlight grey centers (Stover, 1972). These necrotic lesions later coalesce, causing complete drying of the leaves and defoliation, leading to delayed flowering, reduction in the number of hands andfingers, premature ripening and peel splitting of the fruits, small sized unmarketable bunches with subsequent post-harvest spoilage effects (Surridge *et al*., 2003; Selvarajan *et al*., 2001).Conidial morphology mostly cylindric, occasionally obclavate, 1-5septate, same thickness throughout length no distinct basal hilum (Borah *et al*., 2020). When leaves with rapidly developing young mass spots of black sigatoka were left lying on the ground to decay, discharge of ascospores was observed within 5 days and continued as long as 23 days. Ascospores of *M. musicola* survived as long as 8 weeks in the shade on leaf tissue above the ground (Stover, 1971). Time taken for ascospore survival on leaves lying on the ground in the shade depends on the rate of decomposition. Fallen leaves on ground if they subjected intermittent rainfall, dew and partial drying in the daytime continue to discharge ascospores in declining amounts for up to 4 weeks. Chemical control by spraying fungicides on a preventive or curative basis isthe only acceptable strategy adopted in the management of this disease.Certain fungicides belonging to groups benzimidazole, dithio carbamates, strobilurins and triazole are currently used in managing Sigatoka disease in India. Continuous use of the systemic fungicides is reported to increase the riskof development of resistance to these fungicides in *Mycosphaerell amusicola* (Hermanto *et al*., 2010; Oliveira *et al.,* 2022) apart from the environmental pollution entailing from the rigorous application ofthese chemicals.There are only few reports on the satisfactory results obtained inmanaging Sigatoka diseases by including non-chemical methods such as the application of *Trichoderma harzianum* and sour butter milk which comprises millions of beneficial *Lactobacillus* bacteriaare component of the integrated diseases management of Sigatoka leaf spot (Aman and Rai, 2016; George and Cherian, 2020). As of now in this particular banana crop, none of the literatures comprised sour butter milk as one among the organic approaches to manage sigatoka disease moreover it is a sustainable approach for crop production. Hence these taking into account, present study was to evaluate of different farming systems management practices to combat Sigatoka leaf spot disease in banana.

Material and Methods

The field trial was carried out at experimental field of Natural farming project (Zone-9), College of Horticulture, Sirsi (Latitude 14.6039° N and longitude 74.8467°E) Uttara Kannada district of Karnataka, India during 2020-2022. The experiment was laid out in randomized block design with 5 replications using Yelakki variety of banana. Tissue culture plantlets were used for planting with the plant to plant and row to row spacing 2.5m x 1.8m, respectively.The fertilizers were applied at the rate of N:P:K- 400:240:500 Kg/ha. and FYM at the rate of 40 T/ha. Other intercultural operations were practiced as recommended by University of Horticultural Science, Bagalkot for commercial cultivation of banana. Treatment were imposed after appearance of symptoms and severity was recorded subsequently at 30 days of intervals. It included spraying of Tebuconazole 50%+ Trifloxystrobin 25% WG @0.5gm/lit. from chemical farming propiconazole 1ml/lit. from recommended package of practice. *Tichoderma harzianum* 10gm/lit. (talc-based formulation) from organic farming andsour butter milk (5liters per 200 liters of water) from natural farming.

Measurement of Sigatoka leaf spot disease severity index in banana

To find out the effect of treatment to combat disease severity, which is expressed in terms of Percent disease index (PDI). For that, plants were selected for observation excluding the border pants in order to avoid the border effect. Observation were taken for two years at monthly intervals amid the time of treatment imposition to harvest of the crop. A 0-6 disease scale was followed for scoring the sigatoka leaf spot symptoms and the schematic representation of Gauhl's modification (1993) of the Stover's severity scale (1972b) (Carlier*et al*.,

2002). Description of each grade is also shown in Table 1. The total number of leaves was scored as per the scale based on the area of infection. Using the score values the extent of infection was estimated based on the proportion of the area affected by the leaf spot infection in relation to the total leaf area and calculated the disease index using the Gauhl's formula (1993) modified from Stover's disease severity scale.

Percent Disease Index (PDI):

$$\frac{sum\ of\ all\ disease\ rating}{Total\ number\ of\ ratingx\ maximum\ grade\ in\ disease\ score}\ X\ 100$$

Table 1: Disease score scale for yellow Sigatoka disease given by Carlier *et al.* (2002)

Score	Description
0	No symptoms
1	Not more than 1% of leaf area affected
2	Less than 5% of the leaf area affected
3	From 6 to 15 % of the leaf area affected
4	From 16 to 33% of the leaf area affected
5	From 34% to 50% of the leaf area affected
6	More than 50% of the leaf area affected

The area under the disease progress curve (AUDPC) is a helpful quantitativemeasurement of disease intensity over period, comparison across years, management strategy.The trapezoidal method, which is the most popular technique for determining the AUDPC, involves discretize time variable (hours, days, weeks, months and years) and determine the average disease's intensity prevalence between each pair of adjacent time points (Madden *et al.* 2007).We can consider the sample time points in a sequence $\{t_i\}$, where the time interval between two time points may be consistent or fluctuate and we also have associated measures of the disease level (y_i). We define y (0) = y_0 as the initial infection or the disease level at t = 0 (*i.e.,* the first disease severity observation in our study). $A(t_k)$, the AUDPC at $t = t_k$, is the total accumulateddisease until $t = t_k$, given

$$A_k = \sum_{i=1}^{N_{i-1}} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

Statistical analysis:

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique as per the method described by Gauthier and Hawley (2015).

Results and Discussion

In the absence of resistant cultivars in banana, use of fungicides to manage the disease is an old-age practice and now it becomes the prominent component of integrated disease management strategy however due to environmental and health hazards, fungicides have been a concern since they were introduced in agriculture hence present study comprised the treatment of sour butter milk, is an alternative to pesticides for resistance management in fungus. Sigatoka is not one among the catastrophic disease of banana and prevalent thought-out the worldwide. an experiment was conducted to evaluate the effectiveness of different management practices. The effects of the different management practices showed consistent trends in efficacy during the two consecutive years of evaluation. The perusal of data pertaining to Table 2 and Table 3 revealed that among the four different management practices to combat Sigatoka leaf spot disease in banana. During 2020-21, chemical farming (Tebuconazole 50%+ Trifloxystrobin 25% WG @ 0.5gm/lit.) found effective against managing disease with significant decrease in disease severity (9.90% PDI) at 210 days after planting which was followed by recommended package of practices (12.50% PDI) and organic farming (18.75% PDI). More severity of disease was noticed in natural farming (21.85% PDI). During 2021-22, similar trend was observed with a least disease severity was observed in chemical farming with a tone of 10.12% PDI.These resultsarein confirmatory with Ruth and Nagalakshmi (2017) were conducted field trial comprises of eleven different fungicides among that tebuconazole 50% + trifloxystrobin 25% WG @ 0.5 g/lit. was found effective against Sigatoka leaf spot of banana. Apart from chemical farming, recommended package of practice (12.52% PDI) showed effective. The recoded observations are in parallel with Pradhan *et al*. (2020) and Paresh (2009) who observed propiconazole was found effective against banana leaf spot disease.In organic farming disease severity was recorded with 15.61% PDI.Dattatray (2013), Castro (2015) and Samuelian (2016) are found *Trichoderma harzianum* potential for controlling yellow sigatoka leaf spot of banana.

Table 2: Evaluation of different management practices against Sigatoka disease of bananaduring 2021& 2022

Treatment		Per cent Disease Index (PDI)									
		2020-21					2021-22				
		Before spray	120 DAP	150 DAP	180 DAP	210 DAP	Before spray	120 DAP	150 DAP	180 DAP	210 DAP
T_1	RPP	29.50±0.58 (5.86)	18.94±0.73 (4.34)	18.06±0.82 (4.24)	17.18±0.77 (4.14)	12.50±0.58 (5.86)	31.50±0.58 (5.86)	17.95±1.25 (4.23)	16.31±0.62 (4.03)	15.74±0.82 (3.96)	12.52±1.18 (3.93)
T_2	Organic farming	31.75±0.72 (6.25)	21.16±0.42 (4.59)	20.12±0.75 (4.48)	19.10±0.69 (4.36)	18.75±0.72 (6.25)	33.75±0.72 (6.25)	19.10±0.69 (4.36)	18.97±0.80 (4.36)	17.15±0.74 (4.13)	15.61±0.73 (4.08)
T_3	Natural farming	28.85±0.84 (5.92)	26.21±0.76 (5.11)	24.11±1.08 (4.91)	22.60±0.67 (4.75)	21.85±0.84 (5.92)	30.85±0.84 (5.92)	27.17±1.12 (5.21)	22.80±0.58 (4.77)	20.99±1.29 (4.57)	18.67±0.82 (4.31)
T_4	Chemical farming	30.90±0.57 (6.05)	16.31±0.62 (4.03)	15.98±0.92 (3.99)	15.73±0.92 (3.95)	9.90±0.57 (6.05)	29.90±0.57 (6.05)	13.89±0.65 (3.72)	13.46±0.60 (3.66)	12.65±0.81 (3.54)	10.12±0.72 (3.33)
S.Em±		0.73	0.06	0.12	0.09	0.10	0.73	0.18	0.14	0.13	0.07
C.D @ 5%		0.21	0.19	0.30	0.24	0.31	0.21	0.37	0.34	0.34	0.22

T1-RPP (Recommended Package of Practice): Propiconazole 25EC @ 1 ml/l T2-OF (Organic farming): *Trichodermaharzianum*(talk based) 10g/lit
T3-NF (Natural Farming): Sour butter milk (5l per 200 l of water) T-4 CF (Chemical Farming):Tebuconazole 50%+ Trifloxystrobin 25% WG @ 0.5 gm/lit.
Note:DAP- Days after planting, Figures in the parenthesis are square root transformed values.

Table 3: Pooled Per cent disease incidence of Sigatoka disease caused by banana

Treatments		Management practices	Per cent Disease Index* (PDI)				
			120 DAS	150 DAS	180 DAS	210 DAS	AUDPC
T_1	RPP	Propiconazole 25EC @ 1 ml/l	18.44±0.54 (23.02)	17.18±0.54 (23.54)	16.46±0.84 (22.10)	16.33±0.58 (20.58)	2637.15
T_2	Organic farming	*Trichoderma harzianum* (talk based) 10g/lit.	20.13±0.37 (24.19)	19.54±0.69 (25.18)	18.12±0.72 (26.23)	17.33±0.49 (26.65)	2899.50
T_3	Natural farming	Sour butter milk (5lit. per 200 lit. of water)	26.69±0.80 (26.31)	23.45±0.85 (27.81)	21.79±0.75 (28.95)	19.66±0.87 (31.09)	3653.85
T_4	Chemical farming	Tebuconazole 50%+ Trifloxystrobin 25% WG @ 0.5 gm/lit.	15.10±0.46 (23.80)	14.72±0.62 (23.81)	14.19±0.83 (24.74)	12.38±0.68 (24.59)	2185.50
S.Em±			0.47	0.52	0.60	0.35	-
C.D @ 5%			1.45	1.60	1.85	1.10	-

Note:DAS- Days after sowing, PDI- Per cent disease index, Figures in the parenthesis are arc sine transformed values, Figures with same alphabetical superscripts are statistically non-significant (p<0.05) by DMRT. * Pooled data of two years, AUDPC- Area under disease progress

Maximum disease severity was noticed in natural farming (18.67% PDI) at 210 days after planting. (Samuelian2016). Pooled disease severity of two years experimental period resulted that least disease severity was noticed in chemical farming (12.38% PDI) with 2185.50 AUDPC followed by recommended package of practice (16.33% PDI) with 2637.15 AUDPC and organic farming (17.33% PDI) with 2899.50 AUDPC. Maximum incidence was noticed in natural farming treatment with sour butter milk (19.66% PDI) with 3653.85 AUDPC. Chemical farming showed effective management against Sigatoka leaf spot disease of banana with an irrespective time intervals after spraying. While natural farming found least effective when compare to other treatments.

Conclusion

In the present studies, Among the evaluated management practice, the fungicides spraying of tebuconazole + trifloxystrobin was showed effective which is followed by propiconazole under field condition due to specific mode of action of fungicides and translaminar moment of systemic fungicides can effectively suppress the disease by fungistatic and antisporulant mechanisms. However, application of *Tichoderma harzianum* and sour butter milk not found effective compared to fungicides. Even though sour butter milk which is component of natural framing was not found effective but it is an alternative, ecofriendly, sustainable approach to combat disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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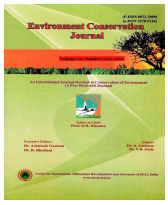

Characterization of a cotton interspecific hybrid of American cotton with wild species *G. armourianum*

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ABSTRACT

Wild species constitute a source of valuable genes for many adverse climatic conditions, disease and pests. To match up the level of quick depleting insect pest and disease resistance and fast evolving pests, it is the urge of the hour to broaden the resistance base. In order to achieve this goal in cotton, wide hybridization was performed between *G. hirsutum* (AADD) cv. MCU5, CO14 and CO17, and *G. armourianum* and interspecific hybrids developed were characterized for several morphological characters for obtaining an idea about the status of the various traits. Interspecific hybrid developed with all three *G. hirsutum* varieties are potential lines for future introgression programs of insect and disease resistance along with other useful traits. The F_1 hybrid displayed intermediate expression for most of the traits. Traits like colour of the stem, leaf colour, position of stigma, nectarines of hybrid completely resembled wild parent-and are considered as dominant in expression. The petal spot was present in the hybrid similar to that wild parent, unlike the cultivated parent; this appeared with different levels of intensity in F_1 along with other characters like colour of the anther, and filament colour. Hybrids had profuse flowering throughout the year with low pollen load and pollen of variable shape and size expressing sterility to partial fertility. Noteworthy differentiation was seen between the leaf size and size of other plant parts of the hybrid.

Introduction

Botanically, cotton belongs to the genus *Gossypium* and the family Malvaceae. About 50 species constitute this genus, including five allotetraploids (2n = 4x = 52) and 45 diploids (2n = 2x = 26) (Gotmare *et al.,* 2000). All four cultivated species are grown commercially in India (Blaise and Kranthi, 2019). Among the D genome diploid wild species counting to 13 in number, *G. armourianum,* is resilient towards a variety of insects, pests, and diseases, especially hazardous pink bollworms and jassid (Narayanan *et al.*, 2014; Pushpam and Raveendran, 2006; Kaur *et al.*, 2016). Numerous instances have demonstrated the significance of cultivated as well as wild *Gossypium* diploid genomes as reservoirs of commercially valuable genes (Mehetre *et al.*, 2002; Kebede *et al.*, 2007). To harness the useful features of the wild germplasm, wide hybridization with a further advanced tool to recover the fertile progeny constituting valuable pre-breeding lines are the method of choice by the breeders.

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The cultivated variety chosen for the study has moderate to high susceptibility to the various sucking pests such as cotton jassid, aphids and whitefly, which not only causes loss directly by sucking the sap and burning the surface of the leaf but also attract secondary infections. Differentiating the hybrids is possible at very early stage of crop growth with the presence of sufficient and strong morphological markers. Previous reports suggest the utility of various qualitative and quantitative characters of different parts like a leaf; stem flower characters in differentiating the triploid progeny from tetraploid and diploid parents (Manickam and Prakash, 2014).

As the aim of the study was to develop prebreeding lines and transfer of the useful resistance genes present abundantly in the wild species, the crosses were made and all the character starting from the leaf related to stem related, all were carefully observed. Morphological observation is given priority in this experiment considering the fact that, in wide hybridization it is rare to achieve the combinations with desirable trait. Hence, before moving for further generation and undergoing any expensive molecular breeding program for further transfer of the traits, morphological characterization can give sound amount of idea about the status of visually expressive traits.

Material and Methods

The wide hybridization was carried out between *G. hirsutum* cv.MCU5, CO14 and CO17, and *G. armourianum* during *Summer,* 2021, *Summer*, 2022, and *Kharif*, 2022. MCU5 is a multi-cross derivative famous for its extra-long staple length obtained from the germplasm maintained at TNAU, Coimbatore. The CO14 and CO17 are the improved high yielding variety of American cotton developed at the Department of Cotton, TNAU, Coimbatore. The wild species *G. armourianum* is maintained as perennial at the wild species garden at Department of Cotton, TNAU, Coimbatore. The flowers were emasculated at the candle stage, the previous day of anthesis by removing anthers by the Doak's method (Doak, 1934). Since huge numbers of crosses were to be attempted in wide hybridization, considering ease of handling, the Doak's method was followed. The next day morning, the emasculated flowers were dusted, and the buds were covered with a white butter paper bag. The dusting date along with the parentage of crosses recorded on jewel tags.

For gathering information on several morphological aspects, the F_1 hybrid and its parents were examined. The traits like growth habit of the plant, coloration, extent of hairiness of stem shape, lobe number, size, pubescence, presence of nectarines in leaf, the shape of the bract, and the number of serrations on it were observed. Apart from these traits, the other aspects of plants like colour, presence of spots in petals, pigmentation in filaments, colour and average no. of the anther, pollen size, and boll shape were also examined. All the characters were observed on fully developed plants. The presence or absence of pubescence on the stem and leaves was recorded. On the abaxial surface of the leaves, at the midrib, the number of nectarines was counted.

Results and Discussion

Table 1 depicts the morphological features of the parents, *G. hirsutum* varieties, *G. armourianum*, and their hybrid progenies. For a series of traits, hybrids displayed either dominance from one of the parents or intermediate expression. It was also revealed that in the hybrid, growth habits, petal coloration, the shape of the leaf, and leaf size and incision were all at the intermediate level (Table 1). Parallel result was obtained in the experiment performed by Mahalingam *et al.* (2020). The intermediate level of expression and availability of a wide range of trait expression for each of them shows probable polygenic nature of these traits.

All the cultivated species used in the crosses were annual and erect type in nature whereas, the diploid male being the wild species is naturally perennial and spreading in growth habit. CO17 parent had the most erect stem and occupied the least area among the cultivated ones. *G. armourianum* is a very spreading one as far as branching is concerned. It was not much tall but heavily spreading. The hybrids have unanimously shown the perennial tendency as that of the wild parent. But the plant spread showed intermediate expression and semi-spreading in nature. The wild parent had a brownish-purple stem whereas, three cultivated parents all had a greenish-brown colour. The F_1 across three crosses had a brownish to the grayish purple stem. Glabrous stem surface was there in the wild parent but, the cultivated parents varied a lot

Table 1: Characterization of the different hybrids produced in combination with *G. hirsutum*-parents and *G. armourianum*

SN	Characters	*G. armourianum*	CO14	CO14 x *G. armourianum*	MCU 5	MCU 5 x *G. armourianum*	CO17	CO17 x *G. armourianum*
1.	Growth habit	Perennial, spreading	Annual, Erect	Perennial and semi spreading	Annual, erect	Perennial, Semi spreading	Annual, Erect	Perennial shrub
2.	Stem colour	Brownish purple	Greenish brown	Brownish purple	Dark green With brown	Brownish purple	Dark green With brown	Brownish green
3.	Stem pubescence	Absent	Moderately pubescent	Moderately pubescent	Sparsely pubescent	Moderately pubescent	Sparsely pubescent	Moderately pubescent
4.	Leaf shape	Ovate	Palmate	Palmate to ovate	Palmate with 3-4 lobes	Palmate with slight lobes	Palmate with 3-4lobes	Palmate with 2-3 lobes
5.	Leaf colour	Dark green	Green	Dark green	Green	Dark green	Green Light green	Dark green
6.	Leaf lobe	No lobe	Check	1-3 lobe	Five	2-4	Five	2-4
9.	Leaf incision	Shallow	Deep	Moderate deep	Shallow to Moderate deep	Shallow	Deep	Shallow or absent
10.	Leaf veins	Thin	Thick and prominent	Thick and Prominent	Thick and prominent	Thick and prominent	Prominent	Medium thick and prominent
11.	Leaf texture	Smooth	Smooth	Moderate	Medium smooth and thin	Smooth and thick	Medium smooth and thin	Smooth and thick
12.	Leaf hairiness	Glabrous	Medium	Moderate	Sparse	Sparsely hairy	Sparse	Sparsely hairy
13.	Leaf size	Absent	Big	Intermediate	Big	Intermediate	Big	Intermediate
14.	Bract type	Caducous	Cordate	Cordate	Cordate	Cordate	Cordate	Cordate
15.	Bract size	Small	Large	Medium	Medium	Small	Medium Large	Small Medium
16.	Petal colour	Yellow	Yellow	Yellow	Creamy white	Creamy white	Yellow	Yellow
17.	Petal size	Medium	Large	Large	Medium	Medium	Large	Medium
18.	Petal spot	Dark pink	Absent	Light to dark pink	Absent	Light to dark pink	Absent	Light to dark pink
19.	Anther density	Dense	Dense	Moderate	Dense	Medium	Dense	Medium
20.	Pollen colour	Yellow	Light yellow	Creamy yellow	Yellow	Yellow	Yellow	Yellow
21.	Filament colour	Absent	Absent	Creamy white to purple	White to creamy White	Variable colouration	Absent	Variable colouration
22.	Position of stigma	Protruded	Protruded	Protruded	Embedded	Protruded	Embedded	Protruded
23.	Nectarines	Absent	Present	Absent	Present	Absent	Present	Absent
24.	Connective	Pink to purple	Colourless	Coloured variable	Colourless	Coloured variable	Colourless	Coloured Variable

for the level of pubescence on the stem. MCU5 and CO17 had sparsely pubescent stems. Combinations with MCU5 and CO17 showed a glabrous stem like wild parent whereas; the CO14 hybrid had a moderately hairy stem. Out of the three hybrids developed, the *G. armourianum* hybrid with CO14 had highest level of stem pubescence followed by MCU5 x *G. armourianm* which seemingly contributed to jassid resistance as the crop grew further. Substantial variations were noticed between each parent and the hybrid in terms of petal and leaf sizes (Fig.1, Fig. 2). In contrast to *G. armourianum*, having cordate-shaped foliage with no lobes, MCU5 has palmate leaves with 3–4 distinct lobes. The number of lobes in the palmate leaves of the F_1 hybrid varied greatly, whereas some plants showed many leaves with no to one or two leaves, some branches even showed lobation similar to that of the cultivated parent but comparatively smaller in size and depth of serration (Fig. 2).The leaves with no lobes mostly have a similar shape to that of the wild parent but are larger than it. The depth of serration in the lobes was shallower than the parent plant. Even some branches had all combinations together. The trend of the hybrid to show higher level of serration is expected to contribute further to the jassid resistance. Dark green colour leaves were found in the F_1 progenies similar to the wild parents, thus showing dominance for the trait. Thick and prominent leaf veins observed in the hybrids of all three crosses were similar to that of the *hirsutum* parent. But, the veins were not as prominent as that of the parent. Leaf nectaries were not present in the *hirsutum* parent. The hybrid progenies had similar characteristics like the wild male parent showing complete dominance for the trait.

The wild parent had glossy leaves but, the *hirsutum* parent is moderately smooth but comparatively has thicker leaves. In the progenies, they had smooth surfaces on the leaf with a thickness higher than the wild parent. CO17 x *G. armourianum* had the thickest leaves among the three hybrids. *Hisrutum* parents had a sparse amount of hair whereas; the wild diploid parent was glabrous. A moderate amount of hair was there on F_1 hybrids. The progenies with CO17 were the least hairy among the three hybrids. Trichome parameters are quite important concerning the sucking pest resistance. Since the wild parent was glabrous in nature, much improvement in trichome character was not observed among the hybrids produced for leaf hairiness. Petal colour in all three F_1 resembled their female parent mainly with a wide range of variation. The colour of the flower in the cross with MCU5 was creamy white to light yellow, whereas CO14 had yellow coloured petals. As far as the size of the petals is concerned, the progenies also had medium-sized petals.

The mature flower of the wild parent was completely devoid of bracts showing caducous nature, where bracts shed at a preliminary phase of flower development, even before the opening of the flower. Both the seed parent and hybrid had three normal bracts with teeth-like serrations. The female parental bracts were bigger than that of the hybrid (Fig.2). Petal spot, as usual, was lacking in the parent American cotton but visible in *G. armourianum*. In the F_1hybrid, various flowers from the same plant and the offspring of the same cross varied in petal spot size and intensity. The colour ranged from pale to dark pink, and the degree of development as a male parent (Fig.3). The petal spot, a crucial morphological indicator for confirming hybridity, exhibited variable expression strength. On the other hand, intra-*hirsutum* crosses between wild type and mutant strains revealed complete dominance of the petal spot (Ahuja and Dhayal, 2007).Other researchers have noted that *G. hirsutum* x *G. arboreum* hybrids had less colour intensity in their petal spots than F_1hybrids (Tahir *et al.*, 2011; Ahmad *et al.*, 2011, Kaur *et al.*, 2016). Mixed variable expression of petal spots was also observed in *G. hirsutum* and *G. barbadense* interspecific hybrids grown in India (Kaur*et al.*, 2016).The probable reason for the expression of petal spot might be because of variable expressivity for the trait. In the current investigation, an analogous response was observed in the case of filament coloration ranging from creamy white to white in both parents. The F_1 hybrids, however, had filaments that spanned from colourless to varied colours in various flowers. Within the same flower, few anthers displayed colourless anthers while others had coloured anthers. Similar results have been reported by Pushpam and Raveendran (2006), Mahalingam *et al.* (2020). Pollen colour of the F_1 was seen to be light yellow to yellow in colour, intermediate between two parents in all three crosses.

MCU5 x *G. armourianum* CO14 x *G. armourianum* CO17 x *G. armourianum*

Figure 1: Flower structure of different *G. armourianum* based hybrid with *G. hirsutum*

MCU5 x *G. armourianum* CO14 x *G. armourianum* CO17 x *G. armourianum*

Figure 2: Variation among the *G. armourianum* hybrid with different *G. hirsutum* varieties.

The connective tissue interconnecting the pollen-carrying anther and the filament were not having any colour in the female parent but was coloured in the male. However, the hybrid had both colourless and coloured connectives present in the different flower and also in same flower. Previous researchers hypothesized that the varied expression may be caused by epigenetic changes in the DNA (Kaur *et al.*, 2016). Mahalingam *et al.* 2020 has also reported similar results regarding the colour of connective tissue. The dark colouration in the connectives seems to have role in attracting the pollinators and aiding to the pollen dissemination.

The current findings regarding growth habit, petal colour, and leaf shape do not complement those of the dominant expression, because the hybrids exhibited variable and intermediate types of expression rather than a specific expression identical to one of the parents. For certain traits like plant stem colour, position of stigma, leaf colour and nectarines, the wild diploid parent was revealed to be dominant since the hybrid closely matched with it. These observations are in line with those noted by Mahalingam *et al.* (2020). But the traits like stem pubecence, pollen colour were found to be intermediate. These traits expression are in contrast to dominance as reported by previous author (Mahalingam *et al.*, 2020). Polygenic and epistatic interactions might be present in plant concerning these traits yielding intermediate expression. The current findings support data previously revealed by Pushpum and Raveendran (2006), Kaur *et al.* (2016), Muthuraj *et al.* (2019), and Mahalingam *et al.*(2020), who established that hybrids of *G. hirsutum* and *G. armourianum* had intermediate leaf shape and size. Other interspecific *Gossypium* species hybrids, including those between *G. herbaceum* and *G.australe* (Liu *et al.*, 2015), *G. hirsutum* and *G. arboreum* (Ahmad *et al.*,

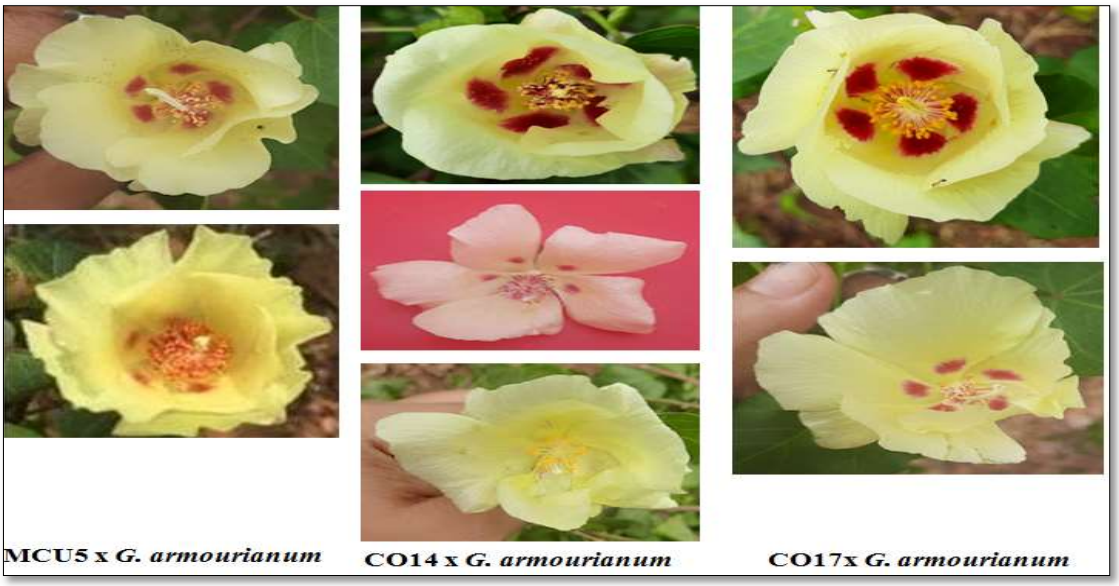

Figure 3: Variation for petal spot trait among the different *G. armourianum* hybrid

2011; Tahir *et al.,* 2011), and *G. arboreum* and *G. thurberi*, have also been reported to exhibit comparable intermediary manifestation of the growth habit of the plant, leaf size, and petal coloration (Manisha *et al.*, 2007).These characters show dominance, according to some researchers though. Saravanan *et al.* (2007)have shown the paternal parent's growth habits to be there in the triploid hybrid of American cotton with diploid wild species, *G. raimondii*. The hybrids form important genetic material for further development through different breeding methods and subsequent transfer of useful genes to cultivated background.

For the trait considered in the study, either they had dominant expression similar to one of the parent or intermediate variable in the F_1 progeny. For those traits showing complete dominance, transfer of trait may be little simpler compared to polygenic ones.

Hence while deciding the further breeding program using such lines, the program should be designed keeping in view the trait to be improvised. The wide range of variation available for each trait provide ample scope for further breeding process such as conventional backcross breeding or molecular breeding programs.

Conclusion

In the present study of characterization of the hybrids in the interspecific hybridization involving the wild cotton, *G. armourianum,* leaf shape, size, intensity of petal spot and the size of the bract were seen to have much amount of variation. Though belong to same species of cultivated cotton the three varieties used as female parent behaved substantially different in their progeny performance. These progenies constitute a valuable collection of prebreeding material which can be further harnessed for crop improvement programme.

Conflict of interest

The authors declare that they have no conflict of interest.

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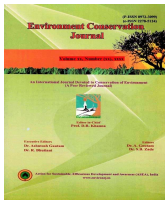

Response of nitrogen scheduling and weed management on growth and yield attributes of wheat (*Triticum aestivum* L.)

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ABSTRACT

A field experiment was conducted to study response of nitrogen scheduling and weed management on growth and yield attributes of wheat (*Triticum aestivum* L.) at the Experimental Farm, Mata Gujri College, Shri Fatehgarh Sahib during *Rabi* season of year 2018-2020. The experiment laid out in Split Plot Design (SPD) with three replications. The nitrogen scheduling includes N_1 -½ Basal + ¼ at 4WAS + ¼ at 8 WAS, N_2 -⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS, N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS while weed management treatment were W_2 -clodinafop @ 60 g/ha, W_3 -sulfosulfuron @ 25 g/ha, W_4-carfentrazone @ 20g/ha along with weed free and weedy check. The results revealed that the maximum growth and yield attributes were recorded of N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS which was at par N_2 -⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS and found significantly superior over N_1-½ Basal + ¼ at 4WAS + ¼ at 8 WAS.N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS + W_3 -sulfosulfuron @ 25 g/ha recorded significantly maximum which was significantly superior over all the treatments.

Introduction

Wheat (*Triticum aestivum* L.) is one of the major cereal crop and most important staple food in the world. It provides more protein than any other cereals crop. Wheat has a relatively high content of niacin and thiamine. It has higher food value over rice is that it contains 12% protein, 1.72% fat, 69.60% carbohydrates and 27.20% minerals matter (BARI, 1997). The insufficient fertilizer application, weed management and crop management are limiting factor for both growth and yield attributes. Consequently, to get more crop production, nitrogen application is indispensable and unavoidable (Massignam *et al.,* 2009). Nitrogen is vital constituent of protein, enzymes, coenzymes, nucleic acid and chlorophyll. Chlorophyll is primary observer of sunlight for photosynthesis (Leghari, 2016). The nitrogen availability has been reduced due to leaching, volatilization, surface runoff and denitrification (Gehl *et al.,* 2005). Fertilizer would not be taken by the plants if it is applied at wrong time or in the wrong place. Therefore, there is need to improve N use efficiency through maximizing N uptake at critical growth stages. So, the split application of nitrogen makes the nitrogen availability to the crop and increase the nitrogen use efficiency resulted growth and yield attributes also increase (Kumar *et al*., 2022). Nitrogen Scheduling not only increase the crop growth but also decrease the weed density and biomass (Kim *et al.,* 2006). Split application nutrients mostly taken by the crop than weeds. Application of sulfosulfuron + metsulfuron (POE) @ 30+2g a.i./ha were found most effective control of the all types of weeds than the rest of the

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treatments and improving growth and yield of wheat (Singh *et al.,*2020). Punia *et al*., 2018, reported that the higher crop yield and weed control were observed with application of carfentrazone 20 g/ha compared to carfentrazone 10 g/ha. A suitable combination of herbicides with nitrogen scheduling is to make out the effect on growth and yield of wheat (Singh *et al.,* 2015). Information of herbicide and split application of nitrogen in wheat is necessary to maximize yield and improve nitrogen use efficiency. Moreover, different N rates and application timings suggested that the application of 140 kg N ha^{-1} with triple splits timings, i.e., 25% at the sowing, 50% at the tillering, and 25% at the booting stage of the crop, resulted in the maximum yield and N recovery for different commercial wheat varieties (Khan *et al*., 2022).

Material and Methods

A field experiment was conducted at the Experimental Farm, Mata Gujri College, Fatehgarh Sahib during *Rabi* season of year 2018-2020. The experiment laid out in split plot design with three replications. The nitrogen scheduling was subjected to main plot, viz. N_1- ½ Basal + ¼ at 4WAS + ¼ at 8 WAS, N_2- ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS, N_3- ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS while, weed management was kept in sub plots, viz. W_0- weedy check, W_1- weed free, W_2- clodinafop @ 60 g/ha, W_3- sulfosulfuron @ 25 g/ha and W_4- carfentrazone @ 20 g/ha. The total treatment combinations were fifteen. The town lies between at 76^0-22'E and 76^0-46'E longitude and 30^0-36'N and 30^0-39'N latitude. The soil of experimental field was Gangetic alluvial having clay loam texture with pH 7.1. It was moderately fertile, with available nitrogen (392 kg N/ha), available phosphorus (18.31 kg P_2O_5/ha), available potassium (168.5 kg K_2O/ha), organic carbon (0.69 %) and electrical conductivity (0.57 dS/m). A uniform dose of 120 kg N/ha, 60 kg P_2O_5/ha, 40 kg K_2O/ha was applied to all treatments. Nitrogen was applied as per treatments and full dose of P and K was applied as basal at the time of sowing. Source of N, P and K were urea, single super phosphate and muriate of potash, respectively. The sowing of wheat variety "PBW 725" was sown in the experimental field on 10^{th} November 2018 as well as 11^{th} November 2019. The wheat crop was sown manually using seed rate 100 kg/ha at row to row distance of 22.5 cm. The first and second irrigation was applied after 21 days of sowing at CRI stage and at milking stage, respectively. Some shower of rains also occurs in the month of January, February, March and April. Regular biometric observations were recorded at periodic intervals of 30, 60, 90 DAS and at harvest stage viz., plant height was recorded from the ground level to the base of last fully opened leaf, the groundmass of plant to a length of running meter harvested from the border row then sun dried and oven dried at 60 ± 2 ^{0}C till a constant weight was obtained. After weighing the material, the dry matter of plant was recorded and leaf area index was measured by using assimilatory surface area occupied by the plants with the help of leaf area meter. Yield parameters were observed just before the harvesting of crop. The grain yield and straw yield of each plot was recorded and converted in hectare. The data obtained on various parameters were tabulated and subjected to analysis of variance techniques as described by Cochran and Cox, (1957).

Results and Discussion

The growth parameters of plant were significantly influenced by nitrogen scheduling and weed management practices. Among nitrogen scheduling, the maximum plant height (Table-1), number of tillers (Table-2), leaf area (Table-3) and dry matter accumulation (Table-4) was recorded with application of N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS which was at par N_2-⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS and found significantly superior over N_1 -½ Basal + ¼ at 4WAS + ¼ at 8 WAS at all growth stages of crop except 30, 60 DAS. This could be split application of nitrogen helps in more availability of nitrogen which might have encouraged high protein synthesis means more chlorophyll content leading to higher photosynthesis which increased growth parameters. Nitrogen split improved wheat growth and its competitive ability against weeds. Initially weed germination was increased by N use at sowing date. The minimum values were recorded under N_1-½ Basal + ¼ at 4WAS + ¼ at 8 WAS due to low nitrogen availability at the later stages because of nitrogen losses through leaching,

denitrification and volatilization etc which was applied as basal application. Kaur & Kumar (2018) reported similar finding and they reported the maximum growth attributes were recorded with the application of ZT +. N@125 kg (4 splits) applied at ¼, ¼, ¼ and ¼ at basal, 4, 6 and 8 WAS gave the best result in the terms of growth character which was statistically at par to the application of ZT + N@125 kg (4 splits) applied at ¼, ¼, ¼ and ¼ at basal, 4, 6 and 8 WAS and it was significantly superior over rest of treatments. However at 30 and 60 DAS, the maximum plant height (Table-1), number of tillers (Table-2), leaf area (Table-3) and dry matter accumulation (Table-4) was recorded with the nitrogen scheduling as N_1 -½ Basal + ¼ at 4WAS + ¼ at 8 WAS which was at par with N_2 -⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS. It due to higher doses of nitrogen were applied as basal which contributes more nitrogen available to crop.

Table 1: Effect of nitrogen scheduling and weed management on plant height (cm) at different growth stages of wheat crop

Treatments	30 DAS		60 DAS		90 DAS		At Harvest	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)								
N_1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	17.28	18.10	36.61	36.14	77.31	77.89	78.79	78.58
N_2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	15.70	16.44	33.37	33.02	83.99	85.41	84.25	84.03
N_3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	15.17	15.96	35.36	35.46	91.97	91.42	93.05	92.82
SEm±	0.41	0.43	0.59	0.59	2.06	1.70	2.10	2.18
CD 5 %	1.63	1.67	2.31	2.31	8.08	6.67	8.24	8.55
SUB PLOT (Weed Management)								
W_0= Weedy check	13.86	14.42	27.60	29.04	68.90	69.66	67.04	70.37
W_1= Weed free	18.44	19.26	39.45	40.06	92.77	95.80	93.19	96.98
W_2= Clodinafop@60g/ha	14.64	17.00	33.98	32.94	81.18	82.99	83.98	82.17
W_3= Sulfosulfuron@25g/ha	15.87	17.96	38.36	36.65	92.00	88.59	92.00	88.66
W_4= Carfentrazone@20g/ha	17.45	15.51	36.18	35.68	87.26	87.48	90.61	87.55
SEm±	0.43	0.59	0.96	0.87	2.40	1.84	2.31	2.00
C`D 5 %	1.26	1.71	2.80	2.55	7.00	5.38	6.76	5.83
N×W	NS	NS	NS	NS	NS	NS	NS	NS

Table 2. Effect of nitrogen scheduling and weed management on number of tillers (in running meter) at 30, 60, 90 DAS and at harvest stage

Treatments	30 DAS		60 DAS		90 DAS		At Harvest	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)								
N_1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	72.60	83.63	99.60	100.80	113.20	115.80	104.60	110.20
N_2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	66.20	74.52	82.80	84.40	127.20	134.80	114.80	129.40
N_3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	59.40	68.87	87.40	89.60	141.20	149.40	132.00	143.60
SEm±	2.27	2.59	3.27	3.06	4.65	4.44	4.90	4.63
CD 5 %	8.90	10.18	12.84	12.03	18.26	17.43	19.26	18.18
SUB PLOT (Weed Management)								
W_0= Weedy check	55.00	63.43	54.33	56.00	66.00	73.33	54.67	68.67
W_1= Weed free	75.00	86.96	118.33	113.67	180.33	195.00	174.00	186.00
W_2= Clodinafop@60g/ha	61.00	70.72	74.00	80.33	107.00	113.33	94.33	109.33
W_3= Sulfosulfuron@25g/ha	70.67	79.87	104.33	107.33	149.67	149.00	136.33	143.33
W_4= Carfentrazone@20g/ha	68.67	77.39	98.67	100.67	133.00	136.00	126.33	131.33
SEm±	3.75	4.69	5.12	4.42	6.35	6.78	5.85	6.58
CD 5 %	10.94	13.70	14.95	12.90	18.55	19.79	17.07	19.19
N×W	NS	NS	NS	NS	NS	NS	NS	NS

***SEm± - Standard error mean *CD- Critical difference *N×W- Interaction between nitrogen scheduling and weed management *NS- Non significant**

Table 3: Effect of nitrogen scheduling and weed management on leaf area index at 30, 60 and 90 DAS

Treatments	30 DAS		60 DAS		90 DAS	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)						
N_1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	0.69	0.71	4.14	4.83	3.53	3.76
N_2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	0.64	0.66	2.35	3.45	4.36	4.68
N_3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	0.58	0.63	3.16	4.41	5.34	5.73
SEm±	0.02	0.02	0.29	0.14	0.29	0.34
CD 5 %	0.08	0.06	1.13	0.55	1.12	1.34
SUB PLOT (Weed Management)						
W_0= Weedy check	0.29	0.32	1.66	1.57	2.81	3.16
W_1= Weed free	0.87	0.88	4.63	5.79	5.83	6.64
W_2= Clodinafop@60g/ha	0.57	0.76	2.77	3.39	3.97	3.85
W_3= Sulfosulfuron@25g/ha	0.71	0.81	3.67	5.41	4.87	5.52
W_4= Carfentrazone@20g/ha	0.76	0.55	3.36	4.98	4.56	4.44
SEm±	0.02	0.03	0.25	0.25	0.25	0.47
CD 5 %	0.06	0.08	0.74	0.73	0.74	1.36
N×W	NS	NS	NS	NS	NS	NS

Table 4: Effect of nitrogen scheduling and weed management on dry matter accumulation (g/running meter) at 30, 60, 90 DAS and at harvest stage

Treatments	30 DAS		60 DAS		90 DAS		At Harvest	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)								
N_1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	2.97	3.08	33.38	36.25	289.60	293.47	724.00	739.63
N_2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	2.74	2.83	25.38	29.93	309.33	321.80	773.33	786.40
N_3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	2.09	2.14	31.77	34.40	330.13	336.90	825.33	836.32
SEm±	0.06	0.11	0.58	1.11	5.73	5.73	14.32	13.50
CD 5 %	0.23	0.43	2.29	4.36	22.49	22.51	56.22	53.00
SUB PLOT (Weed Management)								
W_0= Weedy check	1.23	1.24	19.42	25.80	238.22	260.01	595.56	623.56
W_1= Weed free	3.11	3.22	37.79	39.46	352.00	353.99	880.00	890.40
W_2= Clodinafop@60g/ha	2.80	2.91	27.63	30.05	297.33	305.49	743.33	749.07
$4W_3$= Sulfosulfuron@25g/ha	2.91	3.04	34.59	37.09	338.67	339.87	846.67	854.67
W_4= Carfentrazone@20g/ha	2.95	3.02	31.45	35.24	322.22	327.60	805.56	819.56
SEm±	0.14	0.12	1.97	2.04	6.65	6.82	16.61	18.22
CD 5 %	0.42	0.35	5.75	5.95	19.40	19.92	48.49	53.18
N×W	NS	NS	NS	NS	NS	NS	NS	NS

***SEm± - Standard error mean *CD- Critical difference *N×W- Interaction between nitrogen scheduling and weed management *NS- Non significant**

The result were confirmed by Akhter *et al.* (2017) and they reported that application of nitrogen in three splits with reduced basal dose in the ratio of 25 : 50 : 25 improved growth and yield as compared to recommended practice. Among the weed management practices, the maximum plant height, number of tiller, dry matter accumulation and leaf area index were recorded with the application of W_3- sulfosulfuron @ 25g/ha which was at par with theW_4- carfentrazone @ 20g/ha at 60, 90 DAS and at harvest stage due to less crop-weed competition for nutrients, light, space and soil moisture resulted nutrients were available constantly to crop ultimately photosynthetic rate was increased which increase the supply of carbohydrates ultimately increased growth attributes of crop. These both herbicides significantly lowering the population of weed density at initial stage. However, the minimum growth parameters were recorded in weedy check due to more weed flora which competes with the crop. Similar results were also validated by Singh *et*

al., 2019, reported that the application of pendimethalin + metribuzin (1.0 + 0.175 kg/ha) as pre-emergence being at par with weed free and pendimethalin (1.0 kg/ha) followed by sulfosulfuron (0.025 kg/ha) significantly reduced the density of weeds as compared to other treatments. The interactions between nitrogen scheduling and weed management were found non-significant effect on growth attributes of crop during the experimentation.The data showed that the yield parameters of crop were significantly influenced by nitrogen scheduling. Amongst the nitrogen scheduling, the maximum number of grains/spike, number of effective tillers, spike length, test weight (Table-5) were recorded with the nitrogen scheduling as N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS which was at par with the nitrogen scheduling as N_2 -⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS. It might be due to the resultant of that splitting of nitrogen at the different stages of crop period which improves nutrient use efficiency and also increased growth attributes. Due to higher performance of growth attributing character which increase yield attributing characters of crop. Further Similar results were also validated by Dubey *et al*. (2018) they reported that the higher growth attributes were recorded under nitrogen scheduling (T_2 - 1/3 at sowing and 2/3 after first irrigation) which was significantly superior over rest of the treatments. The minimum value of yield attributing characters was recorded with the nitrogen scheduling asN_1-½ Basal + ¼ at 4WAS + ¼ at 8 WAS because sufficient amount of N was not available due to maximum losses of nitrogen resulted reduced translocation of photosynthates which was responsible for poor yield attributing characters. Similar results were recorded by Litke *et al.*(2018) and they reported that application of nitrogen fertilizer rate (altogether eight rates: N0 or control, N60, N90, N120 (90+30), N150(90+60), N180 (90+60+30), N210 (90+70+50), and N240 (120+ 60+60). All spilt of nitrogen treatments have significant effect on improve nitrogen use efficiency as well as higher crop growth of wheat crop and least performance in no split application of nitrogen fertilizer. The maximum number of grains/spike, number of effective tillers, spike length, spike weight were recorded with the application of W_3- sulfosulfuron @ 25g/ha which was at par with theW_4- carfentrazone @ 20g/ha but under test weight highest value was recorded with the application of W_3- sulfosulfuron @ 25g/ha which was at par with theW_4- carfentrazone @ 20g/ha and W_2- clodinafop @ 60 g/ha (Table-5). It might be due to better weed management practice resulted less crop-weed competition for nutrients and provides the better environmental condition to the root zone of crop resulted maximum inputs used by the crop for their growth. Therefore, growth attributes were increased; ultimately yield attributes were also increased. The minimum value was recorded in weedy check treatment because weed density was high and more competition between weed and crop for nutrients resulted poor performance of growth attributes. Similar results were found by Singh *et al*., 2020, they reported that the application of sulfosulfuron + metsulfuron (POE) @ 30+2g a.i./ha were found most effective control of the all types of weeds followed by hand weeding control of weeds than the rest of the treatments and improving growth, yield attributes of wheat followed by hand weeding than the rest of the treatments (Singh *et al.,*2020). The interactions between nitrogen scheduling and weed management were found non- significant effect on growth attributes of crop during the experimentation.The highest grain yield (q/ha), straw yield (q/ha), biological yield (q/ha) and harvest index (%)(Table-6) was recorded with the nitrogen scheduling N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS which was at par with the nitrogen scheduling as N_2 -⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS. The reason for higher values of grain yield can be discussed in light of fact that there was positive correlation between grain yield and yield components like numbers of effective tillers and grains/spike which were increased in nutrients availability.Yield attributes was increased with the increasing nitrogen splitting as well as increased nutrient use efficiency and also less chance of nitrogen losses due to leaching, immobilization and denitrification as well as weed population were less ultimately competition was less. Split application of nitrogen maintained continuous supply of nutrients resulted better translocation of photosynthates from source to sink which was responsible for good growth and yield

Table 5: Effect of nitrogen scheduling and weed management on yield attributes of crop

Treatments	Spike Length (cm)		Spike Weight (g)		Grain/Spike		Effective tillers (m^2)		Test Weight (g)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2018-19	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)										
N_1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	11.26	11.73	3.22	3.57	62.02	63.72	95.80	107.00	38.31	38.78
N_2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	12.06	12.54	3.38	3.79	67.43	67.94	107.80	127.80	38.35	38.82
N_3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	12.70	13.35	3.39	3.93	69.81	70.18	123.20	142.00	39.97	39.99
SEm±	0.25	0.24	0.11	0.28	1.42	1.17	5.12	3.62	0.70	0.60
CD 5 %	0.96	0.95	0.44	1.11	5.59	4.60	20.11	14.22	2.74	2.36
SUB PLOT (Weed Management)										
W_0= Weedy check	9.39	9.36	2.56	2.97	47.94	48.87	50.33	66.00	37.32	37.78
W_1= Weed free	14.27	15.01	3.79	4.27	78.12	78.30	165.00	184.67	40.53	40.69
W_2= Clodinafop@60g/ha	10.91	11.18	2.96	3.36	60.80	64.21	84.00	104.33	37.99	38.01
W_3= Sulfosulfuron@25g/ha	13.25	14.06	3.74	4.19	74.66	73.82	127.00	142.00	39.44	39.92
W_4= Carfentrazone@20g/ha	12.19	13.09	3.60	4.03	70.58	71.21	118.33	131.00	39.10	39.57
SEm±	0.42	0.56	0.24	0.30	1.68	2.02	6.13	5.93	0.67	0.67
CD 5 %	1.21	1.63	0.69	0.87	4.91	5.91	17.88	17.30	1.96	1.97
N×W	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 6. Effect of nitrogen scheduling and weed management on yield (q/ha) and harvest index (%)

Treatments	Grain Yield (q/ha)		Straw Yield (q/ha)		Biological Yield (q/ha)		Harvest Index (%)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
MAIN PLOT (Nitrogen Scheduling)								
N1= nitrogen scheduling as ½ Basal + ¼ at 4WAS + ¼ at 8 WAS	37.74	39.23	57.58	61.30	95.31	100.53	39.36	38.83
N2= nitrogen scheduling as ⅓ at 4 WAS + ⅓ at 8 WAS + ⅓ at 10 WAS	42.62	43.20	64.29	67.09	106.90	110.29	39.58	39.08
N3= nitrogen scheduling as ¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS	46.06	47.23	68.97	69.34	115.03	116.57	39.67	40.33
SEm±	1.10	1.20	1.77	1.21	2.57	2.14	0.62	0.53
CD 5 %	4.31	4.73	6.94	4.76	10.09	8.41	2.44	2.08
SUB PLOT (Weed Management)								
W0= Weedy check	26.76	27.62	53.20	47.59	79.96	75.20	33.56	36.75
W1= Weed free	52.85	53.50	71.65	79.43	124.50	132.93	42.59	40.32
W2= Clodinafop@60g/ha	40.03	40.26	59.95	58.84	99.98	99.09	40.13	40.58
W3= Sulfosulfuron@25g/ha	46.04	47.82	67.54	72.21	113.58	120.02	40.65	39.78
W4= Carfentrazone@20g/ha	45.01	46.91	65.72	71.49	110.73	118.40	40.75	39.63
SEm±	0.82	1.71	2.40	1.89	2.47	2.76	1.15	0.63
CD 5 %	2.40	3.41	7.01	5.51	7.22	8.05	3.35	1.84
N×W	NS	NS	NS	NS	NS	NS	NS	NS

***SEm± - Standard error mean *CD- Critical difference *N×W- Interaction between nitrogen scheduling and weed management *NS- Non significant**

attributes. On the other hand, the lesser yield was recorded with the nitrogen scheduling asN_1 -½ Basal + ¼ at 4WAS + ¼ at 8 WAS due to insufficient supply of nutrients and more nutrients losses occurred due to higher weed population inthis treatment. Further reported that the application of 140 kg N ha^{-1} with triple splits timings, i.e., 25% at the sowing, 50% at the tillering, and 25% at the booting stage of the crop, resulted in the maximum yield and N recovery for different commercial wheat varieties (Khan *et al.*, 2022). Earlier finding also reported by Belete *et al.*, 2018 and they state that the split application of nitrogen (¼ at sowing, ½ at tillering and ¼ at

booting) produced the highest crop yield and also nitrogen use efficiency traits. The maximum value of grain yield, straw yield and biological yield were recorded with the application of W_3- sulfosulfuron @ 25g/ha which was at par with the W_4-carfentrazone @ 20g/ha (Table-6). The reason for higher values of crop yield can be discussed in light of fact that the yield attributes increased due to very less competition for nutrients and improved dry matter accumulation in crop as well as high weed control efficiency resulted grain yield, straw yield and biological yield increased. The minimum value of crop yield was recorded in weedy check treatment due to high weed population as well as higher competition for nutrients, space and light as well as soil moisture. Similar results were recorded by the results revealed that application of ready mix of sulfosulfuron + metsulfuron (32 g/ha) and mesosulfuron + iodosulfuron (14.4 g/ha) gave higher yield attributes and yield of crop (Meena *et al*., 2019). Further, Punia *et al*., 2018, reported that application of different herbicides significantly reduced the dry weight of weeds compared to weedy check at different growth stages of crop. The higher crop yield and weed control were observed with application of carfentrazone 20 g/ha compared to carfentrazone 10 g/ha. The interactions between nitrogen scheduling and weed management were found non- significant effect on growth attributes of crop during the experimentation.

Conclusion

The present study helps to minimize the overuse nitrogen in wheat crop in central zone of Punjab and also help in find out the suitable herbicide in wheat crop. Based on results obtained in the present investigation it was concluded that application of N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS + W_3 -sulfosulfuron @ 25 g/ha performed better concerning growth and yield attributes of the wheat. Along with treatment consisted N_3 -¼ at 4 WAS + ¼ at 6 WAS + ¼ at 8 WAS + ¼ at 10 WAS + W_4 -carfentrazone @ 20 g/ha which also showed good results in growth and yield attributes.

Conflict of interest

The authors declare that they have no conflict of interest.

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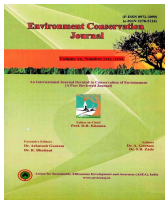

In vitro management of Fusarium wilt of linseed using phytoextract, fungicides and bioagents

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ABSTRACT

Fusarium wilts of linseed caused by *Fusarium oxysporum* f.sp. *lini* have been identified in nearly all linseed-producing countries of the world. A comparison of phytoextract, chemical, and bio control agents against *Fusarium oxysporum* f.sp. *lini* was conducted. Among the phytoextracts tested, Neem extract exhibited the highest antifungal activity in inhibiting the growth of *F. oxysporum* f.sp. *lini* at 5, 15, and 30% concentrations. In terms of biocontrol agents, *T. virens* was identified as the most efficient antagonist against *F. oxysporum* f.sp. *lini.* It significantly inhibited pathogen mycelial growth, displaying the highest level of inhibition. Among the chemical fungicides assessed, propiconazole exhibited the lowest mycelial growth of the pathogen and outperformed the other fungicides, with difenoconazole following as the next most effective.

Introduction

Linseed is an important oilseed crop used largely for commercial oil in India. Linseed (*Linum usitatissimum* L.), also known as Alsi/Flaxseed, is a plant in the Linaceae family that belongs to the genus Linum. The oil content of the seeds varies from 33 to 47 percent depending on the variety. It is high in soluble fiber mucilage and one of the main sources of alpha-linolenic acid (ALA) (Cunnane *et al*., 1993). In terms of yield and acreage, Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Karnataka, and West Bengal follow Madhya Pradesh. Madhya Pradesh and Uttar Pradesh generate more than 70 percent of the nation's linseed (Anonymous, 2015). However, it is hampered by various biotic and abiotic stresses. Considering the present scenario, it is a challenging task to achieve sustainable global food security with the growing human population and shifting global food consumption patterns brought on by climate change (Kumar *et al*., 2021). Among the different diseases, major diseases include Fusarium wilt (*Fusarium oxysporum* f.sp. *lini*), rust (*Melampsora lini*), powdery mildew (*Oidium lini*), Alternaria blight (*Altenaria linicola*), foot rot (*Rhizoctonia solani*, Pythium spp.), damping off of seedlings (Pythium spp.), etc. Wilt caused by *Fusarium oxysporum* f. sp. *lini* is a severe barrier to linseed industry output and productivity (Kishore *et al*., 2021). Sattar and Hafiz (1952) reported crop losses of 80-100 percent due mainly to wilt. *Fusarium oxysporum* f. sp. *lini*, one of the most dangerous fungal pathogens in flax, is a causative agent of Fusarium wilt. It invades the plant through roots and spreads inside the vascular bundle. After germination, it develops microconidia, blocking water and nutrient flow,

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which leads to plant wilt, yellowing of lower parts, and death (Michielse and Rep 2009, Rozhmina and Loshakova 2016). Fusarium is a genus of filamentous, seed, and soil-borne ascomycetes with numerous pathogenic members that have been reported to cause disease in over 100 major crop species worldwide (Ma *et al.,* 2013, Purohit *et al*., 2022). Infection occurs through the roots, invading the water-conducting tissues, which impairs water transport and results in wilting, necrosis, and chlorosis of aerial parts (Ma *et al*., 2013). *Fusarium oxysporum* can persist in the soil for 5–10 years. Fusarium wilt of linseed has management options varying from preventive to curative interventions. To date, various strategies for plant disease management have been advocated, and for eco-friendly management, the use of biocontrol agents has been successfully utilized in different crops against various plant pathogens. Among different bioagents, *Trichoderma* is a potential agent that not only successfully controls plant pathogens (Kumar *et al.*, 2013a; Kumar *et al.*, 2014; Jain *et al.*, 2017; Kharte *et al.*, 2022) but is also used as a biofertilizer (Srivastava *et al*., 2009) and in the production of several secondary metabolites (Kumar *et al*., 2009). They also serve as plant growth-promoting agents (Kumar and Sahu, 2014; Kumar *et al*., 2019) and in bioremediation (Kumar *et al*., 2015). Furthermore, their use as native isolates has proven to have better potential in local areas for successful biocontrol agents after proper identification and characterization (Kumar *et al*., 2013b; Kumar and Sahu, 2015; Kumar *et al*., 2016). They can also be used in combination with fungicides for more effective control of the disease (Kumar *et al*., 2019).Each control mechanism is important, but none can function alone. Identifying the most effective concentration dose of various phytoextracts, as well as the chemical and antagonistic activity of bioagents against the pathogen. The purpose of this study was to determine the most efficient biocontrol and ideal concentrations of phytoextracts, which are fungicides that are widely utilized in disease control.

Material and Methods

In vitro evaluation of leaf extract

The poisoned food method (Sreenu and Zacharia, 2017) was used to assess *F. oxysporum* f. sp. *lini* sensitivity to phytoextracts. Seven phytoextracts, viz., neem, eucalyptus, tulsi, ginger, garlic, gokhru, and mint, were examined to test their effectiveness against Fusarium wilt. By using a wide range of concentrations, the study aims to identify the most effective concentration(s) of these plant extracts for further investigation while also ensuring that any potential toxicity to the plants is minimized. The standard procedure employed by Gerard *et al.* (1994) was utilized to create extracts of plant components such as leaves, bulbs, and clove, among others. Fresh plant parts were cleaned with tap water followed by sterile distilled water before being processed with a mortar and pestle at 1 ml g^{-1} of plant tissue (1:1 v/w) and filtered through a double-layered muslin cloth. The resulting filtrate was the typical plant extract solution. Solutions from stock were used to generate 5, 15, and 30 percent concentrations of plant extract, and 5, 15 and 30 ml were combined with 95, 85, and 70 ml of sterilized molten potato dextrose agar (PDA) medium, respectively. The extracted material was gently shaken to ensure uniform mixing. In sterilized Petri plates, 20 ml of poisoned PDA was poured. The plates were inoculated with a five mm diameter mycelium disc from an actively developing pure culture of *F. oxysporum* f. sp. *lini* and incubated at 27±2°C for 168 hours. The mycelial growth was recorded, and the percent growth inhibition was calculated using the following formula by Vincent, 1947.

$$\mathbf{I = \frac{C-T}{C} \times 100}$$

where I= mycelial growth inhibition (%); C=mycelial growth in control (mm); and T= mycelial growth in treatment (mm)

In vitro evaluation of fungicides

Six fungicides, Mancozeb + Carbendazim, Difenconazole 3% FS, Copper oxychloride, Azoxystrobin 23% SC, Propiconazole, and Fluxapyroxad + Pyraclostrobin, were tested against the pathogen using the poisoned food method, as reported by Sreenu and Zacharia, 2017, at doses of 300 and 500 ppm. In a completely randomized experimental design, Petri plates containing PDA amended with the appropriate concentration of fungicides were infected with five mm mycelial discs of the active culture of fungus and kept at

27±2°C by maintaining a suitable control, and each treatment was replicated three times (More 2016). In each treatment, mycelial growth was measured, and growth inhibition was estimated.

***In vitro* evaluation of bioagents**

Trichoderma viride, T. harzianum, T. virence, Pseudomonas florescence, and *Bacillus subtilis* were tested *in vitro* against *Fusarium oxysporum* f.sp. *lini* utilizing the dual culture method proposed by Vani (2019). Five-millimeter discs of actively growing pathogen and antagonist cultures were placed at a distance of 5 cm on PDA, and plates were incubated at 27±2°C. As a control, PDA medium inoculated with pathogen alone was used. Following the entire development of the pathogen in the control, the mycelial growth of the pathogen and bioagents was recorded, and growth inhibition was calculated.

Results and Discussion

Effect of various phyto extracts on *Fusarium oxysporum* f.sp. *lini*

Seven plant extracts, *viz*., Neem, Tulsi, Eucalyptus, Ginger, Garlic, Gokhru and Mint, at 5, 15 and 30 percent concentrations were evaluated against *F. oxysporum* f.sp. *lini* (Tables 1, 2, 3 and Figures 1, 2 and 3). Among them, the maximum percent inhibition of Neem (36.02%) was recorded as the most effective, showing maximum inhibition of mycelial growth of the pathogen, followed by Garlic (34.75%), Tulsi (28.87%) and Ginger (24.95%). Mint recorded minimum growth inhibition (07.18%) of the pathogen. Neem was superior to all other tested plant extracts at the 5, 15 and 30 percent concentrations after 168 hours of inoculation. Thus, the results clearly indicated that plant extracts reduced the radial growth of *F. oxysporum* f. sp. *lini*. The ability of garlic and neem extract to inhibit mycelial growth may be attributable to the antifungal substances they contain, such as diallyl disulfide, diallyl trisulfide and azadirachtin. Unlike in Tulsi, Ginger and Eucalyptus, antifungal compounds such as oleanolic acid, ursolic acid, zingiberence, gingerol, flavonoids, and catechins have already been reported to have biofungicidal efficacy against various fungi. Each plant extract contains a unique blend of phytochemicals, and their relative concentrations may differ depending on the plant species, location, and growth stage. Some phytochemicals may exhibit potent antifungal properties, while others may be ineffective or even toxic to the plant. Thus, the variation in the chemical composition of plant extracts could lead to differences in their antifungal activity against Fusarium species. According to Singh *et al*. (2010), *Datura festilosa*, *Tagetes erecta*, *Eucalyptus citridora, Aegle marmelos,* and *Mimusops elengi* were the plants that inhibited the growth of *F. udum* mycelium the least effectively. Similarly, Khaleel *et al*. (2014) investigated the fungitoxic effects of six methanolic plant extracts at nine different concentrations: garlic, ginger, oak leaf, neem leaf, moringa leaf, and parthenium leaf. The best control was found to be 1000 g/ml Neem leaf extract, followed by Ginger extract. At the highest concentration (1000 g/ml), parthenium leaf extract was shown to be the least effective. carbendazim (70.23%). However, copper oxycloride (30.33%) exhibited the least percent growth inhibition over the control, followed by azoxystrobin (46.42%) after 168 hours at 300 ppm (Table 4, plate 4 and figure 4).

Table 1: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 5 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	16.26	40.90	42.69	40.90	55.50	28.98
Tulsi	21.11	28.46	47.13	34.75	63.37	18.91
Eucalyptus	24.48	17.04	56.30	22.06	72.61	07.08
Ginger	22.54	24.97	50.29	30.38	65.36	16.36
Garlic	17.53	40.59	46.23	36.00	58.14	25.60
Gokhru	26.53	10.09	59.20	18.05	74.41	04.78
Mint	26.99	08.53	60.35	09.53	77.11	01.33
Control	29.51	00.00	72.24	00.00	78.15	00.00
CD (5%)	0.84		1.91		1.63	
SE(m)	0.28		0.63		0.54	

Table 2: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at 15 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	12.58	54.69	40.56	43.38	46.51	38.09
Tulsi	16.13	41.91	46.26	35.76	53.38	28.94
Eucalyptus	22.14	20.27	54.39	24.47	59.97	20.17
Ginger	19.78	28.77	49.25	31.62	56.52	24.77
Garlic	13.79	50.34	42.83	40.53	48.52	35.41
Gokhru	23.34	15.95	58.25	19.12	65.12	13.32
Mint	26.36	05.07	60.49	16.00	66.72	11.20
Control	27.77	00.00	72.02	00.00	75.13	00.000
CD (5%)	0.80		2.48		1.48	
SE(m)	0.26		0.82		0.49	

Table 3: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at 30 percent concentration

Treatment	72 hours		120 hours		168 hours	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Neem	11.99	56.62	38.40	36.47	46.37	36.02
Tulsi	15.05	45.54	45.13	25.34	51.55	28.87
Eucalyptus	19.12	30.82	51.73	14.42	59.22	18.29
Ginger	17.13	38.02	47.12	22.05	54.39	24.95
Garlic	12.82	53.61	41.03	32.12	47.30	34.75
Gokhru	21.44	22.43	54.61	09.66	63.36	12.58
Mint	23.22	15.99	57.46	04.94	67.27	07.18
Control	27.64	00.00	60.45	00.00	72.48	00
CD 5%)	1.30		2.24		2.39	
SE(m)	0.43		0.7		0.79	

Table 4: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *lini* at 300 ppm

Treatment	72 hours		120 hour		168 hour	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Mancozeb+ Carbendazim	00.00	100.00	18.00	59.84	22.00	70.23
Difenconazole 3% FS	00.00	100.00	15.50	65.42	19.67	73.39
Copper oxychloride	24.50	11.45	42.00	06.31	51.50	30.33
Azoxystrobin 23% SC	16.33	40.98	28.66	36.06	39.60	46.42
Propiconazole	00.00	100.00	00.00	100.00	00.00	100.00
Fluxapyroxad+ Pyraclostrobin	15.00	45.78	27.00	39.77	36.49	50.63
Control	27.67	00.00	44.83	00.00	73.92	00.00
CD (5%)	1.32		1.62		1.92	
SE(m)	0.43		0.53		0.63	

Propiconazole exhibited a percent growth inhibition over the control, followed by difenconazole (71.74%),

In vitro evaluation of fungicides

Six fungicides, *viz.,* mancozeb + carbendazim, difenconazole (3% FS), copper oxychloride, azoxystrobin (23% SC), propiconazole, and fluxapyroxad + pyraclostrobin, were evaluated at 300 and 500 ppm each against *F. oxysporum* f.sp. *lini* (Table 4 and 5). Of the six fungicides, no radial growth of the test pathogen was observed with propiconazole, followed by difenconazole (19.67 mm), mancozeb + carbendazim (22.00 mm), and fluxapyroxad + pyraclostrobin (36.49 mm). However, the maximum growth of the test pathogen was observed with copper oxychloride (51.50 mm), followed by azoxystrobin (39.60 mm). Propiconazole exhibited cent percent growth inhibition over the control, followed by difenconazole (73.39%) and mancozeb+

Table 5: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *lini* at 500 ppm

Treatment	72 hours		120 hour		168 hour	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Mancozeb+ Carbendazim	00.00	100.00	15.50	65.28	19.16	68.15
Difenconazole 3% FS	00.00	100.00	09.50	78.72	18.00	71.74
Copper oxychloride	22.83	08.68	40.33	09.67	53.33	11.35
Azoxystrobin 23% SC	9.33	62.68	17.50	60.80	29.00	51.79
Propiconazole	00.00	100.00	00.00	100.00	00.00	100.00
Fluxapyroxad+ Pyraclostrobin	08.00	68.00	21.67	51.46	31.00	48.47
Control	25.00	00.00	44.65	00.00	60.16	00.00
CD(5%)	1.591		2.24		1.29	
SE(m)	0.519		0.73		0.42	

Table 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f. sp. *Lini*

Treatment	**72 hours**		**120 hours**		**168 hours**	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
T. virens	21.16	36.49	32.33	42.94	43.16	32.73
T. viride	17.00	48.97	25.00	55.87	35.15	45.21
T. harzianum	28.83	13.47	41.50	26.75	51.66	19.48
P. fluorescence	31.16	06.48	46.83	17.35	57.00	11.15
B. subtilis	26.16	21.48	47.00	17.04	48.17	24.92
Control	33.32	00.00	56.66	00.00	64.16	00.00
CD (5%)	1.62		2.98		1.45	
SE(m)	0.52		0.95		0.46	

mancozeb+carbendazim (68.15%) and azoxystrobin (51.79%); however, copper oxycloride (11.35%) exhibited the least percent growth inhibition over the control, followed by fluxapyroxad+ pyraclostrobin (48.47%) after 168 hours at 500 ppm (Table 5, plate 5 and figure 5). Sharma *et al*. (2002) also investigated the effects of Mancozeb, Thiram, Copper Oxychloride, Rovral, Bavistin, Ridomil, Kavach, Benomyl, and Captan against *F. oxysporum* f.sp. *lini*, they discovered similar results, which caused lins regardless of concentration, and every fungicide outperformed the untreated control in a considerable way. Both carbendazim and benomyl inhibited the mycelial growth of *F. oxysporum* f.sp. *lini*. Taskeen Un-Nisa *et al*. (2011) and Arunodhayam *et al*. (2014) found similar results under *in vitro* assessment of fungicides against *Fusarium* spp. Similarly, fungicide effects against test pathogens were reported by earlier workers; for example, Ravichandran and Hegde (2015) noticed that Carbendazim 12% + Mancozeb 63% inhibited almost half of the growth of *F. oxysporum* f. sp. *ciceri*. Patra and Biswas (2016) indicated that carbendazim, propiconazole, and two combination products (carbendazim + mancozeb and tebuconazole + trifloxystrobin) were most effective in completely inhibiting the mycelial growth of the fungus at different concentrations. Dahal *et al.* (2018) and Niwas *et al.* (2020) found similar results *in an in vitro* assessment of fungicides against *Fusarium* spp. Similarly, Bhujbal *et al*., 2021 reported that Mancozeb + Carbendazim and Thiram + Carbendazim were the most effective, inhibiting 100% and 93.63% of pathogen growth, respectively.

Evaluation of bioagents against *Fusarium oxysporum* f.sp. *lini*

T. viride, T. harzianum, T. virens, P. fluorescens, and *B. subtilis* were evaluated *in vitro* for their bio efficacy against *F. oxysporum* f.sp. *lini.* It was observed that all bioagents exhibited antifungal activity against *F. oxysporum* f.sp. *lini* and significantly inhibited its mycelial growth. *T. viride* was shown to be the most efficient pathogen inhibitor, with considerably less mycelial growth (35.15 mm) and the greatest mycelial growth

inhibition (45.21%) after 168 hours, because *T. viride* interacts directly with pathogens by hyperparasitism or antibiosis. Hyperparasites attack and kill the mycelium, spores and resting structures of pathogens. The pathogen's growth suppression was lowest in *P. fluorescens* (11.15%), followed by *B. subtilis* (19.48%) after 168 hours (Table 6, plate 6 and figure 6). Similarly, *T. viride* and *T. harzianum* were also tested *in vitro* for antagonistic activity against *F. oxysporum* in a dual culture experiment, and *T. viride* inhibited the mycelial development of all pathogens. These studies are supported by Jagraj *et al*., 2018, who reported that *T. harzianum* inhibited the maximum radial growth of *F. oxysporum,* followed by *T. viride.*

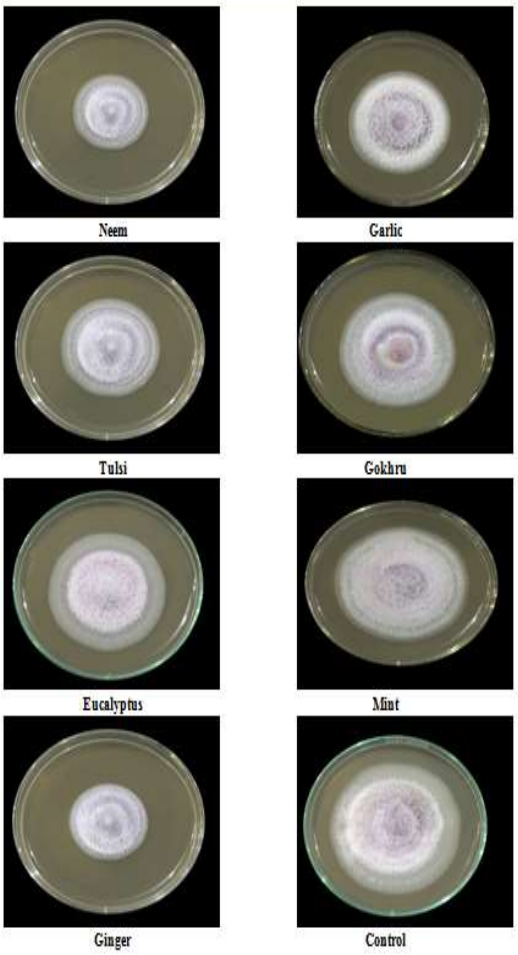

Plate 1: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 5 percent concentration after 168 hours

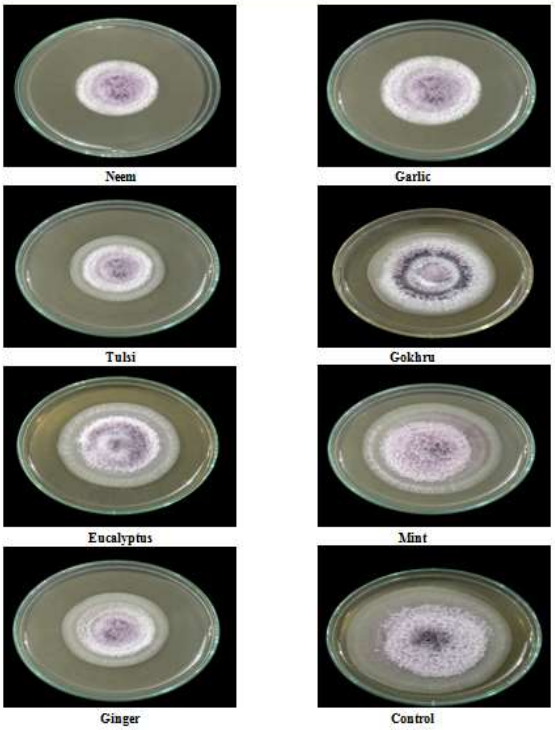

Plate 2: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 15% concentration after 168 hours

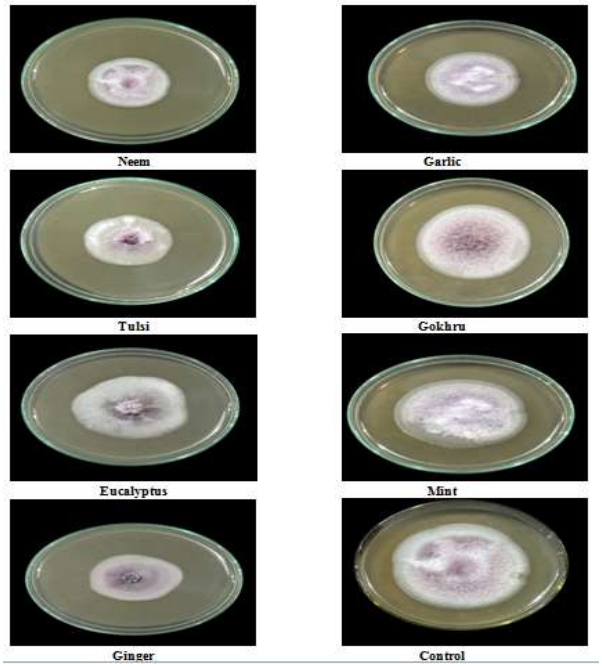

Plate 3: Effect of plant extracts on mycelial growth of *Fusarium oxysporum* f.sp. *lini* at a 30% concentration after 168 hours

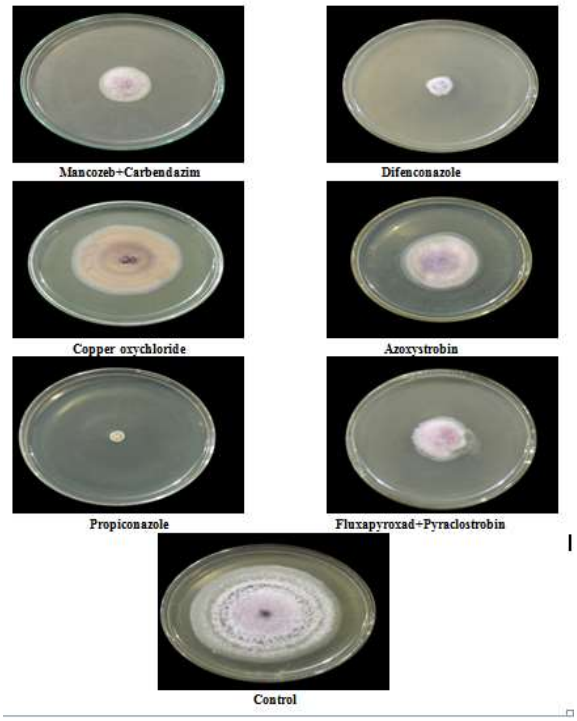

Plate 4: Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lini* at 300 ppm after 168 hours

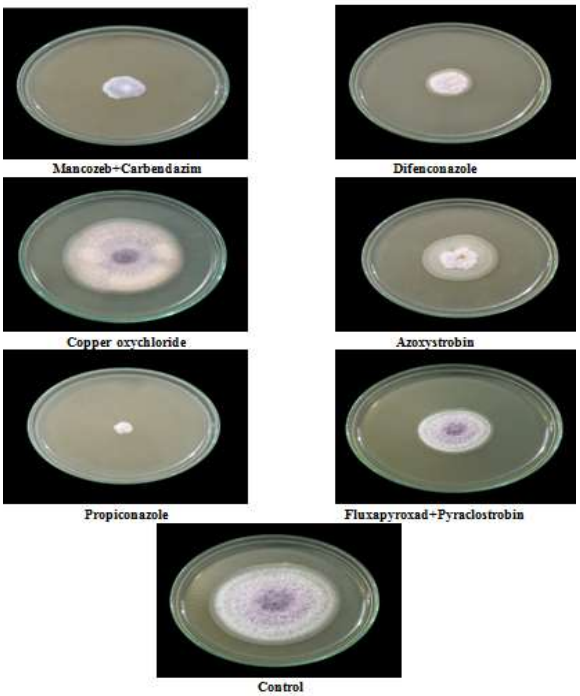

Plate 5: Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lini* at 500 ppm after 168 hours.

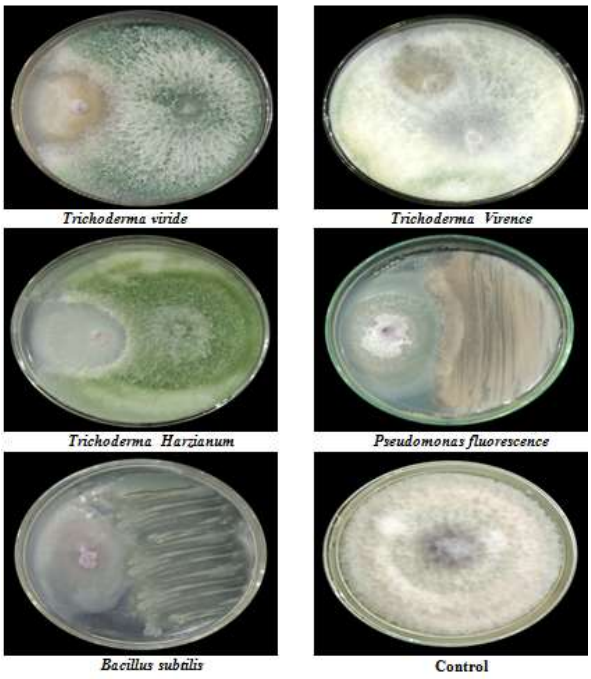

Plate 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f. sp. *Lini*

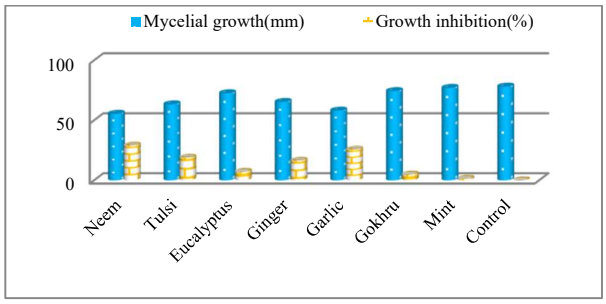

Figure 1: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at a 5 percent concentration

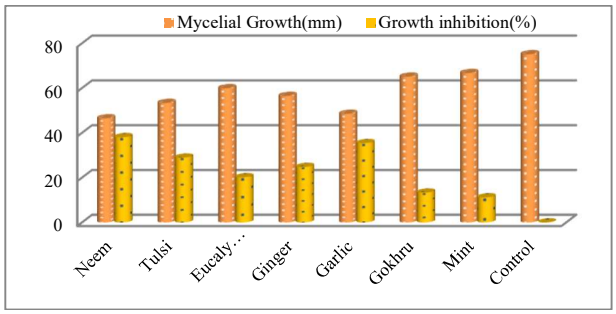

Figure 2: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at 15 percent concentration

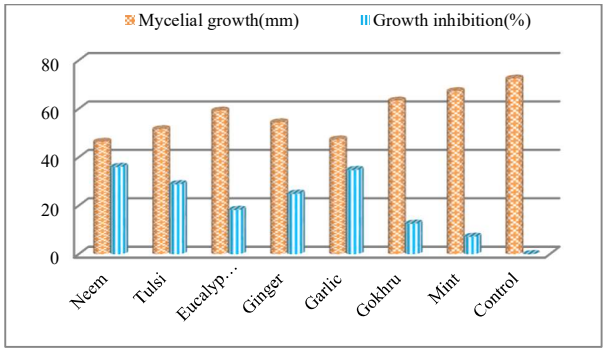

Figure 3: Influence of plant extracts on mycelial growth and growth inhibition of *Fusarium oxysporum* f.sp. *lini* after 168 hours at a 30 percent concentration

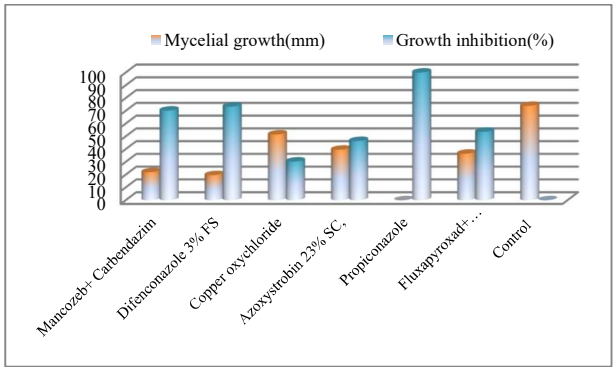

Figure 4: Efficacy of different fungicides on mycelial growth and growth inhibition of *Fusarium oxysporum* f. sp. *lini* at 300 ppm

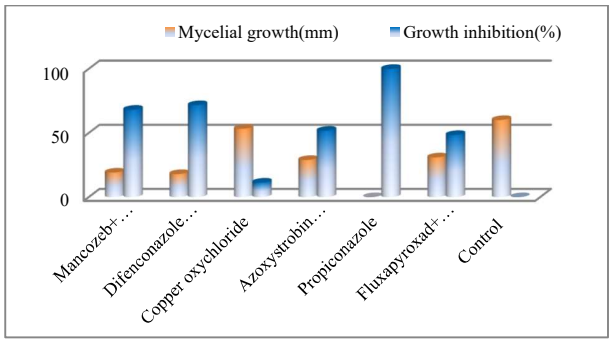

Figure 5: Efficacy of different fungicides on mycelial growth and growth inhibition of *Fusarium oxysporum* f. sp. *lini* at 500 ppm

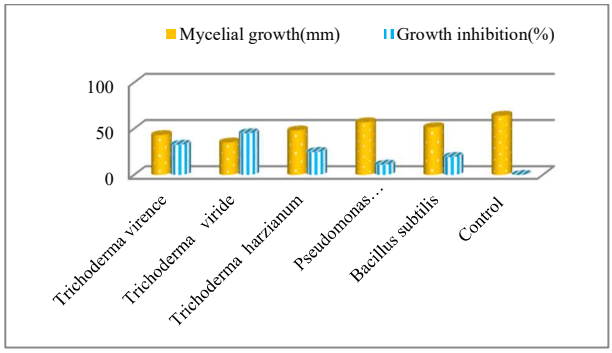

Figure 6: Antagonistic efficacy of bioagents against *Fusarium oxysporum* f.sp. *lini*

Conclusion

In conclusion, this study investigated the effectiveness of different bioagents, phosphoextracts and fungicides against *F. oxysporum* f. sp. *lini*. The findings demonstrated that Neem extract exhibited high efficacy as an antifungal agent, effectively inhibiting the growth of the pathogen *in vitro*. Propiconazole fungicide was also identified as a potent inhibitor of Fusarium wilt, with recommended concentrations of 300 ppm and 500 ppm, respectively. Among the bioagents tested, *T. viride* showed the highest effectiveness in inhibiting the test pathogen. These findings suggest that Neem extract, propiconazole, difenoconazole, and *T. viride* can be considered valuable options for controlling Fusarium wilt of linseed. However, further research is needed to validate their efficacy under field conditions and evaluate potential environmental impacts before implementing them as best practices for disease management.

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Conflict of interest

The authors declare that they have no conflict of interest.

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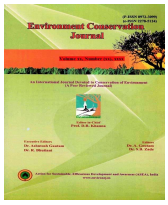

Performance of herbicides for managing weed flora in transplanted *aman* paddy (*Oryza sativa L.*)

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ABSTRACT

A field investigation was performed during the rainy seasons of 2018 and 2019 at the Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Jaguli, Nadia, West Bengal, India ($22^0$56'N and $86^0$48'E, 9.75m above mean sea level) with the aim of determining the comparative effectiveness of different herbicides in controlling various kinds of weeds (grass, sedge and broad-leaf) in the transplanted *aman* paddy. The experiment was laid out in Randomized Block Design having sixteen treatments with three replication, that includes application of either pre-emergence [butachlor, pretilachlor, pyrazosulfuron ethyl and ready mix (RM) of bensulfuron methyl + pretilachlor at 2 days after transplanting (DAT)] or post-emergence [bispyribac sodium and bispyribac sodium + penoxsulum at 20 DAT] herbicides followed by hand weeding at 40 DAT; application of both pre-emergence and post-emergence herbicides; hand weeding at 20 and 40 DAT and weedy check. Hand weeding at 20 and 40 DAT registered significantly lower weed density, weed dry matter and the highest weed control efficiency. Among the herbicidal treatments, ready-mix formulation of bensulfuron methyl 0.6% + pretilachlor 6% (Londax power 6.6%) performed better in controlling weeds of all categories and recorded higher paddy (3.96 t/ha) and straw yield (4.92 t/ha) with the lowest weed index which were statistically at par with the hand weeded treatment. Hence, to fetch the effective suppression of weed, application of Londax power 6.6% @ 0.66 kg a.i./ha as pre-emergence (at 2 DAT) with hand weeding at 40 DAT can easily replace additional one hand weeding at 20 DAT.

Introduction

Paddy (*Oryza sativa* L.) is an essential food grain for majority of the human population of the world, specifically in South East Asia. After wheat, it is the second most widely consumed cereal in the world (Anonymous, 2014a). The world's 112 rice-producing nations cover all seven continents, and 2.5 billion people living in developing nations consume it, with 90% of them residing in Asia and the remaining 10% in Africa, Australia, North and South America and Europe. It contributes roughly 45% of the nation's total grain production and grows on 44.1 million hectares of land, yielding 106.64 million tons of product each year at 2.42 tons/ha of productivity (Bhatt *et al.*, 2017). By 2040, 96 million tons more of milled rice will be required to supply the world's demand for rice than in 2015 (Valera and Belie, 2020). Globally, India ranks first in paddy in terms of acreage and second in terms of production next to China. India accounts for 21% of global rice production from 28% of rice area and West Bengal is the leading state, contributing 13.8% to all India rice production.

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However, weeds are pervasive and insidious oppressor on Earth and are known as important biological obstacles which prevent the yield of paddy with the optimum quality and productivity (Rao & Nagamani, 2013). Thus, weed control at right time is crucial for achieving the desired amount of productivity. One of the major obstacles to effective rice cultivation is heavy weed infestation (Parthipan *et al.*, 2013). They have a significant impact and, on average, lower agricultural productivity by more than 35% (Sattin and Berti, 2003). Under transplanted conditions, unchecked weeds compete with paddy and reduced grain yields by 76% (Mukherjee & Singh, 2005). The efficient control of weeds at early phases [0-40 days after transplanting (DAT)] can help in enhancing the productivity of this crop. Weed removal by manually is labor-intensive, and tiresome. An herbicide is chosen on the basis of type and the extent of weed infestation in the rice field. Herbicides are efficient against different weed species, however the majority of them target a limited number of weed species (Mukherjee and Singh, 2005). Since manual and other weed control procedures are time consuming, cumbersome and expensive, while chemicals are the absolute alternative and indispensable weed management methods. Thus, effective weed management usually calls for a combination of chemical and manual control in order to avoid development of herbicide resistance and lessen the herbicide load in the agro-ecosystem (Rao *et al*., 2007). Therefore, the present study was carried out to evaluate the efficacy of herbicides with proper dose for broad spectrum control of weed flora and to identify the effective weed management practice which will ensure satisfactory yield of transplanted *aman* rice.

Material and Methods

Experimental Site

A two-year investigation entitled 'Performance of herbicides for managing weed flora in transplanted aman paddy (*Oryza sativa L.*)' was performed during the *kharif* (Aman) period of 2018 and 2019 at the Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Jaguli, Nadia, West Bengal, India (22^0 56' N and 86^0 48' E, 9.75m from mean sea level). The investigation was carried out just south of the tropic of cancer under tropical humid climate in fairly uniform topographical condition having sandy clay-loam texture with excellent water retention capacity, neutral in reaction and moderate soil fertility during the months from July to November. The rainfall was distributed throughout the experimental period in both the year. The average maximum temperature for the course of investigation varied from 30 to 33℃ and the range of average minimum temperature for the similar time period was 14 to 23℃. Some important meteorological parameters during the time of experiment are presented in the Figure 1.

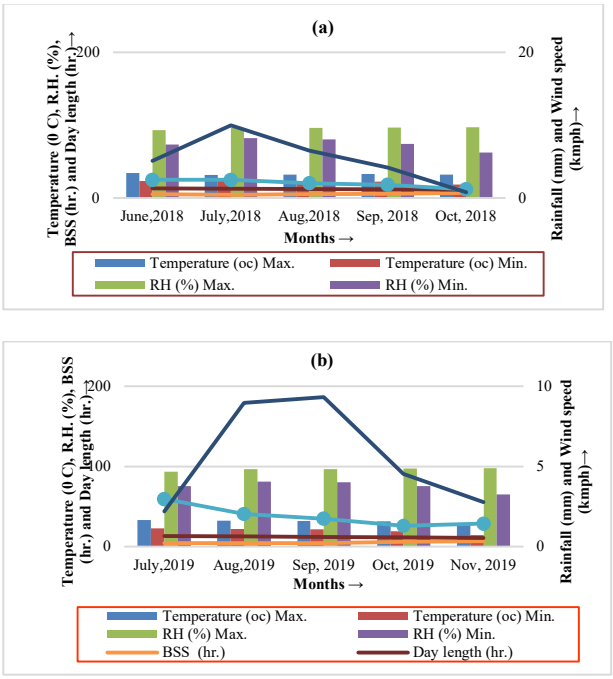

Figure 1: Meteorological observations of (a) 2018 and (b) 2019 during the course of investigation

Experiment details

The semi-dwarf, short duration (110-115 days) and high yielding rice variety Satabdi (IET-4786) was selected and 25 days old seedlings were transplanted with the spacing of 20 cm × 15 cm. Fertilizers used were Urea, Single Super Phosphate and Muriate of Potash with the recommended dosage of N: P_2O_5: K_2O @ 60: 30: 30 kg/ha. Randomized Block Design (RBD) was adopted for the lay out with sixteen treatment combinations (Table 1) replicated three times with the plot size 4 m × 5 m each. Weed control measures include four pre-emergence herbicides (pyrazosulfuron ethyl,

Table 1: Treatment details – herbicide dosage, application time and their combination with hand weeding

Treatment	Herbicide combinations
T_1	Butachlor 50 EC @ 1500 g a.i./ha at 2 DAT + hand weeding at 40 DAT
T_2	Pretilachlor 50 EC @ 750 g a.i./ha at 2 DAT + hand weeding at 40 DAT
T_3	Pyrazosulfuron ethyl 10 WP @ 25 g a.i./ha at 2 DAT + hand weeding at 40 DAT
T_4	Londax power 6.6 % (Bensulfuron methyl + Pretilachlor) [RM] @ 660 g a.i./ha at 2 DAT + hand weeding at 40 DAT
T_5	Bispyribac sodium 10 SC @ 25 g a.i./ha at 20 DAT + hand weeding at 40 DAT
T_6	(Bispyribac sodium 9.5 SC + Penoxsulum 7.8 SC) [RM] @ (23.75 + 19.50) g a.i./ha at 20 DAT + hand weeding at 40 DAT
T_7	Butachlor 50 EC @ 1500 g a.i./ha at 2 DAT + Bispyribac sodium 10 SC @ 25 g a.i./ha at 20 DAT
T_8	Pretilachlor 50 EC @ 750 g a.i./ha at 2 DAT + Bispyribac sodium 10 SC @ 25 g a.i./ha at 20 DAT
T_9	Pyrazosulfuron ethyl 10 WP @ 25 g a.i./ha at 2 DAT + Bispyribac sodium 10 SC @ 25 g a.i./ha at 20 DAT
T_{10}	Londax power 6.6 % [RM] + Bispyribac sodium 10 SC @ 25 g a.i./ha at 20 DAT
T_{11}	Butachlor 50 EC@ 1500 g a.i./ha at 2 DAT + (Bispyribac sodium 9.5 SC + Penoxsulum 7.8 SC) [RM] @ (23.75 + 19.50) g a.i./ha at 20 DAT
T_{12}	Pretilachlor 50 EC @ 750 g a.i./ha at 2 DAT + (Bispyribac sodium 9.5 SC + Penoxsulum 7.8 SC) [RM] @ (23.75 + 19.50) g a.i./ha at 20 DAT
T_{13}	Pyrazosulfuron ethyl 10 WP @ 25 g a.i./ha at 2 DAT + (Bispyribac sodium 9.5 SC + Penoxsulum 7.8 SC) [RM] @ (23.75 + 19.50) g a.i./ha at 20 DAT
T_{14}	Londax power 6.6 % [RM] + (Bispyribac sodium 9.5 SC + Penoxsulum 7.8 SC) [RM] @ (23.75 + 19.50) g a.i./ha at 20 DAT
T_{15}	Hand weeding at 20 and 40 DAT
T_{16}	Weedy check

***DAT: Days after transplanting; EC: Emulsifiable concentrate; WP: Wettable powder; SC: Soluble concentrate**

pretilachlor, butachlor and ready mix (RM) of Bensulfurron methyl + Pretilechlor i.e londax powder. two post-emergence herbicides bispyribac sodium and ready mix (RM) bispyribac sodium + penoxsulum) and hand weeding (HW) at 20 and 40 DAT.

Sampling and measurement on weeds

Weeds appeared in the experimental field were identified and by keeping a quadrate of 0.5 m × 0.5 m size at random places of the experimental plots, weed count or density (number/m^2) and biomass (g/m^2) were collected category wise - grass, sedges and broad leaved at the interval of 15 days (from 30 to 90 DAT). From this, total weed count (number/m^2) and biomass (g/m^2) were worked out. Following are the various weed indices that were calculated using the weed data and standard methods:

Weed control efficiency (WCE):

It was computed using the methodology given by Mani *et al*., (1973) and it is expressed as percentage (%)-

$$\mathbf{WCE} = \frac{(\mathbf{DWC} - \mathbf{DWT})}{\mathbf{DWC}} \times \mathbf{100}$$

Where,
WCE = Weed control efficiency (%)
D_{WC} = Weed dry matter (unit/m^2) in control plot
D_{WT} = Weed dry matter (unit/m^2) in treated plot

Weed index (WI):

Weed index was calculated on the basis of yield drop in comparison to weed-free treatment and expressed in percentage. It was determined utilizing the formula provided by Gill and Kumar (1969).

$$\mathbf{WI} = \frac{\mathbf{X} - \mathbf{Y}}{\mathbf{X}} \times \mathbf{100}$$

Where,
WI = Weed Index (%)
X = Crop yield (t/ha) from weed free plot (hand weeded plot)
Y = Crop yield (t/ha) from treated plot

Yield and harvest index:

The harvesting was done at 94 DAT and 91 DAT during the year 2018 and 2019 respectively with the aid of a sickle. Grain and straw yield (t/ha) yields were documented when the crops were harvested. Harvest index (HI %), given by Donald (1962), was determined using the formula below:

$$\text{HI (\%)} = \frac{\text{Economic yield (grain yield)}}{\text{Biological yield (grain + straw yield)}} \times 100$$

Statistical analysis

The data were statistically analyzed utilizing Randomized Block Design of Panse & Sukhatme (1985). Data regarding weed count (number/m^2) were analyzed after transforming this using 'Square root transformation (SQRT)' method i.e., $\sqrt{(x + 0.5)}$. Fisher & Snedecor's F-test, with a 5% level of probability, was used to determine the significance of various sources of variation. The Fisher & Yates (1953) statistical table was used for the ascertainment of critical difference (C.D.) at the 5% level of significance. In this tables, mean values were compared with the result of standard error of mean S.Em. (±).

Results and Discussion

Weed flora

The rate of reduction of plant growth and yield mainly depends on the type of weeds prevalent in the field. In both of the experimental years, the field was dominated with mixed weed flora *viz.* grasses, sedges and broad leaved weeds. Eleven (11) species of weeds had been identified in the experimental field among which four (4) species were found to be grassy weed category, three (3) species from sedges and four (4) species from broad leaved category of weeds which were barnyard grass (*Echinochloa crusgalli*), jungle rice (*Echinochloa colonum*), bermuda grass (*Cynodon dactylon)* and southern cut grass (*Leersia hexandra*) among grassy weeds; rice flatsedge (*Cyperus iria*), tall fringe rush (*Fimbristylis miliacea*) and softstem bulrush (*Scirpus juncoides*) among sedge weeds; blistering ammannia (*Ammannia baccifera*), swamp morning-glory (*Ipomea aquatica*), banana plant (*Nymphoides indica*) and pickerel weed (*Pontederia cordata*) among broad leaved weeds which might be due to their seasonal preferences and favourable condition of growth. These outcomes are in agreement with the findings of Ghosh & Ghosh (2005); Mukherjee *et al*. (2008) and Pal *et al*. (2009a) where they observed that the rice fields were seriously infested by similar weed species (like *Echinochloa sp., Cynodon sp., Cyperus sp., Ammannia sp. Fimbristylis sp.* etc.) under lowland condition because of their long emergence profile under ideal soil and climatic condition.

Weed density (number/m^2)

Broad leaved weed population was found to be higher than grassy and sedge weeds for all the weed management methods (Table 2). Increment in weed population was also observed with the advance of growth stages in all weed management techniques, with the exception of twice HW (T_{15}) as the grassy and broad leaved weed counts were decreased in this treatment at 45 DAT and thereafter, it also increased subsequently. The highest weed count was registered in weedy check (T_{16}) and was substantially greater than any other treatments whereas hand weeded plot (T_{15}) registered the lowest count which was at par with the treatment where ready mix (RM) herbicide Londax power (bensulfuron methyl 0.6% + pretilachlor 6%) was used as pre-emergence herbicide followed by one HW (T_4).

Among herbicidal treatments, Londax power [RM] (at 2 DAT) with one HW (at 40 DAT) registered minimum number of total weed population (Table 3) in all the observations (2.82, 3.25, 4.47, 5.6 and 6.83 /m^2 at 30, 45, 60, 75 and 90 DAT, respectively) as it is an acetolactate synthase (ALS) inhibiting herbicide which provides effective solution for weed control in rice by inhibiting the growth of the most important perennial and annual species of weeds and it was statistically at par with pre-emergence application of pretilachlor or pyrazosulfuron ethyl with HW at 40 DAT (T_2 and T_3), these outcomes are in compliance with Shekhra *et al.* (2011) and Mishra (2019). The RM of bispyribac sodium and penoxsulum at 20 DAT with HW at 40 DAT (T_6) showed profound effect in controlling weeds as compared to bispyribac sodium (at 20 DAT) followed by HW at 40 DAT (T_5). Londax power [RM] followed by RM of bispyribac sodium and penoxsulum (T_{14}) performed well as compared to Londax power [RM] followed by only bispyribac sodium (T_{10}). Butachlor at 2 DAT followed by bispyribac sodium at 20 DAT (T_7) recorded the highest total weed density (Table 3).

Weed dry matter (g/m^2)

Hand weeded plot (two HW at 20 and 40 DAT) registered the least dry weight of all categories of

Table 2: Effect of weed control treatments on density (number/m^2) of different categories of weeds in transplanted winter paddy (pooled data over 2018 and 2019)

Treatments	Density of Grassy Weeds (number/m^2)					Density of Sedge Weeds (number/m^2)					Density of Broad leaved Weeds (number/m^2)				
	30DAT	45DAT	60DAT	75DAT	90DAT	30DAT	45DAT	60DAT	75DAT	90DAT	30DAT	45DAT	60DAT	75DAT	90DAT
T_1	3.28 (10.23)	4.02 (15.63)	4.38 (18.31)	5.66 (31.58)	6.19 (37.86)	2.61 (6.31)	2.83 (7.50)	3.00 (8.51)	4.11 (16.42)	4.63 (20.94)	1.91 (3.14)	2.3 (4.79)	3.07 (8.93)	3.34 (10.68)	3.84 (14.25)
T_2	2.64 (6.45)	3.03 (8.68)	3.65 (12.82)	4.78 (22.34)	5.40 (28.70)	1.93 (3.22)	2.09 (3.86)	2.49 (5.71)	3.28 (10.25)	3.97 (15.23)	1.63 (2.17)	1.92 (3.19)	2.53 (5.91)	2.8 (7.32)	3.47 (11.52)
T_3	3.02 (8.62)	3.65 (12.82)	4.16 (16.82)	5.18 (26.34)	5.79 (32.98)	2.3 (4.79)	2.46 (5.56)	2.71 (6.87)	3.84 (14.26)	4.28 (17.81)	1.84 (2.89)	2.11 (3.94)	2.76 (7.12)	2.94 (8.14)	3.55 (12.13)
T_4	2.17 (4.22)	2.59 (6.21)	3.48 (11.59)	4.31 (18.10)	5.03 (24.80)	1.69 (2.36)	1.59 (2.03)	1.91 (3.15)	2.74 (7.03)	3.38 (10.96)	1.18 (0.89)	1.53 (1.84)	2.3 (4.78)	2.49 (5.71)	3.31 (10.44)
T_5	4.04 (15.79)	4.62 (20.84)	5.30 (27.64)	6.34 (39.65)	6.73 (44.76)	3.09 (9.03)	3.38 (10.94)	3.42 (11.21)	5.33 (27.87)	5.54 (30.20)	2.51 (5.83)	2.84 (7.57)	3.46 (11.54)	3.54 (12.03)	4.17 (16.92)
T_6	3.51 (11.79)	4.16 (16.82)	4.94 (23.92)	6.00 (35.56)	6.36 (39.94)	2.82 (7.47)	3.06 (8.90)	3.21 (9.79)	4.61 (20.73)	4.96 (24.12)	2.17 (4.21)	2.41 (5.31)	3.21 (9.83)	3.36 (10.81)	4.03 (15.78)
T_7	5.19 (26.45)	6.23 (38.32)	7.84 (61.02)	8.41 (70.26)	8.71 (75.39)	4.48 (19.61)	4.72 (21.82)	5.43 (28.97)	6.54 (42.27)	7.27 (52.38)	3.57 (12.23)	4.29 (17.91)	4.51 (19.89)	4.91 (23.67)	5.67 (31.64)
T_8	4.81 (22.67)	5.78 (32.88)	7.31 (52.91)	8.01 (63.68)	8.23 (67.28)	4.13 (16.53)	4.35 (18.45)	4.86 (23.10)	6.35 (39.79)	6.71 (44.58)	3.34 (10.63)	4.05 (15.93)	4.18 (16.98)	4.52 (19.90)	5.01 (24.62)
T_9	5.03 (24.82)	6.01 (35.66)	7.59 (57.05)	8.23 (67.26)	8.45 (70.89)	4.33 (18.25)	4.44 (19.21)	5.09 (25.45)	6.37 (40.11)	6.84 (46.32)	3.47 (11.55)	4.23 (17.38)	4.28 (17.84)	4.68 (21.38)	5.29 (27.48)
T_{10}	4.35 (18.45)	5.18 (26.31)	6.01 (35.66)	6.94 (47.64)	7.13 (50.34)	3.47 (11.57)	3.82 (14.16)	4.04 (15.87)	5.71 (32.14)	6.03 (35.87)	2.84 (7.57)	3.28 (10.28)	3.69 (13.12)	3.92 (14..86)	4.47 (19.45)
T_{11}	4.68 (21.45)	5.67 (31.64)	6.93 (47.56)	8.17 (60.22)	7.92 (62.27)	4.02 (15.68)	4.26 (17.61)	4.68 (21.40)	6.14 (37.17)	6.54 (42.31)	3.23 (9.97)	3.9 (14.68)	4.08 (16.18)	4.45 (19.31)	4.82 (22.74)
T_{12}	4.50 (19.79)	5.30 (27.64)	6.23 (38.32)	7.22 (51.62)	7.40 (54.25)	3.68 (13.02)	4.01 (15.62)	4.34 (18.32)	5.91 (34.43)	6.23 (38.32)	2.96 (8.25)	3.51 (11.82)	3.84 (14.23)	4.14 (16.63)	4.57 (20.38)
T_{13}	4.62 (20.87)	5.55 (30.31)	6.54 (42.23)	7.53 (56.27)	7.67 (58.27)	3.84 (14.28)	4.12 (16.51)	4.47 (19.52)	5.98 (35.29)	6.36 (39.98)	3.09 (9.04)	3.68 (13.02)	4.01 (15.54)	4.22 (17.34)	4.71 (21.69)
T_{14}	4.02 (16.78)	4.81 (23.64)	5.46 (30.31)	6.66 (43.56)	6.75 (47.09)	3.29 (10.31)	3.66 (12.92)	3.91 (14.77)	5.52 (30.02)	5.75 (32.58)	2.68 (6.70)	3.1 (9.11)	3.64 (12.79)	3.66 (12.93)	4.34 (18.34)
T_{15}	1.93 (3.22)	1.18 (0.89)	2.45 (5.52)	3.47 (11.52)	4.11 (16.44)	1.18 (0.89)	0.71 (0)	1.56 (1.92)	2.43 (5.41)	2.95 (8.23)	1.08 (0.67)	0.71 (0)	1.69 (2.37)	2.04 (3.68)	2.73 (6.94)
T_{16}	6.43 (40.81)	7.20 (51.34)	8.85 (77.89)	9.71 (93.79)	11.59 (133.84)	4.89 (23.40)	5.75 (32.57)	6.43 (40.87)	8.29 (68.29)	9.84 (96.38)	4.14 (16.67)	4.59 (20.54)	6.15 (37.33)	6.87 (46.37)	8.31 (68.23)
S. Em. (±)	0.36	0.29	0.32	0.34	0.33	0.14	0.17	0.18	0.21	0.27	0.12	0.13	0.16	0.14	0.15
C.D. at 5 %	1.03	0.84	0.90	0.96	0.95	0.41	0.50	0.53	0.61	0.77	0.34	0.39	0.46	0.41	0.44

Figures in parentheses indicate original values of weed count/ m^2. Square Root transformed data [√(x + 0.5)] has been used for analysis.

Table 3. Effect of weed control treatments on total weed density (number/m^2) in transplanted winter paddy (pooled data over 2018 and 2019)

Treatment	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
T_1	4.49 (19.68)	5.33 (27.92)	6.02 (35.75)	7.69 (58.68)	8.58 (73.05)
T_2	3.51 (11.84)	4.02 (15.73)	4.99 (24.44)	6.35 (39.91)	7.48 (55.45)
T_3	4.1 (16.3)	4.77 (22.32)	5.59 (30.81)	7.01 (48.74)	7.96 (62.92)
T_4	2.82 (7.47)	3.25 (10.08)	4.47 (19.52)	5.6 (30.84)	6.83 (46.2)
T_5	5.58 (30.65)	6.31 (39.35)	7.13 (50.39)	8.94 (79.55)	9.61 (91.88)
T_6	4.89 (23.47)	5.61 (31.03)	6.64 (43.54)	8.22 (67.1)	8.96 (79.84)
T_7	7.67 (58.29)	8.86 (78.05)	10.51 (109.88)	11.69 (136.2)	12.64 (159.41)
T_8	7.09 (49.83)	8.23 (67.26)	9.67 (92.99)	11.13 (123.37)	11.7 (136.48)
T_9	7.42 (54.62)	8.53 (72.25)	10.04 (100.34)	11.37 (128.75)	12.05 (144.69)
T_{10}	6.17 (37.59)	7.16 (50.75)	8.07 (64.65)	9.75 (94.64)	10.30 (105.66)
T_{11}	6.9 (47.10)	8.03 (63.93)	9.25 (85.14)	10.82 (116.7)	11.30 (127.32)
T_{12}	6.45 (41.06)	7.45 (55.08)	8.45 (70.87)	10.16 (102.68)	10.65 (112.95)
T_{13}	6.68 (44.19)	7.77 (59.84)	8.82 (77.29)	10.46 (108.9)	10.97 (119.94)
T_{14}	5.85 (33.79)	6.79 (45.67)	7.64 (57.87)	9.33 (86.51)	9.92 (98.01)
T_{15}	2.3 (4.78)	1.18 (0.89)	3.21 (9.81)	4.59 (20.61)	5.92 (34.61)
T_{16}	9.02 (80.88)	10.24 (104.45)	12.51 (156.09)	14.45 (208.45)	17.29 (298.45)
S.Em. (±)	0.25	0.29	0.26	0.31	0.32
CD at 5%	0.72	0.84	0.75	0.88	0.91

Figures in parentheses indicate original values of weed count/m^2. Square Root transformed data [$\sqrt{(x + 0.5)}$] has been used for analysis.

weeds (Table 4), which, however, was followed by of Londax power [RM] with one HW (T_4) as it is highly selective to most varieties of Indian rice and most of the annual and perennial weed species can effectively be controlled.Increasing trend of total weed biomass with advancement of growth stages had been noticed (Table 5). Whereas, it was decreased at 45 DAT in HW at 20 & 40 DAT (T_{15}), however it has been increased from 60 to 90 DAT due to occurrence, growth and development of several late flushes of weeds in the rice field. The maximum total weed biomass was acquired from weedy check (T_{16}) at all the five observations (21.51, 26.71, 31.03, 35.64 and 41.24 g/m^2 at 30, 45, 60, 75 & 90 DAT, respectively) that was considerably greater than rest of the treatments. Londax power 6.6% [RM] at 2 DAT with HW at 40 DAT (T_4) recorded the minimum total weed biomass among the herbicidal treated plots which reflect its superiority of controlling weeds than the other herbicides and it was also reported by Singh *et al.* (2010) and Mishra (2019).

Effect on weed control efficiency (WCE, %)

The highest WCE was obtained at 45 DAT, however, it started to decline from 60 DAT onwards and this trend was found in both the years of investigation (Table 5) which might be due to emergence of some new weed species at later by different weeding treatments. Among various weed control practices, HW at 20 and 40 DAT (T_{15}) was the most efficient and registered highest WCE (85.54, 92.18, 85.08, 75.62 and 69.18 % at 30, 45, 60, 75 and 90 DAT, respectively) at all the intervals followed by Londax power at 2 DAT with HW at 40 DAT (T_4) recorded the highest WCE (81.68, 85.02, 80.15, 70.29 and 64.99 % at 60, 75 and 90 DAT, respectively) which confirmed the opinion of

Table 4: Effect of weed control treatments on weed dry matter (g/m^2) of different categories of weeds in transplanted winter paddy (pooled data over 2018 and 2019)

Treatments	Dry weight of Grassy Weeds (g/m^2)					Dry weight of Sedge Weeds (g/m^2)					Dry weight of Broad leaved Weeds (g/m^2)				
	30DAT	45DAT	60DAT	75DAT	90DAT	30DAT	45DAT	60DAT	75DAT	90DAT	30DAT	45DAT	60DAT	75DAT	90DAT
T_1	2.12	2.18	3.21	5.48	7.13	1.62	1.82	2.55	3.76	5.37	1.60	1.88	2.28	3.77	4.84
T_2	1.78	1.83	2.71	4.92	6.88	1.36	1.48	2.07	3.07	4.65	1.38	1.52	1.98	3.12	3.96
T_3	1.95	2.08	2.97	5.00	6.92	1.48	1.66	2.34	3.41	5.05	1.44	1.68	2.14	3.48	4.54
T_4	1.53	1.41	2.48	4.75	6.32	1.25	1.16	1.81	2.90	4.24	1.16	1.43	1.87	2.94	3.88
T_5	2.46	2.54	3.54	5.56	7.43	1.98	2.25	3.33	4.56	5.84	2.05	2.52	2.92	4.21	5.17
T_6	2.24	2.33	3.35	5.41	7.24	1.77	2.02	2.84	3.98	5.51	1.82	2.04	2.54	3.96	4.98
T_7	3.89	4.48	4.82	6.91	9.89	4.01	5.11	6.78	7.86	8.92	3.49	4.32	5.23	6.12	7.64
T_8	3.45	3.91	4.42	6.55	9.20	3.42	4.48	5.86	6.93	7.98	3.18	3.87	4.50	5.41	6.78
T_9	3.64	4.05	4.66	6.77	9.56	3.78	4.82	6.33	7.41	8.23	3.26	4.10	4.94	5.87	7.13
T_{10}	2.84	2.95	3.88	5.95	8.05	2.56	2.73	4.21	5.48	6.72	2.45	2.83	3.32	4.69	5.72
T_{11}	3.38	3.68	4.27	6.43	8.94	3.15	3.94	5.32	6.75	7.71	3.09	3.56	4.29	4.98	6.55
T_{12}	2.98	3.04	3.95	6.09	8.18	2.84	3.04	4.78	5.91	6.96	2.64	3.01	3.68	4.88	5.91
T_{13}	3.13	3.22	4.13	6.36	8.62	2.97	3.37	4.95	6.34	7.35	2.89	3.28	3.95	5.03	6.24
T_{14}	2.75	2.86	3.72	5.85	7.81	2.13	2.42	3.86	4.96	6.23	2.18	2.69	3.11	4.48	5.53
T_{15}	1.22	0.64	1.86	3.73	5.82	0.77	0.58	1.45	2.58	3.44	1.12	0.87	1.32	2.38	3.45
T_{16}	6.96	8.17	9.73	11.58	13.42	8.32	10.43	12.05	13.24	14.98	6.23	8.11	9.25	10.82	12.84
S. Em. (±)	0.12	0.11	0.15	0.21	0.28	0.10	0.11	0.16	0.19	0.21	0.10	0.11	0.13	0.17	0.21
C.D. at 5 %	0.33	0.32	0.44	0.62	0.79	0.28	0.29	0.47	0.54	0.62	0.29	0.32	0.39	0.49	0.62

Table 5. Effect of weed control treatments on total weed biomass (g/m^2) and weed control efficiency (%) in transplanted winter paddy

Treatments	Total weed biomass (g/m^2)					Weed control efficiency (%)				
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
T_1	5.34	5.88	8.04	13.01	17.34	75.17	77.99	74.09	63.5	57.95
T_2	4.52	4.83	6.76	11.11	15.49	78.99	81.92	78.21	68.83	62.44
T_3	4.87	5.42	7.45	11.89	16.51	77.36	79.71	75.99	66.64	59.97
T_4	3.94	4	6.16	10.59	14.44	81.68	85.02	80.15	70.29	64.99
T_5	6.49	7.31	9.79	14.33	18.44	69.83	72.63	68.45	59.79	55.29
T_6	5.83	6.39	8.73	13.35	17.73	72.9	76.08	71.87	62.54	57.01
T_7	11.39	13.91	16.83	20.89	26.45	47.05	47.92	45.76	41.39	35.86
T_8	10.05	12.26	14.78	18.89	23.96	53.28	54.1	52.37	47	41.9
T_9	10.68	12.97	15.93	20.05	24.92	50.35	51.44	48.66	43.74	39.57
T_{10}	7.85	8.51	11.41	16.12	20.49	63.51	68.14	63.23	54.77	50.32
T_{11}	9.62	11.18	13.88	18.16	23.20	55.28	58.14	55.27	49.05	43.74
T_{12}	8.46	9.09	12.41	16.88	21.05	60.67	65.97	60.01	52.64	48.96
T_{13}	8.99	9.87	13.03	17.73	22.21	58.21	63.05	58.01	50.25	46.14
T_{14}	7.06	7.97	10.69	15.29	19.57	67.18	70.16	65.55	57.1	52.55
T_{15}	3.11	2.09	4.63	8.69	12.71	85.54	92.18	85.08	75.62	69.18
T_{16}	21.51	26.71	31.03	35.64	41.24	--	--	--	--	--
S. Em. (±)	0.26	0.27	0.36	0.53	0.61	2.30	2.06	2.03	1.70	1.58
C.D. at 5 %	0.74	0.78	1.04	1.52	1.76	6.64	5.96	5.87	4.94	4.61

Mishra (2019) whereas butachlor at 2 DAT followed by bispyribac sodium at 20 DAT (T_7) recorded the lowest (47.05, 47.92, 45.76 , 41.39 and 35.86 % at 30, 45, 60, 75 and 90 DAT, respectively) WCE among the herbicidal plots. Post-emergence herbicidal plots with HW at 40 DAT (T_5 and T_6), registered lower WCE than the pre-emergence herbicidal plots with HW at 40 DAT (T_1, T_2, T_3 and T_4). However, application of pre-emergence herbicide or post-emergence herbicide followed by HW at 40 DAT were recorded higher WCE than the both pre- and post-emergence herbicides treated plots (T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13} and T_{14}). So, it was evident that application of pre-emergence herbicide with HW at 40 DAT was the effective weed control strategy which might be attributed to lowering down of weed density which ultimately increase the weed control efficiency. This result corroborated the findings of Hasanuzzaman *et al*. (2007 and 2009).

Effect on yield (t/ha), harvest index (%) and weed index (WI, %)

According to study, in weedy check plot, weed infestation led to 47.17 % loss of rice grain yield in comparison to HW at 20 and 40 DAT (Table 6). Similar yield reduction in rainy season rice due to crop-weed competition in lateritic belt of West Bengal was also reported by Duary *et al*. (2009) and Mandal *et al*. (2013). Whereas, HW at 20 and 40 DAT (T_{15}) registered maximum grain as well as straw yield (t/ha) that was proceeded by application of Londax power (RM) with HW at 40 DAT (T_4). This could be due to less crop-weed competition for limited resources which promotes good crop growth and its development resulted into higher number of effective tillers per plant and maximum grain this is in compliance with the result registered by Reddy *et al.* (2012) and Mishra (2019). Harvest index of the rice crop during the experimentation varied between 37.72 to 45.02% and the highest value had been registered from plot with twice HW at 20 and 40 DAT whereas the lowermost was observed in weedy check (Table 6). Among herbicides, the maximum HI (44.59 %) was recorded from Londax power (RM) with single HW (T_4) followed by pretilachlor with HW once (T_2). This is due to higher grain as well as straw yield of rice obtained from T_4 which was statistically at par with the treatment with two HW at 20 and 40 DAT. The least value of Weed Index was recorded under T_4 (1.57 %) followed by T_2, T_3 and T_1 (2.90, 4.22 and 5.88 % respectively) among the herbicides showing a wide range of effectiveness in weed control. The results were in conformity with Partipan *et al*. (2013).

Table 6: Effect of weed control treatments on yields (grain and straw), harvest index and weed index in transplanted winter paddy (pooled data over 2018 and 2019)

Treatment	Grain yield (t/ha)	Straw yield (t/ha)	Harvest index (%)	Weed Index (%)
T_1	3.77	4.72	44.41	5.88
T_2	3.9	4.86	44.52	2.90
T_3	3.84	4.8	44.44	4.22
T_4	3.96	4.92	44.59	1.57
T_5	3.51	4.55	43.55	10.64
T_6	3.62	4.62	43.93	8.65
T_7	2.71	3.77	41.82	28.12
T_8	2.91	3.99	42.17	23.47
T_9	2.8	3.87	41.98	26.02
T_{10}	3.31	4.36	43.16	14.95
T_{11}	3.02	4.1	42.42	21.04
T_{12}	3.18	4.25	42.80	17.61
T_{13}	3.09	4.17	42.56	19.49
T_{14}	3.41	4.46	43.33	12.74
T_{15}	4.07	4.97	45.02	0.0
T_{16}	2.15	3.55	37.72	36.75
S.Em. (±)	0.17	0.30	---	2.88
CD at 5%	0.48	0.87	---	8.31

Conclusion

The outcomes of our research showed that even though highest WCE and yield was recorded from treatment with HW at 20 and 40 DAT however, it was laborious and time consuming while use of chemicals or herbicides for weed management was both highly effective and lucrative. The aforementioned experimental out comes indicated that pre-emergence application of Londax power (bensulfuron methyl 0.6% + pretilachlor 6%) [RM] @ 0.66 kg a.i./ ha at 2 DAT with HW at 40 DAT can one of substitute of one HW. The granular formulation of Londax Power gives farmers an additional benefit of easy hand dispersal in the puddled rice field. This low dose herbicide has the potentiality to replace other voluminous and costly herbicides (like butachlor 50 EC @ 1.5 kg a.i./ha and bispyribac sodium 10 SC @ 25 g a.i./ha). This is a newly introduced herbicide and control weeds very effectively than the older herbicides which are used for long time repeatedly. Hence, application of bensulfuron methyl 0.6% + pretilachlor 6% (RM) @ 0.66 kg a.i./ha (2 DAT) with HW at 40 DAT may be suggested for better weed control with higher productivity which can easily replace the tedious and lingering HW (twice) practice. Also, farmers have to follow the rotational use of herbicide followed by hand weeding at 40 DAT to avoid any selection pressure towards weeds.

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Conflict of interest

The authors declare that they have no conflict of interest.

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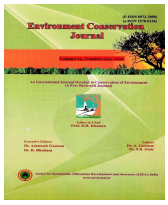

Analysis of the probability of rainfall in the Fingeshwar Tehsil of the Gariyaband District for crop planning

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ABSTRACT

Rainfall probability analysis improves predictions of the minimum assured rainfall to aid crop planning. An attempt has been made to look into the patterns of rainfall distribution, including weekly, seasonal, and annual rainfall, using data collected from the Fingeshwar tehsil of the Gariyaband district, Chhattisgarh, over a ten-year period (2011-2020). Using the Weibull plotting position function, expected weekly, monthly, seasonal, and yearly rainfall values were calculated for various probability levels. Based on a 10-year yearly average, the data revealed that 1074.4 mm of rain were actually seen, following an average of 52.2 rainy days. A rainfall amount with a 75% probability level predicts 862.9 mm annually. The largest amount of weekly rainfall, 49 mm, was predicted to fall in the 35th week, followed by 32.1 mm in the 25th standard week and the least amount, 0.0 mm, in the 20-22nd, 29, 37, 40-42nd SMW. This prediction was made at a 75% chance level, same like the one before. According to a study of monthly rainfall at 70, 75, and 80% probability levels, the three crucial wet months are July, August, and September, with probabilities of getting a monthly rainfall between 0 and 50 mm. At a 70% probability level, the seasonal rainfall report projects 833 mm for the *Kharif* season. Thus, it can conclude that the kharif season's activities could start between the 22nd and the 23rd standard week and farmers can properly produce paddy crops in highland areas followed by any rabi crop in rabi season.

Introduction

One of the main climatic elements that directly influences the water balance of the soil is rainfall. The crop planning and crop calendar for a particular agroclimatic zone are influenced by the quantity, timing, and spatial variability of the rainfall that falls on the soil surface. The start and end of the rainy season are clearly associated with the agricultural calendar, which in turn directly affects agricultural productivity. It is essential to comprehend the pattern based on past data in order to be able to predict the future. The probability of rainfall and its occurrence has been studied by a number of researchers. For a number of locations in India, rainfall data have been subjected to frequency analysis. Raju *et al.* (2021) studied the weekly probability analysis of rainfall using statistical methods to forecast the lowest assured rainfall that aids in crop planning and management in Anakapalle, it gives the length of the growth season taking a 50% chance of rain into account. In spite of the amount of rainfall expected, a study of the coefficient of variation of weekly rainfall showed that reliable rainfall would occur during the time period. Sinha *et al.* (2018) investigated the rainfall probability for crop planning in the Raipur region of Chhattisgarh state and discovered that despite the need for more irrigation for Kharif crops, there is at least a 70% chance that there will be enough rainfall to support the growth of high-value fruit crops. Based on data collected over a 13-

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year period (2000–2012) at Shivri, Lucknow, Uttar Pradesh, S. Singh *et al.* (2016) attempted to assess the distribution of rainfall and forcast the rainfall using plotting-position formula. Baweja (2011) found that Solan, Himachal Pradesh, India's constant rainfall period spanned from the 24th to the 37th standard meteorological week based on an analysis of 38 years of rainfall data. In the Orissa area of Kandhmal, Subudhi *et al.* (2012) did a study on the probability analysis of rainfall for crop planning. They discovered that there is a 75% chance that there will be enough rain to water crops.He demonstrated that the season's rainfall distribution, not its total amount, has an impact on agricultural productivity. Krishnamurthy *et al*. (2009) reveals that the intensity and frequency of extreme events have typically decreased in the north and centre of the Indian Subcontinent, while they've surged in the coastal regions of the peninsula and the immediate area west of Bangladesh.This shows that understanding the overall quantity of rainfall that falls in a particular year and how it is distributed is ultimately necessary for effective crop planning, estimating the irrigation and drainage needs of crops, and devising and constructing buildings for soil and water conservation. A probability and frequency analysis of rainfall data can be used to estimate the projected rainfall at various percent chances. It is the most reliable method of predicting the probability of possible rainfall data since it is based on the behaviour of rainfall in the past. When analysing rainfall data, several probability distributions are typically employed to calculate the predicted rainfall for a certain frequency. This demonstrates the value of using local rainfall data to study the localised pattern of rainfall. As a result, information from the region was collected to conduct a 10-year rainfall probability study for the Fingeshwar tehsil in the Chhattisgarh district of Gariyaband.

Material and Methods

The Fingeshwar Tehsil in the Gariyaband district of Chhattisgarh, which is located at 20°57'56"N latitude and 81°53'19"E longitude, provided the daily rainfall data for the previous 10 years (2011-2020) Anonymous, (2019) Rainfall patterns are looked at and analysed on a weekly, monthly, seasonal, and annual level. The entire year is classified into the three seasons of summer, kharif, and rabi in order to assess the seasonal tendency. The Standard Week Nos. 17 to 19 (from 23 April to 13 May) are regarded to be the Summer (Zaid) Season, the Nos. 20 to 44 (from 14 May to 4 November) are considered to be the Kharif (Monsoon) Season, and the Nos. 45 to 16 (from 5 November to 22 April) are considered to be the Rabi Season (Winter). The daily totals for each of the 12 months have been combined in the case of rainfall, starting at the start and finish of each month. To anticipate the behaviour that these occurrences are likely to display in the future, the data were changed to match an appropriate probability distribution. Using the Weibull graphing function, the probability of rainfall at various levels and the amount of precipitation were computed and predicted. The following formula can be used to calculate the normal probability density function from the mean and standard deviation:

$$P = \frac{m}{n+1} \ldots\ldots\ldots (1)$$

Where,
P = Probability
m = Rank
n = Number of years

The predicted rainfall levels were estimated using this equation for various probabilities of exceedance. Additionally, a second-degree power equation was fitted to predict rainfall at different probability levels, and probability (P%) was displayed in relation to weekly, monthly, seasonal, and annual time intervals using a semi-log scale.

Results and Discussion

Rainfall trend

Annual rainfall

The 10-year trends in average rainfall and number of rainy days are displayed in Figure 1. Based on an analysis of the yearly rainfall pattern from 2011 to 2020, the lowest amount of rain, 445 mm, was recorded in 2015, the result of 44 rainy days. This was closely followed by 2014, which had 48 rainy days and a total of 687 mm of average rainfall. 65 wet days contributed to the 1417.2 mm average annual rainfall in 2013. 1074.4 mm of rain have been recorded over the past ten years, with an

average of 52.2 rainy days. The results show that rainfall in this region fluctuates substantially from year to year. While preparing for the region's future development and management, it is crucial to take this variability into account (Sinha, 2019).

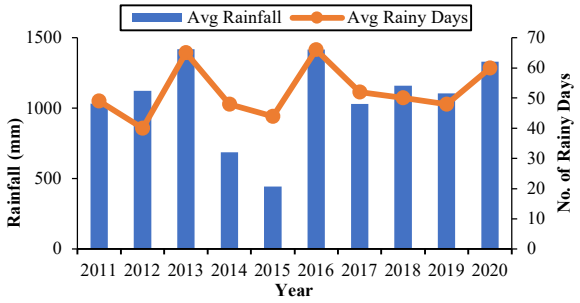

Figure 1: Annual rainfall and rainy days variability at Fingeshwar

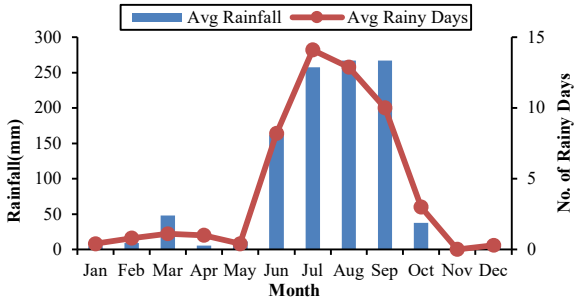

Figure 2: Average monthly rainfall (mm) and rainy days for 10 years

Mean monthly rainfall

The average monthly rainfall for the preceding ten years is depicted in Figure 2 together with the number of rainy days. It demonstrates that pre-monsoon rains beginning in May cause the majority of the region's annual rainfall to fall in the months of June, July, August, and September. The average monthly total of rainy days showed the same trends. According to the rainfall pattern, the number of rainy days each month in May, June, July, August, and September was 0.4, 8.2, 14.1, 12.9, and 10, respectively. As a result, May, June, July, August, and September experienced average monthly rainfalls of 0.4, 8.2, 257.38, 267.02, and 267.08 mm, respectively. With an average rainfall of 1074.4 mm and average number of wet days of 52.2 days over the last ten years. The end of the monsoon season can start in October. On the other hand, there are signs that it rained during the winter, although in very little amounts and mostly just during the months of December through February. The average annual rainfall of 1074.4 mm during the previous 10 years has been observed to fall between May and October with the monsoon months of June, July, August, and September contributing for 93% of this total amount. From this finding, it may be inferred that while there is sufficient rainfall throughout the Kharif season, less irrigation is required during the Rabi season due to a lack of precipitation. This finding is supported by the fact that this area, which receives less rainfall during the Rabi season than other area in India, has a lower incidence of drought than most others. Tomar, (2006)

Mean weekly rainfall

The pattern of rainfall during the last 10 years is illustrated in Figure 3. The monsoon rains, with the exception of pre-monsoon rains, often fall between the 19th and 22nd standard weeks, occasionally between the 25th and 29th standard weeks (18th June to 22th July) rainfall begins. Between the 38th and 40th standard weeks, the monsoon departs (17th Sept to 7th Oct.). According to the 10-year average, the rainy time of the year falls between the 26th and 37th standard weeks (25 June to 16 September). The biggest chance of receiving the weekly average rainfall is during this time period.

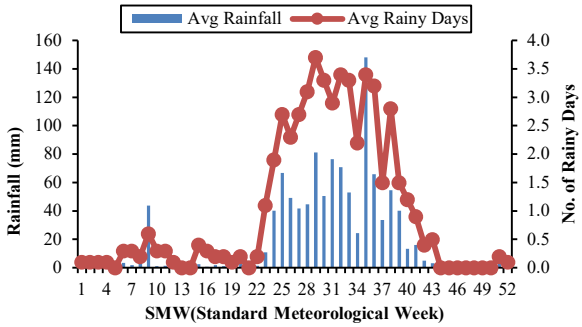

Figure 3: Average weekly rainfall for 10 years

Rainfall Probability Estimation

Weekly rainfall probability estimation

Table 1 illustrates the results of the analysis of rainfall data from the previous 10 years together with a weekly chance of occurrence. The choice of crop, timing of sowing, irrigation strategy, and efficient use of rainwater for optimal output can all be assisted by this forecast. We focused on calculating the probability of weekly rainfall for the monsoon season weeks (the 22nd to the 40th),

which are shown in Table 1. The predicted values of rainfall show that the value of rainfall declines as the probability level for a certain week rises. According to variety of studies, (Sinha *et al.* (2018), Singh *et al.,* (2016)) a minimum guaranteed number should be used for crop planning and should be the projected amount of rainfall with a 75% possibility of exceeding it. Figure 4 illustrates the anticipated rainfall amounts for a 70 to 90 percent probability level. The probability of 70% is at the top of the graph, with the curve with greater probabilities coming next, showing that the probability of 90% got the least amount of rainfall. The maximum rainfall, 39 mm, was recorded in the 35th week, followed by 32.1 mm in the 25th standard week, both at a 75 percent probability level. The weeks 20-22, 29, 37 and 40-42 saw the lowest rainfall, of 0.0 mm. Similar to this, the greatest observed rainfall values for 70, 80, and 90 percent probabilities were 51.6 mm (35th week), 29.2 mm (38th week), and 25.6 mm (38th week), while the lowest observed rainfall values were 0 mm (20-22, 29, 37 and 40-42 standard week). The graphical trend between the 26th, 28th, 30th to 31st, 33rd to 35th, and 38th standard weeks shows five peaks. This suggests that there is a higher chance of rainfall between the 26th and 38th standard weeks, while at other periods irrigation can be provided as needed utilising the available irrigation sources.

Monthly rainfall probability estimation

The monthly rainfall changes at various probabilities are displayed in Table 2 and Figure 5. The three major rainy months are predicted to be July, August, and September, with probabilities of monthly precipitation ranging from 210 to 260 mm at 70, 75, and 80% probability levels.

As a result, measures for harvesting surface runoff can be considered during this time in order to effectively use captured rainfall during the next dry period. Tomar (2006) advised that rain with a 70% probability of occurring is safe to presume to be guaranteed rain, while risk-taking should be limited to 50%. In the months of July, August, and September, it was expected to rain between 210 and 270 mm, with a 70% chance of doing so. With the expected rainfall from the table taken into

Table 1: Prediction of rainfall at different probability level (mm) on basis of SMW (Standard Meteorological Week)

SMW	Probability Level									
	90%	80%	75%	70%	60%	50%	40%	30%	20%	10%
22	0.0	0.0	0.0	0.0	1.7	7.1	13.7	22.2	34.3	54.8
23	0.0	0.8	2.8	5.0	9.8	15.6	22.6	31.7	44.4	66.3
24	0.0	6.2	11.3	16.7	28.8	43.1	60.6	83.2	115.0	169.4
25	8.3	19.7	25.9	32.6	47.4	65.0	86.6	114.3	153.4	220.2
26	27.3	34.3	38.2	42.4	51.6	62.6	76.0	93.3	117.6	159.3
27	6.1	12.8	16.5	20.5	29.3	39.7	52.4	68.9	92.0	131.6
28	29.5	37.7	42.2	47.0	57.6	70.3	85.7	105.7	133.8	181.8
29	4.1	24.4	35.5	47.3	73.8	105.2	143.6	193.0	262.8	382.0
30	23.0	31.5	36.1	41.1	52.2	65.3	81.3	102.0	131.2	181.1
31	9.5	26.9	36.4	46.6	69.4	96.3	129.2	171.7	231.5	333.9
32	5.9	13.4	17.5	21.8	31.6	43.2	57.3	75.6	101.3	145.2
33	30.7	38.5	42.8	47.4	57.6	69.7	84.5	103.6	130.5	176.5
34	29.1	34.9	38.1	41.5	49.1	58.1	69.1	83.3	103.3	137.6
35	20.8	34.2	41.6	49.5	67.1	87.9	113.4	146.2	192.5	271.6
36	6.6	21.5	29.7	38.4	58.0	81.1	109.4	145.9	197.3	285.3
37	6.8	13.4	17.0	20.9	29.5	39.8	52.3	68.4	91.2	130.1
38	8.7	24.5	33.2	42.4	63.1	87.6	117.6	156.2	210.7	303.7
39	0.8	3.1	4.4	5.8	8.9	12.6	17.1	22.9	31.0	45.0
40	0.8	4.9	7.2	9.6	14.9	21.3	29.1	39.1	53.2	77.3

Table 2: Prediction of monthly rainfall (mm) at different probability level

Month	Probability Level									
	90%	80%	75%	70%	60%	50%	40%	30%	20%	10%
Jan	0.0	0.0	0.0	0.0	0.0	0.5	1.2	2.0	3.2	5.2
Feb	0.0	0.0	0.0	0.0	0.0	2.1	5.6	10.1	16.5	27.5
Mar	0.0	0.0	0.0	0.0	2.1	5.1	8.8	13.5	20.2	31.6
Apr	0.0	0.0	0.0	0.0	2.9	6.7	11.4	17.4	25.9	40.5
May	0.0	0.0	0.0	0.3	6.6	14.0	23.1	34.8	51.3	79.6
Jun	117.5	118.8	119.5	120.2	121.9	123.8	126.2	129.3	133.7	141.2
Jul	208.3	237.9	254.2	271.6	310.5	356.4	412.6	485.1	587.3	762.0
Aug	222.1	245.6	258.5	272.2	303.0	339.4	384.0	441.4	522.3	660.7
Sep	194.7	211.3	220.5	230.2	252.0	277.9	309.4	350.1	407.5	505.6
Oct	16.5	25.2	30.0	35.2	46.7	60.2	76.9	98.3	128.5	180.1
Nov	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dec	12.5	14.9	16.2	17.6	20.8	24.5	29.0	34.9	43.1	57.2

consideration, it can be seen that the three rainy months of July, August, and September are likely to contribute, with a 70, 21 and 17% of the annual rainfall, respectively. As a result, this accounts for 70% of total annual rainfall. Because the majority of the rice plant's water-dependent stages take place between July and September, Singh *et al.,* (2016) transplantation should be carefully timed.

Cropping season rainfall probability estimation

Table 3 and Figure 6 show the crop season probabilities. Both the rainfall trend and the data's seasonal analysis imply that rainfall value falls as probability rises. Planning agricultural operations around the seasonal cycle is thought to be safer at a 70% probability level. Barman *et. al* (2016) At a 70% Probability level, the seasonal rainfall projection for the kharif season predicts 1237.24 mm of rain annually, more than the other two seasons combined. Using this strategy, it is possible to plan harvests throughout the Kharif season and repurpose irrigation during drought spells to protect crops throughout critical growth stages. The 4.84 mm rainfall predicted for Rabi, which has a 70% chance of occurring, demonstrates the need for a solid irrigation infrastructure for other crops in addition to pulses, such as wheat and oilseeds.

Table 3: Prediction of seasonal rainfall (mm) at different probability level.

Season	Probability Level									
	90%	80%	75%	70%	60%	50%	40%	30%	20%	10%
Zaid	0.0	0.0	0.0	0.0	2.4	7.7	14.3	22.7	34.7	55.0
Kharif	1155.7	1193.9	1214.9	1237.2	1287.3	1346.4	1418.8	1512.2	1643.8	1868.7
Rabi	0.0	0.0	1.8	4.8	11.7	19.9	29.8	42.7	60.8	91.8

Annual rainfall probability estimation

To estimate the relative frequency of occurrence of a particular amount of annual rainfall with adequate accuracy and to visualise the probability distribution of annual rainfall. It is evident from Figure 7 that the value of rainfall decreases as the probability level rises. At 90, 80, 75, 70, 60, 40, 30, 20, and 10% chance levels, the values of rainfall projected to occur are 1877.64, 1668.86, 1546.74, 1460.09, 1392.88, 1337.9, 1291.53 and 1270.75 mm. IMD defines a place or area as drought-affected if its seasonal or annual total rainfall is less than 75% of the average (Appa Rao, 1986; Sinha B.L., 2019). Hence, based on the yearly rainfall over the previous ten years, the four years of 2011, 2012, 2015, 2016, and 2017 were hit by drought.

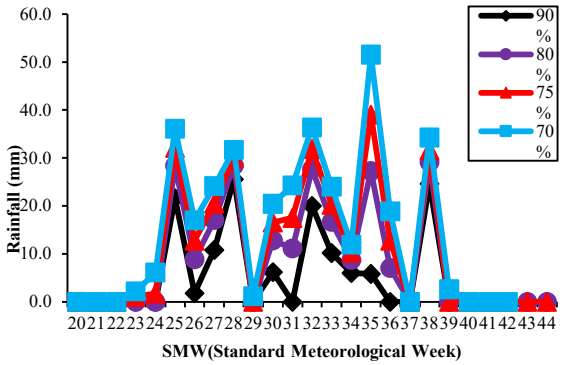

Figure 4: Predicted weekly rainfall at different probability level of monsoon week (*Kharif*)

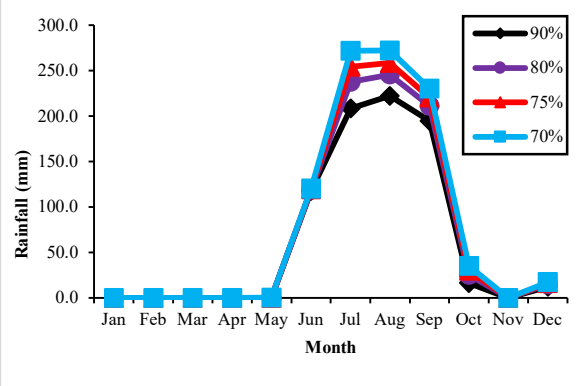

Figure 5: Predicted monthly rainfall at different probability level

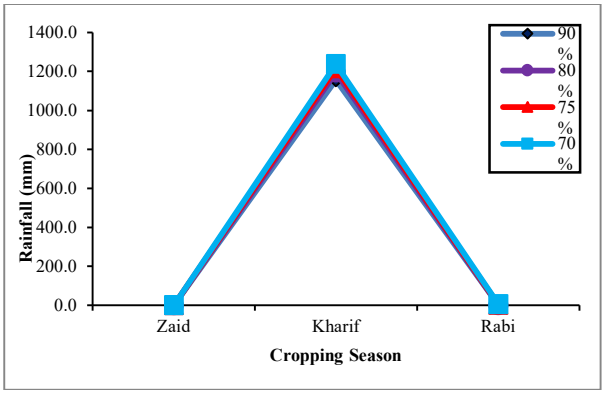

Figure 6: Estimation of rainfall at different probability level on the basis of cropping season

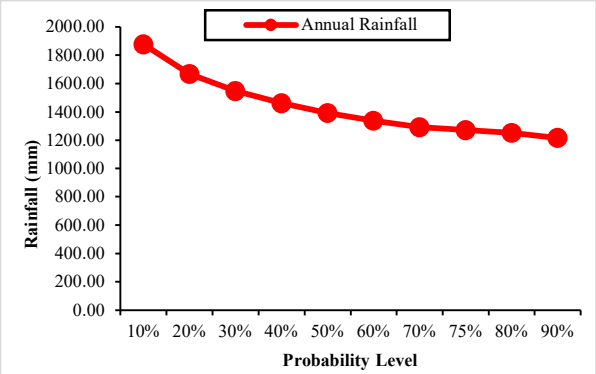

Figure 7: Annual expected rainfall at different probability level.

Conclusion

Possessing exact and consistent rainfall pattern information is useful for managing and executing irrigation techniques during drought spells. Knowledge of successive days of return periods is a crucial parameter of safe, sound, and effective economic planning, as well as in the design of various structural and non-structural measures for small and medium hydraulic structures such as culverts, bridges, check dams, and ponds. It has been observed that the June, July, August, and September got more than 100 mm, thus farmers in these places can produce paddy crops in highland areas followed by any rabi crop in rabi season. At a 50% probability level, the annual rainfall in Fingeshwar Tahsil of the Gariyaband district is 1005.9 mm. It is reasonable to conclude that the kharif season's activities could start between the 22nd and the 23rd standard week and additional runoff should be collected and can be used in post-monsoon crops in this area. It also becomes imperative to offer farmers with high yielding crop types as well as those that use less water and mature earlier.

Conflict of interest

The authors declare that they have no conflict of interest.

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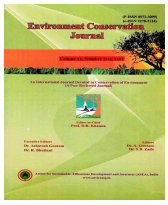

Spatial variability analysis of soil properties of Gwalior District, Madhya Pradesh

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ABSTRACT

The application of fertilizers can be optimized to maintain crop yield while reducing the amount of fertilizer input. One way to achieve this is by using soil fertility maps and fertilizer recommendations to regulate fertilizer application. In this study, statistical techniques were employed to analyze the physical-chemical quality of soils in the Gwalior region of Madhya Pradesh. The study involved collecting 95 soil samples (0-15 cm) from four blocks in the Gwalior area using GPS (Bhitarwar, Morar, Ghatigao, and Dabra), and conducting laboratory analyses. The results of the analysis showed that the pH, EC, and OC of the soil samples ranged from 7.10 to 8.90, 0.21 to 0.83 dS/m, and 0.23 to 0.98%, respectively. The soil samples also had varying levels of N, P, and K, with values ranging from 194 to 336, 6.10 to 45.00, and 69.89 to 751.30 kg/ha, respectively. The study revealed significant differences in the physicochemical properties of soil in the study area

Introduction

If farmers wish to increase crop yield and nutrient efficiency, they must first understand the regional diversity and distribution of soil features. Fertilizer guidelines suggest applying fertilizers based on the characteristics of the soil to sustain productivity while using less fertiliser. Geo statistics is a popular tool for analysing geographical variability, interpolating between point data, and calculating interpolated values with a given error from a limited number of observations (Long *et al.,* 2014; Cambule *et al.,* 2014). For crop selection , designing a cropping system and adoption of crop management practices, knowledge of geographic diversity in soil's physical, chemical, and biological properties, was essential (Liu *et al.,* 2013. Researchers looked at spatial variation in pH, organic matter, total and available NPK, and micronutrients under various soil and management strategies to improve and implement site-specific nutrient Managemnet (Franzen *et al.,* 2002; Li *et al.,* 2011). One of the most crucial elements of a system for producing food sustainably is the soil. Crop production ultimately reflects the quality of the soil, which is determined by its physicochemical characteristics and capacity to supply nutrients. Because of the different elements that go into their production and the complex interactions between these elements, soils are naturally heterogeneous (Maniyunda *et al.,* 2013). It is important to characterise the spatial variability of soil nutrients in relation to site characteristics such as climate, land use, landscape position, and other factors in order to comprehend how ecosystems function and predict how future land use change will affect soil nutrients (Wang *et al.,* 2001; Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022).

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Material and Methods

Located at latitudes 26.0283 and 78.2020 the Gwalior district of Madhya Pradesh covers a total area of 4560 km^2 and is divided into four blocks: Bhitarwar, Ghatigao, Morar, and Dabra. The district is situated at an elevation of 211.5 m above mean sea level, with the region's elevation ranging from 200 to 211.5 metres. To better understand the physical-chemical properties of the soil in the Gwalior region, a study was conducted using GPS to collect 95 soil samples (0-15 cm) from various locations across the four blocks. Figure 1 and table 1 depict the location of the study area and the sampling sites.

Description of Experimental Site:

Laboratory analysis was done at School of Agriculture, ITM, University, Gwalior (M.P.)

Soil sample collection and preparation:

GPS based Ninety-five random surface soil samples were collected at depths ranging from 0 to 15 cm from various blocks in the Gwalior district, Madhya Pradesh. The samples were then combined to form a composite sample weighing approximately 1 kg. Following air drying, the larger aggregates were

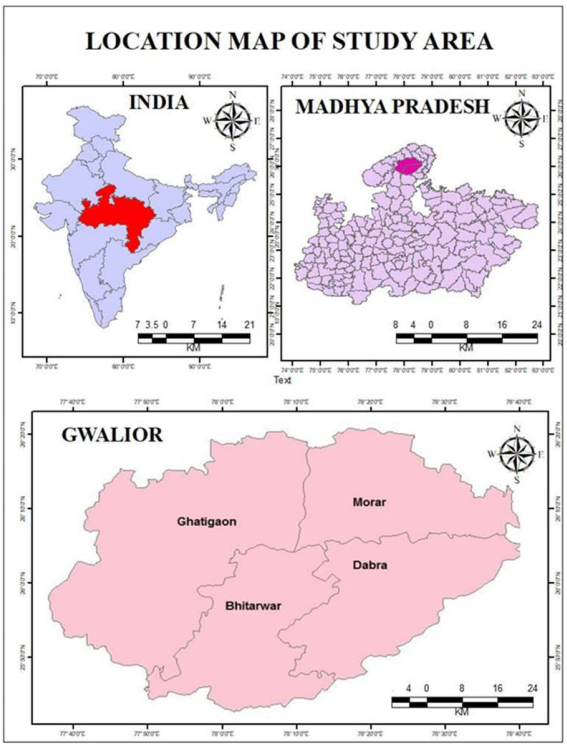

Figure :1 Location map of collecting soil samples

Table 1: Location of soil sample collection site

Block	Latitude	Longitude	Block	Latitude	Longitude	Block	Latitude	Longitude	Block	Latitude	Longitude
M1	26^{0}15’20.63’’	78^{0}15’50.97’’	D1	25^{0}57’11.58’’	78^{0}18’58.72’’	B1	26^{0}00’28.03’’	78^{0}10’25.44’’	G1	26^{0}04’50.25’’	78^{0}08’51.99’’
M2	26^{0}15’18.36’’	78^{0}1545.34’’	D2	25^{0}57’07.17’’	78^{0}19’14.88’’	B2	26^{0}00’31.38’’	78^{0}10’21.96’’	G2	26^{0}06’03.7’’	78^{0}09’07.7’’
M3	26^{0}15’20.91’’	78^{0}15’45.23’’	D3	25^{0}57’09.76’’	78^{0}19’12.26’’	B3	26^{0}00’36.02’’	78^{0}10’29.95’’	G3	26^{0}06’02.5’’	78^{0}09’04.4’’
M4	26^{0}17’24.7’’	78^{0}11’26.2’’	00444	25^{0}57’10.62’’	78^{0}19’02.46’’	B4	26^{0}00’31.91’’	78^{0}10’34.00’’	G4	26^{0}06’02.2’’	78^{0}09’15.8’’
M5	26^{0}17’20.5’’	78^{0}11’19.0’’	D5	25^{0}57’12.83’’	78^{0}19’18.50’’	B5	26^{0}00’38.02’’	78^{0}10’56.28’’	G5	26^{0}06’27.6’’	78^{0}09’04.9’’
M6	26^{0}14’01.6’’	78^{0}23’24.6’’	D6	25^{0}58’28.68’’	78^{0}20’02.26”	B6	26^{0}03’35.80’’	78^{0}12’37.55’’	G6	26^{0}07’11.0’’	78^{0}08’39.9’’
M7	26^{0}14’03.2’’	78^{0}23’13.5’’	D7	25^{0}58’25.71’’	78^{0}20’01.44”	B7	26^{0}03’32.99’’	78^{0}12’39.92’’	G7	26^{0}07’14.9’’	78^{0}08’45.3’’
M8	26^{0}14’00.6’’	78^{0}23’31.3’’	D8	25^{0}58’23.00’’	78^{0}19’57.40”	B8	26^{0}03’36.74’’	78^{0}12’38.54’’	G8	26^{0}07’22.7’’	78^{0}08’47.8’’
M9	26^{0}14’00.6’’	78^{0}23’31.3’’	D9	25^{0}58’31.84’’	78^{0}20’00.93”	B9	26^{0}03’31.40’’	78^{0}12’41.56”	G9	26^{0}07’19.4’’	78^{0}08’50.0’’
M10	26^{0}14’04.9’’	78^{0}23’17.7’’	D10	25^{0}58’27.59’’	78^{0}20’10.05”	B10	26^{0}03’31.44’’	78^{0}12’34.14’’	G10	26^{0}07’58.5’’	78^{0}08’36.7’’
M11	26^{0}13’58.0’’	78^{0}23’18.9’’	D11	25^{0}54’55.07’’	78^{0}18’38.40	B11	26^{0}00’12.99’’	78^{0}08’28.90’’	G11	26^{0}07’39.5’’	78^{0}01’55.5’’
M12	26^{0}13’57.8’’	78^{0}23’09.7’’	D12	25^{0}54’55.77’’	78^{0}18’36.94”	B12	26^{0}00’02.19’’	78^{0}08’09.92’’	G12	26^{0}07’38.8’’	78^{0}01’17.2’’
M13	26^{0}13’54.0’’	78^{0}23’12.6’’	D13	25^{0}54’57.70’’	78^{0}18’40.82”	B13	26^{0}00’02.79’’	78^{0}08’45.82’’	G13	26^{0}07’39.5’’	78^{0}01’49.3’’
M14	26^{0}13’31.4’’	78^{0}23’40.3’’	D14	25^{0}54’59.29’’	78^{0}18’39.85”	B14	26^{0}00’04.00’’	78^{0}08’32.94’’	G14	26^{0}07’42.9’’	78^{0}01’11.4’’
M15	26^{0}13’20.4’’	78^{0}23’40.3’’	D15	25^{0}54’57.84’’	78^{0}18’37.21”	B15	26^{0}00’15.08’’	78^{0}08’04.63’’	G15	26^{0}07’37.4’’	78^{0}01’49.8’’

gently crushed with a wooden hammer and passed through a 2 mm sieve. The sieved soils samples were in appropriately labelled sample bags and taken to laboratory for further soil analysis.

Analytical procedure:

The following procedures were used to analyze various physical and chemical parameters of soil. The electrical conductivity (EC) of soil was measured using an EC metre at a soil-to-water ratio of 1:5, and the result was converted to a soil-to-water ratio of 1:1 as recommended by USDA, Jackson 1967. The pH of soil samples was measured using a pH meter while keeping the soil-to-water ratio of 1:2.5 as suggested by Jackson 1962. The organic carbon content of soil samples was determined using Wet Digestion method Walkley and Black,1934. The amount of available nitrogen (N) was calculated using Alkaline potassium permanganate method Subbiah and Asija, 1956 , while the amount of available phosphorus (P) was determined using Sodium bicarbonate as an extractant given by Olsen,1954 and available potassium was determined using Neutral ammonium acetate extractant method by Jackson (1973).

Results and Discussion

A study was conducted to examine the physicochemical properties and nutrient content of soils in the Gwalior district. The results showed that the bulk density of the soils in Gwalior ranged from 1.26 to 1.64 Mg/m^3, with of 1.42 Mg/m^3 as the mean and a 5.74 percent variability (Fig. 2). It is well-established that the increase in bulk density is caused due to conversion of forest land into arable land and it is a reliable indicator of soil degradation severity. Higher bulk density may have resulted from increased compaction in the lower horizons of the soil profiles over time, which may have occurred as a result of varying levels of soil erosion, depending on slope and management techniques. The results showed the less porous nature of soils in the region, which ranged from 1.29 to 1.49, 38.11 to 52.45, 40.38 to 51.70, and 40.38 to 51.70 % in different (Bhitarwar, Ghatigao, Morar, and Dabra), had mean values ranging from 46.89 to 46.11, 46.11 to 46.49% (Fig. 3). Low soil porosity and compaction are indicators of high bulk density, it might hinder root development and impede the flow of air and water through the soil, These results are in line with the findings of Jain and Sigh (2013). According to statistics presented in table 2, the average percentages of sand, silt, and clay in the Gwalior district are 52.4%, 23.6%, and 24%, respectively. These values indicate that the textural class of soil in the Gwalior district is sandy loam. Typically, finer soil fractions (silt and clay) increase with depth, while sand percentages decrease. However, Boke (2004) suggested that farming activities may have led to changes in soil texture, despite being an inherent quality. Chima (2007) suggested that plant modification could also have played a role in altering particle size distribution over time, despite the natural texture of soil. Analysis the data showed that the soils in several blocks of the Gwalior district were alkaline in nature (Fig. 4). The mean soil pH in the Gwalior district varied 7.10 to 8.90, with a range of 7.20 to 8.90 across the different blocks (Bhitarwar, Ghatigao, Morar, and Dabra). The mean values for the blocks were 8.26, 8.15, 8.35, and 7.72, respectively. Variation in soil pH under different blocks of district, as whole might be due to variations in the parent material of soil, management practices & land uses. Similar variations in soil pH in different regions were reported by Yadav *et al.* (2018). Figure 5 shows electrical conductivity of soils across different blocks of the Gwalior district ranged from 0.21 to 0.83 dS/m at 25°C, with an average of 0.54 dS/m, which is within the normal range (<1 dSm^{-1} at 25°C). The low electrical conductivity in soil under study area might be due to deep water table which is certainly possible by commencement of sufficient rainfall, Similar results for different soils were also reported by Dilliwar *et al.* (2014) and Singh *et al.* (2017).

Furthermore, the organic carbon (OC%) content of soils in the Gwalior district's different blocks (Bhitarwar, Ghatigao, Morar, and Dabra) varied from 0.23 to 0.98%, with an average value ranging from 0.27 to 0.65% (Fig. 6). Variation in organic carbon content in soil samples was may be due to addition of organic matter in the soil, variability in use pattern of land, cropping sequence and crop species. Findings of that Mandal *et al.*, (2011) also revealed that, crop species and cropping systems

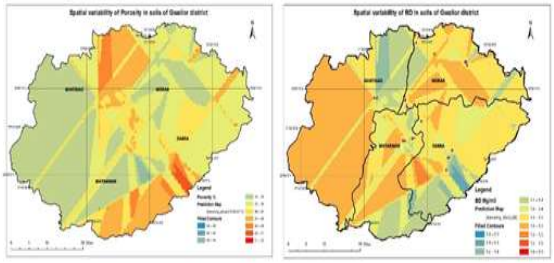

Fig:2 Fig:3

Figure 2-3: Spatial variability maps of physical properties of Gwalior district

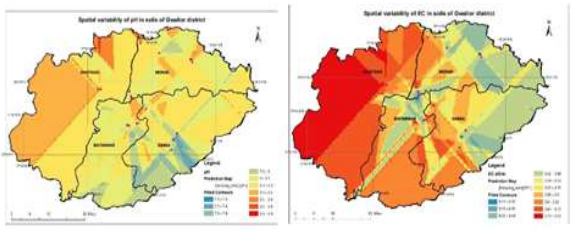

Fig:4 Fig:5

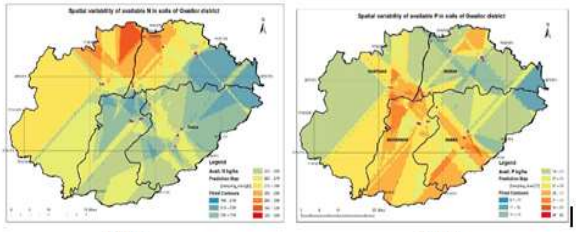

Fig:6 Fig:7

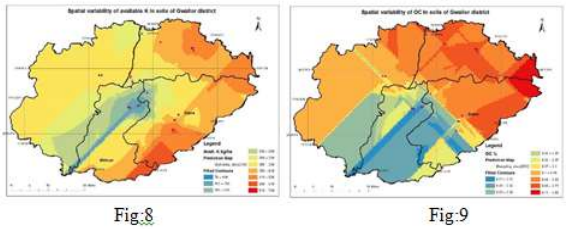

Fig:8 Fig:9

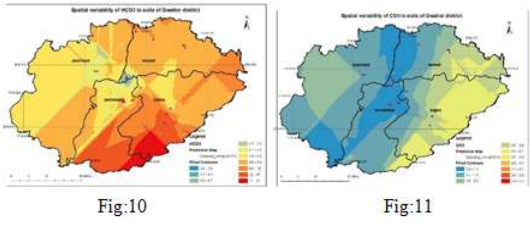

Fig:10 Fig:11

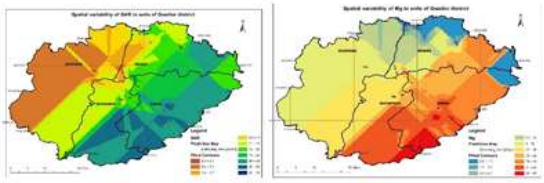

Fig:12 Fig:13

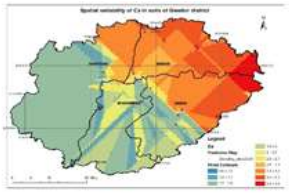

Fig:14

Figure 4-14: Spatial variability maps of Chemical properties of Gwalior district.

may play an important role for variations in soil organic carbon. Findings of Singh *et al.* (2014) and Yadav *et al.*, (2018) also support the findings of present studyWhereas, the bicarbonate content of soils in the Gwalior district ranged from 1.40 to 27.40 Cmol(P^+)/kg (Fig. 7), with an average value of 7.60 Cmol (P^+)/kg. The high coefficient of variance indicated the significant variation in bicarbonate content among the soil samples this might be due to calcareous parent materials, poor drainage and improper leaching process, similar results were reported by Dilliwar *et al.* (2014) Singh *et al.* (2014) and Yadav *et al.*, (2018) .The carbonate content of the soils varied between Gwalior, Bhitarwar, Ghatigao, Morar, and Dabra, with the highest value reported in the Dabra block (11.29 Cmol (P^+)/kg) and the lowest in the Ghatigao Block (4.85 Cmol (P^+)/kg). The carbonate concentration in Gwalior Distict ranged from 0.80 to 4.50 Cmol (P^+)/kg, with an average value of 1.77 Cmol (P^+)/kg, a standard deviation of 0.83, and a variation of 46.68%, as shown in table 2 and fig. 8. Soil carbonate contents ranged from 0.90 to 1.80 Cmol (P^+)/kg in Bhitarwar, 0.40 to 1.60 Cmol (P^+)/kg in Ghatigao, 0.80 to 2.80 C mol (P^+)/kg in Morar, and 0.90 to 4.50 C mol (P^+)/kg in Dabra.

The calcium content of soils in the Gwalior district varied widely, with a range of 1.90 to 27.60 Cmol (P^+)/kg, as shown in Table 3 and Figure 14. The mean value was 8.89 Cmol (P^+)/kg, with a standard

deviation of 6.65 and a coefficient of variation of 73.88%. Looking at the data in Table 2, we see that the calcium content in soils across the different blocks of Gwalior (Bhitarwar, Ghatigao, Morar, and Dabra) ranged from 1.90 to 8.10 Cmol (P^+)/kg, 3.60 to 13.70 Cmol (P^+)/kg, 3.20 to 9.50 Cmol (P^+)/kg, and 3.50 to 8.50 Cmol (P^+)/kg, respectively. Among these, Ghatigao village had the highest calcium content (7.32 Cmol (P^+)/kg), while Bhitarwar Block had the lowest (3.91 Cmol (P^+)/kg). pH of the soils increased with Increase in the availability of Ca and Mg. Table No. 2 and Fig. 13 present data on magnesium levels in the soil of the Gwalior District, which ranged from 0.60 to 9.50 Cmol (P^+)/kg, with an average value of 2.76 Cmol (P^+)/kg, a standard deviation of 1.91, and a coefficient of variation of 69.08%. The four blocks in the district (Bhitarwar, Ghatigao, Morar, and Dabra) exhibited magnesium levels that varied from 0.70 to 3.10 Cmol (P^+)/kg, 0.60 to 3.90 Cmol (P^+)/kg, 0.80 to 3.20 Cmol (P^+)/kg, and 1.00 to 2.60 Cmol (P+)/kg, respectively.

Table 2: **Status of Physical properties of soils of different blocks of Gwalior district**

Blocks		Sand %	Silt%	Clay %	Bulk density (Mg/m^3)	Porosity %
Bhitarwar	Min	52.40	20.10	20.30	1.29	43.77
	Max	59.40	24.10	24.00	1.49	51.32
	Mean	56.27	22.02	21.71	1.41	46.89
	SD	2.03	1.38	1.14	0.06	2.15
	CV	3.61	6.29	5.24	4.04	4.58
Ghatigao	Min	51.30	20.50	19.00	1.26	38.11
	Max	60.10	23.90	25.40	1.64	52.45
	Mean	56.27	22.37	21.37	1.43	46.11
	SD	2.48	1.11	1.96	0.09	3.51
	CV	4.40	4.98	9.19	6.51	7.60
Morar	Min	30.30	33.20	24.30	1.28	40.38
	Max	40.20	39.50	33.40	1.58	51.70
	Mean	36.09	36.18	27.73	1.43	46.11
	SD	2.97	2.03	2.09	0.09	3.49
	CV	8.22	5.61	7.54	6.47	7.56
Dabra	Min	33.20	32.20	27.10	1.28	40.38
	Max	39.60	38.20	30.60	1.58	51.70
	Mean	36.03	35.07	28.91	1.42	46.49
	SD	1.74	1.88	0.99	0.09	3.21
	CV	4.83	5.36	3.44	6.00	6.91

The maximum magnesium level (2.03 Cmol (P^+)/kg) was reported in the Ghatigao block, while the minimum (1.66 Cmol (P^+)/kg) was reported in the Morar block. As the data indicate, magnesium levels significantly affected the soil health and agricultural productivity.According to Table 3, the soil in the Gwalior District has a sodium content that ranges from 0.80 to 88.80 Cmol (P^+)/kg, with an average value of 21.28 Cmol (P^+)/kg, a standard deviation of 24.07, and a coefficient of variation of 113.10%. The exchangeable sodium content of the soil also varied across the four blocks of the district. Specifically, Bhitarwar had a range of 6.30 to 26.39 Cmol (P^+)/kg, Ghatigao had a range of 4.40 to 21.60 Cmol (P^+)/kg Morar had a range of 8.20 to 27.60 Cmol (P^+)/kg, and Dabra had a range of 5.60 to 88.80 Cmol (P^+)/kg. It is important to note that high levels of exchangeable sodium in soil can negatively impact soil structure and plant growth, and therefore it is important for farmers and land managers to be aware of the sodium levels in their soil.The nitrogen content in the soil of different blocks in the Gwalior District, including Bhitarwar, Ghatigao, Morar, and Dabra, varied from 194.00 to 295.00, 195.00 to 314.00, 194 to 336.00, 198.00 to 298.00 kg/ha, with average values ranging from 257 to 274.67 kg/ha (Fig. 9). The results also indicate that the minimum and maximum values of nitrogen in the soil were found in Bhitarwar and Morar blocks, respectively. Similar findings were also reported by Talib and Verma (1990), indicating low to medium levels of available nitrogen in all soil profiles, with a decreasing trend in nitrogen content with depth due to a reduction in organic matter content.

In the different blocks of the Gwalior District (Bhitarwar, Ghatigao, Morar, and Dabra), the nitrogen level in the soil varied from 194 to 295, 194 to 314, 194 to 336, and 198 to 298 kg/ha, with average values ranging between 257,258.07 and 274.67,258.20 kg/ha. Additionally, according to the findings, the Bhitarwar and Morar Blocks had the minimum and maximum soil nitrogen values. Talib and Verma (1990), who also reached similar conclusions, concluded that all of the soil profiles contained low to medium levels of available nitrogen, and due to the decline in organic matter with increasing depth, all of the profiles displayed a decreasing trend.

Table 3: Status of Chemical properties of soils of different blocks of Gwalior district

Blocks		pH	EC (dS/m^{1})	OC (%)	N	P	K	Co3	Hco3	Ca	Mg	Na	Sar
					(Kg/ha)			Cmol (P^{+}) kg^{1}					
Bhitarwar	Min	7.20	0.21	0.23	194.00	15.00	69.89	0.90	3.50	1.90	0.70	6.30	6.26
	Max	8.90	0.75	0.36	295.00	43.00	340.70	1.80	8.70	8.10	3.10	31.30	26.39
	Mean	8.26	0.59	0.27	257.40	26.60	169.48	1.20	6.45	3.94	1.77	17.58	15.21
	SD	0.56	0.16	0.03	31.77	8.92	66.10	0.44	1.73	1.75	0.75	7.29	6.38
	CV	6.75	26.86	12.32	12.34	33.53	39.00	36.60	26.77	44.41	42.39	41.49	41.95
Ghatigao	Min	7.50	0.27	0.35	195.00	14.00	227.14	0.90	1.40	3.60	0.60	4.40	2.40
	Max	8.90	0.83	0.49	314.00	45.00	393.12	1.90	6.90	13.70	3.90	21.60	18.42
	Mean	8.15	0.57	0.41	258.07	27.53	308.67	1.45	4.85	7.32	2.03	11.31	7.76
	SD	0.45	0.19	0.05	36.43	10.11	49.93	0.46	1.48	2.91	0.92	5.18	3.99
	CV	5.46	33.84	11.76	14.12	36.73	16.18	31.98	30.53	39.74	45.37	45.82	51.37
Morar	Min	7.40	0.30	0.45	194.00	6.10	192.19	0.80	5.00	3.20	0.80	8.20	5.91
	Max	8.90	0.61	0.98	336.00	44.00	751.30	2.80	9.70	9.50	3.20	27.60	22.50
	Mean	8.35	0.49	0.65	274.67	19.08	416.42	2.00	7.80	5.39	1.66	18.15	14.04
	SD	0.46	0.08	0.17	41.06	11.70	149.85	0.68	1.50	1.93	0.78	6.24	5.18
	CV	5.50	17.33	26.31	14.95	61.31	35.99	33.96	19.20	35.84	46.82	34.37	36.93
Dabra	Min	7.10	0.28	0.32	198.00	13.00	139.78	0.90	1.80	3.50	1.00	5.60	3.96
	Max	8.50	0.64	0.48	298.00	43.00	716.35	4.50	27.40	8.50	2.60	88.80	76.43
	Mean	7.72	0.50	0.39	258.20	28.87	408.26	2.43	11.29	6.13	1.84	54.57	38.96
	SD	0.45	0.12	0.05	30.58	10.20	187.82	1.01	7.93	1.46	0.47	25.29	18.80
	CV	5.88	23.89	12.22	11.84	35.32	46.00	41.72	70.23	23.85	25.56	46.34	48.25

Phosphorus level in the soil of different blocks (Bhitarwar, Ghatigao, Morar, and Dabra) varied from 15.00 to 43.00, 14.00 to 45.00, 6.10 to 44.00, and 13.00 to 43.00 kg/ha with average values ranging from the results showed that the Morar and Dabra Blocks had the lowest and highest soil phosphorus ,respectively (Fig. 10). Surface soils are categorised as medium in terms of accessible P concentration, exactly as N. Similar results have also been reported byTodmal *et al.*, (2008).

The range of the available potassium status (kg/ha) in the study area was 69.89–751.30 kg/ha, with an average value of 363.14 kg/ha (Fig. 11). These results are consistent with those of Prasad and Rokima (1991), who found that potassium accumulation is due to its stable buildup, as a result of its addition through fertilizers, organic manures and bio-fertilizers.

Conclusion

It was determined that the prevalent soil texture in the Gwalior district is sandy loam, with a pH range that varies from neutral to alkaline. The soils were classified as having low organic carbon levels, low to medium accessible levels of nitrogen and phosphorus, and medium to high levels of available potassium. The research findings indicate that the soils in the Gwalior district suffer from extensive nutrient deficiencies, with some regions also facing salinity and alkalinity problems. To express the geographic variability, nutrient scarcity, and availability in key blocks, GIS-based maps were created, which will be beneficial in developing crop planning and providing advice on nutrient use efficiency.

Conflict of interest

The authors declare that they have no conflict of interest.

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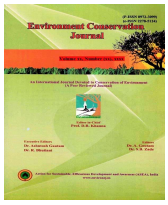

Effect of molybdenum trioxide nanoparticle-mediated seed priming on the productivity of green gram (*Vigna radiata* L.)

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ABSTRACT

A field experiment was undertaken in the post-*Rabi* season of 2019-2020 to reveal the response of greengram (*Vigna radiata* L.) to seed dressing and seed priming with nano molybdenum trioxide (MoO_3). The experiment was laid out in randomized block design (RBD) consisting of 10 different treatments i.e., M_0 (no seed treatment with Mo); M_1 (seed dressing with Sodium molybdate @ 400 ppm); M_2, M_3, M_4, and M_5 (seed dressing with nano Molybdenum trioxide-MoO_3 @ 50, 100, 200 and 400 ppm, respectively); and M_6, M_7, M_8 and M_9 (seed priming with nano MoO_3 @ 50, 100, 200 and 400 ppm, respectively). Inoculation of greengram seeds cv. *Shreya* (IPM 2-14) with *Rhizobium sps*. was undertaken in all treatments as per the recommended practice, except in M_0 (control). The MoO_3 nanoparticles (NPs) synthesized from Ammonium molybdate through calcination at 600 ^{0}C for 5 hours indicated globular-shaped NPs of 68.55 nm in TEM and XRD. Nanopriming with MoO_3 @ 200 ppm (M_8) was most promising in recording significantly superior growth and yield attributing parameters and yield, whereas Mo (control) produced the least. Crop height, number of branches, root length, shoot dry matter, pods/plant and seeds/plant and root nodulation at harvest in M_8 were 39.4% and 22.6%; 39% and 5.6%; 23% and 9.3%; 43.9%, and 16.3%; 28.2% and 5.3%; 28.1% and 0.8%, and 73.3% and 36.5% higher than M_0 (control) and M_1 (farmers' practice), respectively. Superior growth and yield attributing characters in M_8 treatment produced the highest grain and stover yield of 0.88 and 3.74 t/ ha that was 32.53% and 8.37%, and 35.5% and 14.7% higher than M_0 (control) and M_1 (farmers' practice), respectively. Seed priming with nano MoO_3 @ 400 ppm (M_9) and seed dressing with nano MoO_3 @ 400 ppm (M_5) were of second and third order in recording grain and stover yield but M_0 recorded the lowest among all the treatments.

Introduction

Pulses are part of a healthy and balanced diet and have special importance for vegetarians in fulfilling their daily requirements for body proteins. India leads the world in terms of area as well as

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production under pulses with about 35-37% (23.63 m ha) and 27% (14.76 m tons) of global coverage and output, respectively (Movalia *et al.,* 2020). However, pulse productivity is appallingly low (506 kg/ha), resulting in an annual import of about 2-3 m tons. Among the pulses, greengram (*Vigna radiata* L. Wilczek), popularly known as *mung* bean or golden gram, has a special position due to its yielding ability, high nutritive values and wider adaptability to arid and semi-arid agro-ecosystems. It contains 24.3% protein, a good source of riboflavin, thiamine (Awomi *et al.,* 2012) and vitamin C (ascorbic acid). Mung bean contributes 7% (1.7 M tons) of total production from 14% (3.6 M ha) of the area under all pulses in India and the productivity is about 500 kg/ha. It fixes atmospheric N of about 35 kg/ha with the help of symbiotic *Rhizobium*, a soil bacterium (Movalia *et al.,* 2020). The element molybdenum (Mo) is crucial for functions of nitrogenase and nitrate reductase, the two important enzymes that act as a catalyst for N fixation. Plants get Mo from soils and can assimilate in Molybdate (Grant 2018). In low pH, the MoO_4^- adsorbs onto positively charged metal oxides (Fe, Al, and Mn) and the maximum adsorption occurs between pH 4 to 5 (Smith *et al.,* 1997) which leads to its deficiency. Soil application of Mo may require large quantities depending on the crop, soil type and inherent soil contribution. It is subjected to losses through percolation, leaching, runoff and weed uptake. Foliar application is another viable option but is practicable only after crop canopy development; till then, the crop may suffer irreparable loss. Foliar application is prone to weather parameters (Hidayatullah *et al.,* 2016). One more option for supplementing crop requirement for Mo is through seed pelleting and seed dressing but it may impair seed respiration, reduce survival of Rhizobia, plant nodulation and efficiency of N_2 fixation (Almeida *et al.,* 2013; Dwibedi *et al*., 2018). The proportion of applied Mo that enters into the seeds and actively plays a role in plant metabolism is also inadequate. However, no other alternative mode of application of Mo such as the use of nanoparticles of Mo to avert the above-mentioned negative effects of soil, foliar and seed application is traceable. Therefore, the present experiment was conducted to standardize the concentration of hydro priming of nano Mo.

Material and Methods

Experimental site

The experiment was conducted in the Research farm of Odisha University of Agriculture and Technology, Bhubaneswar, India during post-*Rabi*, 2019-20 with a geographical position of 20^0 15'N Latitude and 85^0 52' E Longitude at an altitude of 25.9 m above sea level, which is about 64 km away from the Bay of Bengal. The total rainfall received at the experimental site during the cropseason was 16.1 mm. The range of monthly average maximum temperatures during the experimental period varied from 27.7 to 34.2 ^{0}C and the monthly average minimum temperatures varied from 16.1 to 22.4 ^{0}C.

Agronomic practices

A short duration (62-65 days) variety, *Shreya* (IPM-2-14) was used as a test variety in this experiment. Sowing was done in lines with a seed rate of 25 kg/ha on a well-pulverised field. The crop was supplied with $N:P_2O_5:K_2O$ @ 20-40-40 kg/ha. The seeds were inoculated with the strains of *Rhizobium spp.*@ 20 g/kg of seeds just before sowing. The plots were irrigated just after sowing with 5 cm of irrigation water for early and uniform germination of the seeds. The weeds thus emerged were effectively managed with the pre-emergence application of herbicide i.e. Pendimethalin @ 0.5 kg a.i./ha. Subsequently, two irrigations were applied at 20 and 40 DAS for mitigating the water requirement of the greengram crop. The harvesting of greengram was done at the physiological maturity stage by cutting just above the ground surface. The harvested crop samples were labelled properly and sun-dried before threshing as per the treatments.

Nanoparticle synthesis and characterization and XRD analysis

The nanoparticles of MoO_3 were synthesized taking commercially available ammonium ortho-molybdate $\{(NH_4)_2MoO_4\}$ in a silica crucible and calcinated at 600 °C in a muffle furnace for 5 hours. The MoO_3 nanoparticles thus formed were kept inside an airtight zipper polythene bag and then stored inside a refrigerator at 4 ^{0}C to prevent agglomeration. The nanoparticles were characterized by Transmission Electron Microscope (TEM) and Power X-ray diffraction (XRD) analyses. The average particle size in the calcined MoO_3 nanoparticles was estimated by the Scherrer equation (Dinesh 2012).

The equation is given by D= kλ / β cosθ, where, D is the crystallite size, k is the shape factor, which usually takes a value of about 0.89, λ is the wavelength of X-ray source used (0.15406 nm for Cu Kα), β is the full width at half-maximum (FWHM) in radians (0.00885231), and θ is the Bragg diffraction angle in radians (0.2259381077) (Figure 1).

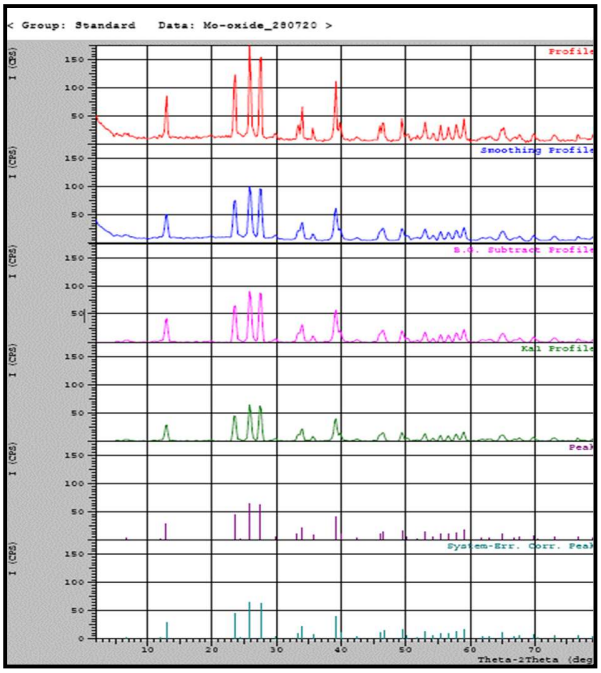

Figure 1: X-ray diffraction (XRD) analysis of Molybdenum trioxide nanoparticles

Treatments details

The treatments consisted of 10 different seed treatment methods *i.e.* control (without seed dressing or priming of Mo); farmers' practice (seed dressing with sodium molybdate @ 400 ppm by weight of seeds); seed dressing with Mo-NPs @ 50, 100, 200 and 400 ppm and seed priming with Mo-NPs @ 50, 100, 200 and 400 ppm. The fieldexperiment was carried out in a randomized block design with 10 different treatments (*i.e.* M_0, M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8 and M_9) and 3 replications, giving rise to 30 experimental units. The details of the treatments used in the experiment are given in Table 1. The plant height at 15, 30, and 45 days after sowing (DAS) and at harvest was measured from the base of the plant up to the tip by using a meter scale and was expressed in cm.

Table 1: Details of treatments and symbols used (field experiment)

Treatments	Symbols used
Control (noseed treatment with Mo or seed inoculation with *Rhizobium*)	M_0
Farmers' practice (seed dressing with sodium molybdate @ 400 ppm + seed inoculationwith *Rhizobium*)	M_1
Seed dressing with MoO_3-NPs @ 50 ppm + seed inoculationwith *Rhizobium*	M_2
Seed dressing with MoO_3-NPs @ 100 ppm + seed inoculationwith *Rhizobium*	M_3
Seed dressing with MoO_3-NPs @ 200 ppm + seed inoculationwith *Rhizobium*	M_4
Seed dressing with MoO_3-NPs @ 400 ppm + seed inoculationwith *Rhizobium*	M_5
Seed priming with MoO_3-NPs @ 50 ppm + seed inoculationwith *Rhizobium*	M_6
Seed priming with MoO_3-NPs @ 100 ppm + seed inoculationwith *Rhizobium*	M_7
Seed priming with MoO_3-NPs @ 200 ppm + seed inoculationwith *Rhizobium*	M_8
Seed priming with MoO_3-NPs @ 400 ppm + seed inoculationwith *Rhizobium*	M_9

The length of the longest root at 15, 30, and 45 DAS and at harvest was measured from the base of the plant up to the tip of the longest root by using a meter scale and was expressed in cm. The total shoot and root dry weight at 15, 30, and 45 DAS and at harvest was weighed after drying the plant samples in a hot air oven at 70 ^{0}C for 48 hours and expressed in grams (g). The total number of leaves/plant at 15, 30, and 45 DAS and at harvest was recorded by counting the number of healthy leaves with less than 30% non-green area. The number of nodules/plant at 15, 30, and 45 DAS and at harvest was recorded by counting the number of healthy nodules present on the root irrespective of their size. The number of pods of 10 randomly selected plants of greengram from each plot was counted and harvested at the maturity stage and an average number of pods/plant was produced. Randomly selected 10 pods from each plot were used to count the number of seeds produced in each pod. For comparison, the average number of seeds/pod was considered. The sun-dried 1,000 well-filled seeds per plot were counted at random from the composite seed sample of each treatment which was then cooled in the shade for recording their weight (g) to find out the test weight. The seed

weights derived from the harvest area of 5 m X 1 m selected at random within each plot were collected and recorded accurately after threshing, cleaning and sun-drying. The weights of seeds thus recorded were then converted into t/ha. Standard error of means i.e., S.Em. (±) were used in all cases. The significance of variance was tested by the 'Error mean square" method of Fisher Snedecor's F-test at the probability level of 0.05 for appropriate degrees of freedom. Statistical analyses were done by using R-studio version 4.2.1 to elucidate the treatment effects.

Results and Discussion

Characterization of molybdenum trioxide nanoparticles

Figure 2 illustrates the TEM image of MoO_3-NP. From the image it is observed that a small amount of agglomeration is present in the synthesized ash sample. The MoO_3 particles are nearly monodisperse oblong-shaped crystalline with the average size seen in the micrograph below 100 nm at least in one dimension. Upon calcination of Ammonium molybdenum, the particles were transformed into orthorhombic (α) MoO_3. The XRD pattern of the MoO_3 sample, calcined at 600 ^{0}C for 5 hr indicated an average particle size of 68.55 nm (Figure 1). Such a low-cost nanoformulation technology, as characterized by TEM and XRD, could produce oblong shaped crystalline nano MoO_3 with concurrent results in TEM and XRD. However, the presence of a small amount of metallic agglomeration in the submitted sample as observed from the TEM image could possibly be due to the absorbance of moisture during storage and characterization. Similar results were also obtained by Muthamizh *et al.* (2015).

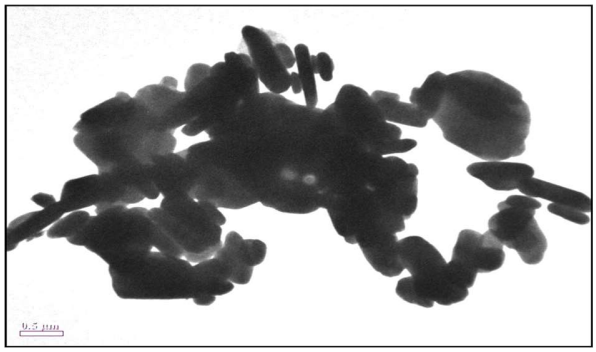

Figure 2: TEM image of Molybdenum trioxide nanoparticles

Growth traits

Seed treatment with molybdenum had no significant effect on the crop height at 15 DAS, but at subsequent growth stages, the impact was significant and positive. Seed priming with MoO_3 @ 200 ppm + seed inoculation with Rhizobium spp. (M_8) resulted in the tallest plants with a height advantage of 39.4% and 22.6% over the control (M_0) and farmers' practice (M_1), respectively (Table 2). The influence of seed dressing on crop height was positive but the increments were lower than priming at all four levels of MoO_3. No significant difference in crop height at 15 DAS was observed but at subsequent growth stages, the treatments with Mo supplementation gained an advantage in recording taller plants. An increase in crop height might be because Mo increases the biosynthesis of chlorophyll and the stability of photosynthetic apparatus, which results in increased biomass and crop height. Similar results were obtained by Singh *et al.* (2014) who studied the effect of seed priming with molybdenum on the performance of rainfed chickpea and observed that with the increase in level of seed priming with molybdenum up to 500 ppm, there was increased plant height. This corroborated the earlier findings by Srinivasan *et al*. (2007) and Arpit *et al*. (2016) researching foliar Mo application on greengram and soybean, respectively.

Table 2: Physicochemical properties of the soil of the experimental site

Physicochemical traits	Values
Bulk density (g/cm^3)	1.67
Particle density (g/cm^3)	2.65
Sand (%)	80.2
Silt (%)	7.4
Clay (%)	12.4
pH	5.3
Organic Carbon (%)	0.64
EC (ds/m)	0.16
Available N (kg/ha)	290.5
Available P_2O_5 (kg/ha)	10.24
Available K_2O (kg/ha)	143.52
Available Mo (ppm)	0.017

In all four stages, M_8 (seed priming with nano MoO_3 @ 200 ppm + seed inoculation with *Rhizobiumsps*.) recorded the highest number of leaves but plants under M_0 (control) had fewer leaves (Table 3). This could be due to increased nitrogen availability in M_8. Moreover increased

chlorophyll content due to Mo seed priming resulted in higher photosynthetic activity and higher biomass production, which might have produced more leaves in the plant. This corroborated the earlier results of Khan *et al.* (2019) who evaluated different priming methods and molybdenum applications on various cultivars of mung bean and similar positive effects of conventional Mo application in greengram by Srinivasan *et al*. (2007), and Anwarulla and Shivashankar (1987) corroborated the present result. The branching of greengram did not start up to 15 DAS, but subsequently started and continued up to 45 DAS faster (Table 3). But the rate of increase was very slow during maturity due to ageing. Among the treatments, M_8 and M_5 produced the maximum number of branches/plant whereas untreated control produced the least at 45 DAS and harvest. More branches might be due to the availability of growth factors in adequate quantity and in suitable proportions. Research results of Anwarulla and Shivashankar (1987), and Srinivasan *et al*. (2007) on the application of conventional Mo on greengram also corroborated the positive influence of Mo in the present investigation. At harvest, nano-priming with MoO_3 at 50, 100, 200 and 400 ppm significantly affected shoot dry matter accumulation with 26.7%, 36.4%, 43.9%, and 40.0% increments over the unprimed control, respectively. But only seed dressing with nano MoO_3 @ 400 ppm (M_5) was significantly superior to the control (M_0) and all other levels of seed dressing were at par with the unprimed seeds in recording shoot dry biomass. Nano priming was also reported to produce more branches and higher shoot biomass accumulation which could be due to better availability of Mo in plants that might have supported nutrient metabolisms such as N, P, K, S, and Fe as suggested by Awomi *et al*. (2012), Kumar *et al*. (2015), and Kumar *et al*. (2018). The root length recorded from 15 DAS till harvest increased up to the harvest but the rate of increment was higher from 30 to 45 DAS but beyond that, the rate slowed down (Table 4). The longest roots were recorded under M_8 but the shortest ones were recorded in M_0 (control) at all four observations. The increase in the concentration of nanopriming from 200 to 400 ppm resulted in the shortening of roots but remained at par with the other priming levels. Roots are dynamic and their growth is strongly affected by the environmental conditions in the root zone. Microbial association and non-toxic soil chemistry results in longer roots in plants. Mo plays a crucial role in the symbiotic association between the root of legume plants and the Rhizobium bacteria. This might be the reason for the longer roots produced in M_8. Increase in concentration of nano priming from 200 to 400 ppm resulted in shortening of roots. The rate of root biomass accumulation was faster up to 45 DAS but the rate slowed down towards the harvest. At harvest, M_8 accumulated 53.1% and 22.8% more root biomass than M_0 and M_1. Nanopriming at 200 ppm (M_8) and 400 ppm (M_9) levels did not differ significantly throughout the crop growth despite higher root biomass accumulation in M_8. The reason might be that with increased level of Mo, there is an increased shoot uptake than root uptake, resulting in higher shoot growth than root growth (Zakikhani *et al*., 2014). This corroborated the earlier findings of Kailash et al. (2019) and Gewehr et al. (2019) by applying nano Mo in pigeon pea and conventional Mo in soybean, respectively. Root nodule count increased from 15 DAS up to 45 DAS but it declined at harvest. Higher rate of increment in nodulation between 30 to 45 DAS matched with the active growth stage of the crop. Irrespective of growth stages, nano priming with MoO_3 showed positive results and the highest nodule counts/plant were recorded under M_8. At 45 DAS, M_8 could produce 78.1% and 36.8% higher nodules in plant roots over the control (M_0) and farmers' practice (M_6), respectively. As nodulation in greengram usually begins at around two weeks after sowing no significant difference in the treatment effects was recorded at 15 DAS but at subsequent growth stages, the treatment effects on nodule count were significant due to Mo application. Nano priming with MoO_3 positively influenced nodule number possibility because of the higher accumulation of Mo in seeds that favoured nodulation compared to other modes of Mo application. Seed priming with MoO_3 significantly impacts the root nodule count irrespective of the growth stage and the highest nodule count per plant was recorded in M_8. Root nodules are formed in leguminous plants due to symbiotic association with N-fixing bacteria Rhizobium. As Mo plays a key role in this symbiotic nitrogen fixation, its application might

Table 3: Plant height, number of leaves and number of branches/plant of greengram at different stages as influenced by seed treatments with molybdenum

Treatments	Plant height (cm)				Number of leaves/plant				Number of branches/plant			
	15 DAS	30 DAS	45 DAS	Harvest	15 DAS	30 DAS	45 DAS	Harvest	15 DAS	30 DAS	45 DAS	Harvest
M_0	7.0	21.4	29.0	31.5	4.3	11.5	16.2	12.1	1.1	1.2	3.9	4.1
M_1	7.4	24.3	32.9	35.8	4.6	14.6	20.8	15.4	1.0	1.5	5.1	5.4
M_2	7.2	22.2	30.1	33.4	4.4	11.7	17.5	14.4	1.1	1.4	4.6	5.0
M_3	7.3	22.9	31.1	34.1	4.5	12.1	18.1	14.5	1.0	1.5	4.7	5.2
M_4	7.5	25.3	34.4	37.5	4.9	12.9	19.2	15.8	1.1	1.7	4.7	5.4
M_5	7.7	26.5	35.7	39.3	4.9	14.2	22.4	15.8	1.1	1.8	5.3	5.7
M_6	7.6	26.7	36.3	39.6	5.1	13.5	18.5	16.5	1.0	1.6	4.7	5.1
M_7	7.8	28.0	37.8	41.5	5.4	15.4	20.4	18.4	1.0	1.8	5.1	5.4
M_8	8.0	29.8	40.4	43.9	5.8	16.7	23.4	19.8	1.1	1.9	5.3	5.7
M_9	7.9	27.3	37.1	40.8	5.6	16.4	21.4	18.3	1.0	1.9	5.2	5.5
S.Em. (±)	0.48	0.24	0.36	0.27	0.19	0.81	1.55	1.01	0.00	0.06	0.22	0.26
CD (0.05)	NS	0.7	1.1	0.8	0.6	2.4	4.6	3.0	NS	0.2	0.7	0.8

Table 4: Dry matter of shoot, longest root length and root dry matter of greengram at different stages as influenced by seed treatments with molybdenum

Treatments	Dry matter of shoot (g)/plant				Longest root length (cm)				Root dry matter (g)			
	15 DAS	30 DAS	45 DAS	Harvest	15 DAS	30 DAS	45 DAS	Harvest	15 DAS	30 DAS	45 DAS	Harvest
M0	1.56	3.59	7.16	10.15	4.8	9.8	26.2	29.5	0.102	0.191	0.423	0.622
M1	1.69	3.96	8.39	11.89	5.6	10.9	31.2	33.2	0.123	0.223	0.505	0.775
M2	1.62	3.75	7.71	10.92	4.9	10.6	28.2	32.1	0.107	0.204	0.455	0.699
M3	1.69	3.84	7.98	11.31	5.2	10.7	28.3	32.5	0.110	0.208	0.505	0.776
M4	1.68	4.04	8.67	12.28	5.9	10.9	30.3	33.3	0.113	0.232	0.505	0.776
M5	1.75	4.13	8.94	12.67	6.0	11.4	31.3	34.1	0.127	0.234	0.555	0.853
M6	1.68	4.17	9.08	12.86	5.0	10.2	29.8	34.2	0.110	0.196	0.504	0.721
M7	1.76	4.37	9.76	13.84	5.2	10.5	30.5	36.1	0.107	0.223	0.604	0.865
M8	1.85	4.54	10.31	14.61	6.3	12.0	33.3	36.3	0.130	0.235	0.632	0.952
M9	1.84	4.41	10.13	14.21	6.0	11.0	30.6	35.9	0.130	0.221	0.613	0.914
S.Em. (±)	0.043	0.166	0.370	0.859	0.27	0.39	1.17	0.97	0.0078	0.0076	0.0177	0.0151
CD (0.05)	0.13	0.49	1.10	2.55	0.8	1.1	3.5	2.9	NS	0.022	0.053	0.045

Table 5: Number of root nodules/plant, yield and yield attributing charactersof greengram at different stages as influenced by seed treatments with molybdenum

Treatments	Number of root nodules/plant				Pod/ plant	Seeds/ pod	Test weight (g)	Grain yield (t/ha)	Stover yield (t/ha)
	15 DAS	30 DAS	45 DAS	Harvest					
M_0	0.6	5.1	9.6	8.1	12.4	9.6	31.32	0.654	2.76
M_1	0.7	7.2	12.5	10.4	14.5	12.4	32.01	0.812	3.26
M_2	0.7	6.7	11.4	9.5	13.8	10.2	31.28	0.710	3.06
M_3	0.7	7.1	12.1	10.1	14.2	11.3	31.42	0.752	3.09
M_4	0.8	7.5	13.0	10.6	14.8	11.7	31.54	0.788	3.25
M_5	0.8	8.0	14.8	11.0	15.3	11.6	32.42	0.829	3.35
M_6	0.7	8.3	14.3	11.0	14.3	11.4	31.45	0.769	3.45
M_7	0.8	9.1	15.9	12.3	14.8	11.9	31.86	0.826	3.65
M_8	0.8	9.5	17.1	14.2	15.9	12.5	32.42	0.880	3.74
M_9	0.9	9.4	16.3	13.8	15.8	12.3	32.24	0.854	3.57
S.Em. (±)	0.11	0.34	1.28	0.99	0.55	0.60	0.77	0.0325	0.13
CD (0.05)	NS	1.0	3.8	2.9	1.6	1.8	NS	0.096	0.39

have resulted in increased nodule count. Evidence of enhanced nodulation due to elevated Mo concentration has also been reported by Kumar *et al*. (2015), Velmurugan and Mahendra (2015), and Kumar *et al*. (2018) which corroborated the present investigation.

Yield and yield attributing characters

Seed priming with MoO_3 at all four levels had significantly increased the number of pods/plant over M_0 but M_1 (farmers' practice) remained at par with both treatment effects of nano priming and dressing. Seed priming with MoO_3 @ 200 (M_8) and 400 ppm (M_9), and seed dressing @ 400 ppm (M_5) were of first, second and third order in pod count. Seeds/pod significantly showed positive (30.2%) effects of Mo seed treatment over control (Table 5). But no significant difference in seed number was recorded between farmers' practice (M_1) and seed dressing or priming with nano MoO_3, except under M_3 i.e. seed dressing at 50 ppm which recorded a significantly lower number of seeds. Similar results were obtained in the case of test weight as well. Yield attributing characters like pod number, seeds/pod and test weight are influenced by Mo seed treatment, either seed dressing or seed priming. This result might be due to the availability of Mo in adequate amounts towards the crop maturity stage irrespective of the sources. Seed treatment with Mo could be attributed to the adequate availability of plant growth elements in appropriate proportions. The above results on pods/plant, seeds/pod, and seed weight confirmed the earlier findings from the research conducted by Manjili *et al*. (2014), Gad *et al*. (2013), Heidarzade *et al*. (2016), Kumar *et al*. (2018), studied greengram and other pulses by using conventional and nano Mo.

The grain yield of greengram was influenced significantly due to the difference in molybdenum seed treatment. Seed priming with nano MoO_3 @ 200 ppm along with *Rhizobium* seed inoculation (M_8) was the most promising in recording significantly the highest grain yield of 0.88 t/ha that provided an additional yield of 0.068 t/ha (8.37% more) over farmers' practice (M_1 i.e. seed dressing with sodium molybdate @ 400 ppm and seed inoculation with *Rhizobium*) and 0.226 t/ha (32.53% more) over the control (M_0 i.e. no Mo seed treatment or *Rhizobium* seed inoculation). Seed priming with nano MoO_3 @ 400 ppm and *Rhizobium* seed inoculation (M_9) resulted in second highest grain yield of 0.854 t/hathat produced an additional yield of 0.042 t/ha(5.17% more) over farmers' practice (M_1) and 0.2 t/ha(30.58% more) over the control (M_0). Seed dressing with nano MoO_3 @ 400 ppm along with *Rhizobium* seed inoculation (M_5) resulted in third highest grain yield of 0.829 t/ha. The grain yield/plant followed a similar trend as grain yield in the field. The stover yield of greengram was influenced significantly due to the difference in seed treatment with molybdenum. Seed priming with nano MoO_3 @

200 ppm along with *Rhizobium* seed inoculation (M_8) recorded significantly the highest stover yield of 3.74 t/ha which provided an additional yield of 0.48 t/ha (14.7% more) over farmers' practice (M_1) and 0.98 t/ha (35.5% more) over control (M_0). Seed priming with nano MoO_3 @ 100 (M_7) and 400 ppm (M_9) occupied the second and third ranks in recording stover yield of 3.65 and 3.57 t/ha, respectively.

Priming treatment with MoO_3 results in rapid seedling emergence and crop establishment with increased growth and yield attributing characters, and ultimately higher yield. Moreover, Mo plays a crucial role in symbiotic nitrogen fixation in leguminous crops, increasing the availability of nitrogen. This might be the reason for the treatment M_8 has attributed to the maximum grain and stover yield (Maroufi *et al*., 2011). Higher grain yield in treatments over control except M_2 and M_3 could be due to the synergistic effects of all yield attributes such as pods/plant, seeds/pod, and test weight so also the effect of *Rhizobium* inoculation that might have supported nutrient metabolisms such as N, P, K, S, and Fe as suggested by Awomi *et al*. (2012). The present result was in line with earlier reports by Manjili *et al*. (2014), Velmurugan and Mahendra (2015), Arpit *et al*. (2016), Heidarzade *et al*. (2016), Hossain *et al*. (2018) and Kumar *et al*. (2018). The highest stover yield in the case of M_8 might be due to its higher growth attributing characters like plant height, number of branches, number of leaves etc. Research findings of Arpit *et al*. (2016), Kumar *et al*. (2018), and Movalia *et al*. (2020) in greengram and other pulse crops also corroborate the above results.

Conclusion

From the analysis of the observations recorded in the present experiment, it can be concluded that molybdenum seed treatment, irrespective of mode and level of application, positively influenced the growth, development, and productivity of the greengram crop. The overall performance of seed priming with Molybdenum trioxide nanoparticles (MoO_3-NPs) was better than either seed dressing with MoO_3-NPs or Sodium molybdate. Preconditioning of greengram seeds by nanopriming with MoO_3 @ 200 ppm + Rhizobium inoculation was the best option for achieving higher grain yield, and productivity under the present investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Elucidating genetic diversity and variability in Chickpea (*Cicer arietinum* L.) using yield attribution traits

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ABSTRACT

Fifty-six *desi* chickpea (*Cicer arietinum* L.) advance breeding lines were evaluated in order to explore the possibility of genetic divergence for yield and its contributing traits using Mahalanobis's D^2 Statistics and Principal Component Analysis. High estimates of heritability, genetic advance, GCV and PCV were recorded for seed yield per plant (92.2%, 12.4%, 37.1% and 38.7%), biological yield per plant (88.1%, 21.9%, 29.1% and 31.0%) and harvest index (87.3%, 25.0%, 22.7% and 24.3%). All the test genotypes were sort into five discrete clusters. Biological yield/plant (23.5%), days to maturity (17.3%), harvest index (14.6%), seed yield/plant (11.3%), total number of pods/plant (7.4%) and 100 seed weight (6.49%) were found to have highest percentage contributions to genetic diversity in the present research. The first six principal components (PC1 19.7%, PC 16.2%, PC3 11.2%, PC4 9.69%, PC5 7.2% and PC6 6.69%) could explain 70.68% of the total of the interaction variation and have Eigen value more than one. Genotypes JG 2016-1411, JG 2016-9605, JG 2017-46, ICCV 16105, ICCV 16109, ICCV 16112 and ICCV 16116 were present in more than one PCs hence contributed maximum towards yield and can be used in various breeding programmes for yield improvement.

Introduction

India has gained astounding headway in improving pulse production during last 15 years. Total pulse production in India during 2005-06 was 13.38 million MT, by 2020-21 the production increased to 25.58 million MT (Gaur, 2021). Chickpea had an overwhelming majority share of 49.3% in total pulse production in 2020-21. India had leapfrogged towards attaining self sufficiency in pulses and is essential for supplying reasonably priced protein to the world's expanding population (Bankoliya *et al*., 2022). Adaptation and implementation of innovative breeding programs has increased the total yield of chickpea from 1.27 million MT in 1979 to 6.95 million MT in 2019 in central and southern India, which accounts for 445% increase in production. It was possible due to 177% area growth (2.42 to 6.71 million ha) and 97% yield growth (527 to 1036 Kg/ha) over the last 40 years (1979-2019) (Gaur, 2021). Chickpea is a valued crop and possess huge wealth of vitamins, minerals, proteins, fiber and complex carbohydrates. It is consumed all over the world, especially in the Asian and African countries and is referred to as poor man's meat as it contains rich source of protein. Chickpea is grown in rotation

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with cereal crops in India due to its ability to fix areal nitrogen into the soil and intern improves soil fertility. Due to diverse role of this crop in farming system, research on chickpea crop will have significant impact on nutritional security and soil fertility. Narrow genetic base of cultivated chickpea is the major limiting factors for initiating the breeding programme for increasing the yield potential. Understanding genetic variation is crucial for recognize its availability and its potential application in breeding. Improvement in yield can be achieved by selecting the genotypes with desirable characters either alone or in combination with other morphological traits (Shivwanshi and Babbar, 2019).

Material and Methods

Fifty six genotypes of *desi* chickpea, received from ICRISAT, Patancheru and JNKVV, Jabalpur along with two checks, JG 16 and JG 36, were evaluated during post rainy season, 2018-19, Department of Plant Breeding and Genetics, Seed Breeding Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. Geographically, the experimental site is located at 23°21'N latitude and 79°94" E longitude at an altitude of 411.8m above the sea level. Tested genotypes were laid out in three replications based on Randomized Complete Block Design (RCBD), with genotypes spaced 30 cm by 10 cm apart on 4.0 m by 1.2 m plot. Weather data such as precipitation, was also obeseved throughout the cropping seasion since weather has profound influence on crop growth, production, pest and disease incidence, irrigation and fertilizer requirement. Throughout the growing season, the chickpea crop was grown according to the prescribed agronomical package of practice of chickpea. Five randomly chosen plants from each plot's were selected to record yield and yield attributing attributes. Mean value of 56 chickpea genotypes were computed for determining principals of variance, Principal Component Analysis (PCA) and cluster analysis were also performed to assess genetic diversity among chickpea accessions.

Results and Discussion

Throughout the cropping seasons, there was a total of 19.4mm rainfall, a relative humidity range of 31.4 – 82.8% with an average maximum temperature of 27.9°C and lowest temperature of 9.6°C. There was no occurrence of erratic weather change. Hence the weather requirement for crop growth was optimum.

For all the parameters considered in the study, the analysis of variance (ANOVA) indicated high significant differences ($P<0.001$) among the tested genotypes, suggesting presences of genetic variability among the genotypes and revealing the significance of chickpea germplasm in crop improvement programmes. Previous studies (Mohammed *et al*., 2019) reported presence of variability in similar traits, while some research (Pandey *et al*., 2013) reported exception for days to maturity and number of seeds/pods.

To assess the level of genetic variability existing in the tested population, the study of phenotypic range of variation alone is insufficient. Both genotypes and the environment in which they are grown play a combine role in determining phenotypic variation. To evaluate genetic variability and to determine the amount of any potential improvement in various traits, one should employ genetic parameters such as heritability, components of variance, coefficient of variation and genetic advance. The traits under research were less influenced by environment since genotypic coefficient of variation (GCV) closely followed phenotypic coefficient of variation (PCV) in all the traits. For seed yield/plant (38.7% & 37.1%), biological yield/plant (31.0% & 29.1%) and harvest index (24.3% & 22.7%), estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were higher, indicating a high level of variability. Rest of the trait (Table 1) showed moderate to low GCV and PCV indicating the need for improvement of based population.The estimated PCV and GCV value simply provides information on the degree of variability present for different traits; it contains no details on heritability component. The information of heritability enables the researcher to choose the selection strategy to attain potential selection gain (Ramanappa *et al*., 2013). High heritability is also a good indicator for characters inheritance from parents to their off-springs. For seed yield/plant, harvest index, biological yield/plant, number of effective pods/plant and total number of pods/plant, high estimates of heritability were found combined

Table 1: Estimates of genetic parameters of chickpea genotypes

Traits	Range	Mean	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
DFI	44-63	52.9	7.2	6.3	77.2	7.7	14.7
D50% F	52-72	60.7	6.3	5.3	72.2	7.3	12.0
DPI	61-77	67.4	5.0	3.8	57.9	5.1	7.6
DM	94-114	105.0	4.3	4.0	87.0	10.4	9.9
PH (cm)	43.5-71.9	53.4	9.5	8.4	77.3	10.4	19.5
HFFN (cm)	15.1-23.1	18.8	14.1	11.1	61.7	4.36	23.1
NPBPP	2.33-3.88	3.09	15.6	6.98	19.8	0.25	8.20
NSBPP	5.33-9.10	7.08	17.0	12.6	55.4	1.76	24.9
TNPPP	35.5-70.0	52.5	18.4	15.9	74.9	19.1	36.4
NEPPP	29.9-69.9	47.6	19.5	17.0	75.5	18.5	19.0
NSPP	1.03-1.24	1.09	5.3	3.3	38.8	0.06	5.48
100 SW (g)	14.8-34.2	26.1	19.2	16.0	69.5	9.23	35.3
BY (g)	15.1-53.4	30.3	31.0	29.1	88.1	21.9	51.2
HI (%)	26.6-76.5	44.6	24.3	22.7	87.3	25.0	46.0
SYPP (g)	6.50-30.3	13.2	38.7	37.1	92.2	12.4	48.2

Where,

PCV- phenotypic coefficient of variation, GCV-genotypic coefficient of variation, h^2-heritability, GA-genetic advance, GAM-genetic advance as percentage of mean, DFI-Flower initiation, D50%F-Days to 50% flowering, DPI-Days to pod initiation, DM-Days to maturity, PH-Plant height (cm), HFFN-Height of first fruiting node (cm), NPBPP-Number of primary branches/plant, NSBPP-Number of secondary branches/plant, TNPPP-Total number of pods/plant, NEPPP-Number of effective pods/plant, NSPP-Number of seeds/pod, 100SW-100 Seed weight (g), BY-Biological yield (g), HI-Harvest index (%), SYPP-Seed yield/ plant (g).

with moderate to high variation. Similar conclusion was reported by (Mohammed *et al*., 2019).

Heritability estimates have a limited scope as it varies with change in environment, experimental materials or treatments (Swarup and Chaugale, 1962). Hence, using heritability in association with genetic advance will give an insight into the nature of gene action governing a certain character (Johnson *et al*., 1955). High broad sense heritability coupled with high genetic advance as percent mean was recorded for biological yield/plant, seed yield/plant, harvest index and total number of pods/plant indicating that these traits were governed by additive gene action and would respond favorably to selection. Conclusion of some researches such as Meena *et al*., (2014) summarized that traits; seed yield per plant, biological yield, number of pods per plant exhibited high heritability coupled with high expected genetic advance. Findings of Dhuria and Babbar, 2015 and Dehal *et al*., 2016 also revealed that seed yield per plant is highly heritable coupled with high genetic advance. These results are similar to the present findings. Cluster-IV and cluster-V showed greater divergence, based on the relative divergence of inter-cluster distance (D^2 = 140.0) (Table 2). Genotypes of cluster-IV was characterized by maximum number of primary branches/plant, high biological yield, high number of seeds/pod and low 100 seed weight, whereas short duration advance breeding lines were accommodated in cluster V which was characterized phenological traits such as days to flower initiation, days to 50% flowering, days to pod initiation and days to maturity but has low primary branches per plant. Inter-cluster distance of cluster-III and cluster-V was D^2 = 139.5.The genotypes of cluster-III were characterized by maximum height for first fruiting node and secondary branches/plant also highest seed yield/plant and harvest index. Based on D^2statistics, the inter cluster distance suggests hybridization program between genotypes of cluster-IV (JG 24) with cluster-V (JG2016-9218 -14) and cluster-III (JG 2016-1411, JG 2017-50, JG 14) with cluster-V (JG2016-9218 -14) is expected to generate desirable sergeants for various yield attributing traits. Parent line should be chosen from these three different clusters because hybridization between divergent parent is likely to induce variability and transgressive segregants with significant heterotic effect.

Table 2: Mahalanobiseuclidean inter (diagonal) and intra cluster D^2 values

Cluster	I	II	III	IV	V
I	**27.3**	62.2	77.4	69.7	76.7
II		**0.0**	35.0	111.1	114.9
III			**33.0**	132.5	139.5
IV				**0.0**	140.0
V					**0.0**

The number of contrasting alleles at the desired loci increases with parental distance. These loci will recombine in the F_2 and F_3 generation through successful breeding of unrelated parents, increasing the opportunity for selection of yield related factors. The current study confirmed that any pair of cluster having sufficient genetic variability, could be exploited through hybridization. Similar research was conducted by Jakhar *et al*. (2016), suggesting that, crossing between the genotypes of clusters with high inter cluster would yield good segregates for selection.However, cluster-II and cluster-III had the minimum inter cluster distance (D^2 = 35) followed by cluster-II and cluster-V (D^2 = 114.9). Because the genotypes in these two clusters are relatively closer, breeding between them might not produce high vigor F_1's or high yielding segregants. Maximum intracluster distance was recorded for cluster-III (33.0) followed by cluster-I (27.3), whereas cluster-II, cluster-IV were mono-genotypic hence, showed zero value for intra cluster distance, however cluster-III and cluster-I are poly-genotypic with high diversity. Genetic divergence among the parental lines participating in crossing programme is typically credited for heterosis. Less intra-cluster distance than inter cluster distance was also reported by Kujur *et*. *al*., (2017), indicating homogeneity within the clusters and heterogeneity between the cluster. Cluster-I was largest among all the clusters containing 50 genotypes (Table 3 and Table 4) and have characteristics such as height of plant (53.0 cm), number of primary branches (3.1) and pods per plant (51.1) and test weight (26.2 g). Cluster-III is trigenotypic and has following features: height of first fruiting node (22.4 cm), number of secondary branches (7.29) and number of seeds/pod (1.14) as well as high percentage of harvest index (73.4%) with high seed yield/plant (26.9 g). On the other hand, cluster-II is mono genotypic (JG 2016-9605) and was characterized by lowest height of first fruiting node (15.1 cm), maximum number of pods (65.5) and effective pods per plant (59.5). Cluster-IV is also mono genotypic(JG 24) and this genotype was characterized by phenological traits such as late emergence of flowers (63 days), delayed 50% blossoming of flowers (72.3 days), delayed pod initiation (77 days), late maturity duration (115 days), tallest plant height (71.9 cm), number of primary branches (3.33), number of seed per pod (1.14), biological yield (53.4 %), test weight (29.8 g) and harvest index (26.6 %). There is only one genotype in cluster V, JG2016-9218 -14, and it was characterized for early flower initiation (50 days), early blooming of 50% flowers (57.6 days), early pod initiation (66 days), early maturity (102 days), number of secondary branches (6.33), effective pods (42.4), number of pods per plant (1.06), biological yield (25.2 g), seed yield/plant (11.3 g) and number of primary branches/plant (3.33).In the present findings days to 50% flowering (0.13%), Days to pod initiation (0.06%), number of primary branches per plant (0.19%) and number of effective pods per plant (0.71%) had minimal contribution towards genetic divergence. However Babbar *et al*. (2015) observed that pod initiation, 100SW (seed weight), days to 50% blossoming of flowers, BW and HI contributed most to divergence. Nevertheless, Shrivastava *et al*. (2012) revealed that days to 50% flower emergence, days to flower commencement, pod initiation, days to maturity, primary branches and seed/pod showed little contribution towards genetic divergence. The fundamental idea of PCA is to eliminate the redundancy in large number of interrelated data set into uncorrelated new set of variables or principal components, and which are arranged, so that the first few components retains most of the variation present in all of the original variables. PCA was used in the current study to analyze fifteen yield and attributing traits in chickpea genotypes. Brejda *et al*. (2000) stated that data were taken into account for each component with an Eigen value greater than 1 which accounts for at least 10% of the variation. Attributes in principal component are most accurately represented by those with higher Eigen values. Six components (PCs) were the only ones to exhibit more than one Eigen value and having cumulative variability of 70.7%, therefore they were given due importance for additional

Table 3: Clustering of 56 *desi* chickpea genotypes using D^2 statistics

Cluster	Genotypes (number)	Genotypes (Name)
I	50	ICCV 15117, ICCV 15109, JG 2016-74315, ICCV 15102, ICCV 16111, JG 14, JG 2016-3-1205, ICCV 15115, ICCV 15104, JG 74315-2010-14, JG 2016-14226, ICCV 16101, ICCV 16110, JG 2016-1307, ICCV 16113, JG 2016-45, ICCV 16105, ICCV 16106, ICCV 15105, ICCV 16107, JG 2016-3-1, ICCV 15107, JG2016-16-14, JG 2016-11-1, ICCV 15111, ICCV 16103, ICCV 16114, ICCV 16112, ICCV 16115, ICCV 16117, ICCV 16109, JG 2017-46, ICCV 16118, ICCV 16116, ICCV 15108, JG 2016-44, JG 2016-94, ICCV 15118, JG 2016-11551, JG2016-63-4958, ICCV 16113, ICCV 16104, ICCV 16108, JG 36, ICCV 16102, JG 2017-47, JG 2016-43, JG 2016-1416-11, JG 16, JG 2016-1216
II	1	JG 2016-9605
III	3	JG 2016-1411, JG 2017-50, JG 14
IV	1	JG 24
V	1	JG2016-9218 -14

Table 4: Cluster mean of 15 quantitative traits of 56 *desi* chickpea genotypes

Traits	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
DFF	53	53	56	63**	50*
D50% F	60	59	62	72**	58*
DPI	67	67	70	77**	66*
DM	104	108	112	115**	102*
PH (cm)	53.0*	53.4	53.8	71.9**	53.3
HFFN (cm)	18.7	15.1*	22.4**	22.2	16.5
NPBPP	3.10*	2.55	3.14	3.33**	3.33**
NSBPP	7.09	6.55	7.29**	7.22	6.33*
TNPPP	51.1*	65.5**	61.3	57.1	62.7
NEPPP	47.0	59.5**	54.4	51.4	42.4*
NSPP	1.10	1.10	1.14**	1.14**	1.06*
100 SW (g)	26.2**	24.3	25.7	23.8*	23.9
BY (g)	29.2	48.9	36.8	53.4**	25.2*
HI (%)	43.0	54.9	73.4**	26.6*	44.5
SYPP (g)	12.1	26.8	26.9**	14.2	11.3*

Where, "" and "*" indicates highest value and lowest value, respectively.**

explanation. The PC1 had the highest variability of 19.7%, followed by PC2, PC3, PC4, PC5 and PC6 exhibiting 16.2%,11.2%, 9.69%, 7.20% and6.69% variability respectively for traits under study (Table 6).Based on rotated component, the PC1 was found to have the highest variability, 19.7% (Table 5) and it was primarily associated with phenological parameters including days to flower initiation, days to 50% flowering, days to pod initiation and days to maturity. When choosing genotypes based on duration, significant difference in flowering time and maturity period can be crucial. The second major component, which make up 16.2% of the overall variability, was highly loaded with yield related characteristics like total of pods/plant, number of effective/per plant, harvest index and seed yield/plant. The genotypes, ICCV 16102, JG 2017-50, JG 2016-9605, ICCV 15105, ICCV 16110, ICCV 16114, ICCV 16107, JG 2016-94, ICCV 16105, JG JG 2016-1411, ICCV 16108, ICCV 16115, ICCV 15108, ICCV 16112 and ICCV 16116 may be considered as promising advance breeding lines because they have more pods/plant and effective pods/plant, a high harvest index, and increased seed yield per plant. They were found to be better yielding under timely sown condition and may be used further in breeding programme to improve seed yield. The PC3 was highly loaded with number of seeds/pod and exhibited 11.2% of the variability, while PC4 was dominated for seed yield/plant. The PC5 showed 7.2% variability and consisted of trait *viz*. height of first fruiting node indicating usefulness of this PC for mechanical harvesting. The PC6 was dominated for 100 seed

Table 5: Contribution of different attributes towards clustering

Traits	Times ranked 1st	Contribution towards divergence (%)
Days to flower initiation	84	5.45
Days to 50% flowering	2	0.13
Days to pod initiation	1	0.06
Days to maturity	270	17.3
Plant height (cm)	61	3.96
Height of the first fruiting node (cm)	52	3.38
No. of primary branches/plant	3	0.19
No. of secondary branches/plant	23	1.49
Total no. of pods/plant	114	7.4
No. of effective pods/plant	11	0.71
No. of seeds/pod	57	3.7
100 seed weight (g)	100	6.49
Biological yield/plant (g)	363	23.5
Harvest index (%)	225	14.6
Seed yield/plant (g)	174	11.3

Table 6: Eigen value, variability percent and cumulative percent for the principal component axes

	Principal Components (PCs)					
	1	**2**	**3**	**4**	**5**	**6**
Eigen value	2.95	2.43	1.68	1.45	1.08	1
Variability (%)	19.7	16.2	11.2	9.69	7.2	6.69
Cumulative (%)	19.7	35.9	47.1	56.8	64	70.7
Traits	**Factor loading after Varimax rotation**					
DFI	**0.762**	-0.496	0.011	0.123	0.003	-0.052
D50% F	**0.782**	-0.424	0.079	0.102	-0.002	-0.036
DPI	**0.752**	-0.384	-0.080	0.038	-0.074	0.075
DM	**0.741**	0.060	0.212	0.080	0.165	0.086
PH (cm)	-0.202	-0.361	-0.258	0.413	-0.050	0.147
HFFN (cm)	-0.052	0.006	-0.119	0.148	**0.660**	**0.690**
NPBPP	0.113	0.329	0.353	-0.401	-0.024	0.204
NSBPP	0.319	0.347	0.300	-0.174	-0.425	0.239
TNPPP	0.474	**0.572**	-0.456	-0.192	0.029	-0.060
NEPPP	0.455	**0.539**	-0.542	-0.225	0.026	-0.020
NSPP	0.024	0.100	**0.656**	0.418	0.036	-0.154
100 SW (g)	-0.001	0.185	0.138	0.191	-0.522	**0.573**
BY (g)	0.184	**0.690**	0.192	0.390	-0.182	-0.163
HI (%)	-0.101	-0.090	-0.582	0.424	-0.347	0.040
SYPP (g)	0.133	**0.593**	-0.068	**0.637**	0.003	-0.083

weight and height of first fruiting node. PC3, PC4 and PC6 are associated to yield related traits. Genotypes JG 2016-1411 was common in PC1,PC2 and PC4. Genotypes JG2016-9605 was common in PC1, PC2 and PC 4, genotype JG 2017-46 was common in PC3, PC5 and PC6 and genotypes ICCV 16105, ICCV 16109, ICCV 16112 and ICCV 16116 was common in PC2, PC3, PC6, and PC1, PC3, PC6 and PC1, PC2, PC3and PC 2, PC5, PC6 respectively. These genotypes accounts for the entire yield attributing traits. With the help of principal component analysis phenotypic characterization can

be accredited, which is responsible for the observable genetic variation present in each component. Hence, when using these attributes in breeding programmes, characteristics that come together in different principal components, contribute to elucidating the variability, and have a tendency to persist together may be taken into account. Similar analysis was observed in groundnut (Patil *et al*., 2020) where PC1 was considered as most important component which consists to traits like number of pods per plant, pod yield per plant and seed yield per plant,

Conclusion

Genotypes JG 2016-1411, JG 2016-9605, JG 2017-46, ICCV 16105, ICCV 16109, ICCV 16112 and ICCV 16116 were common in three PCs. These genotypes contribute maximum towards yield. Genotypes ICCV 16109 performs best in comparison to other genotypes and can be considered as promising breeding material for precise selection to the development of suitable genotypes and also for the transfer of suitable traits. Five clusters were created out of 56 genotypes. Cluster-IV (JG 24) and cluster-V (JAKI 9218) had the greatest intercluster distance, followed by cluster-III (JG 2016-1411 and JG 2017-50 and JG 14) and cluster-V (JAKI 9218), cluster-II (JG 2016-9605) and cluster-V (JAKI 9218). The chances for the occurrence of a high frequency of heterotic cresses and with high values of heterosis are more when the parent are chosen from these clusters compared to the crosses between parents whose divergence is narrow.

Conflict of interest

The authors declare that they have no conflict of interest.

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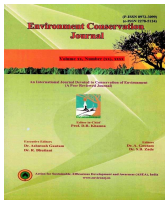

Response of organic black gram to botanical seed pelleting and row spacing

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ABSTRACT

The field trial was carried in *Zaid* season of 2021 at SHUATS Model of Organic Farm (SMOF), Naini Agricultural Institute, SHUATS, Prayagraj (U.P.). The objective was to study the growth and yield of Black gram as influenced by crop geometry and seed pelleting with botanicals under certified organic production system. The experiment was laid out in a randomized block design to study the effect of seed pelleting with leaf powders of three botanicals, *viz*., *Pongamia pinnata*, *Prosopis juliflora,* and *Albizia lebbeck,* and sown in three different row spacings of 20 cm, 30 cm, and 40 cm, on the growth and yield of black gram. The results revealed that black gram seeds pelleted with *Albizia* leaf powder and sown at row spacing of 30 cm reported maximum plant height (43 cm), dry weight (12.49 g/plant), number nodules per plant (27.50), seeds per pod (7.07), test weight (42.19 g), grain yield (872.96 kg/ha), and haulm yield (2511.11 kg/ha) which was significantly superior to other treatments.

Introduction

In India, black gram (*Vigna mungo* L.) is the third-most significant pulse crop. It is rich in protein (~27.75 %), which is about two third of the protein content of soybean, twice that of wheat and thrice that of rice (Kamani and Meera, 2021). Hence, a diet combining black gram and cereal grains forms a balanced amino acid diet.This crop is important in terms of sustaining soil fertility through improving soil physico-chemical properties. In 2020-21,India produced about 2.34 m t of urad annually from about 4.67 m ha of area, with an average productivity of 501kg per hectare (DACFW, 2021). Because the crop is mostly cultivated in rain-fed circumstances with poor management practices, as well as due to other physiological, biochemical, and intrinsic characteristics related to crop, the yield potential of black gram is extremely low. Several strategies have been introduced to boost the productivity of black gram. One of them is seed pelleting with botanicals, which helps to overcome the adverse environmental conditions. They also boost the emergence and enhances growth of seedling's root and shoot, thereby resulted in increased productivity. In addition, pelleting improves the water holding capacity of the soil in the rhizosphere and supply nutrients to the germinating seed (Srimathi *et al*., 2013). Nutrient applications to dry lands are troublesome; instead, pre-treating the seed with the nutrients will increase its viability and vigour, resulting in improved yield. In any crop, maintaining the ideal plant population is crucial for obtaining optimum yield from the crop. In black gram, optimum plant population can be achieved by adjusting inter- and intra-row spacing. The optimum spacing encourages both above and below ground plant growth, and also provides favourable environment to have maximal light interception during crop growth. This research study will provide insight on how seed pelleting

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and row spacing affect the development, output, and economics of black gram cultivation.

Material and Methods

The experiment was conducted during *Zaid*, 2021, in SMOF (SHIATS Model of Organic Farm), Department of Agronomy, Sam Higginbottom University of Agriculture, Technology, and Sciences, Prayagraj (U.P.). The experimental land was uniform in topography and soil texture was sandy loam with nearly neutral soil reaction (pH 7.1), medium organic carbon (0.58%), low available N (225 kg/ha), available phosphorous (19.50 kg/ha), and medium available potassium (129.7 kg/ha). The experiment was plotted in Randomized Block Design with ten treatments, consists of seed pelleting with three different botanical's powder (*Pongamia pinnata*, *Prosopis juliflora,* and *Albizia lebbeck*) and were sown at three different row spacings (20 cm, 30 cm, and 40 cm) and one control (non-pelleted seed sown at 35 cm row spacing) which were replicated thrice. The trial field was carefully ploughed, harrowed, and levelled. For nutrient supply, well decomposed farmyard manure is applied at the rate of 5 t/ha to fulfil the required amount of nutrients for the production of organic black gram. Seeds were sown after being pelleted with powdered botanicals as per the treatments. Seed pelleting refers to the coating of seeds with inert material just large enough to produce a globular unit to facilitate precision planting. The fresh leaves of three botanicals (*Pongamia pinnata*, *Prosopis juliflora,* and *Albizia lebbeck*) were collected, shade dried, powdered and sieved individually. The seeds were first coated with 10% maida solution (adhesive) and then rolled in leaf powder (200g/kg seeds) until a uniform coating has been done. Seeds were pelleted thoroughly, avoiding the formation of any lumps (Fig. 1). Seeds were dried under shade before sowing (Prakash *et al*., 2020).

Results and Discussion

Growth parameters

The experimental results for growth parameters (Table 1) of black gram recorded significantly highest plant height (43.00 cm) at 45 DAS in *Albizia* leaf powder pelleted seeds, sown at 30 cm row spacing and found statistically at par with T_3, T_6, T_7, T_9, and T_{10}. This outcome could be attributed to the improved metabolic activity in plumule and radicle cells of the seed induced by the micronutrients supplied by *Albizia* powder. This enables better nutrient absorption, initiating seedling growth, and, ultimately, stimulating rapid growth and increased plant height. The inert material improves natural water holding capacity and provides initial nutrients to emerging plantlets (Krishnasamy, 2003). Similar to this, optimal spacing assures the availability of resources for crop establishment and growth, increasing crop height. At 45 days after sowing significantly highest nodules in a plant (51.17) was recorded in seed pelleting with *Albizia* powder at 200g/kg seeds and sown at 30cm row spacing, which was on par to T_1 and T_6. This could be because of the optimum spacing available for the enhanced root proliferation and the favourable rhizosphere environment for nodule development brought up by the seeds pelleted with *Albizia* powder. At 45 DAS, the significantly highest dry weight per plant (12.49 g/plant) was recorded in seeds pelleted with *Albizia* powder at 200 g/kg seeds and sown at the line spacing of 30 cm. The embryo and other associated structures might have been activated by the physiologically active substances resulting in the absorption of more water due to cell wall elasticity and development of stronger and efficient root system which in turn favours the derivation of more nutrients thus enabling better growth of the plants (Prakash *et al*., 2020). The presence of bioactive compounds like auxins in the *Albizia* leaf powders aids seedling growth to reach the autotrophic stage and also create relatively more dry matter with an increase in vigour index. It might also be attributed to the increased growth of the plant (Narayanan *et al*., 2016). Wider plant spacing might have been the cause of the higher plant height and nodules per plant because it intercepted more photosynthetically active radiation. This led to vigorous plant growth, more branches and leaves, and more dry matter per plant (Murade *et al*., 2014).

Yield attributes and yield

The data pertaining to yield attributes (Table 2) reported that except seeds per pod, other yield attributes differed significantly with seed pelleting and varied row spacing. Seed pelleted with *Albizia* leaf powder and sown in lines 30 cm apart recorded higher pods in one plant (24.55), which was

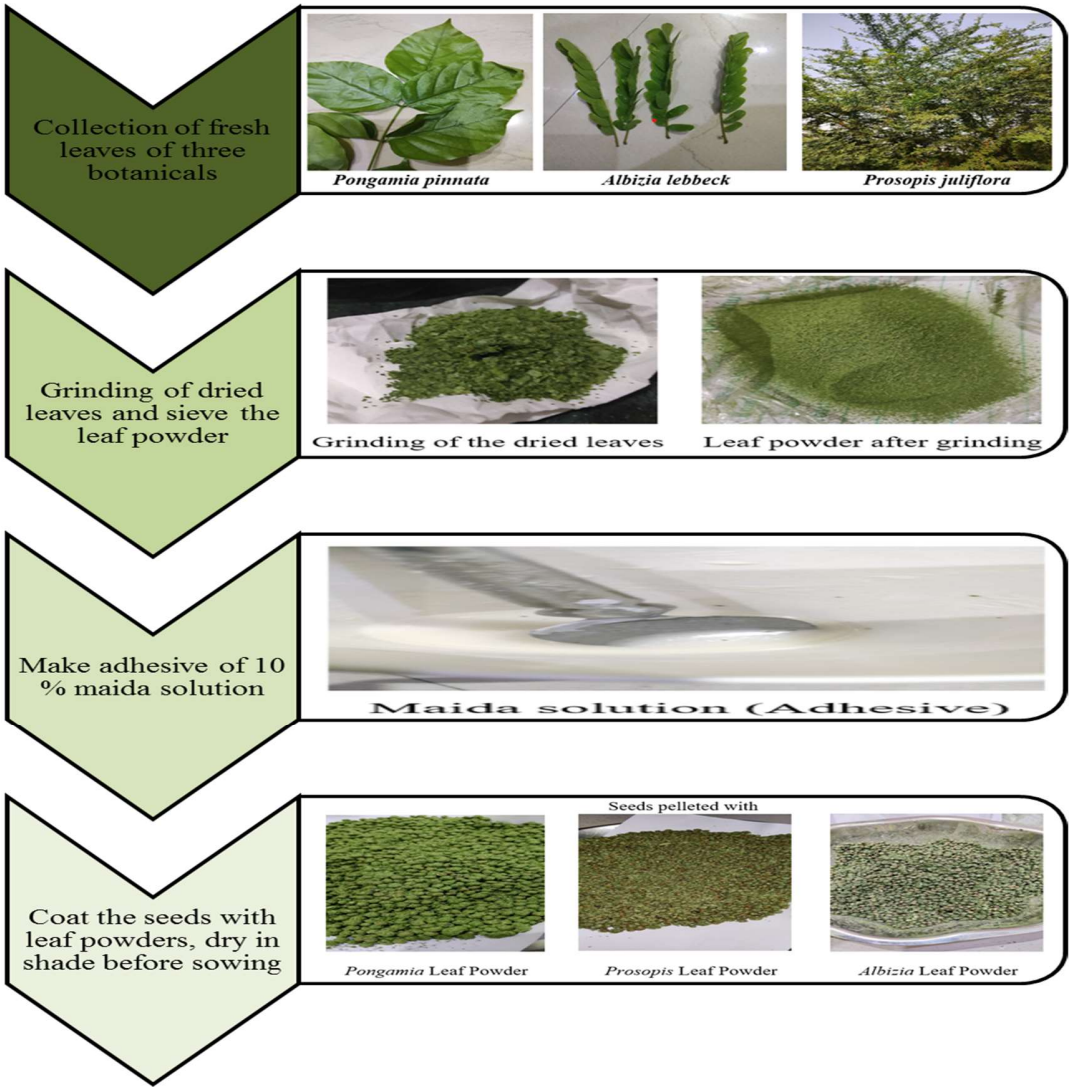

Figure 1: Process of seed pelleting with leaf powder of three botanicals

Table 1: Effect of organic seed pelleting and row spacing on the growth of Black gram

Treatments	At 45 DAS		
	Plant Height (cm)	Nodules/Plant (No.)	Dry Weight (g/plant)
1. Seed pelleting with *Pongamia* L.P. + 20 cm x 10 cm	30.60	50.67	10.66
2. Seed pelleting with *Pongamia* L.P. + 30 cm x 10 cm	28.82	37.83	11.78
3. Seed pelleting with *Pongamia* L.P. + 40 cm x 10 cm	40.47	41.17	12.23
4. Seed pelleting with *Prosopis* L.P. + 20 cm x 10 cm	29.90	38.33	11.89
5. Seed pelleting with *Prosopis* L.P. + 30 cm x 10 cm	32.83	39.17	9.85
6. Seed pelleting with *Prosopis* L.P. + 40 cm x 10 cm	34.98	48.33	11.28
7. Seed pelleting with *Albizia* L.P. + 20 cm x 10 cm	34.97	40.00	12.07
8. Seed pelleting with *Albizia* L.P. + 30 cm x 10 cm	43.00	51.17	12.49
9. Seed pelleting with *Albizia* L.P. + 40 cm x 10 cm	36.23	41.50	12.30
10. No seed pelleting + 35 cm x 10 cm (Control plot)	38.84	40.33	10.08
S. Em. (±)	2.71	2.56	0.06
C. D. (P = 0.05)	8.05	7.60	0.18

Table 2: Effect of organic seed pelleting and row spacing on yield attributes of black gram

Treatment	Pods/Plant (No.)	Seeds/Pod (No.)	Test Weight (g)
1. Seed pelleting with *Pongamia* L.P. + 20 cm x 10 cm	18.33	5.60	37.28
2. Seed pelleting with *Pongamia* L.P. + 30 cm x 10 cm	20.55	5.87	38.52
3. Seed pelleting with *Pongamia* L.P. + 40 cm x 10 cm	21.56	6.33	41.05
4. Seed pelleting with *Prosopis* L.P. + 20 cm x 10 cm	20.33	6.07	40.05
5. Seed pelleting with *Prosopis* L.P. + 30 cm x 10 cm	18.89	5.40	36.20
6. Seed pelleting with *Prosopis* L.P. + 40 cm x 10 cm	22.11	6.67	39.22
7. Seed pelleting with *Albizia* L.P. + 20 cm x 10 cm	21.78	6.73	39.68
8. Seed pelleting with *Albizia* L.P. + 30 cm x 10 cm	24.55	7.07	42.19
9. Seed pelleting with *Albizia* L.P. + 40 cm x 10 cm	22.11	6.87	40.92
10. No seed pelleting + 35 cm x 10 cm (Control plot)	21.56	6.53	38.24
S. Em. (±)	1.16	0.24	0.39
C. D. (P = 0.05)	-	0.71	1.16

Table 3: Effect of organic seed pelleting and row spacing on yield of black gram

Treatments	Seed Yield (kg/ha)	Stover Yield (kg/ha)	Harvest Index (%)
1. Seed pelleting with *Pongamia* L.P. + 20 cm x 10 cm	726.30	1497.78	32.65
2. Seed pelleting with *Pongamia* L.P. + 30 cm x 10 cm	751.48	1724.81	30.36
3. Seed pelleting with *Pongamia* L.P. + 40 cm x 10 cm	794.44	1911.11	29.38
4. Seed pelleting with *Prosopis* L.P. + 20 cm x 10 cm	773.70	1802.22	30.04
5. Seed pelleting with *Prosopis* L.P. + 30 cm x 10 cm	722.59	1428.15	33.60
6. Seed pelleting with *Prosopis* L.P. + 40 cm x 10 cm	801.11	2174.81	26.92
7. Seed pelleting with *Albizia* L.P. + 20 cm x 10 cm	848.52	2410.74	26.03
8. Seed pelleting with *Albizia* L.P. + 30 cm x 10 cm	872.96	2511.11	25.82
9. Seed pelleting with *Albizia* L.P. + 40 cm x 10 cm	852.22	2482.96	25.56
10. No seed pelleting + 35 cm x 10 cm (Control plot)	799.63	2119.26	27.39
S.Em. (±)	10.54	34.51	0.38
C. D. (P=0.05)	31.31	102.54	1.12

Table 4: Economics of different treatments on black gram

Treatments	Total cost of cultivation (INR/ha)	Gross return (INR/ha)	Net return (INR/ha)	B:C
1. Seed pelleting with *Pongamia* L.P. + 20 cm x 10 cm	36,450.00	89,466.20	53,016.20	1.45
2. Seed pelleting with *Pongamia* L.P. + 30 cm x 10 cm	36,450.00	94,562.00	58,112.00	1.59
3. Seed pelleting with *Pongamia* L.P. + 40 cm x 10 cm	36,450.00	76,859.20	40,409.20	1.11
4. Seed pelleting with *Prosopis* L.P. + 20 cm x 10 cm	36,450.00	78,297.90	41,847.90	1.15
5. Seed pelleting with *Prosopis* L.P. + 30 cm x 10 cm	36,450.00	90,550.50	54,10050	1.48
6. Seed pelleting with *Prosopis* L.P. + 40 cm x 10 cm	36,450.00	75,055.70	38,605.70	1.06
7. Seed pelleting with *Albizia* L.P. + 20 cm x 10 cm	36,450.00	82,723.80	46,273.80	1.27
8. Seed pelleting with *Albizia* L.P. + 30 cm x 10 cm	36,450.00	96,676.10	60,226.10	1.65
9. Seed pelleting with *Albizia* L.P. + 40 cm x 10 cm	36,450.00	89,419.30	52,969.30	1.45
10. No seed pelleting + 35 cm x 10 cm (Control plot)	36,450.00	79,105.30	42,655.30	1.17

statistically at par to all treatments. However, a significantly higher seeds in a pod (7.07) and test weight (42.19 g) were obtained in treatment 8. Higher number of pods in a plant, seeds in a pod, and test weight might have been possible as a result of the plants' improved photosynthetic processes, which were made possible by adequate light availability and the provision of balanced nutrients to crop. The grain (872.96 kg/ha) and haulm yield (2511.11 kg/ha) of *Albizia* leaf powder pelleted seeds, sown at 30 cm row spacing were significantly higher, whereas significantly higher harvest index (33.60%) was recorded in seed pelleted with *Prosopis* powder at 200 g/kg seeds and sown at the same spacing. The roots were able to uptake nutrients due to better availability of moisture and moderation of soil temperature for

proper growth and development of plants and ultimately the yield attributes. The plants showed vigorous growth with a stronger root system as a result of the seeds pelleted with *Albizia* leaf powder, which in turn helped the plants to assimilate required moisture and nutrients from soil and enabled increased growth and yield. Seed pelleting increased the photosynthetic rate due to higher nutrient uptake and efficient translocation of photosynthates from source to sink might be attributed for higher yield attributes (Alex *et al*., 2017).

Economics

Observations recorded for the economics of different treatments of black gram are given in Table 4. Maximum gross monetary return (INR 96,676.10 per ha), net monetary return (INR 60,226.10 per ha), and B:C (1.65) were recorded for seed pelleted with *Albizia* powder at 200 g/kg of seeds and sown at the spacing of 30 cm by 10 cm. These effects may be attributable to the treatment's improved expression of growth characteristics and yield qualities, which in turn contributed to higher returns.

Conclusion

It can be concluded that the black gram seeds pelleted with *Albizia lebbeck* leaf powder and sown at 30 cm x 10 cm spacing under organic farming was found to be the most effective treatment, as it enabled better nutrient absorption, initiated seedling growth, and, ultimately, stimulated rapid growth and increased productivity. It also fetched higher economic returns. This conclusion is based on the results of one season of experimentation; for a final recommendation to farmers, further experimentation is required.

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Conflict of interest

The authors declare that they have no conflict of interest.

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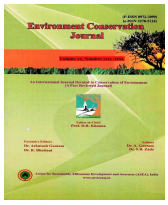

Design and testing of a metering system for fodder seed treatment

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Treater

ABSTRACT

Quality of fodder seeds can be maintained by seed treatment for better production and productivity. It refers to the application of specific physical, chemical, or biological agents to the seed prior to sowing in order to suppress, control, or repel pathogens, insects, and other pests that attack seeds, seedlings, or plants. In this study, the efficient seed and chemical metering system for fodder seed treatment was designed which can be used in different seed coating/treatment machine. Seed metering mechanism was calibrated mechanically and manually at four positions (Full, 3/4th, Half, and 1/4th openings). For manual calibration, it was observed between 0.19 kg/s to 1.08 kg/s for Berseem seeds and 0.15 kg/s to 1.00 kg/s for Cowpea seeds. For mechanical calibration, it was observed between 0.24 kg/s to 1.17 kg/s for Berseem seeds and 0.11 kg/s to 1.04 kg/s for Cowpea seeds. Designed system is useful in developing high capacity, efficient and cost effective seed treaters for treatment/coating of fodder seeds as well as other crop seeds.

Introduction

The most important input for agricultural production is seed. In fact, it is the most cost-effective method of increasing agricultural production and productivity. The effectiveness of other agricultural inputs, such as fertilizers, pesticides, and irrigation, in increasing productivity and production is largely determined by quality of seeds. Seed quality accounts for 20% to 25% of productivity (Annual report 2018, DAC, GOI). Low yields of green fodder are mostly caused by a lack of high-yielding, improved varieties and hybrids of quality fodder seeds (IGFRI, Vision-2050). Agriculture is a very dynamic sector because sudden climatic changes (Patil *et al.,*2023) can significantly alter crop productivity (Satankar *et al.,* 2020) and quality of fodder seeds that ultimate affects quality milk production and health of the livestock (Annual report MoFAHD, 2019-20). The best way to retain the quality of seeds is treatment of seed by polymers, insecticides, pesticides etc. Growers are increasingly using seed treatment to assure the highest quality of supply. The capacity of seeds to flow and handle, germination, and seedling emergence, as well as protection from insects and plant pathogens, are all improved by seed treatment. Seed treatment, which can range from a simple dressing to coating and pelleting, refers to the application of specific physical, chemical, or biological agents to the seed prior to sowing in order to suppress, control, or repel pathogens, insects, and other pests that attack seeds, seedlings, or plants (Singh *et al.,* 2022b). Because of their environmental safety and economic benefits, physical and biological seed treatments are utilised all over the world as an alternative to chemicals or in combination with a chemical treatment (Sharma *et al.*, 2015). In the near future, the biological seed treatment industry is anticipated to grow at one of the fastest rates, in part because it is simpler to register biological seed treatments with the Environmental Protection Agency (EPA).One of the reasons restricting disease management is

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farmer ignorance about seed treatments, hence efforts should be undertaken at the farmer level to implement the technology (Bryant *et al.,* 2021). In order to improve upon and get around some limitations or shortcomings of earlier technologies, advanced seed treatment techniques such film coating, pelleting, priming, etc. were developed. The treatment of seeds must be done as the first step in growing a crop since it is essential to the production of sustainable crops (Kapoor *et al.,* 2022). The term "seed coatings" covers a number of processes and products which are used to alter seeds and ensure a uniform, healthy harvest (Anonymous, 2022). These seed treatment processes include pelleting, encrusting, and film coating. Crops are prey to diseases and insect pests in all life stages, from seeds to seedlings and plants. Soil-borne fungal diseases (Patil *et al.,* 2020) and organisms can cause rotting and blights, fungi-based diseases cause kernels to be replaced by fungal spores, and of course insects eat away at young plants (Johnson *et al.,* 2004).

Seed coatings combat these dangers through two methods: systemic and non-systemic protection. The phrase "seed coatings" refers to a variety of procedures that are used to modify seeds health and ensures the quality and yield. Pelletizing and film coating are some of these seed treatment procedures (TNAU agri-portal, 2021). All life stages of crops, including seeds, seedlings, and plants, are vulnerable to diseases and insect pests.

Table 1: Seed treatment for forage crops

Fodder crop	Seed treatment
Deenanath grass	Soaking of mechanically defuzzed seeds in 0.25% potassium nitrate+200 ppmgibberellic acid (1:1) for 16 hrs
Stylosanthus	Scarification with conc.sulphuric acid@200ml/kg of seed for 4 minutes
Hedge Lucerne	Scarification with conc.sulphuric acid@ 200 ml/kg of seed for 4-5 minutes
Cenchrussp	Soaking of acid scarified seeds in 50 ppm copper sulphate solution for 6 hrs
Fodder sorghum	Soaking of seeds in 0.5 percentage potassium nitrate solution for 2 hrs
Oats	Carboxin + thiram (vitavax 200 – flowable or wettable powder
Maize	2% potassium dihydrogen phosphate for 8hr
Pearl Millet	2% potassium chloride for 16hr
Sorghum	2% potassium dihydrogen phosphate for 6 hr
Ragi	0.5% calcium chloride for 6hr

In addition to insects eating away at immature plants, fungi-based diseases can cause kernels to be replaced by fungal spores and cause rotting and blights (Yadav,2018). Seed coatings defend against these risks in two ways: systemic protection and non-systemic protection (Table1). The machinery used to undertake seed treatment operations is called a seed treater/coater. Any formulas that don't require agitation during application should use liquid treaters. In addition to providing good seed coverage, liquid insecticides can be applied without agitation. Slurry treaters are used with fluid compositions that demand agitation during application. Water can be used to emulsify concentrates or mix wettable powders to create slurry formulations. For dry, powder formulations, dust treaters are used. They do not add moisture to the seed and are simple to clean and use, but they do not evenly distribute chemicals like liquid or slurry treaters do (Copeland *et al.,*1978). Furthermore, dust mixtures frequently drift and need sufficient, regulated airflow.

To research the parameters influencing the design of coating equipment for crop seeds, a wheat coating machine was constructed and tested. The components of the coating machine were the frame, rotating pan, gear box, flame source, and electric motor. A considerable impact on coating quality, cost, and seed germination was discovered to be caused by coating temperature, coating unit speed, and coating polymer (Yehia, 2008). Lucerne nodulation and yield were enhanced by a novel rhizobial seed-coat composition (Zhou *et. al.,*2017). Biogas slurry coating improves the carrot seed germination by recording higher germination than uncoated seeds. Most of the farmers in India are using home-made mixer and shovels for treating seeds in their farms (Patil *et al.,*2021). These mixers are small, low capacity, less efficient, time consuming and tedious to perform seed treatment/coating operation. Many slurry and direct treaters/coaters are available in the market but they are so costly, complex in design and usually used by big industries (Patil *et al.,* 2022b). Precise metering of seeds and chemical agent in the treatment machine is the most important aspect that needs to work efficiently for uniform layering/application of chemical over the surface of seeds. Present study provides solution that can be incorporated in different seed treatment and coating

machine for performing seed treatment operation very effectively to disinfect or save them during germination and storage.

Material and Methods

Design considerations: Cowpea and Berseem seeds were taken into consideration for designing the metering system. Different engineering properties of selected seeds (Singh *et al.,* 2021) i.e. bulk density, angle of repose, roundness; sphericity and size were considered for designing different component of system and determining dimensions based on required capacity and feasibility.

Development of hopper: Physical properties of berseem and cowpea were considered for designing the hopper (Singh *et al.,* 2022a). A hopper was developed (Fig.1) for 35 L volume that can hold 40-50 kg of seeds depending upon the type/density of seeds. A square section of 20 cm length was provided below the hopper for incorporating the seed metering mechanism. Hopper was fabricated using MS sheet of 2 mm thickness.

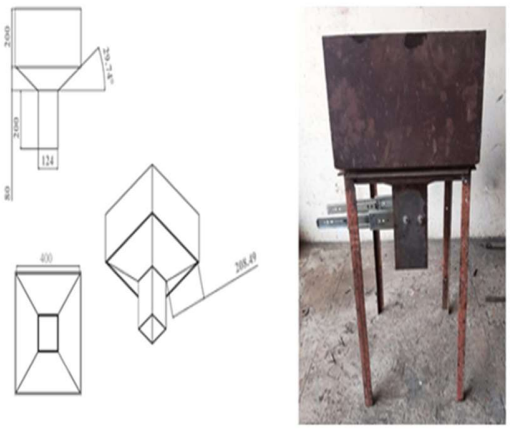

Figure 1 : Developed Hopper

Figure 1: Developed Hopper

Seed metering mechanism: Various types of mechanism used in different fodder production machinery based on suitability and requirement (Sahay *et al.,* 2023).A mild steel sheet gate (200×120×2 mm) was incorporated below the hopper using 8" telescopic channel (loading capacity-50 kg) in such a way that slider gate can be slide freely for opening and closing the a rectangular orifice/opening (76×43 mm). A slider crank mechanism was designed (Fig.2) to slide the gate with the help of DC servo motor. A connecting rod (165mm length) was connected to the crank (98mm dia.) for sliding the gate for 76mm length. Measuring scale was provided for observing the opening of the gate. All the data were analyzed using MS excel- 2010.

Figure 2: Seed metering mechanism

Electronic circuit: Sensors are most important component of every electronic circuit. Here in this setup, different types of sensors were used based on their suitability for treatment capacity. Arduino Uno R3 was used for controlling whole system electronically. Other sensors also connected to this Micro-controller (MC). It processes the data/signal coming from different sensors and according sends signal to other sensors for their smooth operation. Open source IDE software was used to program the MC as per machine requirement. Encoder dc servo motor was used for rotating the crank of sliding mechanism. Electric solenoid valve switch and flow measurement sensor were fitted just below the chemical mixer to measure the flow of chemical to treatment space. 12V-7AH Battery was used to give power supply to electronic circuit. This system consisted of Uno-Arduino R-3 microcontroller (Patil *et al.,* 2022a), sliding gate type metering mechanism, 12V dc servo motor, solenoid valve and fluid flow sensor. In this system, DC servo motor was given the supply from 12 V motor. Servo motor was programmed to displace sliding gate in metering mechanism so that it could detect the seed rate flowing from hopper to coating chamber and sends signal to microcontroller. After processing the signal, micro-controller incorporated

directs the signal to solenoid valve to control the flow from mixer to coating chamber (Fig.3). Solenoid valve allows particular amount of chemical to flow according to signals which was being directed by micro-controller system. In this way, sensor based micro-controller system does the uniform and efficient seed coating.

Metering system for fodder seed treatment: It was fabricated (Fig.4) at ICAR-IGFRI Jhansi. Seed metering mechanism using slider crank mechanism (Patil *et al.*,2021) was fitted below the hopper to control the seed rate. For testing the system, 2 litre graduated plastic cylinder was used as chemical mixer and fitted beside the hopper. Below the chemical container solenoid valve and flow sensor were connected to control the chemical flow from mixer to treatment space. DC power supply was given to all sensors and micro-controller using 12V battery. All the necessary connections were made to operate sensors. Programming was done on IDE software and loaded to run the programme. Technical specification of developed system is shown in Table 2.

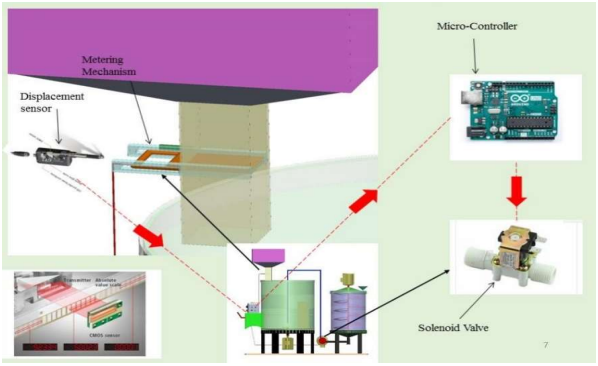

Figure 3: Electronic circuit

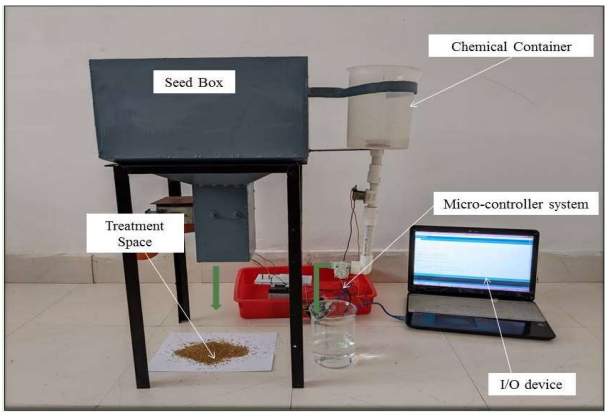

Figure 4: Metering system for fodder seed treatment

Table 2: Technical Specification of developed metering system.

Item	Specification
Overall Size	700×750×720 mm
Micro-controller	Arduino uno R3
Software used	IDE software
Chemical container Material Volume	Plastic 2 ltr
Power supply	12V battery + Laptop
Seed Metering Mechanism	Slider gate type
Hopper Dimension (l×w×h) Volume Material	 400×400×300 mm 35 Ltr or 40-50 kg seed 2 mm Mild Steel
Sensors used	Flow control, solenoid valve, displacement using DC servo
Pipe	PVC 0.5inch
Connections	Jumper wire, USB cable, laptop, break-board

Table 3: Manual Calibration for Berseem seeds (variety-Bardan)

Openings	Time (sec)	Avg. Collected seeds (kg)	Seed rate (kg/s)
Full(76mm)	30	32.6	1.08
3/4th	30	22.8	0.76
Half	30	16.2	0.54
1/4th	30	5.8	0.19

Figure 5: Manual calibration.

Results and Discussion

Calibration of seed metering mechanism: It was done for determining the exact amount of seeds falling from hopper at different positions of

metering gate. Production yield significantly affected by seed rate (Gupta *et al.,* 2021). Calibration data was used for giving the signals to the DC motor for sliding the gate according to the seed rate of metering mechanism. For calibrating the metering mechanism (Patil *et al.,* 2022a), sliding gate was manually opened and closed at different positions (Full, 3/4th, Half and 1/4th openings) of gate for a particular time then the seeds were collected below the mechanism and measured using digital weighing balance. Seed rates were calculated for different sliding positions (Full, 3/4th, Half and 1/4th openings). Three replications at each position were taken for accuracy of seed rate data. By considering coating/treatment Seeds required/batch = 4kg, the opening times for both the selected seeds were calculated and shown in table 4.

The value of bulk density and angle of repose for berseem and cowpea seeds were found 847.5 kg/m3 & 28.5º and 788 kg/m3 & 27º respectively. The

Table 4: Manual Calibration for Cowpea seeds (variety-BL-2)

Openings	Time (s)	Avg. Collected seeds (kg)	Seed rate (kg/s)
Full (76mm)	30	30.0	1.00
3/4th	30	19.5	0.65
Half	30	14.2	0.47
1/4th	30	4.6	0.15

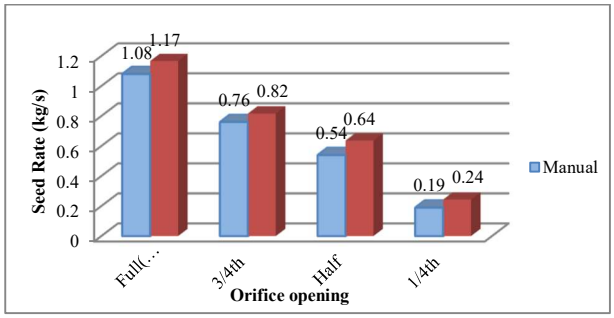

Figure 6: Calibration for berseem seed

seed rate of metering mechanism for manual calibration was observed between 0.19 kg/s to 1.08 kg/s (Fig.6) for Berseem seeds (Table 3) and 0.15 kg/s to 1.00 kg/s (Fig.7) for Cowpea seeds (Table 4) at four positions (Full, 3/4th, Half and 1/4th openings) of metering mechanism. For mechanical calibration, it was observed between 0.24 kg/s to 1.17 kg/s for Berseem seeds (Table 5) and 0.11 kg/s to 1.04 kg/s for Cowpea seeds (Table 6).

Table 5: Mechanical Calibration

Openings	Berseem Seed rate (kg/s)	Cowpea Seed rate (kg/s)
Full(76mm)	1.17	1.04
3/4th	0.82	0.62
Half	0.64	0.52
1/4th	0.24	0.17

Table 6: Opening time of sliding gate

Opening positions	Opening Time (t) in sec for Berseem	Opening Time (t) in sec for Cowpea
Full	3.4	3.9
3/4th	4.9	6.5
Half	6.3	7.7
1/4th	16.4	24.0

Using calibration data, seed metering mechanism could be programmed for delivering particular amount of seeds. 4 kg berseem seed was treated in a batch. For delivering 4 kg of seeds, the opening time for seed metering mechanism to treat was obtained 16.4 sec for Berseem and 24 Sec for Cowpea at 1/4th opening. These data were used in DC servo motor for operating metering system so that it could deliver the particular amount of seeds as desired for treatment. Liquid flow sensor along with solenoid valve was also programmed to deliver a requisite amount of chemical liquid. For seed treatment, both the seed and chemical metering system were programmed to deliver 4 kg berseem seeds with 100 ml chemical liquid. The treated berseem seeds were obtained at treatment space (Fig 4).

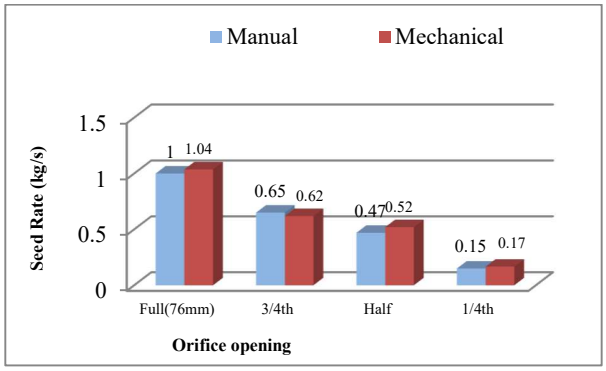

Figure 7: Calibration for cowpea seed

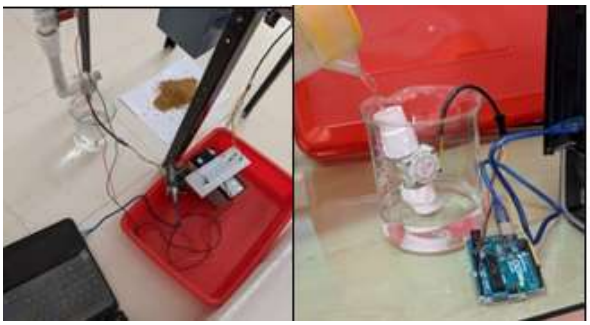

Figure 8: Testing of setup

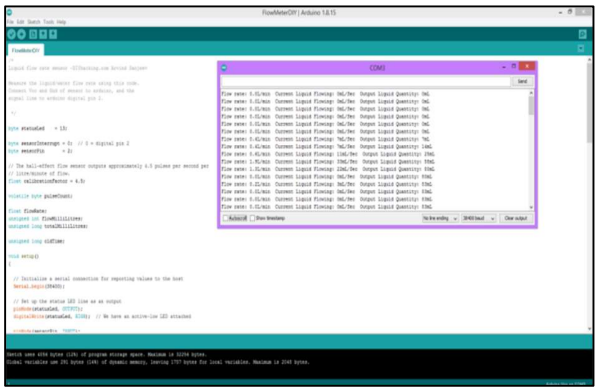

Figure 9: Output of liquid flow sensor

Testing of system

All the connections were made and programming was done to test each sensor. Firstly mechanical calibration was done to check the seed rate using displacement sensor and compare with manual calibration (Fig.5) it was found that metering setup using crank slider mechanism working fine and also seed rate obtained from mechanically was approximately same as manual one.

Then liquid flow sensor also tested. For this testing, known amount of liquid i.e. water was passed through flow sensor (Fig.8) and output results were matched with known one. It was found that same flow rate & volume were shown in laptop screen as known one (Fig.9). These results showed the precise workability of the developed system.

Conclusion

The sensor-based metering system was designed for seed treatment to handle the cowpea and berseem seeds. For the purpose of optimizing the developed system, the seed metering mechanism was mechanically and manually calibrated. Designed setup can be useful to develop high capacity seed treaters which will be precise and higher capacity in operation and also cost effective.

Conflict of interest

The authors declare that they have no conflict of interest.

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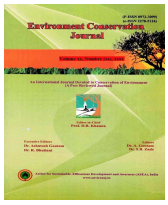

Identification and genetic assessment of transgressive segregants for yield and its contributing traits in wheat (*Triticum aestivum* L.)

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ABSTRACT

Two crosses *viz*., K 1006 x LOK 1 and PBW 343 x HUW 234 derived from four diverse parents were examined during *Rabi* 2018-19 and 2019-20 at Agricultural Research Farm, Institute of Agricultural Sciences, BHU in order to identify and assess the robust transgressive segregants (TS) in the segregating F_2 and F_3 population for yield/plant and its contributing traits respectively. Findings reveal that individuals transgressed beyond the parents in both the crosses for grain yield/plant (9 to 9.52 %). The maximum frequency of TS are found for AL (74.29%) in the PBW 343 x HUW 234 and it was higher for 1000 grain weight (72%) in the K 1006 x LOK 1 cross. The highest number of simultaneous TS for grain yield/plant was found in the F_2 for the PBW 343 x HUW 234 (89.5%) followed by K 1006 x LOK 1 (79.0%). The frequency of simultaneous transgression for grain yield coupled with SLPS, GPS, 1000 GW in K 1006 x LOK 1 cross along with NET and AL in PBW 343 x HUW 234 cross was found very frequently. Hence, it is presumed that either grain yield is dependent on these traits or there may be linkage drag among the genes for such traits so that responsible gene(s) could be inherited together. The most promising TS tagged in F_2'^{s} were plant No. 36 in the K 1006 x LOK 1 and plant No. 30, 68 and 100 in the other cross. Based on high frequency of TS, it is inferred that transgressive breeding could be used as an excellent tool to improve the crop yield and other desirable traits by recovering the transgressive segregants.

Introduction

Among the cereal crops, wheat (*Triticum aestivum* L.) is considered as one of the most economically important crop as it is widely grown and consumed by large people (Prasad, 2022). Poudel *et al.*(2020) also stated that wheat is one of the major as well as the most consumed cereal crops in the world. Production of wheat supports almost 35 percent of the world's population (Mohammadi-joo*et al.,* 2015) and it is known as one of the most economically essential cereal crops in the world as well (Bedada *et al.,* 2022). Published report by Rangare*et al.*(2010) indicates that it is an important cereal crop and accounts about 33 percent of the nation's total food grain production**.** In India, it is cultivated in an area of 31.6 mha with the production of 108.75 mt of wheat grain with productivity of 34.41 quintal per hectare (Department of Agriculture, Cooperation and Farmers Welfare, 2021). There are three wheat species likeBread wheat (*Triticum aestivum*), Macaroni wheat (*Triticum durum*) and Emmer wheat (*Triticum dicoccum*) is widely cultivated in India, comprises of 86, 12 and 2 percent of the total areas of wheat (Ukani *et al.,*2015). Besides having satisfactory crop yield, wheat is also good source of nutritional profile like of protein, minerals, vitamins and dietary fiber (Kumar *et al.,*2011; Prasad, 2022; Rashmi *et al.,* 2022). Since, human population is continuously increasing as compared to the total population remained at the time of green

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revolution so, in parallel because efforts of scientists, farmers, policymaker and use of updated technology crop yield has been enhanced significantly however, still there is need to improve the crop yield of wheat because of huge demand of rapidly growing population. The ultimate goal of any plant breeding program is to develop the potential and reliable genotypes/cultivars perform better across the environments. The selection of the parent for hybridization in order to develop the superior genotypes is depending to great extent on great adaptation, with considerable yield potential. Plant progeny derived from diverse crosses are expected to throw a wide range of heterogeneity, thereby providing better scope for the isolation of high yielding segregants in the segregating generations.Transgressive segregants in F_2 population may be selected because of accumulation of favorable genes for trait of interest from the parents involved in hybridization (Putri *et al*., 2020). The F_2 generation depicts maximum genetic variation and furnished the first opportunity for selection of individual plants, any one of which may end up into a new cultivar (Reddyyamini *et.al.* 2019). Hence, transgressive breeding could be used as a robust approach to improve the yield and its associated traits by recovering/accumulating the linked genes in tothe segregating plants (Singh, 2000). Exploring the information about the transgressive segregants also helps to find out their proportions for desirable genes responsible for yield and its contributing traitsbecause the traits having high heritability would be very usefulfor population improvement for targeted traits and other future breeding programmes. It is therefore, plant breeders/researchers are more concerned with getting the higher frequency of transgressive segregants as it gives a better scope for exercising the selection of superior lines in order to improve the productivity of wheat crops and supplying the demand of rapidly growing population in 21st century.

Material and Methods

The experiment was carried out in the *Rabi* season 2018-19 and 2019-20 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. F_1 population (seeds) of the two crosses *viz*., K 1006 x LOK 1 and PBW 343 x HUW 234 was obtained from my supervisor and the experimental materials (F_2 generation) of these two crosses along with parents were sownin *Rabi* 2018-19 in un-replicated plots having enough distance. The F_2 plants were grown in 20 rows of 2m length and parents in 4 rows of 2m length. Data were recorded on 100 randomly selected individual plants in cross K 1006 x LOK 1 and 105 plants in PBW 343 x HUW 234 cross and 10 plants in each parent for 15 quantitative traits viz., DF, DM, GFD, NET, SPL, AUSDC (Rosyara *et al*., 2007), AL, PL, MTI (I) at flowering and MTI (II) grain filling stage followed by Blum and Ebercon (1981), PLH, SPLS, GPS, TW and GYPP in each cross.

In second season (*Rabi* 2019-20), 19 transgressive segregants tagged in F_2population (based on yield performance) and four parents were grown with aim to test their performance in F_3 generation in Randomized complete block design (RCBD) in three replications. The recommended agronomic practices were followed to raise the good crops. The parental lines and the F_2 and F_3 plants were sown in the line spaced 22.5 cm apart with plant to plant distance of 10 cm. The mean value of ten randomly selected plants of each F_3 families (transgressive segregants) were compared with performance of F_2 generation for GYPP by using F-test for degree of two variances and two sample t-test for equal mean at (n_1+n_2-2) degree of freedom followed by Dhole and Reddy (2011) using Microsoft excel office.

Results and Discussion

In findings, desirable transgressive segregants found in the studied crosses (K 1006 x LOK 1 and PBW 343 x HUW 234) for all the fifteen traits. The frequency of transgressive segregants was differed from cross to cross presented in table 1a, 1b and figure 1 respectively. For grain yield per plant, 9 to 9.52% individuals transgressed beyond the increasing parents in both the crosses, Dahat *et al*. (2017) reported 51 to 55 percent proportion of variation in transgressive segregants. Similarly, range of transgressive segregants (in %) were 23 to 39.05for DF, 42 to 54.29 for DM, 28 to 48.57 for GFD, 19.05 to 20 for NETs, 51.43 to 62, for AUSDC, 39-42 for MTI (I) at flowering stage, 45.71 - 72.0 for MTI (II) at grain filling stage, 14 -

Table 1a:Frequency of transgressive segregants for 15 traits in F_2 Population of K 1006 × LOK 1 crosses

Trait/ cross	F_2 Generation		Parents		Transgressive Segregants	
	Highest value	Lowest value	Higher value	Lower value	Higher than highest parent	Lower than lowest parent
DF	88	70	83 (K 1006)	78.4 (LOK 1)	23 (23%)	42 (42%)
DM	121	106	116.3 (K 1006)	115.4 (LOK 1)	42 (42%)	54 (54%)
GFD	48	23	37 (LOK 1)	33.3 (K 1006)	28 (28%)	40 (40%)
NETs	14	2	10.7 (K 1006)	9.8 (LOK 1)	20 (20%)	61 (61%)
AUSDC	506	370.5	415.1 (K 1006)	412.15 (LOK 1)	62 (62%)	29 (29%)
MTI-I (flowering)	65.83	33.33	52.94 (LOK 1)	46.57 (K 1006)	42 (42%)	28 (28%)
MTI-II (grain filling)	65.06	36.91	48.63(K 1006)	48.568 (LOK 1)	72 (72%)	28 (28%)
SPL	12.3	4.4	10.16 (K 1006)	8.73 (LOK 1)	14 (14%)	52 (52%)
AL	8.8	1.8	7.25 (LOK 1)	4.15 (K 1006)	4 (4%)	9 (9%)
PL	22.5	8.8	16.1(LOK 1)	15.85 (K 1006)	38 (38%)	57 (57%)
PLH	101	46.2	87.51 (K 1006)	75.17 (LOK 1)	16(16%)	19 (19%)
SLPS	27	11	24.07 (K 1006)	18.876 (LOK 1)	16 (16%)	55 (55%)
GPS	78	35	72.6 (K 1006)	57.5 (LOK 1)	14 (14%)	55 (55%)
1000 GW	50.12	24.72	36.039 (LOK 1)	35.19 (K 1006)	72 (72%)	22 (22%)
GYPP	30.81	4.454	27.336 (K 1006)	20.308 (LOK 1)	9 (9%)	65 (65%)

Table 1b: Frequency of transgressive segregants for 15 traits in F_2 Population of PBW 343 × HUW 234 cross

Trait/ cross	F_2 Generation		Parents		Transgressive Segregants	
	Highest value	Lowest value	Higher value	Lower value	Higher than highest parent	Lower than lowest parent
DF	96	75	88.4 (PBW 343)	81.9 (HUW 234)	41 (39.05%)	33 (31.43%)
DM	125	109	118.7 (PBW 343)	115.5 (HUW234)	57 (54.29%)	17 (16.19%)
GFD	45	17	33.6 (HUW234)	30.3 (PBW 343)	51(48.57%)	41 (39.05%)
NETs	16	4	10.8 (PBW 343)	10.5 (HUW234)	20 (19.05%)	85 (80.95%)
AUSDC	534.5	301	417.1 (HUW234)	414.7 (PBW 343)	54 (51.43%)	50 (47.62%)
MTI I (flowering)	66.58	22.71	50.88 (HUW234)	49.4 (PBW 343)	41(39.05%)	58 (55.24%)
MTI II (grain filling)	63.36	40.71	51.46(PBW 343)	50.02 (HUW234)	48 (45.71%)	50 (47.62%)
SPL	13	4.4	10.34 (PBW 343)	9.57(HUW234)	21 (20%)	71 (67.62%)
AL	9.8	2.2	4.81 (PBW 343)	4.75 (HUW234)	78 (74.29%)	23 (21.90%)
PL	27.6	4.1	14.83 (HUW234)	11.09 (PBW 343)	40 (38.09%)	26 (24.76%)
PLH	113	67.4	91.41(PBW 343)	90.57 (HUW234)	53(50.48%)	49 (46.67%)
SLPS	28	14	23.91(PBW343)	20.81(HUW 234)	33(31.43%)	64(60.95%)
GPS	86	42	72.1 (PBW 343)	62.8 (HUW234)	27 (25.71%)	51 (48.57%)
1000 GW	56.86	29.8	35.75 (PBW 343)	30.42 (HUW234)	72 (68.57%)	3 (2.86%)
GYPP	35.49	6.72	27.84 (PBW 343)	20.06 (HUW234)	10 (9.52%)	63 (60%)

20 for SPL, 4-74.29 for AL, 38-38.09 for PL, 16 - 50 for PLH, 16 - 31.43 for SPLS,14 - 25.71 for GPS, 68.57-72 for 1000 grain weight in both the crosses respectively. Promising transgressive segregants for the traits like spike length, SLPS, GPS, 1000 GW and grain yield per plant which is supported with findings of Mitra and Mehra (2005). Similarly,for PLH, DM, DF, 1000 GW, GPS, SLPS, SPL, productive tiller per plant and effective tillers supported byothers. Publication of Ahmed *et al.* (2022) suggested that identified transgressive segregants have wider range of variations for desirable traits and very useful for crop improvement.The highest proportion of transgressive segregants are remarked for AL (74.29%) followed by 1000 grain weight (68.57 %), AUSDC (51.43%), GFD (48.57 %), PLH (46.67 %), MTI (I) at grain filling stage (45.71 %) in F_2 generation of the PBW 343 x HUW 234.Therefore, it is presumed that, HUW 234(higher parent) contributed desirable allele for AUSDC, GFD, and PLH traits while PBW 343 (higher parent) contributed desirable allele for the trait like AL, 1000 grain weight and MTI (II) at grain filling stage. Similarly, maximum frequency of transgressive segregants identifiedfor 1000 grain weight (72 %), MTI (II) at grain filling stage (72 %), AUSDC (62 %), DM (54 %), days to flowering (42 %) was found in F_2 of cross K 1006 x LOK 1(table 1a). Hence, its appeared that parent K1006 (higher parent) contributed desirable alleles for AUSDC, MTI (II) at grain filling stage while LOK 1contributed desirable alleles for DF, DM, 1000 grain weight. Transgressive segregants having lower value than lower parent was accounted high in GYPP, NET in F_2 of cross K 1006 x LOK 1

Table 2: Frequency distribution of transgressive segregants for yield and combination of traits in F_2 generation in K 1006 x LOK 1 and PBW 343 × HUW 234 crosses

SN	Character(s)	Frequency of transgressive segregants	
	Grain yield with other traits	K 1006 x LOK 1	PBW 343 × HUW 234
1	DF	6(6%)	6(5.71%)
2	DM	5(5%)	2(1.90%)
3	GFD	3(3%)	7(6.67%)
4	NETs	4(4%)	9(8.57%)
5	AUSDC	5(5%)	6(5.71%)
6	MSI I (Flowering stage)	3(3%)	2(1.90%)
7	MSI II (Grain filling stage)	5(5%)	5(4.76%)
8	SPL	3(3%)	2(1.90%)
9	AL	-	7(6.67%)
10	PLH	-	2(1.90%)
11	PL	5(5%)	7(6.67%)
12	SLPS	6(6%)	3(2.86%)
13	GPS	3(3%)	2(1.90%)
14	1000 Grain Weight	9(9%)	9(8.57%)
15	GYPP	9(9%)	10(9.52%)
16	DF+DM+GFD+NET	-	2(1.90%)
17	DF+AUSDC+MTI(I)+MTI(II)	-	-
18	DF+SPL+AL+PLH+PL	-	-
19	DF+SLPS+GPS+TW	1(1%)	-
20	DM+AUSDC+MTI(I)+MTI(II)	-	-
21	DM+SPL+AL+PLH+PL	-	-
22	DM+SLPS+GPS+TW	2(2%)	-
23	GFD+MTI(I)+MTI(II)+AUSDC	-	-
24	GFD+SPL+AL+PLH+PL	-	-
25.	GFD+SLPS+GPS+TW	1(1%)	-
26	NET+AUSDC+MTI(I)+MTI(II)	-	-
27	NET+SPL+AL+PLH+PL	-	-
28	NET+SLPS+GPS+TW	1(1%)	1(0.95%)
29	AUSDC+MTI(I)+MTI(II)+SPL	-	-
30	AUSDC+AL+PLH+PL	-	1(0.95%)
31	AUSDC+SLPS+GPS+TW	1(1%)	1(0.95%)
32	MTI(I)+MTI(II)+SPL+AL	-	1(0.95%)
33	MTI(I)+MTI(II)+PLH+PL	-	-
34	MTI(I)+MTI(II)+SLPS+GPS+TW	1(1%)	1(0.95%)
35	SPL+AL+PLH+PL	-	-
36	SPL+SLPS+GPS+TW	2(2%)	2(1.90%)
37	AL+PLH+PL	-	1(0.95%)
38	AL+SLPS+GPS+TW	-	1(0.95%)
39	PLH+PL+SLPS+GPS+TW	-	-
40	PL+SLPS+GPS+TW	1(1%)	2(1.90%)
41	SLPS+GPS+TW	3(3%)	2(1.90%)
	Total transgressive segregants	(79)79%	(94) 89.52%

Table 3: Promising transgressive segregants in F_3 having combination of desirable traits in the cross K 1006 xLOK1

SN	Transgressive Segregants	Grain Yield Per Plant (GYPP) in combination with	Combination of desirable traits with GYPP
1	L2	DF, DM, AUSDC, TW	4
2	L6	DF, GFD, NET, AUSDC, PL, MTI(II), TW	7
3	L25	DM, SPLS, GPS, TW, MTI(I)	5
4	L36	DF, DM, NET, SPL, PL, SPLS, GPS, TW, MTI (I), MTI(II)	10
5	L59	DF, DM, NET, SPLS, GPS, MTI(II), TW	7
6	L88	GFD, SPL, AUSDC, SPLS, MTI(II), TW	6
7	L89	AUSDC, PL, SPLS, TW	4
8	L91	DF, GFD, NET, AUSDC, PL, MTI(II), TW	7
9	L96	DF, DM, SPL, PL, SPLS, MTI(I), TW	7

while for NET and SPL in of cross PBW 343 x HUW 234 respectively. Appearance or occurrence of such transgressions may be due accumulation of matching alleles comes from the parents and unmasking of recessive harmful alleles as well as inbreeding (Reddyyamini *et al*., 2019). The highest proportion of simultaneous transgressive segregation for GYPP i.e., 94 (89.52 %) was observed in F_2 population for the cross of PBW 343 x HUW 234) and 79 (79%) in the F_2 for the cross K 1006 x LOK 1. In majority of individuals, where better parent yield was transgressed, there was simultaneous transgression for one or more of yield contributing traits like SLPS, GPS, 1000 grain weight (40 out of 79 individuals in F_2 of the crossK 1006 x LOK 1 while SLPS, GPS, 1000 grain weight along with NET and AL (55 individuals out of 94 in F_2 of cross PBW 343 x HUW 234) demonstrating reliance of GYPP on above said characters in both the crosses or there might be linkage drag among genes of attributes. Dependency or linkage drag has extraordinary significance in plant breeding for synchronous improvement because plant yield is the complex trait and also come in the category of dependent variable so, exploring of its contributing key component/traits/dependency in order to enhancing the crop yield/output should be main priority of the plant breeders, such findings are agreed with Al-Bakry*et al.* (2011); Kadam *et al*. (2017) and Dahat*et al*. (2017). Plant No. 36 (F_2) was found to be most potential transgressive segregant having combination of desirable traits for GYPP in addition to higher intensity of expression for DF, DM, NET, MTI (I) at flowering stage MTI (II) at grain filling stage, SPL, PL, SLPS, GPS, 1000 grain weight (table 3). In F_2 population of the cross PBW 343 x HUW 234, plant No.100 , 68 and 30 accounted to be most worth noting transgressive segregant which surpassed the better parent in terms of yield, in addition to DF, DM, GFD, NET, AUSDC, AL, PLH, TW along with PL, SPL, SPLS, GPS MTI(I), MTI (II) respectively(table 4). Further assessment is needed for improvement of the most promising transgressive segregants as it is shown that when optimal intensity of a trait is not available in the parents, transgressive breeding can be used to expand the limit of the character. The achievements of obtaining the desired transgressive segregants rely on obtaining genetic recombination between linked and unlinked alleles.

Table 4:Promising transgressive segregants in F_3having combination of desirable traits in cross PBW 343× HUW 234

SN	Transgressive Segregants	Grain Yield Per Plant (GYPP) in combination with	Combination of desirable traits with GYPP
1	K3	DF, GFD, NET, AL, TW	5
2	K5	NET, AUSDC, PL, MTI(I), MTI(II)	5
3	K9	GFD, NET, AUSDC, PL, TW	5
4	K28	DF, GFD, NET, AL, PL, TW, MTI(II)	7
5	K30	NET, SPL, AL, PL, SPLS, GPS. TW, MTI(I), MTI(II)	9
6	K40	DF, GFD, NET, AUSDC, AL, TW	6
7	K52	DF, GFD, NET, AL, PL, SPLS, TW, MTI(II)	8
8	K60	SPL. AUSDC, PL, SPLS, GPS, TW	6
9	K68	DF, DM, GFD, NET, AUSDC, AL, PLH, TW, MTI(II)	9
10	K100	DF, DM, GFD, NET, AUSDC, AL, PL, PLH, 1000 GW	9

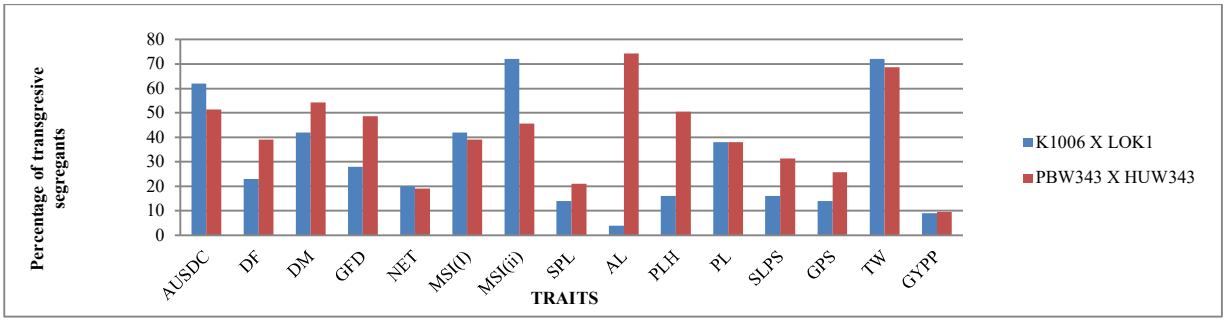

Figure 1:Bar chart representing percentage of transgressive segregants for 15 traits in two crosses (K 1006 x LOK 1 and PBW 343 × HUW 234)

Table 5: Mean performance of transgressive segregants for GYPP in F_2 and F_3 families for the cross K 1006 x LOK 1 and PBW 343 × HUW 234

Crosses	Identified transgressive segregants Plant No.	GYPP (g) in F_2 generation	GYPP (g) in F_3 generation
K 1006 × LOK 1	Line 2	29.52	27.12
	Line 6	28.65	26.44
	Line 25	29.26	27.79
	Line 36	30.60	28.16
	Line 59	30.80	27.92
	Line 88	30.81	31.06
	Line 89	28.65	25.98
	Line 91	30.61	29.86
	Line 96	29.16	26.69
	Mean	29.78	27.89
PBW 343 × HUW 234			
	Line 3	29.52	27.75
	Line 5	28.81	26.81
	Line 9	29.08	27.66
	Line 28	29.82	27.66
	Line 30	35.49	32.62
	Line 40	30.84	29.85
	Line 52	30.49	28.00
	Line 60	30.11	28.47
	Line 68	30.75	28.62
	Line 100	30.96	28.11
	Mean	30.59	28.56

Abbreviations:DF=Days to 50% flowering (days), DM= Days to maturity (days), GFD=Grain Filling Duration (days), NET= Net Effective Tiller (numbers), SPL=Spike length (cm), AUSDC= Area Under SPAD Decline Curve (SPAD value), AL=Awn Length (cm), PL= Peduncle Length (cm), MTI (I) and (II) = Membrane thermo stability index at flowering and grain filling (%), PLH= Plant height (cm), SPLS= No. of spikelets per spike (number), GPS= No. of grains per spike (numbers), TW= 1000 Grain Weight (gm), GYPP= Grain Yield/Plant (gm)

Further evaluation of identified F_2 transgressive segregants was carried out through progeny testing of segregants in F_3 generation for GYPP and there was significant difference between F_2 mean of nine transgressive segregants (29.789 g) and mean of their F_3 population (27.894 g) found for GYPP (table 5) in cross K 1006 × LOK 1. Similarly, significant difference between F_2 mean of ten transgressive segregants (30.592 g) and mean of their F_3 population (28.56 g) was found for GYPP (table 5) in the cross PBW 343 × HUW 234. Such finding may be because of moderate heritability and non-additive gene action in early segregation generation for GYPP which is agreed by Dhole and Reddy (2011). Since, yield is as complex trait and polygenic in nature hence, presence of high frequency of transgressive segregants for grain yield and its components indicates the ample scope for crop improvement in future breeding programme by exploiting such useful findings.

Conclusion

Besides development of high yielding genotypes of crop plants along with genetic improvement for resistance to biotic and abiotic factors. Still there is need to enhance the crop yield production for supplying the demand growing population. In order to this, the assessment of the performance of promising transgressive segregants for yield and its contributing traits in two crosses of wheat is performed. Our finding indicates that parents could have multiple alleles regulating the corresponding traits, showed the potential for the incorporation of beneficial alleles into a solitary genotype by intensive selection. Further evaluation of the most superior transgressive segregants for yield and it contributing traits can be done to achieve the desired plant type by selection in succeeding generations. As per observing the high estimates of transgressive segregants in two crosses of wheat in present study, it is concluded that transgressive breeding can proficiently be utilized to broaden the limits, if desired characters which may not be well expressed/available in parents too.

Since, plant breeding is a key approach to combining the desirable gene(s) into single genotype/line, so making the crosses by using diverse parents and selecting the superior recombinants/segregants for trait of interest in F_2 and advance generations is one of the crucial

approach to develop the genotypes better for yield and other desirable traits in order to produce the enough food for human being in 21st century.

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Conflict of interest
The authors declare that they have no conflict of interest.

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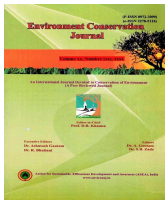

Synthesis and application of biochar in conjunction with various amendments to improve salt-affected soil and crop productivity

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ABSTRACT

Soil salinity is an important abiotic constraint that affects soil quality and crop productivity and has a direct impact on crop yields. Ensuring the sustainable use of saline soils while maintaining environmental integrity is of utmost importance. To achieve this, it is essential to explore and implement methods that can enhance productivity without causing harm to the ecosystem. In the current study, the effect of biochar, Simultaneous inoculation of biomes (Trichoderma harzanium and Pseudomonas fluorescence) and gypsum on soil properties and growth parameters of chickpea was investigated. Of all treatments, the combination of 75 percent GR + biochar@20t/ha and biome @2kg/ha had the greatest effect on lowering pH (9.32 to 7.61), EC (3.65 to 1.6 dSm^{-1}) and SAR (24.22 to 5.9 Cmolc (+) kg^{-1}). As a result, there was a notable improvement in the length of chickpea shoots and roots as well as the overall production of dry matter.

Introduction

A long-lived, self-pollinating, diploid, annual legume with the chromosome number 2N=16, the chickpea (*Cicer arietinum* Linn.) is a member of the Fabaceae family. It has been cultivated in different regions of the world since 7000 BC, as reported by (Tekeoglu *et al.* 2000). Despite its widespread cultivation, chickpea is mainly grown in semi-arid regions (Saxena, 1990). It grades third after the field bean and pea. World's largest producer country is India, accounting for 66% of total global production. Chickpea is cultivated on approximately 11.98 million hectares worldwide, with a yield of 10.91 million tonnes and a productivity rate of 911.2 kg/ha, according to the Food and Agriculture Organization FAO (2010). Salinity has a variety of effects on chickpea, Salinity can have detrimental effects on chickpea growth, including reduced and delayed seed germination, as well as suppression of vegetative plant growth (Yadav *et al.* 1989). Soil salinity is a significant abiotic stress factor that can negatively impact various physiological and metabolic processes in plants, resulting in lower growth and yield (Abbaspoor *et al.* 2009). Several factors, such as germination, survival, plant height, accumulation of suitable solutes in shoots or leaves, and the synthesis of particular metabolites, are typically considered when evaluating a plant's tolerance to salt. (Gamma *et al.* 2009). Salinity causes plants to accumulate sodium (Na^+) and chloride (Cl^-), which can cause critical nutrients like potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) to be displaced., as well as nitrate (NO^{3-}), which can adversely affect their uptake and utilization by the plant. (Sairam *et al.* 2004). Recently, some preliminary research results on the positive effect of biochar as an additive for remediation of sodic soils. An organic soil supplement called biochar

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can improve soil quality, nutritional content, and plant development., Glaser *et al.* (2002), Lehmann *et al.* (2006). As a result, the addition of Ca^{2+} and Mg^{2+} to calcareous soils via biochar improves aggregate stability, hydraulic conductivity and possibly increases Na^{+} leaching from the soil. Furthermore, biochar can enhance the colonization of beneficial microorganisms in the soil, promoting plant growth and overall soil health. (Mukherjee *et al.* 2011). The totaling of old biochar has been exposed to rise microbial activity (Wang *et al.* 2013). Biochar mineralizes faster in soils where it has been previously applied, suggesting that microorganisms play an active role in the mineralization process of biochar (Budai *et al.* 2016). PGPR are soil bacteria that are naturally present and aggressively invade plant roots, benefiting plants by encouraging growth. Early inoculation of crops with specific PGPR strains increases biomass production by directly affecting root and shoot growth (Hamdia *et al.* 1997). There are a variety of ways in which PGPR can affect nutrient uptake, yield and growth (Joseph *et al.* 2011). The application of biochar to soil has numerous benefits, includes enhanced legume nitrogen fixation, encouragement of naturally occurring nitrogen-fixing microorganisms, and improved availability of essential nutrients like iron, copper, phosphorus, and sulphur. PGPRs have attracted a lot of research interest and more are currently being marketed for use in other crops. Many researchers around the world have focused on the biotic strategy of "plant-microbe interaction" to solve salt and salinity problems. Some microorganisms are known for their capability to tolerate and recover the salt tolerance of plants (Ilanghumaran *et al.* 2017). with extremely positive results (Mastouri, 2010). Due to their high success rates, *Trichoderma harzanium* and *Pseudomonas fluorescence* species are extensively used in the experiment to reduce the negative effects of SAS. Trichoderma strains can increase a plant's resistance to biotic and abiotic stresses such as salt and drought (Shoresh *et al.* 2010). However, it has been economically unviable and challenging to implement appropriate management strategies and reclamation practices on a large scale in places affected by salt. This study aims to synthesis biochar and its application in conjunction with various amendments to improve salt-affected soil and crop productivity.

Material and Methods

Experimental Site Information:

A pot experiment was conducted in the year 2021-2022 at Rajmata Vijayaraje Scindia Krishi Viswa Vidyalaya, College of Agriculture in Gwalior (Madhya Pradesh).

Soil sample collection and preparation:

In the Bhind district of Madhya Pradesh's Malanpur, soil sample was taken at depths ranging from 0 to 15 cm. A composite sample was created by combining the samples. The larger aggregates were gently crushed with a wooden hammer after being air dried, and they were then put through a 2 mm filter. Incubation of the sieved soils for the column and pot studies was done in a plastic bag.

Analytical procedure:

The methodologies listed below were used to analyze different physical and chemical characteristics of soil. EC and pH were analyzed using method given by (Jackson 1967), (Jackson1962) respectively, the organic carbon content of soil samples was ascertained using wet digestion method (Walkley and Black1934). The CEC was estimated using Neutral ammonium acetate solution (Jackson,1962). Micro-Kjeldahl method was used to assess the soil's total nitrogen content. (Piper,1950). Ca^{2+} and Mg^{2+} were extracted from a 1 N NH4OAc solution (pH 7.0), as described by Piper and Jackson,1973. the sodium (Na^{+}) content of soil samples was determined separately using a flame emission spectro photometer (Model: Jenway, PEP-7) and a sodium filter (Jackson,1962). The equation $SAR = [Na]/(([Ca]+Mg])/2)^{1/2}$, used to calculate the sodium adsorption ratio, (Bohn *et al.* 2001). These standard methods were used with advanced technologies as followed, (Zeinab *et al.*2016).

Leaching Experiment:

The soil column experiment observed the deterioration of particular salt components and assessed the remediation of saline soils to monitor alterations in soil characteristics for the purpose of leaching trails (Roy *et al.* 2020b). incubating them with diverse combinations of amendments for 30 days., and leaching was carried out in 10 steps (0.5-5 pv) with amounts of water of the desired pore volumes (pv). The amount of water that a saturated soil contains in its pores is known as the pore

volume. After leaching, soil samples from different columns were analyzed.

Pot Experiment:

A greenhouse experiment was carried out, using several treatment combinations to obtain the best possible results. Three Kgs of air-dried soil in various additive combinations were placed inside every container so that the bulk density of the soil was maintained to 1.5 Mgm^{-3} as the volume of the pot was 200 cm^3. To complete the leaching study 30 days period was found sufficient to leach out the salts at room temperature (the average room temperature was arounds 30) For 30 days, these pots were incubated at the necessary temperature in a net house. The chickpea seeds were planted in each pot and grown under different combinations of treatments and recommended dose of fertilizer (RDF). Irrigation and other measures to prevent pests and diseases were taken regularly.

Treatment Details:

T1-Control, T2-100%GR, T-3 75%GR, T4-Biochar, T5-Biomes, T6- 75%GR + Biochar, T7-75%GR +Biochar+ Biomes

***** GR-Gypsum Requirement, Biomes (*Trichoderma harzanium* and *Pseudomonas fluorescence* *Rate of application of biochar-20 t/ha and biomes-2.5kg/ha.

Morphological and growth parameters:

Using a metre scale, the height of four identified plants was measured from the base of the plant to the tip of the main stem, and the data were expressed in centimetres (cm). One morphological measure used to assess plant growth is this one. By averaging the heights of four different plants, each plant's height was determined. Four plants were weighed both fresh and dried, and the average root length of each plant was measured in cm from the tip of the root to the base of the root, including all plant parts (root, shoot and leaves), was recorded. The remaining plants were kept until harvest for additional observations and post-harvest analysis.

Culture collection and Inoculum Preparation:

A potent isolate of Biome was used in this experiment. The pure strain of *Trichoderma harzianum* (NAIMCC-F-1744) and Pseudomonas fluorescence (NAIMCC-B-762) was obtained from ICAR-National Bureau of Agriculturally Important Microorganism (NBAIM) (NAIMCC) Kushmaur, Mau Nath Bhanjan (U.P). Mass propagation of *Trichoderma harzianum* was carried out in PDA media incubated at 250°C for 7-10 days. A similar method of mass propagation was used for the propagation of *Pseudomonas fluorescence* by simply changing the culture medium PDB from PDA. The spore suspension was prepared by harvesting the biomass of a 10–15-day old culture and then adjusting the concentration using a suitable diluent. Then the soil was soaked with the spore suspension and mixed thoroughly.

Biochar preparation:

Freshly harvested stalks of pigeon pea (*Cajanas cajan*) were collected from a field and stalks from a local farm. They were carefully cut into small pieces. The stalks were dried separately in the sun to reduce the moisture content to less than 10-12% to ensure uniform loading of biomass from pigeon pea crop residues and uniform heat transfer between crop residues during the thermal conversion process. The biomass samples were cleaned to eliminate dirt and dust using distilled water, then dried at 105°C for 10-12 hours in a hot air oven. After proper drying In, order to characterise some of the dried raw materials for physical, chemical, and morphological analyses, they were crushed and ground into powder form. The muffle furnace with a digital temperature controller was used to pyrolyze the dried stems at a slow rate. The experiment was conducted at a temperature of 400 degrees Celsius. The experiment was conducted at a heating rate of 13 degrees Celsius per minute for 1 hour to ensure uniform pyrolysis conditions (Lehmann *et al.* 2009). An initial nitrogen purge was performed to create a low oxygen environment. After the biomass remained in the muffle furnace for 10 minutes, the biochar was crushed and passed through a 2-mm sieve to obtain homogenised material for further analytical studies. To get the exact amount of biochar yield from raw material the mathematical calculation was done from given equation: Yield of biochar (%) = (Mass of biochar)/ (Mass of the raw materials) × 100 (Antal and Groni, 2003).

Results and Discussion

The characterization of synthesised biochar was done and yield was also calculated. The results of changes on soil various physico-chemical parameters are represented in various figures from 1-8 and morphological changes are tabulated in table 1.

Table 1: Effect of gypsum and other amendments on the growth of chickpea plants under salinity condition

Treatments	Yield (Kg/ha)	Cost of Cultivation (Rs.)	Gross Return (Rs.)	Net Return(Rs.)	B:C
T1	780	21000	40794	19794	0.943
T2	1430	22500	74789	52289	2.324
T3	1190	21980	62237	40257	1.832
T4	992	21000	51881	30881	1.471
T5	884	21300	46233	24933	1.171
T6	1400	22789	73220	50431	2.213
T7	1570	23000	82111	59111	2.570

Characterization:

The biochar sample was characterized for various composition which are given below:

The (%) Ash and moisture content (%) 4.0 ± 0.05, 6.45 ± 0.09 respectively, the pH- value ranges 6.71 ± 0.16 and the EC was around 2.12 ± 0.04 (dS/m), the percentage elemental composition of biochar was Carbon - 74 %, Nitrogen - 0.49, % Phosphorus 0.41%, Potassium- 0.65% and the resulted biochar yield from pigeon-pea stalks was 28.7 % .

Effect on soil reaction and soluble salt:

Physico-chemical properties of the intial soil samples:

pH-9.32, EC (dS/m)-3.65, OC (%)-0.451, N(kg/ha)-180, P(kg/ha)-13.87, K(kg/ha)-218.4, Ca (Cmolc (+) kg^{-1}) 27, Mg (Cmolc (+) kg^{-1}) - 10 Na (Cmolc (+) kg^{-1})- 247 SAR 24.22

Soil pH:

Soil reaction is considered the most significant physico-chemical property of soil as it determines the availability of nutrients and their uptake by plants. In the current study, soil pH was significantly reduced compared to the early soil pH (9.32) in all treatments that received biochar alone or in combination with the biomes shown in (Figure. 1). Application of 75 per cent GR + biomes + biochar @ 20 t/ha resulted in significantly lower soil pH. The reduction in soil pH caused by the addition of biochar could be due to the replacement of exchangeable Na^+ by Ca^{2+} (Luo *et al.* 2017). noted a similar reduction in the pH of surface and sub-surface horizons of salt-affected soils by the addition of biochar. (Wang *et al.* 2013) found that the addition of biochar lowered soil pH by releasing H^+ ions from exchange complexes through the addition of Ca^{2+} or Mg^{2+}. Another possible explanation for the low pH is the increased CEC of the soil due to the application of biochar, (Hinsinger *et al.* 2003).

Soil EC:

When biochar, biome and gypsum were used alone or in combination, there was a significant difference in EC compared to the control, as shown in (Figure 2). When biochar was used with 75 per cent GR and biome, the EC decreased (from 3.65 to 1.6 dSm^{-1}). Salt leaching may be responsible for the decrease in electrical conductivity (EC), which is subsequently followed by the addition of organic additives. The leaching of salts is caused by the release of organic acids during the breakdown process. The addition of various organic additives (Shoresh *et al.* 2010) significantly reduced the EC of saline soils. By enhancing the physical characteristics of the soil, leaching by organic matter led to a decrease in EC and an increase in the responsiveness of biomes.

Cation exchange capacity of soil:

The use of biochar or gypsum, either alone or in combination, enhanced the soil's ability to exchange cations, as shown in (Figure 3). The CEC was significantly higher (27.73 Cmolc (+) $kg^{-1}$to 35.97 Cmolc (+) kg^{-1}) in the treatments with 75 per cent GR plus biochar @ 20 t ha^{-1} and 2.5 kg/ha. This could be due to the inherent properties of biochar, particularly its large surface area, which may boost soil fertility CEC (Glaser *et al.* 2002). Some studies have consistently found that biochar has a higher intrinsic CEC than total soil, clay or soil organic matter, (Chan *et al.* 2008) likewise reported increases in soil CEC.

Exchangeable Cations in Soil:

Compared to the control, application of biochar or gypsum or their combination had a significant effect on exchangeable cations. The amounts are exchangeable Ca^{2+} and Mg^{2+} were significantly higher in the treatments 75% GR plus biochar @ 20 t/ha and 2.5 kg/ha, respectively. Application of gypsum increased exchangeable calcium and magnesium, while exchangeable Na^+ in the soils decreased (Figure 4, 5 and 6, respectively).

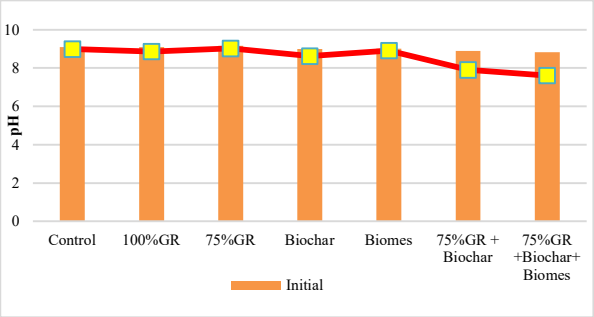

Figure 1: Soil pH for different treatments before and after leaching

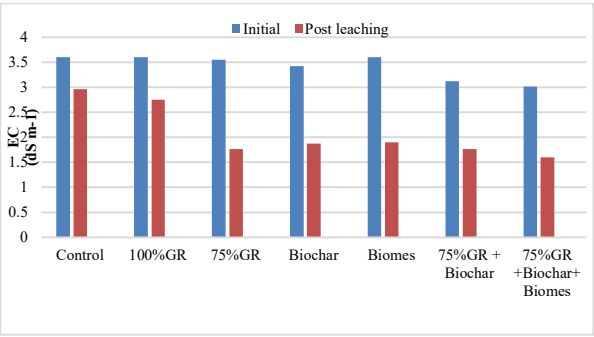

Figure 2: Electrical conductivity of saturation paste extracts of soils before and after leaching for different treatments

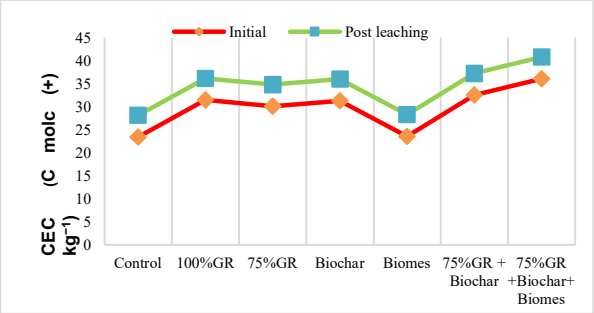

Figure 3: Soil cation exchange capacity for different treatments before and after leaching

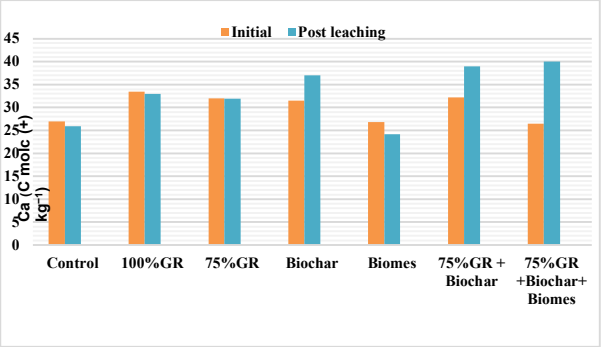

Figure 4: Soil exchangeable, Ca^{2+}concentrations (cmolc/kg) for different treatments, before and after leaching

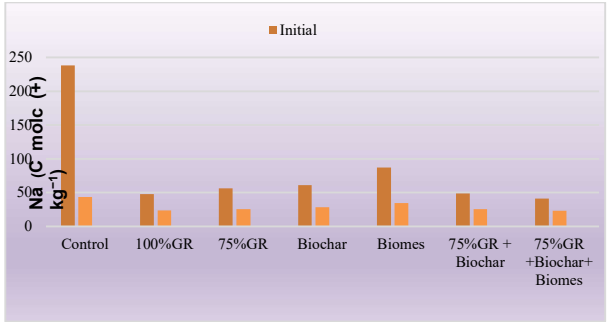

Figure 5: Soil exchangeable Na+ concentrations (cmolc/kg) for different treatments, before and after leaching

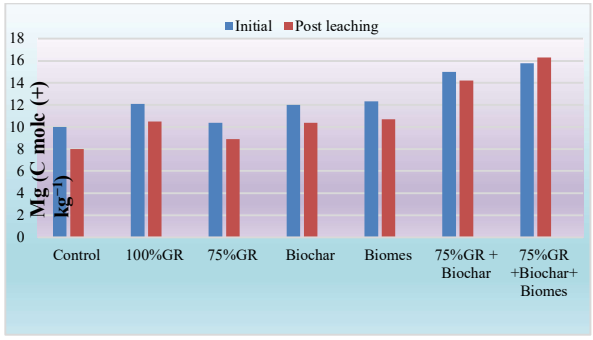

Figure 6: Soil exchangeable Mg^{2+} concentrations (cmolc/kg) for different treatments, before and after leaching

(Major *et al.* 2010) learned that the addition of 20 t ha^{-1} of biochar to a Colombian savanna oxisol increased Ca^{2+} and Mg^{2+} availability. The exchangeable sodium content, on the other hand, decreased with the combination of biochar and 75 per cent GR. This could be due to the higher concentrations of exchangeable Ca^{2+} and Mg^{2+} provided by biochar and gypsum, and their sorption over biochar, replacing Na+ from the soil exchange complex. It should be highlighted that the highest decrease in exchangeable Na+ was seen with the addition of 75% GR plus biochar at 20 t ha^{-1}. Increased Ca^{2+} and Mg^{2+} concentrations due to biochar addition may have reduced the exchangeable Na^{+} concentrations at these sites by enriching the soil profile exchange sites with Ca^{2+} and Mg^{2+}. (Kim *et al.* 2007) discovered a similar decrease in exchangeable Na^{+} concentration (35%) when 5% biochar was applied compared to the control soil and attributed this to the adsorption of Na^{+} on the biochar surface. The findings of (Laird *et al.* 2009) demonstrate that adding biochar raises the concentration of divalent cations and that adding biochar to salt-loaded soils can reduce salt

stress because of the high Na^+ adsorption potential of biochar (Novak *et al.* 2009), (Akhtar *et al.* 2015).

Exchangeable Sodium Percentage in soil:

Compared to the control soil, applying biochar at various rates, either by itself or in conjunction with 75 per cent GR, significantly reduced ESP, shown in (Figure 7). Application of 75 per cent GR plus biochar at a rate of 20 t/ha reduced soil ESP more effectively than biochar alone. The increase in CEC or soil organic matter content was attributed to the significant reduction of ESP by biochar application in sodic soils (Luo *et al.* 2017), that was supported by the negative and significant correlation (0.875*) between soil CEC and ESP in the current study. The decrease in ESP seen with the addition of either gypsum or biochar may also be due to increased Ca^{2+}in the soil solution brought on by the addition of gypsum and/or varying rates of biochar that facilitated Na^+ displacement and subsequent removal during leaching to deeper soil layers., either alone or in combination (Gharaibeh *et al.* 2011). This was also confirmed in the current study where a negative and significant correlation (0.910**) was found between exchangeable Ca^{2+}content and soil ESP.

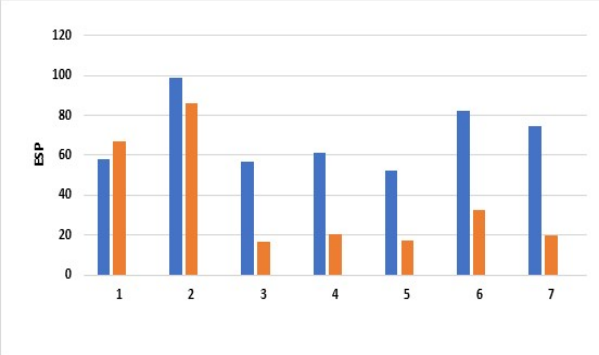

Figure 7: Exchangeable sodium percentage of soils before and after leaching

Soil sodium adsorption ratio:

Application of different amounts of biochar, either alone or in combination, had a significant effect on SAR of post-harvest soils. Significantly less soil SAR was produced by the treatment that got 75% GR along with biochar at a rate of 20 t/ha and various biomes represented in (Figure 8). This could be due to increased Na^+ displacement from the exchange complex as a result of increased Ca^{2+}availability from the combined application of biochar and gypsum. The increase in soil porosity caused by the addition of biochar may also have promoted the leaching of Na^+ from the soil profile and a decrease in SAR (Yue *et al.* 2016). Several studies have confirmed the positive effect of biochar as an additive for saline soils by lowering soil SAR (Amini *et al.* 2016), (Luo *et al.* 2017).

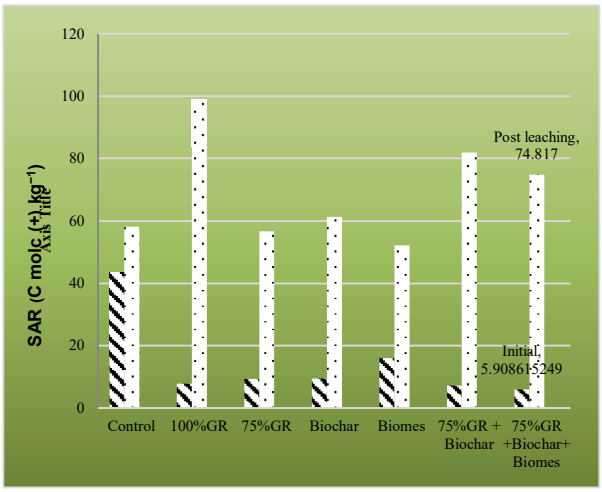

Figure 8: Sodium adsorption ratio of soils before and after leaching for different treatments

Growth and morphological parameters:

The height of each plant was calculated in centimetres from the plant's base to the growing tip of the main branch and represented in centimetres using a metre scale and four marked plants. (cm). To determine the height of each plant, the heights of four plants were averaged. The length of a plant's roots, from root tip to root base, was measured in centimetres Four different plants' roots were measured on average. Using the dry weight formula, the area of the leaf was determined. Salt stress affected the length of chickpea shoots and roots in the current study. The application of the salt-tolerant PGPR strains *P. fluorescens* and *T. harzanium* in combination with nano-gypsum significantly increased the growth and production of chickpea grown under salt stress, according to our results, which are in agreement with previously published studies. All morphological parameters increased statistically significantly compared to the control in biomes with 75 per cent GR + 20 t/ha biochar. Table 1 lists the possible results. In this study, the production of shoot length, root length, leaf area, and dry matter of the chickpea plant was

greatly boosted by the addition of biochar and gypsum. At application of biomes with 75 per cent GR + 20 t/ha biochar the shoot length of plant was 24.9 cm, root length of plant 31.8 cm, shoot dry weight was 2330 mg/plant and root dry weight of plant was 1086 mg/plant. According to the root to shoot length ratio, the shoot was more impacted by salinity than the root (Moud *et al.* 2008). This value was considerably lowered by high salt stress (Akbarimoghaddam *et al.* 2011).

Effect on chickpea output in terms of grain and straw:

Increasing the amount of biochar and gypsum significantly increased grain production from chickpeas. The data range for mean grain yield from chickpeas was 7.39 to 8.52 g/plant. Treatment with 75% GR and biochar at 20 t/ha gave the highest grain yield (8.52 g/plant). How crops respond to biochar application depends on plant species, soil conditions, climate, and the biochar's chemical and physical properties (van Zwieten *et al.* 2010, (Haefele *et al.* 2011). After various treatments, chickpea straw yields showed the same pattern as grain yields. Compared to the other treatments, the 75% GR + 20 t/ha biochar and biome treatments had the highest average straw yields. This may be related to higher biomass yields made possible by biochar additives that mitigate the effects of salt stress on plants. The results are consistent with those of (Drake *et al.* 2016), where biochar application to soils in salinity conditions dramatically greater biomass of both plants that salt-tolerate and salinity-sensitive seedlings.

Economic analysis:

Economic feasibility in financial terms of any innovation or technique has primary importance in deciding its wider adoption among farming community represented in, accordingly, the maximum net benefit was obtained by treatment 7 was applied. Net benefit for the treatment 7 the (75% GR + 20 t/ha biochar) is higher i.e., 59111 Rs. as compared with the rest of the treatments, as it showed an increasing trend compared to other treatments. The benefit cost ratio also showed an increasing trend (2.570), presented in Table 2. Any breakthrough or technology must be economically viable in order for the agricultural sector to adopt it widely. In order to create goods that farmers can easily obtain, an economic analysis was done at the conclusion of the study, and the most effective and economical treatment levels were selected.

Table 2: Effect of gypsum and other amendments on the yield and economic analysis of chickpea plants under salinity condition

Treatment	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)
T1	13	18	1470	486
T2	19.8	30.5	2130	920
T3	18.6	24.7	1690	230
T4	17	20.6	1460	163.4
T5	14	18	1465	490
T6	21	27	2180	586
T7	24.9	31.8	2330	1086

Conclusion

The findings of this investigation suggest that combination of 20 t/ha of biochar along with 75% GR as a supplement to soda ash can effectively reduce soil pH, ESP and SAR, compared to using either gypsum or biochar as a single application. This combination can significantly increase chickpea production, demonstrating the benefits of using biochar as an adjunct to soda soil remediation. Additionally, the pH of the biochar feedstock greatly influences its effectiveness in rehabilitating salinity-degraded soils. Using PGPR as an inoculant and biofertilizer is an effective way to replace chemical fertilizers and pesticides, and is beneficial for plant growth and development, promoting sustainable chickpea agriculture in India and other developing countries. Further studies, including field efficiency trials, are necessary to determine the functionality of PGPR as a viable biofertilizer. Environmental pressures are global variables that negatively impact agricultural

productivity, preventing the introduction of crops into uncultivable areas and reducing yields.

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Conflict of interest
The authors declare that they have no conflict of interest.

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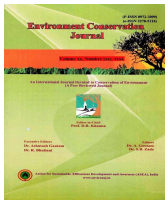

Molecular characterization of selected fungal and bacterial endophytes in acid lime

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ABSTRACT

Endophytes are the microorganisms that are present in living tissue of various plant parts (roots, fruits, stem, seed, leaf *etc*,). Endophytic microorganisms are good source of antibiotics. Endophytic antagonists were isolated from the roots of healthy acid lime plants collected from major acid lime growing areas of Andhra Pradesh. A total of 8 fungal and 10 bacterial endophytic antagonists were isolated. The antagonists were further subjected to preliminary screening, out of which only 6 endophytic fungal antagonists (EFA 1-6) and 8 endophytic bacterial antagonists (EBA 1-8) isolates showed good inhibitory effect on radial growth of *Fusarium solani* causing dry root rot in acid lime *in vitro*. Among them the one of the best fungal and bacterial antagonists that were found to be extremely efficient against *Fusarium solani* in dual culture assay were selected for further molecular identification. The BLAST results revealed that one of the fungal isolate had shown 100% similarity with *Aspergillus fumigatus* and one of the bacterial isolate had shown 95.56% similarity with *Pseudomonas aeruginosa*.

Introduction

Acid lime (*Citrus aurantifolia* Swingle) is one of the largest and most important fruits of tropical and subtropical regions. India is the largest producer of acid lime in the world. Fungi and bacteria are two types of beneficial endophytic microbes that invade internal plant tissues without harming their hosts visibly (Petrini, 1991 and Gouda *et al.,* 2016). They differ from epiphytic microorganisms, which reside on the surface of plant organs and also within the plant tissues, like which they are not harmful, do not infect plants, and do not cause diseases (Hallmann *et al.*, 1997). Endophytic microbes are also capable to produce antimicrobial metabolites and several antimicrobial products were extracted from various plants for various pathogens. These microorganisms were found to be effective, environmentally safe and promising biotic tools in plant disease management. In our study, the endophytes were tested against dry-root rot pathogen *Fusarium solani* in acid lime. A roving survey was conducted to isolate endophytic antagonists from roots of healthy acid lime plants. Isolation of endophytic microorganisms needs the elimination of epiphytic contaminants present on the roots' outer surface. Hence, first the roots were surface sterilized followed by isolation (Araujo *et al.,* 2002). In the present investigation, the sterilization was done using two per cent sodium hypochlorite solution for 5 min with slight changes from the method followed by Saini *et al.,* (2016). The surface sterilized samples were blot dried after washing thrice in sterile water. The sterilized healthy roots were triturated with 8 ml of sterile 0.1M Potassium phosphate buffer (pH -7.0) using a

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sterile mortar and pestle. The triturate was serially diluted in sterile water blanks up to 10^{-7}. One ml of the final buffer wash was pipetted out onto a sterile petri plate with a specified growth medium.

A total of 18 endophytic antagonists were isolated, among which 8 were fungal and 10 were bacterial. On further *in vitro* evaluation, 6 fungal and 8 bacterial endophytic antagonists showed inhibitory effect on the radial growth of the *Fusarium solani* causing dry root rot in acid lime. The isolates EFA 4 and EBA 7 were found to be highly efficient against *Fusarium solani* in dual culture with 66.92% and 63.42% inhibition over control and these isolates were selected for further molecular identification.

Material and Methods

The effective endophytic antagonists EFA 4 and EBA 7 were selected for molecular identification after proper *in vitro* antagonistic assays.

Molecular identification of endophytic fungal and bacterial antagonists

DNA extraction from endophytic fungal and bacterial antagonists

Genomic DNA was isolated from mycelial mat of fungus and single colony of bacterial culture following CTAB method (Li and Yao, 2005; William *et al*., 2012).

PCR amplification and sequencing for endophytic fungal antagonists

The isolated DNA was amplified with universal primers ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) in PCR (White *et al.,* 1990). The PCR initial step was kept at 94°C for five minutes, a denaturation step at 94°C for 60 seconds, an annealing step at 55°C for one minute, an extension step at 72°C for 1.5 minutes and a final extension at 72°C for 5 minutes followed by cooling at 4°C for 30 seconds and repeated for 34 cycles.

PCR amplification and sequencing for endophytic bacterial antagonists

The universal primers 27F (AGAGTTTGATC CTGGCTCAG) and 1492R (GGTTACCTTG TTACGACTT) were used for the amplification of bacterial DNA in PCR (Lane, 1991; Stackebrandt and Liesack, 1993). The PCR was done as an initial step for 5 minutes at a temperature of 95°C, a denaturation step at 95°C for 60 seconds, an annealing step for 1 minute at 56°C, an extension step at 72°C for 1.5 minutes and a final extension for 10 minutes at 72°C followed by cooling at 4°C for 30 seconds and repeated for 30 cycles.

Quantification of Genomic DNA

The total obtained genomic DNA concentration was measured using U.V. Spectrophotometer Nanodrop (ND-1000). Blank was kept against millique water. The optical density was measured at 260 nm to determine the DNA concentration. DNA concentration and optical density were related as follows.To figure out the ratio OD260/OD280, the optical density (O.D.) will be measured at 280 nm. The ratio is thought to be optimal around 1.8, which indicates ideal DNA preparation. A score above 1.8 indicates that the sample contains more RNA, whereas a ratio below 1.8 suggests the presence of proteins in the preparation (Moges *et al*., 2017; Ratanacherdchai *et al*., 2007).

Loading of agarose gel

Gel plates were carefully cleaned using a cleaning agent, then rinsed with distilled water and dried. The plates were sealed with cellophane tape at the two open sides. Then ethidium bromide (1.5 µl) was added to the gel at hand tolerable heat. After that, the solution was put into the gel plate (with a comb) and left to polymerize.

Loading and gel electrophoresis

The inserted comb was delicately removed from the gel after polymerization. The tank of the horizontal electrophoretic apparatus was filled with 1X TBE buffer and the gel plate was set within. With the help of micropipettes, the samples were loaded in the wells. Loading dye of 5 µl was added with the help of micropipettes into each DNA sample and mixed well. After loading, a power pack with a 100V regulated electric power source was connected to the electrophoretic unit. After the gel run was completed, the gel was gently removed, and the gel image was examined on a U.V. transilluminator Gel doc (Alpha Innotech Multi image light cabinet filter positions) and stored in gel documentation system. Gene Ruler100-bp plus DNA ladder (© 2012 Thermo Fisher Scientific Inc.) was used as a molecular weight marker.

Results and Discussion

PCR amplification

In molecular characterization, the DNA obtained from the effective endophytic fungal antagonists EFA 4, was amplified with universal primers ITS 1 and ITS 4, which resulted an amplicon size of 540 to 580 bp fragment of DNA. Further confirmation of pathogen was done by DNA sequencing. The molecular characterization of effective endophytic bacterial antagonists EBA 7 was done using universal primers, 27 F and 1492 R and the DNA was amplified using the universal primers. This resulted an amplicon size of 1000 to 1160 bp, fragment of DNA. The band of DNA pertaining to effective endophytic microbes formed during the gel electrophoresis were displayed in Fig. 1.

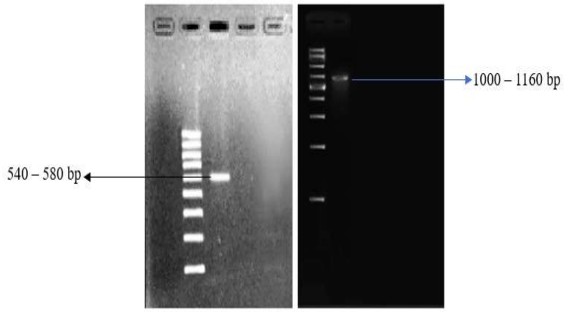

Fig 1. Amplification of DNA of Endophytic Fungal Antagonist (EFA 4) at 540 to 580 bp (left) and Endophytic Bacterial Antagonist (EBA 7) at 1000 to 1160 bp (right)

DNA sequencing

***Aspergillus fumigatus* (EFA 4)**

The BLAST results showed 100% similarity with *Aspergillus fumigatus*. The nucleotide sequence of ITS region of isolate *Aspergillus fumigatus* were submitted to Gen bank under accession number - MN209960. Based on nucleotides homology and phylogenetic analysis the endophytic microbe EFA 4 has shown maximum identity with *Aspergillus fumigatus* strain ZC-2 **(**Gen Bank Accession Number: MK630344.1). *Aspergillus fumigatus* was reported as an endophytic fungus earlier in *Juniperus communis* L. Horstmann (Kusari *et al*., 2009), *Cynodon dactylon* (Liua *et al*., 2004), *Moringa oleifera* (Abonyi *et al.*, 2018). Kumar *et al.* (2012), Savitha and Sriram (2015) and Kannangara *et al*. (2017) also characterized the *Trichoderma* spp. by using universal primers ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and in order to know their antagonistic activity against root rot and foliar pathogens. Zihad *et a.* (2022) identified five *Aspergillus* spp. from Sundarbans forest trees - *Ceriops decandra* and *Avicennia officinalis* using ITS1 and ITS4 primers. Similar findings were done by Singh *et al.* (2020) where they isolated and identified 20 types of fungal endophytes from *Argemone Mexicana* using ITS1 and ITS4 primers. They identified that the endophytes belonged to *Aspergillus* and *Penicillium* spp. Also, Schoch *et al.,* (2012) stated that ITS regions were used frequently as phylogenetic markers for identifying fungi. There have been numerous molecular characterization studies conducted to identify the fungal endophytes from various medicinal plants (Chen *et al.,* 2011; Bhagat *et al.,* 2012 and Yoo and Eom, 2012). Recently, Al-badi *et al.* (2020) characterized five fungal endophytes isolated from Shirazi Thyme using the primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and identified them as *Nigrospora sphaerica* (E1 and E6 isolates), *Polycephalomyces sinensis* (E8 and E10 isolates), and *Subramaniula cristata* (E7 isolate).

***Pseudomonas aeruginosa* (EBA 7)**

The BLAST results showed 95.56% similarity of the isolate with *Pseudomonas aeruginosa*. Based on nucleotides homology and phylogenetic analysis the endophytic microbe EBA 7 has shown maximum identity with *Pseudomonas aeruginosa* strain GIMC5015 **(**Gen Bank Accession Number: CP034429.1). Besides them, based on morphological and physiological characteristics, as well as 16S rRNA gene sequence analysis, the plant growth-promoting bacterial endophyte AL2-14B that was isolated from the leaves of *Achyranthes aspera* L. was identified as *Pseudomonas aeruginosa* (Khaidem, A.D. *et al*., 2017). *Pseudomonas aeruginosa* was identified as the endophytic phosphate-solubilizing bacteria EPR13 that was isolated from the aerial tissues of *Achyranthes aspera* L. (Misra *et al.,* 2012). Similarly, Hassan *et al.* (2016). Amaresan *et al.* (2014) isolated and characterized the beneficial bacteria associated with chilli at molecular level by 16 s rDNA sequencing. The similar line of work was done by Singh *et al*. (2015) on molecular identification and characterization of rhizospheric bacteria, by PCR based 16S rRNA gene

sequencing. Also, Uzair *et al*. (2018) isolated a *Pseudomonas* strain PS24 from soil samples of Balochistan coastline and identified it as *Pseudomonas aeruginosa* by 16srRNA sequence analysis.

Conclusion

In the study, we identified a fungal and a bacterial endophytic antagonist as *Aspergillus fumigatus* and *Pseudomonas aeruginosa,* respectively which were found to be effective in controlling *Fusarium solani* (a dry-root rot causing pathogen in acid lime) under *in vitro*. Further investigations determine their effectiveness under *in vivo* conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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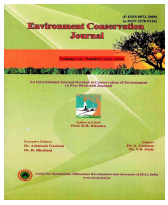

Investigation of trends in basin-scale temperature variables

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Trend analysis

ABSTRACT

This research paper presents an analysis of temperature variables over the West Banas basin in order to detect the presence of underlying trends employing historical temperature data for three points viz., Abu Road, Mount Abu and Pindwara obtained for a period of 40 years (1981 – 2020) from MERRA-2 database. The study aims to investigate the long-term changes in temperature trends and identify any significant patterns or anomalies in mean, maximum and minimum temperatures at monthly, seasonal and annual timescales at the three locations amounting to a total of 162 series. The trends were evaluated using the Mann-Kendall test, a popular and powerful statistical technique formulated for analysing abnormal distributions. Prior to the application of the trend test, autocorrelated time series were identified and the trend test was modified using a variance correction approach to incorporate the influence of autocorrelations upon the resultant trends. The findings of autocorrelation analysis revealed that 11 of the 162 series were autocorrelated, a majority of which were associated with the temperature series at Abu Road. The results of the trend test showed that 27 out of the 162 series possessed significant trends with the mean and maximum monsoon temperatures in most of the series exhibiting a reducing trend while the minimum temperature appeared to be rising. Overall, the research highlights the importance of monitoring temperature trends, particularly in regions that may be more vulnerable to the impacts of climate change. The findings of this study can inform future climate adaptation strategies and support decision-making processes aimed at mitigating the effects of global warming on the natural and built environment.

Introduction

The Earth's climate is a complex and dynamic system that is constantly. One of the most significant changes observed in recent years is the increase in global temperatures. The average global temperature has increased by 1.1°C since the pre-industrial era and it has been predicted that a further rise by another 5°C may occur by the end of this century (IPCC, 2018). This warming of the planet has significantly impacted our environment and society and is the cause of rising sea levels, more intense and frequent extreme weather events and has added to the erratic nature of precipitation (IPCC, 2014). According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (ISPBE), climate change has an impact on a variety of sectors, including agribusiness, public health, and infrastructure (Ebi *et al.,* 2014; ISPBE, 2019). Given these very real

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influences of climate change upon the natural as well as artificial ecosystems of our world, there is an urgent need to better understand and predict changes in temperature patterns and aim at creating policies with the goal of mitigating the consequences of climate change.

Temperature is one of the most widely used indicators of climate change, and long-term temperature data aids in elucidating the effects of global warming on the planet. Recent decades have witnessed a tremendous growth in the research field pertaining to trend analysis of temperature owing to the abrupt changes being observed in global climate. By examining temperature trends over different spatial and temporal scales, this research aims to contribute to our understanding of the variations inherent to Earth's climate system with the aim of directing policymakers towards planning control measures for mitigating the impacts of climate change. Several studies have documented the observed warming trend in the Earth's surface temperatures and the phenomenon of global warming. Hansen et al. (2010) analysed the global surface temperature change from 1880 to 2009 and reported an increase of 0.8°C in the planet's temperature during that period. Although the magnitude of rise in temperature is overstated in various studies, increasing surface temperature of the planet remains an incontrovertible occurrence (Karl *et al.,* 2015).

However, most of these studies have assessed temperature trends over large-scale areas such as countries or continents, but smaller scale analysis is needed to better understand the regional consequences of climate change (Singh *et al.,* 2008). Hence, this study will deal with a comprehensive analysis of basin-scale variations in temperature to better understand the magnitude and direction of temperature changes over time.

Material and Methods

Study area and data acquisition

Rajasthan has 14 major river basins, but most of them have rivers with seasonal flows. The focus of this study is the West Banas basin, which is situated in the south-eastern region of Sirohi district (Fig. 1). The West Banas River originates from Pindwara village in Sirohi district at an elevation of 372.5 m above mean sea level and flows for about 50 km in a south-western direction before entering Gujarat and finally draining into the little Rann of Kachchh. The region is delineated by the Luni basin to the north and northwest, the Sukli basin to the west, and the Sabarmati basin to the east, while its southeastern boundary is contiguous with the state of Gujarat. The river has a total length of 266 km, draining an area of 8,674 km^2, of which 1831.34 km^2 in Rajasthan and the remaining in Gujarat.

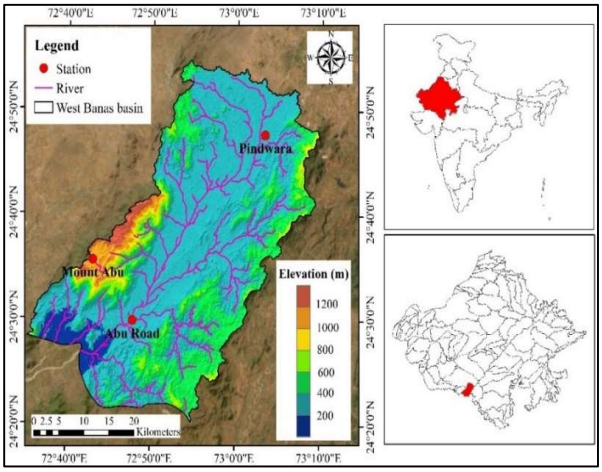

Figure 1: West Banas basin of Rajasthan

Modern-Era Retrospective analysis for Research and Applications, version 2 (MERRA-2) is a widely-used global atmospheric reanalysis product developed by NASA. It provides a comprehensive picture of the planet's climate system from 1980 to the present day at a spatial resolution of approximately 0.5 degrees. The MERRA-2 temperature data refers to the 2D daily land surface temperature data, which is a crucial component in the analysis of the Earth's climate system. The information presented is sourced from the MERRA-2 reanalysis system, which employs sophisticated data assimilation methods to combine data from diverse sources, such as satellite and ground-based instruments, to generate a dependable and consistent account of the Earth's climate. Moreover, the application of this dataset has undergone verification by numerous researchers (Bosilovich *et al.,* 2016; Gupta *et al.,* 2019). The monthly temperatures (mean, maxima and minima) for a duration of 40 years (1981 – 2020) for three points viz., Abu Road, Mount Abu and Pindwara, were obtained from NASA's MERRA-2 dataset. The temporal variation of these temperature variables are shown in Fig. 2.

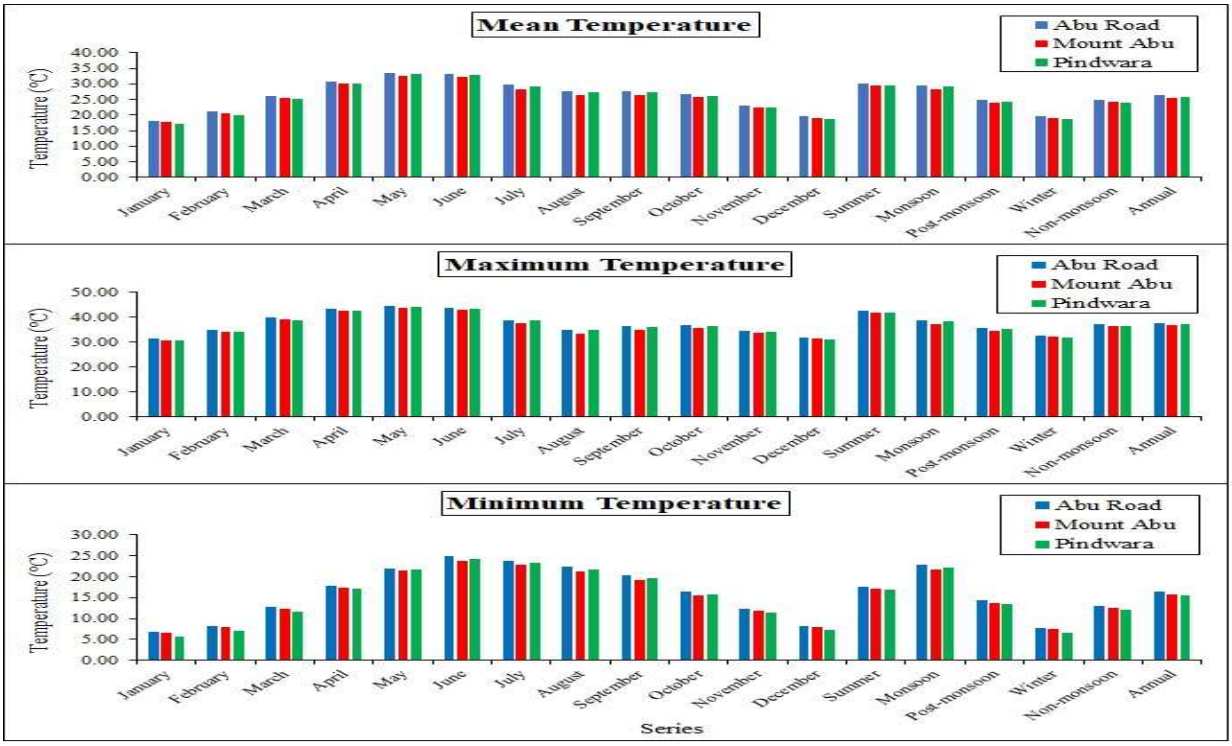

Figure 2: Overview of the temperature dataset

Autocorrelation

The only factor requiring serious consideration while application of MK test is that no autocorrelation (serial correlation) should be present in the time series data as positive autocorrelation in time series tend to increase the possibility of detection of trends whereas that possibility reduces in case of a negative autocorrelation. It has been established that the presence of autocorrelation in time series has an influence upon the variance of MK test statistics. From analysis perspective concerned with the present study, it is imperative to determine the lag 1 autocorrelation coefficients of each time series which can be according to Box and Jenkins (1970) as:

$$r_1 = \frac{\sum(x_t - \bar{x})(x_{t+1} - \bar{x})}{\sum(x_t - \bar{x})^2} \qquad (1)$$

Where, r_1 is the autocorrelation coefficient at lag 1, x_t and x_{t+1} are observations at time t and $t+1$, $\bar{x}$ denotes the time series mean.

The autocorrelation coefficients are usually depicted as a series of stem plots which were developed using Python in this study. Based on a 95% significance level, the time series possessing significant autocorrelations were identified as the individual plots which extended beyond the limits of the confidence interval.

Trend analysis using Mann-Kendall test

Trend in a time series is characterized as the deterministic component which causes successive values to possess increasing or decreasing tendencies with time (Haan, 2002). Trend in a time series may be linear or nonlinear, caused by variations in hydrologic characteristics accounted by natural factors or artificial intervention. A global shift in climatic characteristics and anthropogenic activities are the major factors responsible for the presence of trends in meteorologic variables such as temperature.

The Mann Kendall (MK) method of trend analysis has been extensively used in a plethora of studies (Nalley *et al.,* 2013; Pingale *et al.,* 2016) for analysing trends in rainfall as well as other climatic variables and is viewed as a standard tool for trend detection worldwide. The MK test is a non-parametric statistical technique used to identify patterns in a time series with their nonlinear aspect being derivable from Kendall test statistics (Mann,

1945; Kendall, 1975). The non-parametric attribute of the test provided it with an upper hand over parametric tests because its application omits the prerequisite of the input data being normally distributed and the effects of raw and skewed data are also heavily undermined (Yue *et al.,* 2003).

For time series X_i and X_j where i and j denote the ranks in the range of natural numbers with X_i and X_j being the points of reference being compared in an iteration of the test, a test statistic (S) for the MK test may be derived as:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \boldsymbol{sgn}(\boldsymbol{X_j - X_i}) \quad (2)$$

where n is the range of data points, X_i and X_j are the recorded values of a variable in time series i and j ($j > i$), respectively, such that $j > i$ and $sgn\ (X_j - X_i)$ is termed as the sign function evaluated as:

$$\boldsymbol{sgn}(\boldsymbol{X_j - X_i}) = \begin{cases} +\mathbf{1}, (\boldsymbol{X_j - X_i}) > \mathbf{0} \\ \mathbf{0}, (\boldsymbol{X_j - X_i}) = \mathbf{0} \\ -\mathbf{1}, (\boldsymbol{X_j - X_i}) < \mathbf{0} \end{cases} \quad (3)$$

The variance in the values of test statistic thus obtained is computed as:

$$\boldsymbol{Var(S)} = \frac{\mathbf{1}}{\mathbf{18}}\left[\boldsymbol{n(n-1)(2n+5)} - \sum_{i=1}^{P} \boldsymbol{t_i(t_i-1)(2t_i+5)}\right] \quad (4)$$

with P being the number of tied groups and t_i is called the number of data values in the P^{th} tied group. A tied group comprises a set of sample data containing numerically equal data values. In case the sample data doesn't contain the same data values, the summation portion in eqn. (3) can be omitted (Kisi & Ay, 2014). For a sample size $n >$ 30, the standard normal test statistic Z is computed as:

$$\boldsymbol{Z} = \begin{cases} \dfrac{\boldsymbol{S-1}}{\sqrt{\boldsymbol{Var(S)}}}, & \boldsymbol{S > 0} \\ \mathbf{0}, & \boldsymbol{S = 0} \\ \dfrac{\boldsymbol{S+1}}{\sqrt{\boldsymbol{Var(S)}}}, & \boldsymbol{S < 0} \end{cases} \quad (5)$$

A $|Z| > Z_{1-\alpha/2}$, results in rejection of the null hypothesis of no trend indicating a significant trend. Also, rising trends are characterized by positive values of Z whereas negative values of Z represent a reducing trend over time. In the present study, a confidence levels of $\alpha = 0.05$ was used for which the null hypothesis can be rejected if $|Z| > 1.96$.

Modified Mann-Kendall test

Based on the limitations incurred upon MK test due to presence of autocorrelation in a time series, Hamed and Rao (1998) suggested a variance correction approach to inhibit the effects of autocorrelation upon the time series which required a modification in the variance of a time series by altering its sample size using the following formula:

$$\boldsymbol{V^*(S) = V(S) \times \frac{n}{n^*}} \quad (6)$$

Where, n denotes the actual sample size (ASS) of the data, n^* denotes the effective sample size (ESS) derived from the same data and n/n^* is termed as the correction factor.

In the present study, computation of n^* has been carried out as follows:

$$\boldsymbol{n^* = n \Big/ \left[1 + \left\{\frac{2}{n(n-1)(n-2)} \times \sum_{i=1}^{n-1} (n-i)(n-i-1)(n-i-2)\rho_s(i)\right\}\right]} \quad (7)$$

This MK test, when applied to a time series while following an algorithmic approach (variance correction in present study) to prevent autocorrelations from influencing the trends, is termed as modified MK (MMK) test.

Sen's slope

In order to complement and quantify the results of MK/MMK test, the trend magnitudes in the time series data were derived from the slopes of N pairs of data points using Sen's slope estimator (Sen, 1968) given by:

$$\boldsymbol{Q_i = \frac{X_j - X_k}{j-k}\ (i = 1, 2, \ldots, N)} \quad (8)$$

where X_j and X_k are data values at times j and k, respectively, such that $j > k$.

If each time period corresponds to a single data point, then for n number of time periods, N may be computed as:

$$\boldsymbol{N = \frac{n(n-1)}{2}} \quad (9)$$

The set of slopes thus obtained are arranged in an ascending order and Sen's slope (β) is derived as the median of these ordered values as:

$$\beta = \begin{cases} Q_{(N+1)/2} & N \text{ is odd} \\ \frac{1}{2}\left(Q_{\frac{n}{2}} + Q_{\frac{n+1}{2}}\right) & N \text{ is even} \end{cases} \quad (10)$$

The positive and negative values of Sen's slope are assessed as rising and reducing trends, respectively. The change in magnitude is usually conveyed in the form of percentage of the mean. As per Yue and Hashino (2003), the linear trend is computed as follows:

$$Percentage\ change\ (\%) = \frac{\beta \times length\ of\ year}{Mean} \times 100 \quad (11)$$

Results and Discussion

Arranging the dataset with respect to each combination of location, temperature variable and temporal scale resulted in formation of 162 unique time series that were to be tested for presence of inherent trends. The autocorrelated series were identified and MMK test was applied to these series. The following sections provide a detail of the outcomes of the analysis implemented in the present study.

Autocorrelation

Autocorrelation refers to the degree of similarity between observations of a variable with its past values. In other words, it measures the extent to which a variable is correlated with itself over time. Autocorrelation plots provide an analytical and intuitive visualization of the autocorrelations present in the time series data. The autocorrelation plots created for each set of time series data considered in the present study are shown in Fig. 3. The significant autocorrelations are evaluated as the plots extending beyond the computed confidence intervals. It was found through the autocorrelation plots that 11 of the 162-time series tested were autocorrelated (Fig. 3). The time series pertaining to the maximum temperature at Abu Road consisted of the highest number of autocorrelated time series, i.e., 5, out of all the datasets. At Abu Road, the mean and minimum temperatures for the Non-monsoon period and the month of May were assessed as autocorrelated. The maximum temperature series for January, November, Post-monsoon, Winter and Non-monsoon were also found to be autocorrelated. In the case of Mount Abu, only the maximum and minimum temperatures during May and Non-monsoon, respectively, were identified as autocorrelated. For Pindwara, only the maximum temperature series for January and Non-monsoon showcased and autocorrelation. Temperature series for Abu Road were attributed with the highest number of autocorrelated time series containing 7 out of the total 11 autocorrelated series. It was also observed that the temperature datasets for the non-monsoon period, especially the maximum temperature series, stood out as being the most likely series to have a significant autocorrelation. Through the segregation of the autocorrelated time series from the non-autocorrelated ones, the type of test (MK or MMK) to be utilized for trend analysis was selected and applied to the relevant time series dataset.

Trends in Temperature Variables

The trends in temperature were evaluated for the series of all the individual months as well as seasonal and annual datasets. Furthermore, in addition to the spatial and temporal classification, the temperature dataset was also divided into mean, maximum and minimum temperature dataset adjoined to each spatio-temporal classification. This means that a total of 162 time series were tested.The MMK method of trend analysis was applied to various time series encompassing temperatures at the three stations in the study area. The results revealed the statistical significance of 27 out of the total 162 series tested for trend. The computed test statistics for the MMK temperature trend test at Abu Road were shown in Table 1. At Abu Road station, mean and maximum temperatures in monsoon as well as minimum temperatures in April, May, summer, non-monsoon and annual series were found to be significant out of which, the mean and maximum temperature possessed a reducing trend whereas the minimum temperature seemed to be rising.The nature and significance of trends observed at Mount Abu station, shown in Table 2, resembling those observed at Abu Road. An additional increasing trend was also observed in case of the non-monsoonal mean temperatures at Mount Abu. The significant trends observed at Pindwara, as shown in Table 3, were somewhat similar to those at

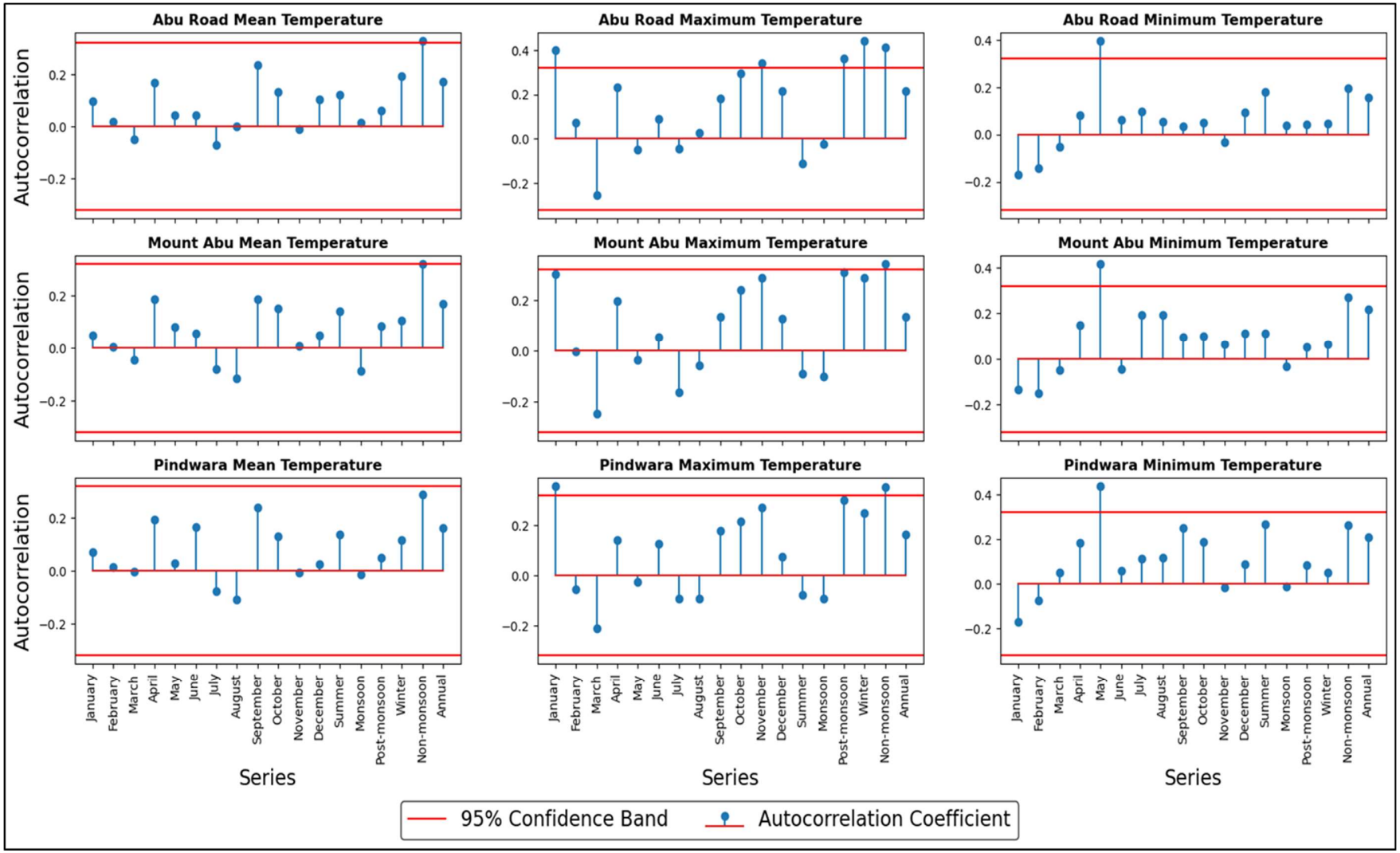

Figure 3: Lag 1 autocorrelation plots for all datasets

Table 1: MMK trends for temperature at Abu Road station

Time series	Mean			Maximum			Minimum		
	Z	↑/↓	%	*Z*	↑/↓	%	*Z*	↑/↓	%
January	-0.589	↓	3.95	-0.317	↓	3.57	0.052	↑	0.82
February	1.570	↑	10.18	1.033	↑	6.66	0.706	↑	25.79
March	1.546	↑	4.83	0.680	↑	3.60	0.209	↑	4.16
April	1.190	↑	4.15	0.837	↑	2.47	**2.171***	↑*	**21.30***
May	1.452	↑	4.34	0.301	↑	1.26	**2.433***	↑*	**17.60***
June	-1.818	↓	6.80	-0.392	↓	1.87	1.177	↑	5.66
July	-1.570	↓	6.25	-1.530	↓	7.85	1.112	↑	2.90
August	-1.177	↓	4.25	-1.779	↓	10.21	0.615	↑	2.37
September	-1.923	↓	11.10	-1.648	↓	15.87	-1.034	↓	9.12
October	0.955	↑	5.49	-1.805	↓	13.18	0.576	↑	6.11
November	0.170	↓	1.03	-0.994	↓	6.55	1.818	↑	23.44
December	-0.105	↓	1.67	0.078	↑	0.16	0.406	↑	9.384
Summer	1.478	↑	3.78	0.889	↑	2.29	**2.119***	↑*	**18.08***
Monsoon	**-2.511***	↓*	**7.12***	**-2.864***	↓*	**8.86***	1.242	↑	2.77
Post-monsoon	-0.275	↓	1.35	-1.373	↓	9.11	1.766	↑	14.86
Winter	0.296	↑	2.19	0.250	↑	2.07	1.462	↑	16.64
Non-monsoon	1.675	↑	2.61	-0.051	↓	0.57	**2.263***	↑*	**16.96***
Annual	-0.510	↓	1.37	-1.622	↓	4.186	**2.498***	↑*	**10.77***

N.B., *significant trend. Z = test statistic; ↑ = increasing; ↓ = decreasing; NT = no trend; % = Sen's slope as percentage change

Table 2: MMK trends for temperature at Mount Abu station

Time series	Mean			Maximum			Minimum		
	Z	↑/↓	%	*Z*	↑/↓	%	*Z*	↑/↓	%
January	-0.353	↓	2.19	0.550	↓	0.02	-0.065	↓	1.58
February	1.661	↑	11.34	1.177	↑	0.04	1.007	↑	29.06
March	1.385	↑	4.84	0.850	↑	0.3	0.144	↑	3.71
April	1.217	↑	4.09	1.020	↑	0.02	**2.093***	↑*	**19.29***
May	1.635	↑	5.02	0.523	↑	0.01	**3.074***	↑*	**23.05***
June	-1.740	↓	7.97	0.327	↓	0.01	0.419	↑	1.60
July	-1.204	↓	4.86	1.177	↓	0.04	1.060	↑	3.39
August	-0.065	↓	0.16	1.112	↓	0.02	1.060	↑	4.68
September	-1.426	↓	8.42	1.151	↓	0.06	-0.955	↓	9.20
October	-0.377	↓	1.19	1.177	↓	0.05	0.798	↑	8.02
November	0.693	↑	5.23	0.275	↓	0.007	1.753	↑	21.21
December	0.118	↑	1.38	1.386	↑	0.02	0.693	↑	23.54
Summer	1.609	↑	4.25	1.073	↑	0.02	**2.393***	↑*	**17.47***
Monsoon	**-2.067***	↓*	**1.14***	**2.132***	↑*	**0.03***	0.837	↑	2.87
Post-monsoon	0.157	↑	1.34	-0.876	↓	0.03	1.805	↑	16.17
Winter	0.907	↑	2.19	0.981	↑	0.02	**2.015***	↑*	**24.09***
Non-monsoon	**2.178***	↑*	**3.91***	0.562	↑	0.01	**4.241***	↑*	**18.24***
Annual	0.000	NT	0.001	0.719	↓	0.01	**2.603***	↑*	**11.73***

Mount Abu with an addition in the form of significantly increasing trends in temperature during the month of November, post-monsoon and winter season as well as during the non-monsoon period.With due consideration to the spatial consistency of trends across different stations, it could be said with certainty that the overall minimum temperatures were predominantly increasing.

Table 3: MMK trends for temperature at Pindwara station

Time series	Mean			Maximum			Minimum		
	Z	↑/↓	%	*Z*	↑/↓	%	*Z*	↑/↓	%
January	-0.188	↓	1.16	-0.396	↓	3.18	0.458	↓	16.72
February	1.530	↑	12.37	0.811	↑	6.14	0.955	↑	35.91
March	0.981	↑	6.36	0.785	↑	5.89	0.249	↑	6.57
April	1.465	↑	4.67	0.667	↑	1.73	**2.302***	↑*	**29.35***
May	1.347	↑	4.00	0.275	↑	1.11	**4.326***	↑*	**21.14***
June	-1.923	↓	8.52	-0.301	↓	0.83	0.353	↑	1.29
July	-1.269	↓	5.32	-1.452	↓	9.08	0.733	↑	2.04
August	-0.471	↓	2.22	-1.086	↓	5.98	0.955	↑	3.59
September	-1.487	↓	11.30	-1.177	↓	11.54	-1.347	↓	10.91
October	-0.794	↓	2.97	-1.426	↓	11.94	0.798	↑	8.46
November	0.798	↑	5.75	-0.262	↓	2.51	**2.210***	↑*	**34.32***
December	0.157	↑	1.28	0.249	↑	0.71	0.850	↑	23.06
Summer	1.530	↑	4.31	1.112	↑	2.50	**2.655***	↑*	**20.91***
Monsoon	**-2.433***	↓*	**7.26***	**-2.158***	↓*	**6.85***	0.327	↑	0.81
Post-monsoon	0.078	↑	0.43	-0.955	↓	6.84	**1.962***	↑*	**20.82***
Winter	1.211	↑	3.61	0.562	↑	2.37	**2.170***	↑*	**31.47***
Non-monsoon	**2.231***	↑*	**4.74***	0.248	↑	0.81	**4.436***	↑*	**22.93***
Annual	-0.013	NT	0.10	-1.321	↓	2.87	**2.603***	↑*	**13.27***

This rise, especially during the summer season, can cause formation of hard pans in regions of heavier soils within the study area, which is a factor boosting the possibility of flash floods as a result of rains in the subsequent monsoon season. Overall, the results apparently indicated an increase in the annual minimum temperatures over the basin. This was corroborated with the findings of Sharma *et al*. (2021) who investigated the temperature trends over five districts of Rajasthan and also reported an increase in annual minimum temperatures across four districts which included Udaipur, the district neighbouring the West Banas basin. The results indicated that overall rising trend in maximum temperatures for summer season and months of post-monsoon and falling trends for individual months during monsoon. Roy (2015) analysed the trends in temperatures in the state of Rajasthan over the past century and reported similar findings. According to Basistha et al. (2009), the observed long-term trends over a basin may be a direct consequence of many relatively smaller-scale factors such as declining forest area (Gupta *et al.,* 2005; Ray *et al.,* 2003; 2006), local land use changes (Pielke Sr. *et al.,* 2007; Ramankutty *et al.,* 2006) and increase in aerosol content due to anthropogenic activities (Ramanathan *et al.,* 2005; Sarkar and Kafkos, 2004). The falling temperatures during the monsoon season observed in the present study contradicted the assessment of Singh et al. (2008) who reported rising mean and maximum temperature trends at Abu Road. This suggested that the influence of aforementioned smaller-scale factors over the temperatures in the basin had become significant since the beginning of last decade (2010s). Increasing summer temperatures also promote the rise in atmospheric water content which may eventually lead up to erratic weather patterns and frequent extreme events especially during the subsequent rainy season (Hardwick Jones *et al.,* 2010; Lenderink *et al*., 2011; Molnar *et al.,* 2015; Utsumi *et al.,* 2011) which in turn may be responsible for the falling trends in temperature observed during monsoon season. The similarities between trends observed in the same time series (April, May and Monsoon) across different stations were consistent with the global climatic shift (Baines, 2006). This suggested that the basin environment and hydrometeorology was bound to gradually face the consequences of climate change. The monsoon season is critical for agriculture and water resources in India especially for an arid state such as Rajasthan, but rising global temperatures due to climate change have led to variabilities in monsoonal patterns that have significant implications for the region. For an agriculture-based economy, this may be viewed as a concerning matter based on the fact that any

extreme climatic variations would influence the spatial and temporal distribution of runoff (Ramanathan *et al.,* 2001), soil moisture and groundwater reserves (Raucher, 2011), and also the frequency of droughts and floods (Mirza, 2002; Sinha Ray and Srivastava, 2000), which can gradually have an impact upon the cropping patterns and productivity (Mall *et al.,* 2007). Malhi *et al.* (2021) also stated that these changes were likely to affect water availability and agricultural productivity.

Conclusion

The trend analysis of temperature conducted in this study provides important insights into the changing climate in the study area. The results indicate significant changes in temperature trends at certain stations, with rising minimum temperatures and decreasing mean and maximum temperatures. These trends may have important implications for various sectors, including agriculture and water resources, and may exacerbate the risk of flash floods during the monsoon season. Further research is required to assess the sensitivity of temperature variables with respect to individual factors whose influence over these variables has already been established through literature. This can aid in assigning priorities to the significant factors based on which, specific action plans may be formulated for each of these factors. Moreover, the interactions between temperature and other hydro-climatic variables (rainfall, discharge etc.) can be studied in order to analyse the sensitivity of one variable with respect to another and the combined effect of variations in these variables upon the entire basin is also of interest. The findings of this study are consistent with previous research on temperature trends in the region and highlight the need to better understand the environmental and socio-economic impacts of these changes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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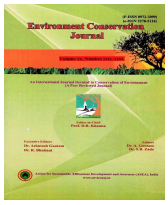

Agronomic biofortification with zinc and iron to enhance nutrient concentrations in mango

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ABSTRACT

Biofortification is a global-scale agricultural approach that can improve human nutrition. Agronomic biofortification is viewed as a quick-fix and supplemental approach. Agronomic biofortification, especially foliar application, is highly effective for zinc and iron. A field experiment on agronomic biofortification of zinc and iron micronutrients in mango cv. Kesar was carried out in 2016-2017 at the Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat). The experiment was arranged in a completely randomized design (CRD) with three replications containing 9 treatments. The results show that foliar application of $ZnSO_4$ and $FeSO_4$ (0.5% each) resulted in higher N (48.73 mg/100 g) and K (94.17 mg/100 g) in the pulp and P (0.056%) in the peel of mango. The iron (Fe) and zinc (Zn) contents in pulp and peel were highest in treatment T_9 (0.50% $FeSO_4$ + 0.50% $ZnSO_4$), which was on par with those in treatment T_8 (0.50% $FeSO_4$ + 0.25% $ZnSO_4$).

Introduction

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae. It is a significant fruit crop in Asia and has gained recognition on a global scale. It has long been associated with culture and religion as a helpful and delectable fruit. Mango is known as "The King of Fruits" due to its great nutritional content. Micronutrients are crucial for plant metabolism and have negative consequences when they are lacking, which makes them important for crop development. Micronutrients have a significant impact on plant development in addition to disease resistance in farmed crop species. Due to the widespread prevalence of micronutrient imbalance throughout India, the situation has taken a troublesome turn. Due to the significance of micronutrients in the diet of humans, it is anticipated that biofortification of horticulture crops will play an important and decisive role in addressing the nation's nutritional security in the years to come (Cakmak,2008). Iron is a crucial mineral and part of several proteins involved in metabolism and oxygen transport. The body typically regulates the amount of iron absorbed from the diet to keep its levels of iron regular. The greatest global nutritional problem, according to the WHO, is iron deficiency. Iron deficiency is responsible for approximately 50% of anemia worldwide. Globally, more than 2 billion people suffer from iron deficiency (Mehansho, 2006). In addition to being a vital component of many enzymes, zinc also plays a crucial role in cellular development and differentiation in tissues with a high rate of differentiation and turnover, such as the immune system and the digestive tract. The skin, brain, central nervous system, and immunological, skeletal, and reproductive systems are all impacted by zinc deficiency. Over 2 billion individuals, according to the World Health Organization

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(WHO), are believed to be zinc deficient. Approximately 20% of the world's population may be at danger of zinc insufficiency, according to estimates of zinc consumption and bioavailability obtained from FAO food balance statistics. Iron and zinc deficits were listed as two of the world's most significant health risks in the World Health Report from 2000.Increasing the content of vitamins in fruit while concurrently enhancing their bioavailability is the dual goal of biofortification, which aims to reduce human micronutrient deficiencies. It is a possible sustainable and cost-effective agronomic method to increase the micronutrient content of food. A future plan is to address micronutrient deficiencies globally. Conferring to estimates from the World Health Organization (Anon., 2000), biofortification of iron might help treat two billion individuals with anemia caused by iron deficiency. Maintaining enough zinc and iron delivery to the fruit throughout the reproductive stage and maintaining a sufficient amount of readily accessible zinc and iron in foliar solution appear to be key agronomic fortification strategies. It is a highly appealing and practical technique for efficiently addressing global health issues connected to iron and zinc deficiencies.

Material and Methods

Study Area: The experiment was carried out on a 20-year-old mango orchard planted at a 10 m×5 m distance, and the site was located at the Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat) from October 2016 to June 2017. The experimental site was situated at 20°57' north latitude, 72° 54' east longitude and has an altitude of 10 meters above the mean sea level. The analytical procedures employed for determining nutrients are presented in Table 1. The climate of this area is characterized by three well-defined seasons: monsoon, mild winter and summer. The monsoon commences from the second week of June and lasts up to the end of September. The intensity of rainfall is high during July and August. The details of the meteorological data with respect to maximum and minimum temperature, relative humidity, rainfall, etc., during the period of experimentation were obtained from the Agricultural Meteorological Observatory, College Farm, N. M College of Agriculture, NAU, Navsari.

Treatment details: The trial was directed in a completely randomized design (C.R.D.) with 9 treatments and replicated three times. The details of the experimental treatment plan employed in the present investigation are as follows: Control (T_1), 0.25% $FeSO_4$ (T_2), 0.50% $FeSO_4$ (T_3), 0.25% $ZnSO_4$ (T_4), 0.50% $ZnSO_4$ (T_5), 0.25% $FeSO_4$ + 0.25% $ZnSO_4$ (T_6), 0.25% $FeSO_4$ + 0.50% $ZnSO_4$ (T_7), 0.50% $FeSO_4$ + 0.25% $ZnSO_4$ (T_8), and 0.50% $FeSO_4$ + 0.50% $ZnSO_4$ (T_9) (Table 2). The foliar spray of these nutrients was performed at the time of flowering (21/12/2016), pea stage (17/02/2017) and egg stage (20/03/2017). For the preparation of Zn and Fe foliar solutions, commercial grade $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ fertilizers were used, respectively. The mandatory quantities of ferrous sulfate and zinc sulfate were weighed and dissolved in water, and then the pH of the solution was adjusted to 6.0 by using saturated $CaCO_3$ solution.

Table 1:- Analytical procedures employed for determining nutrients from pulp and peel of mango

Nutrient	Method employed	Reference
N	Wet digestion (Chromic acid)	Trivedi *et al.* (1999)
P and K	Wet digestion (diacid) followed by P: Spectrophotometric (Vanadomolybdophosphoric yellow color method) K: Flame photometric	Jackson (1973)
Fe, Mn, Zn and Cu	Atomic Absorption Spectrophotometer (AAS)	Elwell and Gridley (1967)

Table 2: Micronutrient fertilizers and water required for foliar spraying

SN	Treatments	Fertilizers required (g/20 Lit)	
		$FeSO_4.7H_2O$	$ZnSO_4.7H_2O$
1.	Control	-	-
2.	0.25% $FeSO_4$	91.4	-
3.	0.50% $FeSO_4$	182.8	-
4.	0.25% $ZnSO_4$	-	89
5.	0.50% $ZnSO_4$	-	178
6.	0.25% $FeSO_4$ + 0.25% $ZnSO_4$	91.4	89
7.	0.25% $FeSO_4$ + 0.50% $ZnSO_4$	91.4	178
8.	0.50% $FeSO_4$ + 0.25% $ZnSO_4$	182.8	89
9.	0.50% $FeSO_4$ + 0.50% $ZnSO_4$	182.8	178

Statistical analysis

The acquired data were statistically evaluated in accordance with the method (Panse and Sukhatme, 1967) suitable for a completely randomized design, and the treatment means were compared using critical differences at a 5% level of probability.

Results and Discussion

The N and K contents in the mango pulp were 48.73 mg/100 g and 94.17 mg/100 g, respectively, which were highest in the combined application of 0.50% FeSO4 + 0.50% ZnSO4 (T9) compared to the other treatments and controls. However, the P content did not show any significant response to foliar spraying on the Fe and Zn contents in the pulp of mango. Furthermore, the N and K contents in the peel of mango did not demonstrate significant variation by the foliar spray treatments applied. However, a significantly increased P content in the peel (0.056 ppm) was found in the foliar spray of 0.50% $FeSO_4$ and 0.50% $ZnSO_4$. Increased nutrient content in pulp and peel may be the result of foliar micronutrient spray, which reduced nutritional deficits and enhanced fruit quality due to absorption of macro- and micronutrients in tissues and organs of mango plants. Singh *et al.* (2020) found a similar effect with mango. The maximum zinc (Zn) content and iron (Fe) content in the pulp and peel of mango cv.

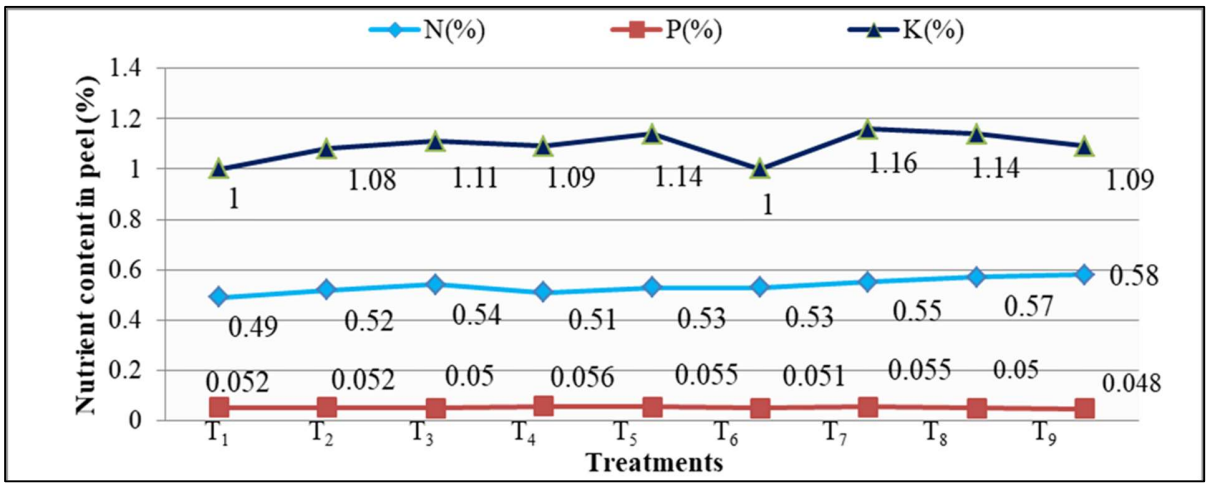

Figure 1: Effect of foliar spray of Zn and Fe on major nutrient content in peel of mango cv. Kesar

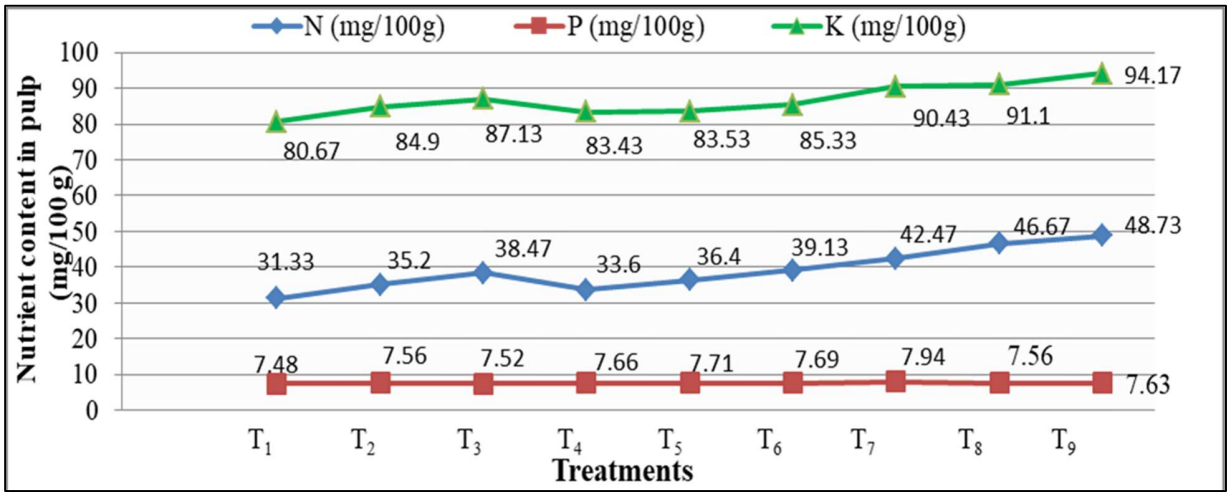

Figure 2: Effect of foliar spray of Zn and Fe on micro nutrient content in pulp of mango cv. Kesar

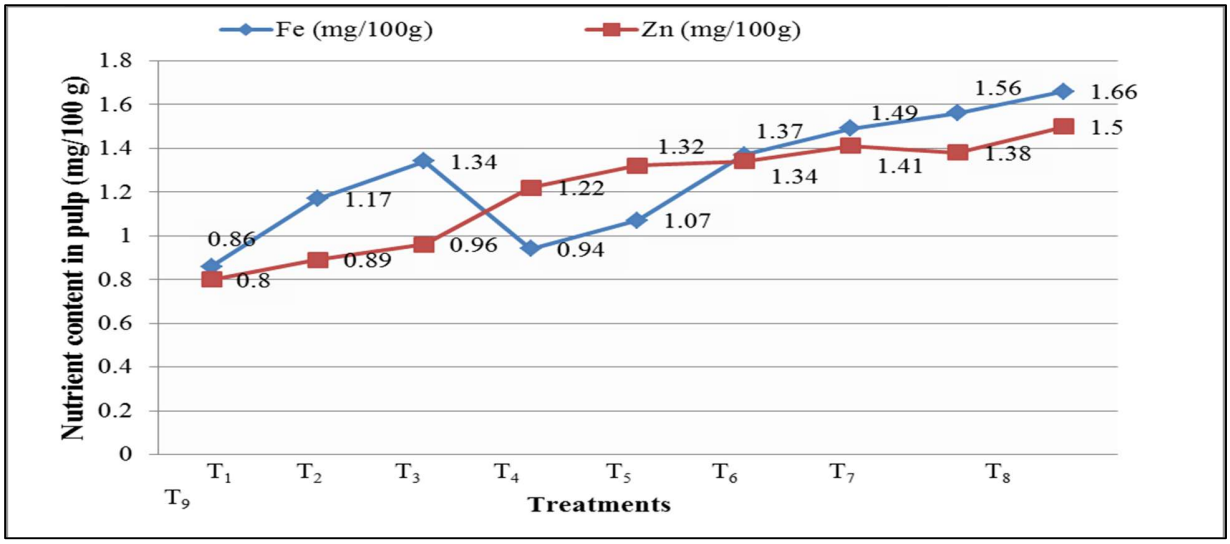

Figure 3: Effect of foliar spray of Zn and Fe on micro nutrient content in pulp of mango cv. Kesar.

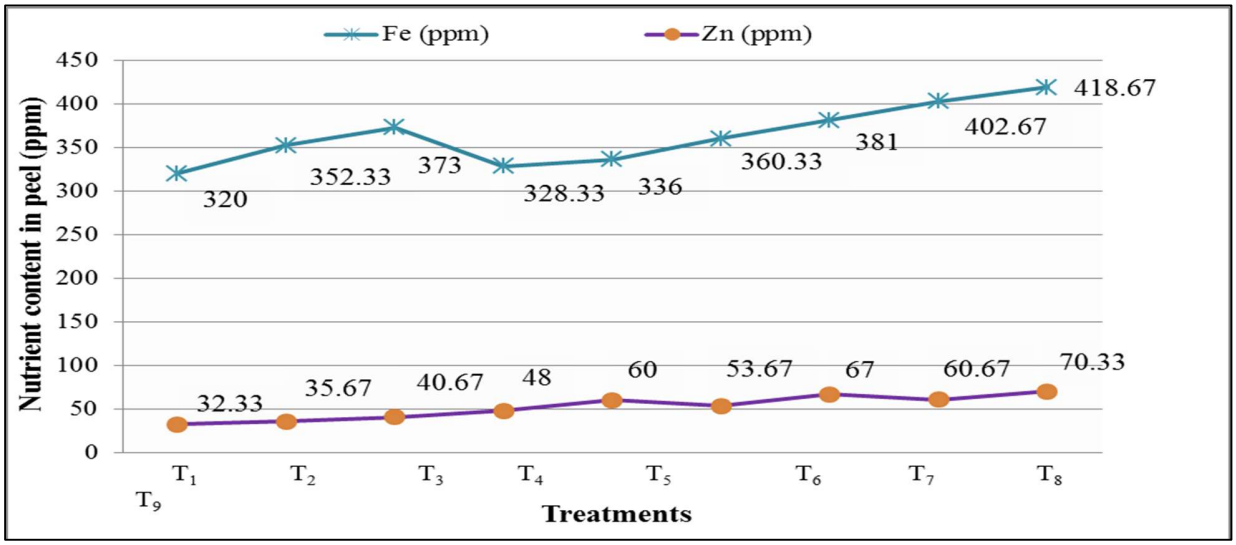

Figure 4: Effect of foliar spray of Zn and Fe on micro nutrient content in peel of mango cv. Kesar.

Kesar were recorded in the treatment combination of 0.50% $FeSO_4$ and 0.50% $ZnSO_4$ (T_9). In 2020, Li Li and his coworkers reported that the pulp may have been a greater sink for zinc than the other mango components. Fruits and leaves quickly absorb iron, increasing their capacity for concentration and uptake. Auxin and protein synthesis, seed development, and the activation of several enzymes are all impacted by zinc. All of these activities promote zinc absorption. The results of nutrient absorption closely support the findings of Sultana *et al.* (2018) in wheat, Chhetri *et al.* (2017) in mandarin orange, and Dhaliwal *et al.* (2021) in lentil.

Conclusion

The results of the study indicate that the foliar application of 0.50% FeSO4 and 0.50% ZnSO4 nearly quadrupled the content of N, K, and P in pulp and peel, as well as Fe and Zn in pulp and peel. The quality of harvested crops pre- and postharvest is one advantage of foliar treatments. For even better results, this experiment can be improved by applying nanotechnology to the

leaves. However, this study will contribute to reducing malnutrition.

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Conflict of interest

The authors declare that they have no conflict of interest.

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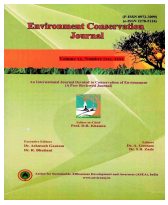

Dynamics of bell pepper using bio nutrient sources in the northwestern Himalayas

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Sustainable agriculture

ABSTRACT

Bionutrients play a vital role in enhancing soil productivity and sustainable agricultural production. In vegetable crops, limited information is available on the relevance of bionutrients in solanaceous crops under protected conditions. Therefore, an experiment was planned to study the response of bionutrients under the modified naturally ventilated polyhouse in mid-hill conditions of Himachal Pradesh for two consecutive years. Various bell pepper varieties, *viz.*, Mekong, Orobelle, Indra and DPCY1, were subjected to a set of bionutrient sources (*beejamrit*, *ghanjeevamrit*, jeevamrit and mulching). The results showed that there was a substantial increase in yield parameters in the treatment module, i.e., Mekong + *beejamrit*@ 200 ml/kg + *ghanjeevamrit*@5q/ha + *jeevamrit* @ 500 lt/ha at 21-day intervals + mulching @ 10 t/ha. This treatment exhibited a minimum number of days to 50% flowering (24.16), maximum number of marketable fruits per plant (28.40), fruit length (7.68 cm), fruit breadth (7.70 cm), pericarp thickness (9.15 mm), average fruit weight (109.53 g), plant height (84.06 cm) and marketable yield per plant (3.11 kg). However, Mekong + *beejamrit* @ 200 ml/kg + *ghanjeevamrit* @5q/ha + *jeevamrit*@ 500 lt/ha at 28-day intervals + mulching @ 10 t/hattreatment proved best for total soluble solids (4.58 °Brix), ascorbic acid (166.50 mg/100 g), capsaicin content (6.64%) and carotenoid content (2.43 mg/100 g). Horticultural and biochemical traits were appreciably enhanced after bionutrient application in bell pepper. Therefore, outcomes from the study point out that it is a feasible and economical approach for farmers.

Introduction

Modern chemical-based crop growing is increasing radically to meet the demands of the ever-growing population by enhancing crop productivity. The use of these chemicals in agriculture leads to enormous environmental and health issues. Considering these facts, various alternative and ancient farming techniques were developed for sustainable agriculture. Sustainable agriculture practices enable food production without compromising the needs of future generations (World Bank, 2017). Among all the techniques, natural farming is a neoteric approach to improve both traditional and modern agricultural practices, which aims to safeguard the environment, public health and communities (Mishra, 2013). Zero-budget natural farming (ZBNF) is also an agroecological approach that has

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attained popularity because it increases agricultural production without any expenses and eliminates the cost of production. *Jivamrit*, *beejamrit,* mulching and *whapasa* –aeration are the main pillars of ZBNF (Khadse and Rosset, 2019). In vegetables, bionnutrients help in the conversion of nutrients from unavailable to available forms in the plant rhizosphere, act as antagonists to pathogens and provide toxic free food to consumers. The demand for fresh vegetables is increasing owing to the growth of a health-conscious population and, more generally, through enhanced income (Ramesh *et al.,* 2005; Sharma *et al.,* 2021).Capsicum or bell pepper (*Capsicum* spp.) is a very important vegetable as well as spice crop belonging to the family Solanaceae. It is also known as night shade and nontraditional vegetable (Kalloo and Pandey, 2002). Bell pepper is thought to have originated in the American tropics, i.e., tropical South America (Hunziker, 2001). Capsicum is cultivated all over the world for fresh, dried, and processed products (Kurubetta and Patil, 2009; Bijalwan *et al.,* 2022a, b). The crop has attained an important status and special significance in the mid-hills of Himachal Pradesh and is also cultivated as an off-season crop during the summer months (Bijalwan *et al.,* 2021). The genus Capsicum includes over 30 species, five of which (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) are domesticated and mainly grown for consumption purposes (Tripodi and Kumar, 2019). It is a high-value and low-volume crop suitable for open fields as well as protected environments in India (Hernandez-Aranda *et al.,* 2021). In both developed and developing countries, there is a need to adopt new approaches to increase food supplies while protecting the resources on which they depend (FAO, 2017). The application of *jeevamrit*, *ghanjeevamrit*, *beejamrit* and mulching enhances soil fertility and increases microorganism activity in soil, which ultimately boosts crop yield. The application of bionutrients improves the total soluble solids, vitamin C, and capsaicin contents and increases the production of bell pepper (Malawadi*et al.,*2003; Sharma *et al.,* 2022). In vegetable crops, very limited research has documented the effect of different combinations of bionutrients on horticultural and biochemical parameters in Capsicum. Therefore, the present study was investigated to identify the best bionutrient combination and variety to maximize the yield under protected conditions in mid-hill conditions.

Material and Methods

Experimental location, plant material and detail of bio nutrient treatments

This study was conducted at the Research Farm, Department of Vegetable Science and Floriculture (N 32°6′, E 76°3′), CSK HPKV Palampur, from 2019-2020. Agro-climatically, it is located in the mid-hill region and has a humid subtemperate climate with 2,500 mm of annual rainfall. The experiment consisted of twelve treatments of foliar application of *jeevamrit*, soil application of *ghanjeevamrit*, seed treatment with *beejamrit* and soil application of mulching (Table 1), and the effects of twelve combinations of bio nutrients on the horticultural and biochemical traits of four Capsicum genotypes (Mekong, Orobelle, Indra and DPCY1) were studied. The experiment was conducted under a randomized complete block design inside a modified naturally ventilated polyhouse (25 m × 10 m) and replicated three times. The plants of each treatment were planted at a spacing of 70 × 30 cm and trained on four stems in each replication.

Observations of horticultural traits

Observations were recorded from five selected plants in each replication in each treatment on various horticultural traits, *viz.*, days to 50% flowering, number of marketable fruits per plant, fruit length (cm), fruit breadth (cm), pericarp thickness (mm), average fruit weight (g), marketable yield per plant (kg) and plant height (cm). All these parameters were taken as suggested by Shilpa *et al*. (2022).

Observations of biochemical traits

Examination of various biochemical traits, viz., total soluble solids (°brix), ascorbic acid (mg/100 g), capsaicin content (%) and carotenoid content (mg/100 g), was performed in the laboratory by Shilpa *et al*. (2022). Total soluble solids (°Brix) were determined using a hand refractometer. The ascorbic acid contents were estimated using 2,6-dichlorophenol indophenol as suggested by Ranganna (1979) and used by Shilpa *et al*. (2022). Capsaicin content was estimated in fresh fruits using Folin-Cicocalteau reagent as described by Gougoulias, (2017).

Table 1: Details of treatment combinations

Treatment code	Treatment combinations
T_1	Mekong + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 14 days interval + Mulching @ 10 t/ha
T_2	Orobelle + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 14 days interval + Mulching @ 10 t/ha
T_3	Indra + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 14 days interval + Mulching @ 10 t/ha
T_4	DPCY1+ Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 14 days interval + Mulching @ 10 t/ha
T_5	Mekong + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 21 days interval + Mulching @ 10 t/ha
T_6	Orobelle + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 21 days interval + Mulching @ 10 t/ha
T_7	Indra + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 21 days interval + Mulching @ 10 t/ha
T_8	DPCY1 + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 21 days interval + Mulching @ 10 t/ha
T_9	Mekong + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 28 days interval + Mulching @ 10 t/ha
T_{10}	Orobelle + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 28 days interval + Mulching @ 10 t/ha
T_{11}	Indra + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 28 days interval + Mulching @ 10 t/ha
T_{12}	DPCY1 + Beejamrit @ 200 ml/kg + Ghanjeevamrit @5q/ha + Jeevamrit @ 28 days interval + Mulching @ 10 t/ha

Carotenoid analysis was performed by HPLC as suggested by Cervantes-Paz *et al*. (2014).

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) by using SPSS Statistical Computer Package (SPSS for Windows, Standard Version 20.0).

Results and Discussion

Effect of bionutrients on horticultural traits of bell pepper

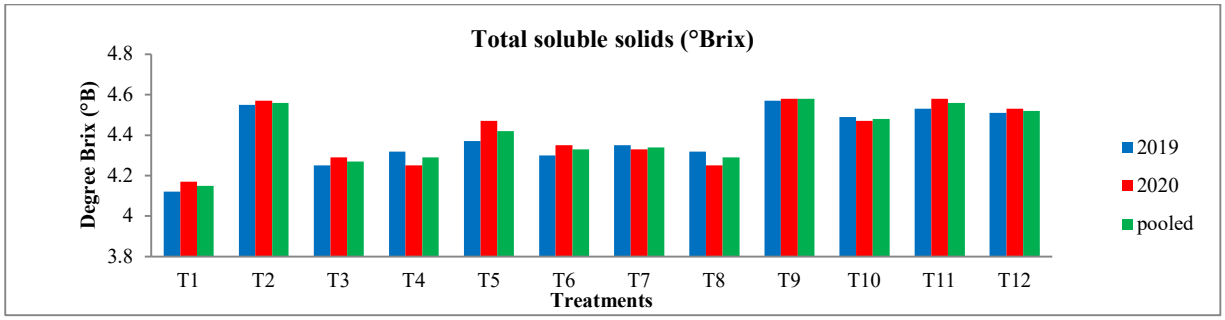
In the current study, pooled data reflect the growth and yield of capsicum, as depicted in Table 2. From the point of view of bionutrient application, beejamrit, ghanjeevamrit, jeevamrit and mulching have significant effects on the growth and yield characteristics of bell pepper. The results shown in Table 2 indicate that the treatment combination comprising Mekong + *beejamrit* @ 200 ml/kg + *ghanjeevamrit*@5q/ha + *jeevamrit*@ 21 days interval + mulching @ 10 t/ha significantly influenced all the evaluated horticultural traits of bell pepper. The application of jeevamrit at 21-day intervals along with other bionutrient sources for the Mekong variety has a significant effect on yield parameters. The analysis of variance showed that the T_5 treatment had a significant effect on the minimum number of days for 50% flowering (24.16), maximum number of marketable fruits per plant (28.40), average fruit weight (109.53 g), fruit length (7.68 cm), fruit breadth (7.70 cm), pericarp thickness (9.15 mm), marketable yield per plant (3.11 kg) and plant height (84.06 cm). Similarly, foliar application of jeevamat at 21-day intervals for the variety Indra resulted in the second highest yield (3.05 kg/ha), minimum number of days for 50% flowering (25.75), maximum number of marketable fruits per plant (27.94), average fruit weight (109.18 g), fruit length (7.39 cm), fruit breadth (7.42 cm), pericarp thickness (9.11 mm), marketable yield per plant (3.05 kg) and plant height (82.58 cm) in the T_7 treatment.

Effect of bionutrients on biochemical traits

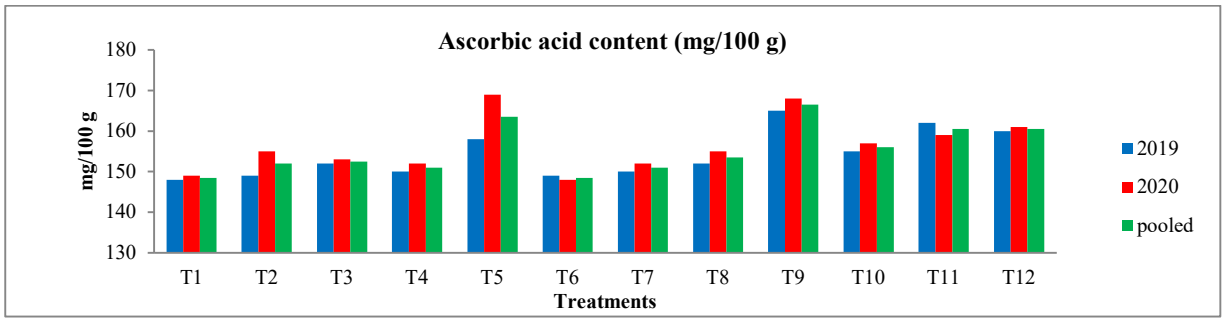
Fig. 1 shows the effects of bionutrient application on total soluble solids, ascorbic acid content, capsaicin content and total carotenoids in bell pepper. From the perspective of the application of bionutrients in the four different varieties, the application of jeevamrit at 28-day intervals for the variety Mekong (T_9) had a significant effect on the biochemical parameters of bell pepper along with other sources of nutrients. Under the conditions of bionutrient spray and drenching, the analysis of variance showed that the T9 treatment had a significant effect on the total soluble solids (4.58 °Brix), ascorbic acid content (166.50 mg/100 g), capsaicin content (6.64%) and carotenoid content (2.43 mg/100 g) of bell pepper. Bionutrients play a vital role in plant growth and yield in vegetable crops. It is an agroecological-based diversified farming approach attaining popularity due to harmony in nature (Rosset and Martinez-Torres, 2012; Kumar *et al.,* 2019; Shilpa *et al.,* 2023; Shilpa *et al.,* 2020). It allows functional biodiversity by reducing the use of agrochemicals

Table 2: Days to 50% flowering, number of marketable fruits per plant, fruit length, fruit breadth, pericarp thickness, average fruit weight, marketable yield per plant (kg) and plant height (cm) of Bell Pepper as influenced by the application of bionutrients.

Sr No.	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
Treatments	**Days to 50% flowering**			**Number of marketable fruits per plant**			**Fruit length (cm)**			**Fruit breadth (cm)**			**Pericarp thickness (mm)**			**Average fruit weight (g)**			**Marketable yield per plant (kg)**			**Plant height (cm)**		
T_1	34.13	31.16	**32.65**	22.63	23.76	**23.20**	6.77	6.79	**6.78**	6.89	6.98	**6.94**	8.46	8.58	**8.52**	87.96	87.54	**87.95**	1.99	2.08	**2.04**	74.25	76.52	**75.39**
T_2	36.49	35.23	**35.86**	21.13	21.69	**21.41**	6.44	6.54	**6.49**	6.59	6.61	**6.60**	8.21	8.27	**8.24**	75.74	82.55	**79.42**	1.60	1.79	**1.70**	71.25	72.25	**71.75**
T_3	35.17	32.18	**33.68**	22.08	22.61	**22.34**	6.64	6.56	**6.60**	6.66	6.87	**6.77**	8.35	8.44	**8.40**	85.62	87.59	**86.84**	1.89	1.98	**1.94**	73.78	75.58	**74.68**
T_4	36.11	34.00	**35.06**	21.56	22.14	**21.85**	6.53	6.60	**6.57**	6.58	6.68	**6.63**	8.22	8.35	**8.29**	82.10	84.91	**83.75**	1.77	1.88	**1.83**	72.81	73.38	**73.10**
T_5	24.32	24.00	**24.16**	28.17	28.63	**28.40**	7.58	7.77	**7.68**	7.55	7.85	**7.70**	9.12	9.17	**9.15**	108.29	110.74	**109.53**	3.05	3.17	**3.11**	82.23	85.89	**84.06**
T_6	27.71	26.16	**26.94**	27.06	27.56	**27.31**	7.02	7.21	**7.12**	7.11	7.25	**7.18**	8.88	8.85	**8.87**	104.23	108.15	**106.21**	2.82	2.98	**2.90**	79.00	79.85	**79.43**
T_7	26.26	25.24	**25.75**	27.87	28.00	**27.94**	7.32	7.45	**7.39**	7.38	7.45	**7.42**	9.10	9.11	**9.11**	107.64	110.71	**109.18**	3.00	3.10	**3.05**	81.87	83.28	**82.58**
T_8	27.04	26.11	**26.58**	27.74	27.99	**27.87**	7.25	7.42	**7.34**	7.28	7.38	**7.33**	8.97	8.98	**8.98**	107.45	102.54	**105.15**	2.98	2.87	**2.93**	80.25	81.10	**80.68**
T_9	27.84	26.54	**27.19**	26.59	27.00	**26.80**	7.00	7.20	**7.10**	7.09	7.25	**7.17**	8.75	8.84	**8.80**	104.19	106.30	**105.24**	2.77	2.87	**2.82**	78.45	79.98	**79.22**
T_{10}	30.12	28.98	**29.55**	25.04	25.50	**25.27**	6.82	6.87	**6.85**	6.98	7.00	**6.99**	8.56	8.67	**8.62**	106.23	105.49	**106.05**	2.66	2.69	**2.68**	75.58	76.68	**76.13**
T_{11}	28.37	27.98	**28.18**	26.13	26.62	**26.38**	6.98	7.00	**6.99**	7.05	7.11	**7.08**	8.68	8.77	**8.73**	103.71	104.06	**103.89**	2.71	2.77	**2.74**	77.49	78.87	**78.18**
T_{12}	29.41	28.63	**29.02**	25.58	26.13	**25.85**	6.85	6.98	**6.92**	7.00	7.20	**7.10**	8.62	8.71	**8.67**	104.79	103.35	**104.06**	2.68	2.70	**2.69**	76.58	77.58	**77.08**
CD (0.05)	**0.75**			**0.40**			**0.07**			**0.07**			**0.04**			**1.57**				**0.07**		**0.71**		



(a)



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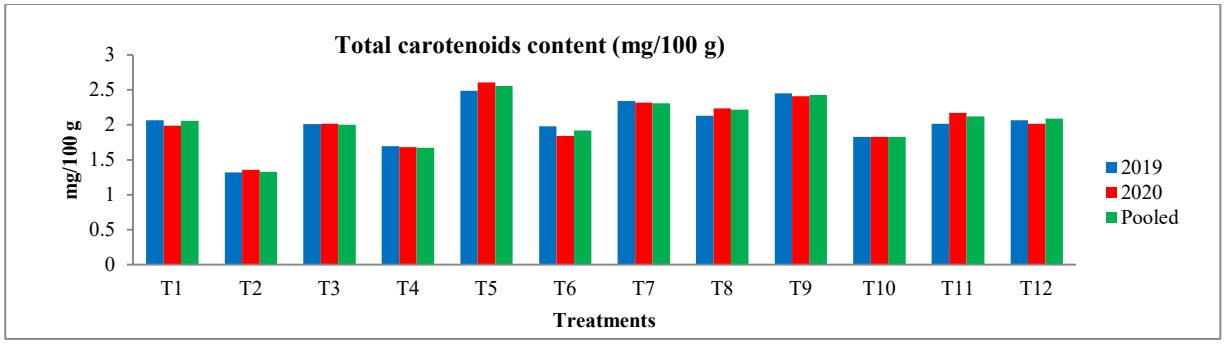

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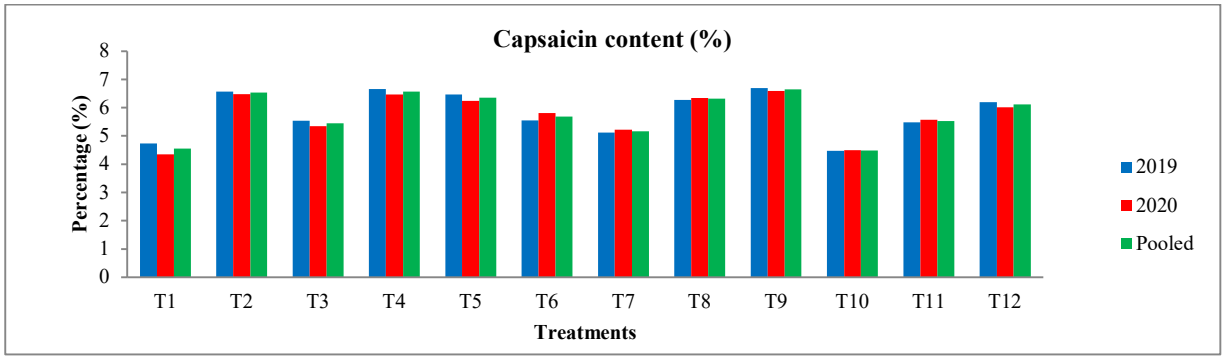

(d)

Figure 1. Application of bionutrients on biochemical traits (a) Total soluble solids (degree Brix) (b) Ascorbic acid content (mg/100 g) (c) Total carotenoid content (mg/100 g) (d) Capsaicin content (%)

for sustainable crop production. *Beejamrit,* heghanjeevamrit, jeevamrit and mulching stimulate microflora, microfauna and nutrient bioavailability and therefore act as antagonists for fungal pathogens (Palekar, 2006; Vasanthkumar, 2006; Devakumar*et al.,* 2008; Lorimer, 2017; Khadse and Rosset, 2019). Very little information is available regarding the application of different bionutrients with varied concentrations at different intervals of days and genotypes, which could enhance yield and improve fruit quality.

The present study demonstrated that the application of *beejamrit* (@ 200 ml/kg), *ghanjeevamrit* (@5q/ha), jeevamrit applied at 21-day intervals and mulching (@ 10 t/ha) in the Mekong variety proved best for horticultural traits, *viz*., number of days for 50% flowering, number of marketable fruits per plant, average fruit weight, fruit length, fruit breadth, pericarp thickness, marketable yield per plant and plant height due to the large amount of beneficial microbes present in *jeevamrit*, higher decomposition of organic matter, availability of nutrients and their utilization by plants from the soil, resulting in better growth and development. Among all the treatments, the combination of *beejamrit* (200 ml/kg), Ghanjeevamrit (5 q/ha), and Jeevamrit at 28-day intervals and mulching (10 t/ha) in the Mekong variety significantly improved biochemical parameters in terms of total soluble solids, ascorbic acid content, capsaicin content and total carotenoids.

Earlier scientists have also documented increased growth, yield and biochemical traits with augmentation of various bionutrients in vegetable crops (Arancon*et al.,* 2005, Shwetha and Babalad, 2008; Joshi and Pal Vig, 2010; Ramesh *et al.,* 2015; Adhikari *et al.,* 2016; Boraiah*et al.,* 2017; Bairwa*et al.,* 2018; Hameedi, 2018; Kumar *et al.,* 2021; Shilpa *et al*. 2022a, b, c). The probable reason for improved quality includes efficient use of nutrients at different growth stages of a plant, higher source to sink ratio and carbohydrate accumulation in the plant tissues. Bionutrients constitute smaller quantities of plant growth regulators (IAA and GA), which create stimuli in plant systems, which in turn enhance growth and development, leading to better fruit yield and quality (*Chandrakala et al.,* 2011 and *Vij et al.,* 2022). Cow urine rich in uric acid, a source of nitrogen, is a readily soluble and liquid form, one of the important compounds in bionutrients that is readily available to plants and directly influences the nitrogen content (Patel *et al.,* 2018). Mulching includes live mulch, soil mulch and straw mulch, which helps to alter the environment surrounding the plant rhizosphere. Mulches have additional utilization, as after their degradation, nutrients are incorporated into the soil; therefore, plant-based residue mulch material ensures a significant enhancement in yield (Jilani*et al.,* 2016; Awal*et al.,*2016; Saroch*et al.,* 2016; Bairwa*et al.,* 2018; Hernandez-Aranda *et al.,* 2021; Shilpa *et al.,* 2021a,b,c,d)

Conclusion

In the present study, various bionutrients showed significant variation in horticultural and biochemical traits in bell pepper. The presence of beneficial microorganisms, synthesis of phytohormones, conversion of unavailable forms of nutrients to available forms and higher organic carbon content in bionutrients predominantly marked satisfactory responses. Therefore, bionutrients meritoriously increased the yield potential and are recommended as an alternative natural farming practice for the cultivation of bell pepper under protected conditions in the northwestern Himalayan region.

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Conflict of interest

The authors declare that they have no conflict of interest.

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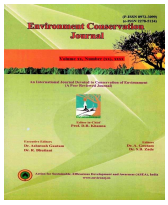

Study on avifauna and species richness in Karanja-Sohol wildlife sanctuary, (MS) India

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ABSTRACT

The present study was carried out during a bird race organized in Karanja-Sohol Wildlife sanctuary Karanja, district Washim Maharashtra, India. The objective of this study was to assess the avifauna of wildlife sanctuaries. Due to the geological and ecological complexity of the area, it has become imperative to conduct research on the region's avian diversity. It is challenging to conduct this assessment, as the wildlife sanctuary is divided into numerous patches by the state highway running through it. Sanctuary is surrounded by numerous villages and agricultural land, which creates human interference and livestock grazing. This checklist will serve as a baseline for further study, as there is no published checklist of this area. In the present investigation, we reported a total of 151 bird species during the exhaustive survey by the authors and accompanied volunteers during the census organized by the wildlife department. The observed species of birds belong to 55 families and 17 orders. They also recorded their residential and IUCN red data status.

Introduction

Birds are environmental indicators, and a slight change in environmental conditions can impact their behavior pattern, population, reproduction and migration. (Harisha and Hosetti, 2009). Ecologically, birds are very important creatures because they help in pollination and perform crucial roles in seed dispersal (Bibi and Ali, 2013). Therefore, it is crucial to comprehend the diversity and structure of birds to describe the local landscape. Species are becoming extinct at an alarming rate, and their conservation has arisen as one of the most significant issues today (Hu *et al*., 2011). Forests provide a safe habitat and plenty of food for birds, so avifauna are attracted to such areas. Wildlife sanctuaries, national parks and biosphere reserves, such as protected areas, serve as supporting systems for biodiversity conservation and play crucial roles in maintaining ecological balance and thereby offsetting adverse climate change (De Fries *et al*., 2007). As one of the super biodiverse nations, 24.62% of India's total territory is covered by forests (India State of Forest Report., 2021), with 1364 extant and extinct bird species (Lepage, Denis, 2021). The third-largest state by geographical size, Maharashtra, has 62952 square kilometers of forest (India, State Forest Report, 2021). The Karanja-Sohol wildlife sanctuary is notified by Govt. of Maharashtra vide notification

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dated 07 December 2000. It covers 1778.40 hectares (17.78 sq km) of forest and grassland (District Survey Report for Washim District, 2019). This Sanctuary is famous for blackbucks, i.e., the Indian Antelopes. According to the Wildlife Protection Act (Anon, 1972), these creatures are listed in Schedule I. The sanctuary is divided into three parts separated by state highways. Two parts are located across the Karanja-Manora highway, and the third part is across the Karanja-Darva state highway (Ingle *et al.,* 2020). Aadan is the main river of the sanctuary. The sanctuary is named after the town Karanja, which is its Tehsil place, and Sohol is the village nearest to it. This sanctuary is also part of the catchment area of the Aadan reservoir, which provides great habitat for water birds. The landscape of the sanctuary is undulating, and it offers an interesting contrast between plateau and plain. The five districts that make up the West Vidarbha area are Akola, Amravati, Buldhana, Washim, and Yavatmal. Dry deciduous woodland is prevalent in this area. According to the 2009 State of Forest Report, the soil is mostly categorized as loamy soil, brown soil, and black cotton soil.The Washim District is home to a wide variety of ecosystems, including wetlands formed by man-made dams, the Karanja-Sohol grassland, and forestland from the Katepurna Wildlife Sanctuary.

Grasslands cover approximately 30% of the Earth's terrestrial portion (Blair, 2014). Grasslands provide ecosystem services, including watershed protection and grazing as well as habitat for wildlife, the occurrence of rare species, and inherent ecological aspects of structure, function, and composition (Faber-Langendoen and Josses, 2010). Birds have been regarded as indicator species of settled regions since they are frequently prevalent occupants of such ecosystems. (Blair, 1999). The selected area is rich in avifaunal diversity. Monitoring bird diversity is planned to undertake a study of this area's bird study.

Material and Methods

Observations for avifaunal diversity of the Karanja-Sohol wildlife sanctuary with coordinates 20.40076936503589 N, 77.49977354742815 E were carried out in two sessions, i.e., morning from 6.00 am to 01.00 pm and evening from 04.00 pm to 07.30 pm when birds were found to be most active, on 7th and 8th November 2020 on the occasion of a bird week. This survey was organized by the Akola wildlife division in collaboration with two NGOs, namely, Nisargakatta Akola and Watsagulma Biodiversity Conservation Society, Washim. Approximately 32 volunteers were present during the census program. All volunteers were divided into four groups led by at least one of the authors. One of the members from each group was equipped with an e-Bird android application developed by Cornell Lab of ornithology (https://www.birds.cornell.edu/home/), and after the survey, the checklist was submitted to the database successfully.https://ebird.org/tripreport/52507.Each track's and team's data were recorded independently and then combined to generate a comprehensive sanctuary checklist. During the study, birds were ascertained by direct sighting and by their calls (for very few species). Bird surveys on both days during their active hours were carried out by adopting the line-transect method (Burnham *et al*., 1980). Birds were observed with Nikon A211 10 X 50 binoculars, Comet, and Zeiss 10x42, and field guides were used for identification. (Ali, 2009; and Grimmett *et al*., 2010). Other details, such as species richness and hazards to the conservation of birds, were also highlighted throughout the surveys. Their residential status and The International Union for the Conservation of Nature (IUCN) status findings were also noted with the help of Kazmierczak *et al*. (2000).

Table 1: Checklist of Bird Species of Karanja Sohol Wildlife Sanctuary

SN	Common Name	Scientific name	Family	Order	R/M	Status
1	Oriental Honey Buzzard	*Pernis ptilorhynchus*	Accipitridae	Accipitriformes	R	LC
2	Pallid Harrier	*Circus macrourus*	Accipitridae	Accipitriformes	W	NT
3	Bar Headed Goose	*Anser indicus*	Anatidae	Anseriformes	W	LC
4	Ruddy Shelduck	*Tadorna ferruginea*	Anatidae	Anseriformes	W	LC
5	Comb Duck	*Sarkidiornis sylviola*	Anatidae	Anseriformes	R	LC
6	Indian Spot-Billed Duck	*Anas poecilorhyncha*	Anatidae	Anseriformes	R	LC
7	Green-Winged Teal	*Anas crecca*	Anatidae	Anseriformes	W	LC

8	Northern Pintail	*Anas acuta*	Anatidae	Anseriformes	W	LC
9	Northern Shoveler	*Spatula clypeata*	Anatidae	Anseriformes	W	LC
10	Common Pochard	*Aythya ferina*	Anatidae	Anseriformes	W	LC
11	Common Pochard	*Aythya fuligula*	Anatidae	Anseriformes	W	LC
12	Gadwall	*Mareca strepera*	Anatidae	Anseriformes	W	LC
13	Eurasian Wigeon	*Mareca penelope*	Anatidae	Anseriformes	W	LC
14	Indian Night Jar	*Caprimulgus asiaticus*	Caprimulgidae	Caprimulgiformes	R	LC
15	Little Ring Plover	*Charadrius dubius*	Charadriidae	Charadriiformes	R	LC
16	Yellow-Wattled Lapwing	*Vanellus malabaricus*	Charadriidae	Charadriiformes	R	LC
17	Red-Wattled Lapwing	*Vanellus indicus*	Charadriidae	Charadriiformes	R	LC
18	Black Tailed Godwit	*Limosa limosa*	Scolopacidae	Charadriiformes	W	NT
19	Common Snipe	*Gallinago gallinago*	Scolopacidae	Charadriiformes	W	LC
20	Common Sandpiper	*Actitis hypoleucos*	Scolopacidae	Charadriiformes	W	LC
21	Common Red Shank	*Tringa totanus*	Scolopacidae	Charadriiformes	W	LC
22	Little Stint	*Calidris minuta*	Scolopacidae	Charadriiformes	W	LC
23	Black Winged Stilt	*Himantopus himantopus*	Recurvirostridae	Charadriiformes	R	LC
24	Eurasian Thick-Knee	*Burhinus oedicnemus*	Burhinidae	Charadriiformes	R	LC
25	Great Thick-Knee	*Esacus recurvirostris*	Burhinidae	Charadriiformes	R	LC
26	Small Pratincole	*Glareola lactea*	Glareolidae	Charadriiformes	R	LC
27	River Tern	*Stema aurantia*	Laridae	Charadriiformes	R	NT (U)
28	Green Sandpiper	*Tringa ochropus*	Scolopacidae	Charadriiformes	W	LC
29	Wood Sandpiper	*Tringa glareola*	Scolopacidae	Charadriiformes	W	LC
30	Barred Buttonquail	*Turnix suscitator*	Turnicidae	Charadriiformes	R	LC
31	Black Headed Gull	*Chroicocephalus ridibundus*	Laridae	Charadriiformes	W	LC
32	Brown Headed Gull	*C. brunnicephalus*	Laridae	Charadriiformes	W	LC
33	Little Egret	*Egretta garzetta*	Ardeidae	Ciconiiformes	R	LC
34	Cattle Egret	*Bubulcus ibis*	Ardeidae	Ciconiiformes	R	LC
35	Gray Heron	*Ardea cinerea*	Ardeidae	Ciconiiformes	W	LC
36	Purple Heron	*Ardea purpurea*	Ardeidae	Ciconiiformes	R	LC
37	Indian Pond Heron	*Ardeola grayii*	Ardeidae	Ciconiiformes	R	LC
38	Painted Stork	*Mycteria leucocephala*	Ciconiidae	Ciconiiformes	R	NT
39	Asian Open Bill	*Anastomus oscitans*	Ciconiidae	Ciconiiformes	R	LC
40	Asian Wooly Necked Stork	*Ciconia episcopus*	Ciconiidae	Ciconiiformes	R	LC
41	Black Headed Ibis	*Threskiornis melanocephalus*	Threskiornithidae	Ciconiiformes	R	NT
42	Red-Naped Ibis	*Pseudibis papillosa*	Threskiornithidae	Ciconiiformes	R	LC
43	Eurasian Spoonbill	*Platalea leucorodia*	Threskiornithidae	Ciconiiformes	R	LC
44	Great Egret	*Ardea alba*	Ardeidae	Ciconiiformes	R	LC
45	Intermediate Egret	*Ardea intermedia*	Ardeidae	Ciconiiformes	R	LC
46	Yellow Footed Green Pigeon	*Treron phoenicoptera*	Columbidae	Columbiformes	R	LC
47	Rock Pigeon	*Columba livia*	Columbidae	Columbiformes	R	LC
48	Eurasian Collared Dove	*Streptopelia decaocto*	Columbidae	Columbiformes	R	LC
49	Spotted Dove	*Spilopelia chinensis*	Columbidae	Columbiformes	R	LC
50	Laughing Dove	*Spilopelia senegalensis*	Columbidae	Columbiformes	R	LC
51	Red Collared Dove	*Streptopelia tranquebarica*	Columbidae	Columbiformes	R	LC
52	White-Throated Kingfisher	*Halcyon smyrnensis*	Alcedinidae	Coraciiformes	R	LC
53	Pied Kingfisher	*Ceryle rudis*	Alcedinidae	Coraciiformes	R	LC
54	Asian Green Bee-Eater	*Merops orientalis*	Meropidae	Coraciiformes	R	LC
55	Indian Roller	*Coracias benghalensis*	Coraciidae	Coraciiformes	R	LC
56	Eurasian Hoopoe	*Upupa epops*	Upupidae	Coraciiformes	R	LC
57	Indian Gray Hornbill	*Ocyceros birostris*	Bucerotidae	Coraciiformes	R	LC
58	Greater Coucal	*Centropus sinensis*	Cuculidae	Cuculiformes	R	LC
59	Cuckoo	*Clamator jacobinus*	Cuculidae	Cuculiformes	M	LC
60	Asian Koel	*Eudynamys scolopacea*	Cuculidae	Cuculiformes	R	LC
61	Common Hawk Cuckoo	*Hierococcyx varius*	Cuculidae	Cuculiformes	M	LC
62	Sirkeer Malkoha	*Taccocua leschenaultii*	Cuculidae	Cuculiformes	R	LC
63	Black-Winged Kite	*Elanus caeruleus*	Accipitridae	Falconiformes	R	LC
64	Black Kite	*Milvus migrans*	Accipitridae	Falconiformes	R	LC
65	Brahminy Kite	*Hiliastur indus*	Accipitridae	Falconiformes	R	LC
66	Shikra	*Accipiter badius*	Accipitridae	Falconiformes	R	LC
67	Eurasian Marsh-Harrier	*Circus aeruginosus*	Accipitridae	Falconiformes	W	LC
68	White Eye Buzzard	*Butastur teesa*	Accipitridae	Falconiformes	R	LC
69	Eurasian Kestrel	*Falco tinnunculus*	Falconidae	Falconiformes	W	LC
70	Indian Peafowl	*Pavo cristatus*	Phasianidae	Galliformes	R	LC
71	Rock Bush Quail	*Perdicula argoondah*	Phasianidae	Galliformes	R	LC
72	Gray Francolin	*Ortygornis pondicerianus*	Phasianidae	Galliformes	R	LC

73	Painted Francolin	*Francolinus pictus*	Phasianidae	Galliformes	R	LC
74	Jungle Bush Quail	*Perdicula asiatica*	Phasianidae	Galliformes	R	LC
75	Western Swamphen	*Porphyrio porphyrio*	Rallidae	Gruiformes	R	LC
76	Eurasian Coot	*Fulica atra*	Rallidae	Gruiformes	R	LC
77	Eurasian Moorhen	*Gallinula chloropus*	Rallidae	Gruiformes	R	LC
78	Crested Lark	*Galerida cristata*	Alaudidae	Passeriformes	R	LC
79	Wire Tailed Swallow	*Hirundo smithii*	Hirundinidae	Passeriformes	R	LC
80	Red Rumped Swallow	*Cecropiis daurica*	Hirundinidae	Passeriformes	R	LC
81	Gray Wagtail	*Motacilla cinerea*	Motacillidae	Passeriformes	W	LC
82	Western Yellow Wagtail	*Motacilla flava*	Motacillidae	Passeriformes	W	LC
83	White Wagtail	*Motacilla alba*	Motacillidae	Passeriformes	W	LC
84	White-Browed Wagtail	*Motacilla maderaspatensis*	Motacillidae	Passeriformes	R	LC
85	Small Minivet	*Pericrocotus cinnamomeus*	Campephagidae	Passeriformes	R	LC
86	Red Vented Bulbul	*Pycnonotus cafer*	Pycnonotidae	Passeriformes	R	LC
87	Common Iora	*Aegithina tiphia*	Irenidae	Passeriformes	R	LC
88	Bay Backed Shrike	*Lanius vittatus*	Laniidae	Passeriformes	R	LC
89	Rufous-Backed Shrike	*Lanius schach*	Laniidae	Passeriformes	R	LC
90	Black Drongo	*Dicrurus macrocercus*	Dicruridae	Passeriformes	R	LC
91	White Bellied Drongo	*Dicrurus caerulescens*	Dicruridae	Passeriformes	R	LC
92	Rufous Treepie	*Dendrocitta vagabunda*	Corvidae	Passeriformes	R	LC
93	House Crow	*Corvus splendens*	Corvidae	Passeriformes	R	LC
94	Eurasian Golden Oriole	*Oriolus oriolus*	Oriolidae	Passeriformes	R	LC
95	Brahminy Starling	*Sturnus pagodarum*	Sturnidae	Passeriformes	R	LC
96	Rosy Starling	*Sturnus roseus*	Sturnidae	Passeriformes	R	LC
97	Common Myna	*Acridotheres tristis*	Sturnidae	Passeriformes	R	LC
98	Oriental Magpie Robin	*Copsychus saularis*	Muscicapidae	Passeriformes	R	LC
99	Indian Robin	*Copsychus fulicatus*	Muscicapidae	Passeriformes	R	LC
100	Brown Rock Chat	*Oenanthe fusca*	Muscicapidae	Passeriformes	R	LC
101	African Stone Chat	*Saxicola torquata*	Muscicapidae	Passeriformes	R	LC
102	Black Redstart	*Phoenicorus ochruros*	Muscicapidae	Passeriformes	W	LC
103	Common Babbler	*Argya caudata*	Leiothrichidae	Passeriformes	R	LC
104	Common Tailorbird	*Orthotomus sutorius*	Muscicapidae	Passeriformes	R	LC
105	Ashy Prinia	*Prinia socialis*	Muscicapidae	Passeriformes	R	LC
106	Great Tit	*Parus major*	Paridae	Passeriformes	R	LC
107	Purple Sunbird	*Cinnyris asiatica*	Nectarinidae	Passeriformes	R	LC
108	Purple-Rumped Sunbird	*Leptocoma zeylonica*	Nectarinidae	Passeriformes	R	LC
109	Indian White-Eye	*Zosterops palpebrosus*	Zosteropidae	Passeriformes	R	LC
110	Creasted Bunting	*Emberiza lathami*	Emberizidae	Passeriformes	R	LC
111	Red Avadavat	*Amandava amandava*	Estrildidae	Passeriformes	R	LC
112	Indian Silverbill	*Euodice malabarica*	Estrildidae	Passeriformes	R	LC
113	Scaly Breasted Munia	*Lonchura punctulata*	Estrildidae	Passeriformes	R	LC
114	Paddy Field Pipit	*Anthus rufulus*	Estrildidae	Passeriformes	R	LC
115	House Sparrow	*Passer domesticus*	Passeridae	Passeriformes	R	LC
116	Baya Weaver	*Ploceus philippinus*	Passeridae	Passeriformes	R	LC
117	White – Bellied Minivet	*Pericrocotus erythropygius*	Campephagidae	Passeriformes	R	LC
118	Common Woodshrike	*Tephrodornis pondicerianus*	Vangidae	Passeriformes	R	LC
119	Brown Shrike	*Lanius cristatus*	Laniidae	Passeriformes	W	LC
120	Indian Bushlark	*Mirafra erythroptera*	Alaudidae	Passeriformes	R	LC
121	Plain Prinia	*Prinia inornata*	Cisticolidae	Passeriformes	R	LC
122	Gray-Breasted Prinia	*Prinia hodgsonii*	Cisticolidae	Passeriformes	R	LC
123	Zitting Cisticola	*Cisticola juncidis*	Cisticolidae	Passeriformes	R	LC
124	Booted Warbler	*Iduna caligata*	Acrocephalidae	Passeriformes	W	LC
125	Blyth's Reed Warbler	*Acrocephalus dumetorum*	Acrocephalidae	Passeriformes	W	LC
126	Barn Swallow	*Hirundo rustica*	Hirundinidae	Passeriformes	W	LC
127	Wire-Tailed Swallow	*Hirundo smithii*	Hirundinidae	Passeriformes	R	LC
128	Red-Rumped Swallow	*Cecropis daurica*	Hirundinidae	Passeriformes	R	LC
129	Streak-Throated Swallow	*Petrochelidon fluvicola*	Hirundinidae	Passeriformes	R	LC
130	Sulphur-Bellied Warbler	*Phylloscopus griseolus*	Phylloscopidae	Passeriformes	W	LC
131	Greenish Warbler	*Phylloscopus trochiloides*	Phylloscopidae	Passeriformes	W	LC
132	Lesser Whitethroat	*Curruca curruca*	Sylviidae	Passeriformes	W	LC
133	Jungle Babbler	*Turdoides striata*	Leothrichidae	Passeriformes	R	LC
134	Large Gray Babbler	*Turdoides malcolmi*	Leothrichidae	Passeriformes	R	LC
135	Tickell's Blue Flycatcher	*Cyornis tickelliae*	Muscicapidae	Passeriformes	R	LC
136	Siberian Stonechat	*Saxicola maurus*	Muscicapidae	Passeriformes	R	LC

137	Pied Bushchat	*Saxicola caprata*	Muscicapidae	Passeriformes	R	LC
138	Yellow-Throated Sparrow	*Gymnoris xanthocollis*	Passiridae	Passeriformes	R	LC
139	Tawny Pipit	*Anthus campestris*	Estrildidae	Passeriformes	R	LC
140	Little Cormorant	*Microcarbo niger*	Phalacrocoracidae	Pelecaniformes	R	LC
141	Great Cormorant	*Phalacrocorax carbo*	Phalacrocoracidae	Pelecaniformes	R	LC
142	Oriental Darter	*Anhinga melanogaster*	Anhingidae	Pelecaniformes	R	NT
143	Indian Cormorant	*Phalacrocorax fuscicollis*	Phalacrocoracidae	Pelecaniformes	W	LC
144	Coppersmith Barbet	*Psilopogon hemacephalus*	Megalaimidae	Piciformes	R	LC
145	Little Grebe	*Tachybaptus ruficollis*	Podicipedidae	Podicipediformes	R	LC
146	Rose Ring Parkeet	*Psittacula krameri*	Psittacidae	Psittaciformes	R	LC
147	Plum Headed Parkeet	*Psittacula cyanocephala*	Psittacidae	Psittaciformes	R	LC
148	Alexandrine Parkeet	*Psittacula eupatria*	Psittacidae	Psittaciformes	R	NT (U)
149	Barn Owl	*Tyto alba*	Tytonidae	Strigiformes	R	LC
150	Spotted Owlet	*Athene brama*	Strigidae	Strigiformes	R	LC
151	Eurasian Eagle Owl	*Bubo bubo*	Strigidae	Strigiformes	R	LC

Results and Discussion

During the avifaunal assessment of Karanja-Sohol Wildlife Sanctuary, a total of 151 bird species (Table 1) from 55 different families (Table 2) and 17 different orders were recorded. Figure 1 shows that the order Passeriformes, known as "perching birds", comprises a maximum of 41% of birds with 62 species, while Charadriiformes shares 12% with 18 bird species. In India, Muscicapidae is the largest family of birds, with 370 species (Manakadan and Pittie, 2001), but in the present investigation, Anatidae (11 species) showed dominance over Muscicapidae (10 species), followed by Accipitridae (08 species), Ardeidae and Scolopacidae (07 species each), Columbidae and Hirundinidae (06 species each), Cuculidae, Estrildidae and Phasianidae (05 species each), Motacillidae (04 species), Charadriidae,Ciconiidae, Cisticolidae, Laniidae, Laridae, Passeridae, Phalacrocoracidae, Psittacidae, Rallidae, Sturnidae and Threskiornithidae (03 species each), and Acrocephalidae, Alaudidae, Alcedinidae, Burhinidae, Campephagidae, Corvidae, Dicruridae, Leothrichidae, Nectarinidae, Phylloscopidae and Strigidae (02 species each). Moreover, Oriolidae, Anhingidae, Bucerotidae, Caprimulgidae, Coraciidae, Emberizidae, Pycnonotidae, Falconidae, Glareolidae, Leiothrichidae, Irenidae, Megalaimidae, Meropidae, Paridae, Podicipedidae, Recurvirostridae, Turnicidae, Sylviidae, Tytonidae, Upupidae, Vangidae & Zosteropidae were were poorly represented in the study areacontaining only one species each (Table 2).The reason behind the maximum number of species occurrence could be the forest type, as it contains grassland, water body and mixed tree forest. On the northern side of Maharashtra, the largest protected area, i.e., Melghat Tiger Reserve (MTR), has 276 bird species (Wadatkar, & *et al*., 2021). When comparing the two areas, the Karanja-Sohal wildlife sanctuary has a higher density of avifauna than the MTR. In Maharashtra, the Vidarbha Region receives 24% more rainfall, the sanctuary area falls under Assured Rain Fall Zone-7 (ARZ-7), and water is available throughout the year (Awatade, *et al*., 2018). It is challenging to conduct this assessment, as the wildlife sanctuary is divided into numerous patches by the state highway running through it. Sanctuary is surrounded by numerous villages and agricultural land, which creates human interference. Overgrazing can result in disturbance and habitat loss and may lead to species extinction (Koli, 2014). According to IUCN red data (IUCN, 2020), 144 birds have the least concern (LC) status, and 05 are in the near threat (NT) category, i.e., Pallid Harrier (*Circus macrourus*), Black Tailed Godwit (*Limosa limosa*), Painted Stork (*Mycteria leucocephala*), Black Headed Ibis (*Threskiornis melanocephalus*) and Oriental Darter (*Anhinga melanogaster*). 02 birds, viz. River terns (*Stema aurantia*) and Alexandrian parakeets (*Psittacula eupatria*) were in the Near Threaten Vulnerable (NT (U)) category, and birds sighted during the survey were categorized based on their migratory status as resident (R), migratory (M) and winter migratory (W). It was observed that the 115 birds were residents, 34 birds were found to be winter migratory and 2 birds viz. Pied crested cuckoo (*Clamator jacobinus*) and common hawk cuckoo (*Hierococcyx varius*) were recorded as migratory. Similar studies were also reported by Kumar (2015), Singh (2013) and Thakur (2012).

Table 2: Reported families of avifaunal diversity of Karanja-Sohol Wildlife Sanctuary

SN	Family	Number of Species	SN	Family	Number of species
1.	Accipitridae	8	29	Leothrichidae	2
2.	Acrocephalidae	2	30	Megalaimidae	1
3.	Alaudidae	2	31	Meropidae	1
4.	Alcedinidae	2	32	Motacillidae	4
5.	Anatidae	11	33	Muscicapidae	10
6.	Anhingidae	1	34	Nectarinidae	2
7.	Ardeidae	7	35	Oriolidae	1
8.	Bucerotidae	1	36	Paridae	1
9.	Burhinidae	2	37	Passeridae	3
10.	Campephagidae	2	38	Phalacrocoracidae	3
11.	Caprimulgidae	1	39	Phasianidae	5
12.	Charadriidae	3	40	Phylloscopidae	2
13.	Ciconiidae	3	41	Podicipedidae	1
14.	Cisticolidae	3	42	Psittacidae	3
15.	Columbidae	6	43	Pycnonotidae	1
16.	Coraciidae	1	44	Rallidae	3
17.	Corvidae	2	45	Recurvirostridae	1
18.	Cuculidae	5	46	Scolopacidae	7
19.	Dicruridae	2	47	Strigidae	2
20.	Emberizidae	1	48	Sturnidae	3
21.	Estrildidae	5	49	Sylviidae	1
22.	Falconidae	1	50	Threskiornithidae	3
23.	Glareolidae	1	51	Turnicidae	1
24.	Hirundinidae	6	52	Tytonidae	1
25.	Irenidae	1	53	Upupidae	1
26.	Laniidae	3	54	Vangidae	1
27.	Laridae	3	55	Zosteropidae	1
28.	Leiothrichidae	1			

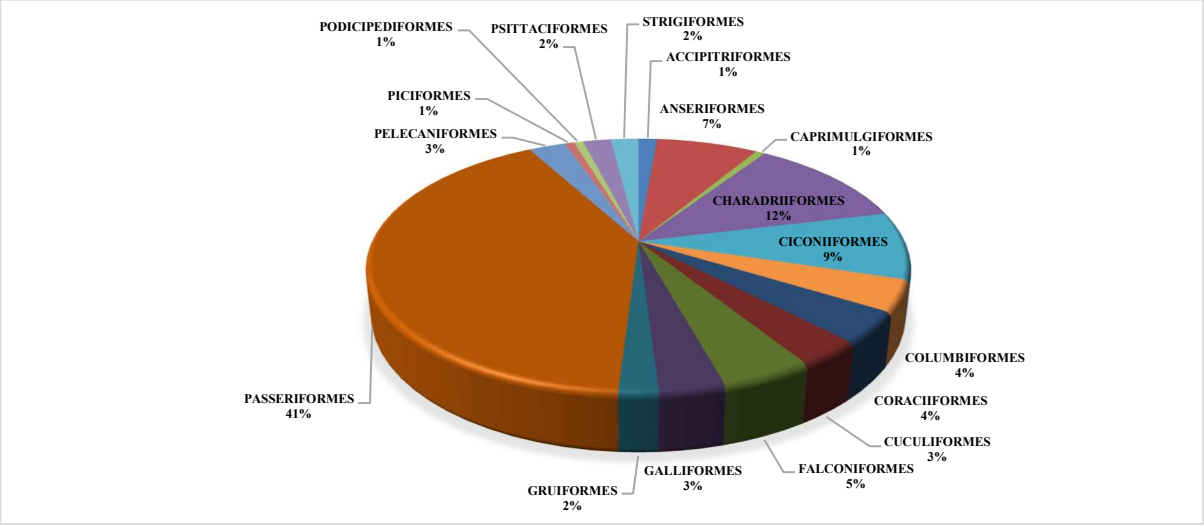

Figure 1: Graphical representation of different orders of avifauna observed in Karanja-Sohol Wildlife Sanctuary

Conclusion

According to the aforementioned study, the Karanja Sohol Wildlife Sanctuary has a rich variety of birds. It is essential to study the region's bird variety because of the region's diverse geology and ecology. The existing checklist will act as the starting point for further study, as there is not a published checklist in this area. The area of the sanctuary makes it an ideal habitat for both water birds and grassland birds. Lesser Folorican

(*Sypheotides indicus*), an IUCN Red List species, has been reported in the Akola, Washim, and Yavatmal areas. One of the goals of the current study was to confirm the presence of lesser florican in the study area. The Karanja-Sohol wildlife sanctuary is the only suitable habitat for lesser floricans in the Vidharbha region of Maharashtra. However, it was concluded that this bird was not observed during the survey in the study area. The number 151 could be higher if human interference and livestock grazing were restricted in this area. It is crucial to document biodiversity in addition to concentrating on the hazards to it. Knowing what actually exists is necessary before conservation planning can be carried out. The preservation of avian biodiversity is crucial for sustainable agriculture.

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Conflict of interest

The authors declare that they have no conflict of interest.

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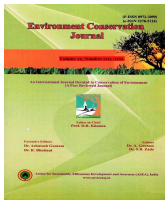

Assessment of different elite mango varieties suitable for North western plain zones of Uttar Pradesh

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ABSTRACT

The evaluation of different mango varieties was conducted during 2019-20 and 2020-21. The experiment was laid out in a randomized block design (RBD) with four varieties, namely, Ambika, Pusa Arunima, Kesar, and Dashehari-51, each replicated three times. The plants were spaced at 4x4 m intervals. The results obtained demonstrated significant variations in various parameters, ranging from minimum to maximum values. The canopy spread ranged from 1.77 to 7.49 m, stem girth from 16.67 to 40 cm, number of fruits per plant from 21 to 118, fruit length from 92.04 to 123.28 mm, fruit width from 56.02 to 77.41 mm, and fruit weight from 202 to 591 g. However, the number of primary branches (2-3) and plant height (1.54-4.35 m) were found to be nonsignificant. Based on the morpho-economic traits, Pusa Arunima appeared to be a superior variety in terms of tree morpho-economic traits, while others were considered moderate. Considering the average number of fruits per plant and fruit weight (g) over a two-year period, Pusa Arunima clearly outperformed the other three varieties in terms of yield. Furthermore, Pusa Arunima, Kesar, and Ambika exhibited more marketable fruit traits. These findings emphasize the significant diversity among the examined mango cultivars. Therefore, it is crucial to protect and preserve these valuable genetic resources for future breeding programs aimed at developing novel and commercially viable cultivars.

Introduction

Mango (*Mangifera indica* L.), renowned and cherished worldwide, stands as one of the most favored fruit crops. Its cultivation thrives in tropical and subtropical regions, as documented by Joshi *et al.* (2013). Belonging to the Anacardiaceae family, this dicotyledonous fruit traces its origin back to the Indo-Burmese region, as observed by Subramanyam *et al.* (1975) and Tjiptono *et al.* (1984). Commercially grown mango varieties owe their existence to meticulous selection processes, encompassing crucial factors such as fruit size, color, shape, flavor, aroma, and taste. Additionally, attributes such as juice content, TSS/acid balance, and maturity time play a significant role in the selection, as stated in various studies. Notably, India's commercial mango varieties have largely emerged as natural chance choices that have evolved over time, according to the research by

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Dey and Singh (2004). India is home to the largest mango germplasm collection in the world, comprising an impressive array of approximately 1500 diverse mango varieties and landraces spread across different regions. Bhalekar *et al.* (2016) highlight mango as the most significant fruit cultivated for export in India, underscoring its economic importance.

Mango production extends beyond India, with several nations standing out as major contributors. Adikshita *et al.* (2018) note that India, China, Thailand, Indonesia, the Philippines, Pakistan, Brazil, Bangladesh, the USA, various African nations, and Mexico rank among the top mango-producing countries globally. Within India, the states of Uttar Pradesh, Andhra Pradesh, Karnataka, Maharashtra, Bihar, Gujarat, Tamil Nadu, Odisha, West Bengal, and Jharkhand take the lead in mango production. With an impressive output of 20.52 million tonnes per year from an area spanning 2.30 million hectares, India solidifies its position as the world's foremost mango-producing nation, as reported by Anon. (2020). The production of mangoes predominantly takes place in developing countries situated within tropical regions. This agricultural pursuit holds immense significance due to the popularity and demand for mangoes worldwide. The cultivation of mangoes in the North Western Plain Zones of Uttar Pradesh faces several challenges and difficulties. The region experiences specific agro-climatic conditions, including variations in temperature, rainfall patterns, and soil characteristics, which can significantly impact mango cultivation. Additionally, the changing climatic conditions due to global warming and the associated risks of extreme weather events further exacerbate the challenges faced by mango growers in this region.

To overcome these difficulties and ensure sustainable mango production, it is crucial to assess and identify elite mango varieties that are well-suited for the specific agro-climatic conditions of the North Western Plain Zones of Uttar Pradesh. These elite varieties should possess desirable traits such as high yield potential, resistance to pests and diseases, tolerance to abiotic stresses, superior fruit quality, and adaptability to local growing conditions. This study aims to evaluate different elite mango varieties and their performance in the North Western Plain Zones of Uttar Pradesh. Through meticulous field trials and rigorous data collection, we will assess various morpho-economic traits of these mango varieties, including canopy spread, stem girth, number of fruits per plant, fruit length, fruit width, and fruit weight. Additionally, we will examine the flowering and fruiting patterns of each variety to determine their suitability for the local climatic conditions.

The performance of mango varieties is greatly influenced by factors such as morpho-economic characteristics. Mango flowering, which is a crucial component of mango productivity and largely a varietal trait influenced by weather, is a complex phenomenon. Because of their propensity to change in reaction to environmental changes, the conclusions drawn from the morphological assessment can be misleading (Sankar *et al.,* 2011). Mango cultivars have been shown to exhibit different flowering patterns in subtropical and tropical environments (Davenport 2003). Shoot initiation is the first step in the mango flowering process, which is followed by floral differentiation and panicle emergence (Murti and Upreti 2000). The majority of mango cultivars go through all of these growth stages between October and December in both tropical and subtropical environments. Environmental factors now in effect as well as the age of terminal resting shoots are related to the induction of floral bud production (Davenport 1997). One of the requirements for effective mango production is the evaluation of mango cultivars for a certain set of ecosystems (Singh and Singh 1996). The weather and the genotypes of the different varieties have a large impact on mango flowering, which is one of the most crucial qualities because it directly influences production. Mangoes only have a brief blossoming cycle that lasts two to three weeks. The mango plant bears both staminate and perfect blooms on the same panicle because it is andromonoecious (Singh *el. al.,*2015). The flowering season in western Uttar Pradesh lasts from February to March. The mango production situation in northwest Uttar Pradesh is significantly impacted by a study analyzing many elite mango varieties suitable for the zone of the northwestern plain. The findings of this study will not only provide valuable insights into the performance and adaptability of

different mango varieties but also contribute to the development of sustainable mango cultivation practices in the North Western Plain Zones of Uttar Pradesh. By identifying elite varieties that are well adapted to the region, mango growers can enhance their productivity, improve fruit quality, and mitigate the risks associated with climate change. This study will serve as a significant step toward ensuring the long-term viability and profitability of mango cultivation in the North Western Plain Zones of Uttar Pradesh. By addressing the major difficulties and challenges faced by mango growers in this region, we aim to provide practical solutions and contribute to the sustainable development of the mango industry.

Material and Methods

Geographically, the experimental field is located at 29°04' north latitude, 77°42' east longitude, and an altitude of 237.75 meters above the mean sea level (coordinates based on Google Earth imagery). The observations focused on various morphoeconomic characteristics during the specified observation period, including date to first flowering, date to full bloom, number of primary branches, canopy spread (m), stem girth (cm), plant height (m), number of fruits per plant, fruit length (mm), fruit width (mm), and fruit weight (g).

To ensure a scientifically sound experimental design, the study employed a randomized block design (RBD). The experiment consisted of four different mango varieties, each replicated thrice, resulting in a total of 12 experimental units. Morphological characterization was carried out following the standard mango descriptors developed by the International Plant Genetic Resources Institute (IPGRI) in 2006 (IPGRI, 2006).

The observation of flowering-related events involved recording daily observations from February to March. The emergence of the date to first flowering and the date to full bloom was determined by closely monitoring the anthesis of the first flower until the last one. The duration of these events was calculated by counting the number of days required for the plant to complete its flowering process.

The number of primary branches per plant was manually counted throughout the trial period. To obtain the average value, the total number of primary branches was divided by the number of plants within each replication of the mango varieties. The canopy spread was measured as the mean diameter in two directions, namely, north–south and east–west. A measuring tape or other appropriate measuring tool was used to determine the distance in metermetres (m). For the mature tree's trunk girth measurement, a measuring tape was wrapped around the trunk at a height of 50 centimeters above the ground, and the circumference was recorded in centimeters (cm).

To measure plant height, a long, straight, premeasured, and marked stick was used. The stick was placed vertically from the base of the tree to the tip of the highest shoot, and the height was recorded in metermetres (m).

The number of fruits per plant was determined by counting the total number of fruits produced by each replication of the mango genotype. For the assessment of morphological fruit characteristics such as fruit length, fruit width (mm), and fruit weight (g), five fruits were randomly collected from each cultivar within each replication. The average length of the five fruits was measured using Vernier calipers, measuring from the fruit's base to its tip. The average width of the five fruits was measured at their widest point using Vernier calipers. Fruit weight was determined by averaging the weights of five randomly selected fruits within each replication. The total fruit weight was divided by the total fruit number to obtain the average fruit weight.

To analyze the acquired observations, the statistical approach recommended by Gomez and Gomez (1984) was employed. This approach is a standard method widely used for statistical analysis in agricultural research.

Results and Discussion

The mango data presented in Table 1 and Figure 1 reveal interesting findings regarding the four studied cultivars. Among them, cv. Dashehari-51 displayed the earliest flower initiation on 28th February, while the latest flower initiation in the same cultivar was observed on 3rd March during the 2019-20 season (Table 1). In the subsequent 2020-21 season, cv. Dashehari-51 once again exhibited the earliest flower initiation on 1st March, followed by Kesar on 6th March and Ambika on 12th March. On the other hand, cv. Pusa Arunima had the latest flower initiation on 14th March. The

Table 1: Flower initiation and full blossom dates for 2019-20 and 2020-21

Cultivars	Date to first flowering						Date to full bloom					
Replication	R1		R2		R3		R1		R2		R3	
Year	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
Ambika	2 Mar	15 Mar	2 Mar	12 Mar	2 Mar	15 Mar	19 Mar	28 Mar	19 Mar	25 Mar	19 Mar	28 Mar
Pusa Arunima	2 Mar	16 Mar	2 Mar	18 Mar	2 Mar	14 Mar	16 Mar	25 Mar	16 Mar	24 Mar	16 Mar	16 Mar
Kesar	1 Mar	9 Mar	1 Mar	8 Mar	1 Mar	6 Mar	18 Mar	19 Mar	18 Mar	15 Mar	18 Mar	20 Mar
Dashehari-51	3 Mar	1 Mar	28 Feb	2 Mar	28 Feb	2 Mar	15 Mar	12 Mar	15 Mar	10 Mar	15 Mar	16 Mar

data further indicate that cv. Dashehari-51 had the earliest full blossom, occurring on 15th March, while cv. Ambika had the latest full blossom on 19th March in the 2019-20 season (Table 1). Similarly, in the 2020-21 season, cv. Dashehari-51 had the earliest full blossom on 10th March, while cv. Ambika once again displayed the latest full blossom on 28th March. These results align closely with the findings of Azam *et al.* (2018), who reported that mango flowering typically begins in the last week of January and continues until the second week of March. It is worth noting that mango flowers are highly influenced by temperature, particularly chilling temperatures, and higher temperatures during flower induction can have a negative impact. Studies by Dambreville *et al.* (2013) and Sukhvibul *et al.* (1999) have demonstrated that elevated temperatures affect flower initiation, full blossom, inflorescence size, and flower quantity per inflorescence in mango. Considering that mango flowering is a critical stage susceptible to climatic variations, unfavorable environmental factors such as rain, humidity, temperature, light, wind, drought, and waterlogging can significantly impact flowering in a negative manner.The key morpho-economic characteristics of the plant samples are summarized in Table 2 and Figure 2. The number of primary branches per plant did not show any significant variation. However, in both 2019-20 and 2020-21, the number of primary branches per plant remained consistent. The highest number of primary branches was observed in cv. Pusa Arunima (3), Kesar (3), and Dashehari-51 (3), while cv. Ambika had the lowest number of primary branches (2). Canopy spread exhibited significant differences between the two years, 2019-20 and 2020-21. In 2019-20, cv. Pusa Arunima had the widest canopy spread (7.08 m), followed by cv. Ambika (6.33 m), Dashehari-51 (5.52 m), and Kesar (1.77 m). Similarly, in 2020-21, cv. Pusa Arunima again showed the maximum canopy spread (8.20 m), followed by cv. Ambika (7.49 m), Dashehari-51 (6.56 m), and Kesar (2.53 m). Stem girth displayed significant variation, with cv. Pusa Arunima exhibiting the maximum stem girth (35 cm) among all four cultivars in 2019-20. It was followed by cv. Ambika (32.33 cm), Dashehari-51 (31.33 cm), and Kesar (16.67 cm). A similar pattern was observed in 2020-21, with cv. Pusa Arunima (40 cm) having the maximum stem girth, followed by cv. Ambika (36 cm), Dashehari-51 (34 cm), and Kesar (19.67 cm). In terms of plant height, cv. Pusa Arunima had the tallest plants (3.03 m) in 2019-20, while cv. Kesar had the shortest plant height (1.54 m) during the same period. Cultivars Ambika and Dashehari-51 had plant heights of 2.48 m and 2.25 m, respectively, in 2019-20. In 2020-21, cv. Pusa Arunima again had the tallest plants (4.35 m), followed by cv. Dashehari-51 (3.06 m), Ambika (3.04 m), and Kesar (2.16 m). However, the observed data for plant height were not found to be significant.

In 2019-20, cv. Dashehari-51 yielded the highest number of fruits per plant (62), followed by cv. Kesar (28), Pusa Arunima (22), and Ambika (21). However, in 2020-21, cv. Pusa Arunima outperformed the other cultivars, producing the maximum number of fruits per plant (118), followed by Dashehari-51 (109), Ambika (84), and Kesar (35). The obtained data were found to be significant.According to Westwood and Blaney (1963), fruit size plays a crucial role in breeding programs for selecting superior genotypes. In terms of fruit length, cv. Pusa Arunima displayed the longest fruits (115.85 mm) in 2019-20. In the same year, Kesar, Ambika, and Dashehari-51 had fruit lengths of 109.58 mm, 104.39 mm, and 92.04 mm, respectively. In 2020-21, cv. Pusa Arunima again

Table 2: Morpho-economic trait values for four mango varieties in 2019-20 and 2020-21

Characters	Ambika		Pusa Arunima		Kesar		Dashehari-51		CD		CV	
Year	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
Number of primary branches	2	2	3	3	3	3	3	3	N.S.	N.S.	26.32	21.65
Canopy spread (m)	6.33	7.49	7.08	8.20	1.77	2.53	5.52	6.56	1.06	1.38	10.31	11.14
Stem girth (cm)	32.33	36.00	35.00	40.00	16.67	19.67	31.33	34.00	10.30	12.68	17.90	19.57
Plant height (m)	2.48	3.04	3.03	4.35	1.54	2.16	2.25	3.06	N.S.	N.S.	21.88	46.03
Number of fruits per plant	21	84	22	118	28	35	62	109	30.40	38.05	45.30	22.02
Fruit length (mm)	104.39	109.44	115.85	123.28	109.58	100.01	92.04	102.65	5.44	8.36	11.45	3.84
Fruit width (mm)	66.47	64.91	76.80	77.41	68.14	62.37	56.02	65.82	6.63	8.29	4.96	6.13
Fruit weight (g)	261.00	403.33	591.67	390.00	316.00	251.67	202.33	208.33	15.23	72.59	2.23	11.60

exhibited the longest fruit length (123.28 mm), followed by Ambika (109.44 mm), Dashehari-51 (102.65 mm), and Kesar (100.01 mm). The data were found to be significant. Regarding fruit width, cv. Pusa Arunima had the widest fruits (76.80 mm) in 2019-20, followed by Kesar (68.14 mm), Ambika (66.47 mm), and Dashehari-51 (56.02 mm). In 2020-21, cv. Pusa Arunima maintained its lead, with fruits having the highest width (77.41 mm), followed by Dashehari-51 (65.82 mm), Ambika (64.91 mm), and Kesar (62.37 mm). The differences observed among the four varieties were significant. Based on the mean value of the number of fruits per plant and fruit weight (g) over the two-year period (2019-20, 2020-21), cv. Pusa Arunima emerged as the highest yielding variety, followed by Ambika, Dashehari-51, and Kesar. The data obtained from this experiment were found to be significant. According to Harada *et al.* (2005), fruit size and weight can be influenced by genetic variables associated with phylogenetic behavior.

Analyzing fruit weight per fruit, cv. Pusa Arunima produced the heaviest fruits (591.67 g) in 2019-20, followed by Kesar (316 g), Ambika (261 g), and Dashehari-51 (202.33 g). In 2020-21, cv. Ambika recorded the heaviest fruits among the four cultivars (403.33 g), followed by Pusa Arunima (390 g), Kesar (251.67 g), and Dashehari-51 (208.33 g). The differences observed were significant. Stanley *et al.* (2000) noted that fruit weight is influenced by genetic, environmental, and cultural factors, all of which interact to determine the final outcome. Genotypes with a higher innate ability to efficiently utilize resources are capable of producing larger fruits. The impact of climate change on agriculture necessitates consideration of the rising levels of atmospheric CO_2, which is a primary driver of climate change. CO_2 plays a crucial role in essential plant processes such as photosynthesis. The fluctuating pattern of cool evenings and relatively warm winters has had a detrimental effect on mango flowering. Additionally, the increasing average temperatures are already affecting mango production. Therefore, rapid climate change should be of paramount concern for mango growers, scientists, and consumers alike.

Mangoes possess a diverse range of genetic resources, providing an advantage for breeding and selection programs aimed at adapting to climate change. Higher temperatures could potentially benefit mango fruit development. However, elevated temperatures also lead to physiological changes in mango fruit. Excessive light, by enhancing photosynthesis, may contribute to larger fruit size (Urban *et al.*, 2003) in mangoes. Furthermore, increased concentrations of CO_2 can improve fruit quality by enhancing fruit dry mass through enhanced photosynthesis. In nonirrigated orchards, it is widely recognized that drought can have both positive and negative effects on fruit quality. While drought decreases fruit size (Spreer

et al., 2009), it can improve fruit quality (Léchaudel *et al.*, 2005) in mangoes by increasing the dry matter content and sugar concentration.

Conclusion

All four varieties of mango tested in the experiment were found to be promising for cultivation under the North Western Plain Zones of Uttar Pradesh and can further be recommended for research trials, mass multiplication programmes and ultimately adoption by farmers and orchardists. On the basis of morpho-economic traits, Pusa Arunima appeared to be a more promising and superior variety in terms of tree morphology, and others were found to be moderate. Furthermore, it can be concluded that the fruits of Pusa Arunima, Kesar and Ambika had more marketable fruit traits. Among the four varieties, the highest-yielding variety was recorded in Pusa Arunima, followed by Ambika and Dashehari-51, based on the mean value of the number of fruits per plant and fruit weight (g) over two years. The present study also confirms that there is much diversity in mango cultivars studied in this experiment, and hence, it becomes necessary to preserve and conserve these unique genetic resources for future breeding programs for the development of innovative, market-driven cultivars.

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Conflict of interest

The authors declare that they have no conflict of interest.

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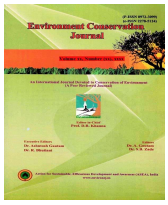

Water quality assessment of Kuwano River, Basti (U.P.) India, with reference to statistical analysis

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ABSTRACT

The present study analyses water quality parameters in the Kuwano River, Basti district, using correlation and regression analysis to establish relationships between variables and provide a comprehensive understanding of the factors influencing water quality. Kuwano is the main river that flows through Basti city. The water samples were collected at three locations. The values of different physicochemical parameters of the river water sample were found to be dependent on the hydrology of the area. The pH was strongly associated with TDS (r = 0.885), DO (r = 0.744), COD (r = 0.969), TH (r = 0.806), and Mg (r = 0.944). The biological oxygen demand (BOD) (-0.345), nitrate (-0.235), and calcium (-0.128) exhibited an inverse correlation with total dissolved solids (TDS), whereas nitrate and calcium had a positive correlation with all other physicochemical parameters. The mean TDS value of the river water sample (81.2) was within the permissible limit for drinking water. The total coliform counts established a negative correlation with most of the parameters studied, e.g., dissolved oxygen (-0.628), BOD (-0.983), chemical oxygen demand (-0.194), total hardness (-0.549), nitrate (-0.955), Ca (-0.918) and Mg (-0.279). The study's findings may provide practical information for decision making in river pollution management.

Introduction

Statistical investigations are a crucial part of science, helping to gather extra information and expand contextual knowledge in an area to make decisions under uncertainties. It is the dynamic science of collecting, analyzing, and interpreting data to make decisions, despite the possibility that the outcomes may differ from reality (Nemade and Shrivastava, 1997). Systematically applying correlation and regression coefficients to water quality variables permits a more comprehensive assessment of the waters as a whole, as well as a better understanding of the relative concentrations of different pollutants in the water, which is crucial for fast execution of initiatives aimed at improving the condition of the water (Khatoon *et al*., 2013; Tyagi *et al.,* 2020). Water is a core component of our economic and social institutions, and its availability is crucial to the maintenance of a vibrant and prosperous civilization (Murali *et al*., 2015; Bhutiani and Ahamad, 2018; Bhutiani *et al.,* 2021a&b; Ahamad *et al.,* 2022). The flowing waters of a stream are dynamic, metabolically rich ecosystem elements (Ouyang *et al*., 2006). It controls the quantity of nutrients as well as contaminants entering the water supply (Meera and Nandan, 2010; Bhutiani *et al.,* 2018; Ruhela *et al.,* 2019). In addition to reflecting their surroundings, the river additionally signifies the culture in which they find themselves and acts as a repository for the collective "sins" of mankind (Yogendra and Puttaiah, 2008). Scientifically supported decisions rely on accurate mathematical inference. Therefore, using adequate statistical methods and experiments is crucial for obtaining reliable results from the analysis of data (Schreiber *et al*., 2022). Quantitative observations of the sustainability of water are just a part of the massive datasets needed for analysis. By using statistical methods, these data

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may be efficiently sorted, analyzed, and interpreted to find undetectable patterns of behavior, tendencies, and connections that would otherwise go unnoticed. Numerous factors, including human negligence and environmental variations, contribute to the inherent inaccuracy of water quality assessments. The variables of underground water quality could be easily correlated, which might provide for more efficient, real-time monitoring (Rao and Naga, 2005). Correlation is an effective method for predicting attributes with a specific level of precision (Chaubey and Patil, 2015).The paper statistically analyses the water quality parameters of the Kuwano River, Basti district, with the objective of quantifying uncertainties in a more precise manner and establishing relationships to infer the value of a variable from a set of other parameters. This is accomplished via the use of regression analysis (Rao and Naga, 2005). It facilitates the creation of prediction models, which may aid in proactive management as well as decision-making to prevent or minimize the effects of potential water quality concerns.Multivariate statistical interpretation of data has been demonstrated to be helpful in assessing riverine water quality variability and exposing temporal and geographical variations due to both natural and human influences in an array of publications (Gajendran *et al*., 2013; Ismail *et al*., 2014; Rizvi *et al*., 2015; Saxena and Saxena, 2015; Ling *et al*., 2017a; Bojago *et al*., 2023). The incorporation of multivariate statistical approaches for evaluating the health of rivers has been the focus of much research across the globe (Kuruppu *et al*., 2013; Khan *et al*., 2014; Tyagi *et al.,* 2020).This research points out the interdependence between inhabitants of Basti and the health of river waters and provides a first-hand report on water quality dynamics and pollutant load flows within the Kuwano River adjacent to Basti. This research also analyzed a correlation data matrix including the various variables.

Material and Methods

Study Area

The district of Basti, which is located in the middle Ganga plain, has a total area measuring 2,771.7 square kilometers. There are an estimated 2,780,683 people residing there as of 2021 (using Aadhar data), and its geographical coordinates are 260 23′ & 270 30′ N and 820 17′ & 830 20′ E. The Kuwano and Ghaghara are the two rivers with the greatest importance in the central and southern parts of the district. In addition to these waters, there are several nalas and ponds in the surrounding region. Within the Basti district, the Kuwano River travels approximately 55 kilometers from northwest to southeast, while it receives water from its numerous tributaries, including Bisuni, Manvar, and Kathinaya. The river serves as the city's main source of water. While passing through Basti, urban sewage, solid waste and industrial effluents are incorporated in it. The Kuwano River flows into Basti district from Siddharth Nagar and feeds the Rapti Zone in the Mid-Western Region. Using a systematic sampling design, we took water samples for analysis from three different locations along the river: upstream at Bandhuwa village near Chandokha (26° 81′ N & 82° 69′ E), in the middle of the district at Amhut (26° 77′ N & 82° 71′ E), and further down the river at Lalganj near the market (26° 65′ N & 82° 82′ E) (Figure 1). Due to the season-dependent nature of pollutant transport pathways, water samples were taken in September 2020, during the monsoon season, in January 2021, during the winter, and in May 2021, during the summer, at three sampling locations in triplicate. On-site measurements of pH, TDS, and EC were taken using digital portable devices; other variables were measured and analyzed in the research lab at Kisan P.G. College, Basti, in accordance with established laboratory protocols within twenty-four hours after collecting the samples. (APHA, 2005; Trivedi and Goel, 1984).

Statistical Analysis

Utilizing a spreadsheet in Excel, the authors determined the minimum, maximum, mean, median, standard deviation, standard error, range, count, confidence level, Pearson's correlation coefficient (r), and equation for regression for each potential combination of the water-related variables. The parameters of statistical significance were computed using the following standard equations:

$$\textbf{\textit{Standard Deviation}}\ (\sigma) = \sqrt{\frac{\Sigma(x-\bar{x})^2}{(n-1)}}$$

x = Sample mean (number1, number2)
n = Sample size

$$\textbf{Standard error} = \frac{\sigma}{\sqrt{N}}$$

σ = Standard Deviation
N = Total number of observations

$$\text{Pearson correlation coefficient (r)} = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 . \ \sum(y-\bar{y})^2}}$$

x and *y* = measurements of two variables
$\bar{x}$ and $\bar{y}$ = Means of two distributions of measurements

Regression Equation

The following formula is used to obtain the straight linear regression line:

$$\boldsymbol{Y = a + bX}$$

where X is the dependent variable, Y is the independent variable, 'a' is the angle of the slope to the line, and 'b' is the y axis intercept.

Empirical component values ('a' and 'b') may be determined using the formula:

$$\boldsymbol{b = \frac{\sum xy - \bar{X}\sum y}{\sum X^2 - \bar{X}\sum y}}$$

$$\boldsymbol{a = \bar{Y} - b\bar{X}}$$

Statistical analyses were performed to determine the importance of associations among a variety of variables. When the score of the coefficient of correlation (r) is substantial and close to one, there is a legitimate relationship between all of the variables. Predictions rely significantly on the concept of correlation, which describes the bond between two variables (Heydari *et al*., 2013). Further strongly linked variables allow for precise forecasting of conclusions. The parameters' associations were determined by regression analysis, which also serves as a framework for making predictions or forecasts (Ghildyal, 2018).

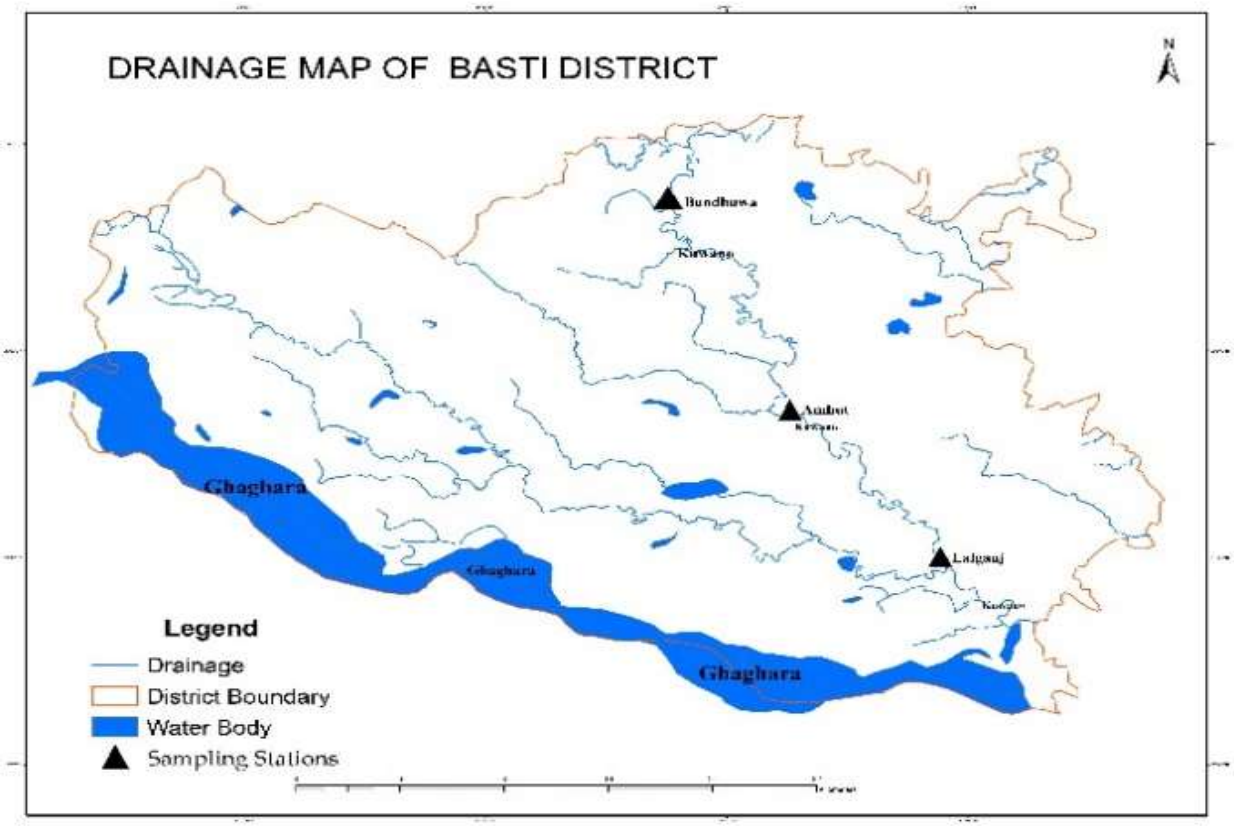

Figure 1: Map showing all three sampling sites in the study area

Results and Discussion

The hydrology of a particular region greatly influences the proportions of multiple physical and chemical variables investigated in the water sample from the Kuwano River. While ponds and lakes are closed systems, rivers are free-flowing structures where water is constantly being exchanged. A river's health may be assessed using a number of physical and chemical indicators. An essential indicator of the acid–base balance of water is its pH value. Animals' immune systems are taxed by the shift in pH of river water, and they also incur physical harm (Ismail *et al*., 2014), which renders them prone to illness. BIS recommends a pH range between 6.5 and 8.0 for water. The acidity level (pH) of Kuwano River water lies between 6.2 and 8.0 (Tables 1 & 2), while the 95% confidence interval (CL) mean value of 0.39 has a positive correlation with all the physical and chemical variables investigated. The pH and Chemical Oxygen Demand (COD) demonstrate a very strong positive correlation with an r value of 0.969. The regression equation suggests that pH increases by 0.0184 units for each unit increase in COD, and the pH value at zero COD is 2.7128. The total dissolved salts or ions in the river water influence the EC. Therefore, EC is a reliable predictor of TDS levels, the quantity of salt that influences the overall flavors of drinking water. The measured EC value for the stream water was between 108 and 186 µmhos/cm (Table 2). At the 95% confidence interval (CL), the mean of EC is estimated as 140.1±18.36. Excluding BOD, nitrate, and calcium, the EC was positively correlated with all other physical and chemical variables. A shift in electrical conductivity, whether increasing or decreasing, may be an indicator of contamination in a water body. In addition, chloride, phosphate, and nitrate ions that come from agricultural land (fertilizer wash) or sewage discharge will contribute to making the water more conductive (Pal *et al*., 2015). TDS, which stands for total dissolved solids, measures the sum of all solutes

Table 1: Seasonal variation in water quality attributes of Kuwano River (mean value)

Parameters	September 2020			January 2021			May 2021		
	Up stream	Mid stream	Down stream	Up stream	Mid stream	Down stream	Up stream	Mid stream	Down stream
pH	7.70	7.65	7.62	7.8	7.8	8.0	6.2	6.74	6.7
TDS	88	95	112	82	88	85	60	55	66
EC	171	171	186	145	136	122	112	108	110
DO	3.8	4.2	4.0	6.6	6.5	6.5	3.1	3.2	4.0
BOD	0.80	1.2	1.2	2.1	2.4	2.3	1.8	1.6	1.8
COD	3.0	3.4	3.4	3.4	3.5	3.8	2.8	2.5	3.2
Total Hardness (TH)	84	88	89	105	117	114	78	76	77
Nitrate	0.22	0.48	0.64	0.82	0.90	1.04	0.45	0.58	0.70
Ca	12.6	12.8	12.8	27.2	25.6	25.0	17.2	16.12	16.9
Mg	17.42	18.34	18.59	18.9	22.2	21.6	14.8	14.6	14.6
Total Coliform (T-coli)	340	384	468	302	312	322	258	378	388

Table 2: Statistical assessment of physical and chemical attributes of water from the Kuwano River

Statistical parameter	pH	TDS	EC	DO	BOD	COD	TH	Nitrate	Ca	Mg	T-coli
Mean	7.4	81.2	140.1	4.7	1.7	3.2	92.0	0.6	18.5	17.9	350.2
Standard error	0.2	5.7	9.4	0.5	0.2	0.1	5.0	0.1	1.9	0.9	19.4
Median	7.7	85.5	136.0	4.0	1.8	3.4	88.0	0.6	16.9	18.3	340.0
SD	0.6	17.0	28.1	1.4	0.5	0.4	15.1	0.2	5.6	2.7	58.1
Range	1.8	57.0	78.0	3.5	1.6	1.3	41.0	0.8	14.6	7.6	210.0
Standard variance	0.3	290.4	790.1	1.8	0.2	0.1	227.1	0.06	30.8	7.2	3378.1
Minimum	6.2	55	108.0	3.1	0.8	2.5	76.0	0.2	12.6	14.6	258.0
Maximum	8.0	112.0	186.0	6.6	2.4	3.8	117.0	1.0	27.2	22.2	468.0
Count	09	09	09	09	09	09	09	09	09	09	09
Confidence level (95%)	0.39	11.11	18.36	0.91	0.33	0.26	9.87	0.13	3.66	1.76	37.96
Upper confidence interval	0.99	28.11	46.46	2.31	0.83	0.66	24.97	0.33	9.26	4.46	96.06
Lower confidence interval	0.21	5.89	9.74	0.49	0.17	0.14	5.23	0.07	1.94	0.94	20.14

that occur in a given volume of water. It adds up all the ions that are less than 2 microns (0.0002 cm) in size. Aquatic creatures, especially fish eggs, may be affected by a surplus amount of overall dissolved solids, primarily due to the ionic attributes of the water. Dissolved substances have a crucial role in maintaining a healthy cell density, which is essential for aquatic organisms (Singh *et al*., 2017). High TDS levels usually imply that the water is fairly alkaline or hard. TDS levels in drinking water cannot exceed 500 mg/l. In this investigation, the average value of TDS with 95% CL was found to be 81.2±11.11. The TDS showed a negative correlation with BOD (-0.345), nitrate (-0.235), and calcium (-0.128) and a strong positive relationship with all other measured physical and biological attributes. The correlation study showed that TDS, EC, and TH are strongly correlated with each other and are likely influenced by the presence of minerals in the water. The significance of dissolved oxygen (DO) is not only from the perspective of addressing water quality issues (Agnieszka *et al*., 2014) but also for comprehending the mechanisms and causes that influence its dynamics in water bodies (Hondzo *et al*., 2013; Zhong *et al*., 2021). Reduced dissolved oxygen (DO) in rivers has a negative effect on physiological functions, leading to a decline in benthic fauna (Bu *et al*., 2021). Decreases in dissolved oxygen (DO) levels may impair pollutant degradation, natural water purification, and overall aquatic ecosystem wellness. The DO value in the current investigation varied between 3.1 and 6.6 mg/l (Tables 1 & 2). BOD was estimated to have a mean score of 4.7±0.91 at the 95% confidence level. The Pearson correlation coefficient analysis revealed a negative relationship between DO and total coliform count (-0.628), while positive interactions were observed for all other variables. DO-TH and DO-Mg show an extremely strong positive correlation with r values of 0.995 and 0.923, respectively (Table 4). The regression equation showed that DO decreased by 0.2054 units for each unit increase in TH and by 0.1369 units for each unit increase in Mg.

BOD (biological oxygen demand) or oxygen content (mg/L), which is used by microbes to breakdown organic materials in water bodies (Bhutiani and Ahamad, 2018). Microorganisms and decomposing biological waste in the water may pose a threat to the ecology of aquatics and human well-being. Dissolved oxygen levels decrease when organic material breaks down, threatening aquatic life by means of hypoxia and disrupting the delicate ecological equilibrium of the water. Therefore, BOD is an indicator of the degree of impurity of water due to organic matter. Based on the results of the current investigation, the BOD concentration was between 0.8 and 2.4 mg/l. The BOD was determined to have a mean score of 1.7 ± 0.33 at the 95% confidence level. In accordance with the results of the correlation analysis, BOD had a positive association with all other parameters investigated but a negative relationship with iron (-0.5) and total coliform (-0.983). The regression equation suggests that BOD decreases by 0.025 units for each unit increase in nitrate, and the BOD value at zero nitrate is 0.62. BOD and calcium also showed a strong positive correlation with an r value of 0.975 (Table 4).

The chemical oxygen demand (COD) is the amount of oxygen required for the breakdown of all bioavailable and non-biodegradable organic material directly into water, carbon dioxide, and other gases. Together, the BOD and COD tests may provide a rough estimate of the quantity of non-biodegradable organic compounds present in a given wastewater sample. The COD value is dependent on the condition of the river; if there is wastewater, the COD could be high, but if the river is clean, it must be low, but the value of COD is always higher than that of BOD. The BOD in the clean river was approximately 3 mg/l. The measured COD loads varied from 2.50 mg/l to 3.80 mg/l (Table 2). COD was determined to have a mean score of 3.2±0.26 at a 95% confidence interval. Although the existing investigation found a negative association between COD and total coliform (-0.194), it found significant correlations between COD and all other variables. The COD-TH pair had a strong positive correlation with an r value of 0.926 (Table 4). The regression equation found that COD increases by 1.2162 units for each unit increase in TH, and the COD value at zero TH is 73.5540. While evaluating the correlated oxygen-depletion impact of waste contaminants, the BOD and COD tests provide similar information. Therefore, both are used to assess the impact of contaminants in water (Prambudy *et al*., 2019;

Qiong, 2009; Sharma and Gupta, 2014). Under tropical climatic conditions, the BOD: COD proportions provide a useful measure of the relationship between BOD and COD concentrations in the water of rivers (Lee and Nikraz, 2015). In this study, BOD and COD were found to be strongly correlated, indicating that high levels of organic matter in the water can increase the amount of oxygen needed for degradation. The negative correlation between BOD and DO suggests that high levels of organic matter can deplete oxygen levels, leading to unfavorable conditions for aquatic life. Scientifically, water high in dissolved minerals, largely calcium and magnesium, is known as hard water. Dissolved metallic components such as aluminum, barium, strontium, zinc and magnesium, along with iron, may also contribute to hardness by generating bivalent or multivalent cations (Sengupta, 2013). The current investigation revealed that the TH levels detected in water samples collected from the Kuwano River varied from 76 mg/l to 117 mg/l, as indicated in Table 2. The 95% confidence interval for the calculated mean value of TH was 92.0 ± 9.87. Apart from the total coliform load (-0.549), all other physical and chemical variables examined correlated positively with TH (Table 2). Both magnesium and calcium compounds are significant contributors to water hardness ("total hardness"), although calcium is of greater significance in water. The hardness of calcium causes cloudiness in water. Scale, a hard and crusty grayish white substance, will form at the surfaces of pipes and other equipment if excessive calcium carbonate is present in water. The results showed that the concentration of Ca hardness varied between 12.6 mg/l and 27.2 mg/l, with a mean score of 18.5 ± 3.66 at a 95% confidence range. Calcium hardness was inversely related to TDS (-0.128), EC (-0.436) and total coliform (-0.918). The samples of water from the Kuwano River were analyzed and found to have a Mg hardness between 14.6 and 22.2 mg/l. With a 95% confidence interval (CL), the mean Mg hardness value was calculated to be 17.9 ± 1.76. While Mg hardness was found to be positively correlated with the remaining physical and chemical variables, it showed a negative correlation with the total coliform count (-0.279).The geological composition of the catchment region, soil class along with category, vegetation diversity, climate variables (precipitation-evaporation, periodical variations), terrain relief, sources of water type and magnitude (surface discharges and underground water inflows), and a number of other factors contribute to the quantities of both calcium and magnesium in rivers (Potasznik and Szymezyk, 2015). Variations in surface water chemistry are triggered by seasonal shifts in the movement of water and activity by organisms (Ling *et al*., 2017). Cations, such as calcium and magnesium in waters, show that environmental and human influences have an impact. Nitrate ions within aquatic systems are produced by both intrinsic and anthropogenic processes. Nitrate content is typically an indication of the nutritional status and extent of organic contamination within the water body; hence, measuring this parameter is an essential aspect of the evaluation of water standards (Maghanga *et al*., 2013). Nitrate nitrogen is essential for the synthesis of peptides and amino acids in all organisms (APHA, 2005). Both nitrite and nitrate are two different forms of nitrogen found in water, but at sufficiently excessive quantities, they may be harmful to human health, particularly to newborns and pregnant women (Panchagnula, 2016). Nitrate concentrations in freshwater are typically between 0.1 and 4 mg/L. Nitrate findings below 1 mg/L are indicative of safe drinking water.

Table 3: Correlation coefficient (r) for several indicators of water quality

Parameter	pH	TDS	EC	DO	BOD	COD	TH	Nitrate	Ca	Mg	T-coli
pH	1										
TDS	0.885	1									
EC	0.692	0.948	1								
DO	0.744	0.348	0.032	1							
BOD	0.132	-0.345	-0.625	0.760	1						
COD	0.969	0.744	0.493	0.885	0.371	1					
TH	0.806	0.437	0.130	0.995	0.693	0.926	1				
Nitrate	0.245	-0.235	-0.531	0.830	0.993	0.475	0.771	1			
Ca	0.349	-0.128	-0.436	0.885	0.975	0.568	0.836	0.994	1		
Mg	0.944	0.682	0.415	0.923	0.451	0.996	0.956	0.550	0.638	1	
T-coli	0.052	0.512	0.758	-0.628	-0.983	-0.194	-0.549	-0.955	-0.918	-0.279	1.000

The levels of nitrates found across various waters are mostly determined by their respective sources (Saha *et al*., 1999). The concentration of nitrate in the water samples of the Kuwano River was determined to be within the limits of 0.2 mg/l and 1.0 mg/l, along with a mean value of 0.6 ± 0.13 when compared at the 95% confidence level. Nitrate showed a negative correlation with TDS (-0.235), EC (-0.531), iron (-0.397) and total coliform (-0.955). Other physicochemical parameters studied showed positive correlations (Table 3). Nitrate showed an extremely strong negative correlation with an r value of 0.994 (Table 4) with calcium (Ca).Total coliform **(***T-coli*) bacteria is a total count or measure of the level of coliform bacteria in a water sample. Total coliform detection may be indicative of environmental contamination. *Escherichia coli* serves as a reliable marker of infestation in water supplies, either from humans or animals. Coliforms constitute an indicator of the water's overall hygienic condition and a potential source of waterborne infections (Sivaraja and Nagarajan, 2014). The total coliform count from the river water sample was found to be in the range of 258 to 468. The mean value of total coliforms at 95% CL was (350 ± 37.96). The total coliform count established a negative association (Table 3) with most of the parameters studied, e.g., DO (-0.628), BOD (-0.983), COD (-0.194), total hardness (-0.549), nitrate (-0.955), Ca (-0.918) and Mg (-0.279).Linear regression studies were performed for water-associated measures, with statistically significant correlation coefficients (R > 0.70). The regression analysis showed that the total coliforms and BOD are positively related, with a regression equation of y = 8081.573x (Table 4). The R square value of 0.2658 indicates that only 26.58% of the variation in total coliforms can be explained by BOD. The significance F value of 0.1554 suggests that the regression model is not significant, and the results should be interpreted with caution. Therefore, further research may be needed to explore other factors that could influence the total coliform count.The results of the statistical correlation study showed which combination of variables was most strongly related, as well as the trend of that link. The results indicate that pH is strongly associated with TDS (r = 0.885), DO (r = 0.744), COD (r = 0.969), TH (r = 0.806), and Mg (r = 0.944). TDS is closely related to EC (r = 0.948) and marginally associated with COD (r = 0.744). EC is strongly correlated with T. coli (r = 0.758). DO was closely linked to TH (r = 0.995) and showed a low degree of association with nitrate (r = 0.830), Ca (r = 0.885), and Mg (r = 0.923). BOD had a weak correlation with nitrate (r = 0.993) and a

Table 4: Linear correlation coefficient (r) and regression equation for some pairs of parameters that have significant correlation values

Pairs of Parameters	r value	Coefficient regression		Regression equation
		a	b	
pH - TDS	0.885	-0.2577	62.0206	pH = -0.2577 (TDS) + 62.0206
pH - DO	0.744	0.8689	-2.2552	pH = 0.8689 (DO) - 2.2552
pH - COD	0.969	0.0184	2.7128	pH = 0.0184 (COD) + 2.7128
pH - TH	0.806	-2.9823	96.5243	pH = -2.9823 (TH) + 96.5243
pH - Mg	0.944	-0.3829	17.1734	pH = -0.3829 (Mg) + 17.1734
TDS - EC	0.948	0.1648	100.0549	TDS = 0.1648 (EC) + 100.0549
TDS - COD	0.744	0.0637	-1.0120	TDS = 0.0637 (COD) - 1.0120
EC – T. coli	0.758	-30	3641.3330	EC = -30 (T. coli) + 3641.3330
DO - BOD	0.760	0.0958	1.4041	DO = 0.0958 (BOD) + 1.4041
DO - COD	0.885	0.6095	0.7404	DO = 0.6095 (COD) + 0.7404
DO - TH	0.995	-0.2054	77.7054	DO = -0.2054 (TH) + 77.7054
DO - Nitrate	0.830	0.2287	-0.2087	DO = 0.2287 (Nitrate) - 0.2087
DO - Ca	0.885	0.1684	16.1615	DO = 0.1684 (Ca) + 16.1615
DO - Mg	0.923	-0.1369	15.1369	DO = -0.1369 (Mg) + 15.1369
BOD - Nitrate	0.993	-0.025	0.62	BOD = -0.025 (Nitrate) + 0.62
BOD - Ca	0.975	4.65	8.68	BOD = 4.65 (Ca) + 8.68
COD - TH	0.926	1.2162	73.5540	COD =1.2162 (TH) + 73.5540
COD - Mg	0.996	-0.0270	14.7432	COD =-0.0270 (Mg) + 14.7432
TH - Nitrate	0.771	-0.065	5.5816	TH = -0.065 (Nitrate) + 5.5816
TH - Ca	0.836	0.54	-24.84	TH =0.54 (Ca) - 24.84
TH - Mg	0.956	0.1	6.9666	TH = 0.1 (Mg) + 6.9666
Nitrate - Ca	0.994	-1.2985	17.4888	Nitrate = -1.2985 (Ca) +17.4888

strong correlation with Ca (r = 0.975). COD is strongly correlated with TH (r = 0.926). Although the correlation analysis minimizes overall decision unpredictability, the regression equation can be used for predictive purposes (Shyamala *et al*., 2008), and both can aid in the evaluation regarding the water's condition.

Conclusion

In the case of rivers, the quality of the water analysis along with the systematic application of correlation and regression techniques can help in ascertaining the overall condition of water and quantifying contaminants, which provides valuable information for decision-making in pollution management. The water flowing in the Kuwano River is suffering owing to the discharge of surrounding home sewage, agricultural runoff, and adjoining business and industrial activities. This study provides a baseline for monitoring the condition of water in the Kuwano River and has potential utility for devising strategies for managing and protecting this important aquatic ecosystem.

Conflict of interest

The authors declare that they have no conflict of interest.

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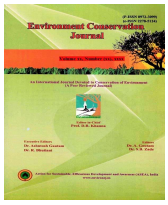

Productivity and nitrogen use efficiency of rice under conventional and organic nutrition

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ABSTRACT

The current study demonstrates the influence of conventional and organic nutrient management practices on nitrogen use efficiency, growth, yield, and physiological and biochemical parameters in four rice varieties, namely, Jaiva, Ezhome 2, Jyothi and Uma. Growth parameters, grain yield per hill, and physiological and biochemical parameters were higher under conventional management for all rice varieties. Although the nitrogen use efficiency of each variety varied significantly with nutrient management practices, the variation was least in Jaiva (23.8%), which is the organic rice variety released by Kerala Agricultural University. The rice varieties Jaiva and Ezhome 2 showed consistency in the grain weight per panicle under both conventional (Jaiva- 4.57 g, Ezhome 2- 5.86 g) and organic (Jaiva, 4.24 g, Ezhome 2, 4.54 g) management. The soil nitrogen content at the tillering stage (0.66**) showed a significantly higher positive correlation with nitrogen use efficiency under organic management. The results of the study provide a better understanding of factors that can lead to a sustained yield in organic rice production in terms of nitrogen use efficiency.

Introduction

The agriculture sector is estimated to be responsible for 60 percent of the projected increase in N pollution by 2050, which is expected to be 150 percent more than that in 2010 (Martinez-Dalmau *et al*., 2021). Rice (*Oryza sativa* L.) is one of the staple cereal food crops for approximately half of the global population. Because of the lower nitrogen use efficiency, rice cultivation has become a fertilizer-intensive process, leading to many environmental implications (Chivenge *et al.,* 2021). Hence, organic farming is quickly gaining popularity as a potential way to ensure ecological

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sustainability and produce healthier food. Despite this, organic farming of rice has a number of limitations, such as decreased yield, lack of suitable varieties, N stress at critical growth stages, shortage of quickly mineralizable organic additions, and crop-weed competition that make it difficult to achieve the potential output (Hazra *et al*., 2016).

Nitrogen (N) is a significant element that plays a vital role in the growth and yield of rice. Suboptimal nutrient input (N in particular) is one of the many yield-limiting factors that causes a noticeable yield difference between conventional and organic production methods for rice (Wild *et al*., 2011; Hazra *et al*., 2014). A better understanding of the nitrogen use efficiency (NUE) of rice genotypes may be helpful in the increased adoption of organic rice farming with a lower yield gap.The current study compared the influence of conventional and organic nutrient management practices on the nitrogen use efficiency of four rice varieties, two popular rice varieties (Uma and Jyothi) in Kerala and two varieties (Jaiva and Ezhome 2) that are recommended for organic cultivation (Vanaja *et al*., 2013; Manjunatha *et al.,* 2016., Vanaja *et al.,* 2017).

Material and Methods

Plant Materials

Rice varieties of *Oryza sativa* ssp. *indica,* namely, Jaiva, Jyothi, Ezhome 2 and Uma, were used for the study. Breeder seeds of rice varieties Jaiva and Ezhome 2 were procured from the Regional Agricultural Research Station, Pilicode, Kerala, India. The breeder seeds of rice varieties Uma and Jyothi were collected from Rice Research Station, Moncompu, Kerala, India and Regional Agricultural Research Station, Pattambi, Kerala, India, respectively.

Experimental Conditions

The experiment was conducted at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India, during the first crop season (June to October) of 2021 in open conditions. The weather data on maximum and minimum temperature (°C), relative humidity (%), total rainfall (mm) and bright sunshine hours (h) were collected from the class B Agromet observatory of the Department of Agricultural Meteorology, College of Agriculture, Vellayani (Supplementary data). Seeds of rice varieties were weighed individually and treated for 12 to 16 hours with *Pseudomonas fluorescens* at 10 g/liter of water before sowing (PoP KAU*,* 2016). Rice varieties were grown under conventional, organic and controlled management conditions. Germinated rice seeds were sown in pots (3 plants per pot) arranged in a completely randomized design with five replicates. The soil used for the experiment (10 kg of soil per pot) was well-pulverized sandy clay loam, acidic in reaction (pH-5), high in organic carbon (1.78%), medium in available nitrogen (313.6 kg/ha), high in available phosphorus (27.52 kg/ha) and medium in available potassium (186.35 kg/ha). Lime was applied at a rate of 600 kg/ha in two split doses., i.e., the first dose (1.75 g per pot) as basal dressing and the second dose (1.25 g per pot) as top dressing at one month after sowing. Lime application and fertilizer application were separated by a week. Straight fertilizers were used in conventional management, and in the case of organic management, nutrients were supplied as farm yard manure (0.5%) and neem cake (4%) on an N equivalent basis (PoP (Organic) KAU*,* 2017). In conventional management, nutrient recommendations of 70:35:35 kg NPK/ha and 90:45:45 kg NPK/ha were followed for short- and medium-duration varieties, respectively (PoP KAU*,* 2016). The control was maintained without manures and fertilizers.

Measurement of traits associated with NUE

Growth, yield and physiological attributes were systematically recorded in different growth stages under conventional, organic and control conditions. Growth attributes such as plant height (cm) and the number of tillers were recorded during the tillering stage, panicle initiation stage and grain filling stage of the crop, and root characteristics such as root biomass (g) and root depth (cm) were also measured. Yield attributes include grain yield per hill (g), number of productive tillers per hill, length of the panicle (cm), grain weight per panicle (g), thousand grain weight (g) and straw yield per hill (g) (*Standard evaluation system of rice*, 2002).

Physiological parameters such as photosynthetic rate (µmol $CO_2/m^2/s$), transpiration rate (mmol $H_2O/m^2/s$), water use efficiency (mmol CO_2/mol H_2O), and stomatal conductance (mmol/m^2/s) were measured at 45 and 60 days after sowing using a portable photosynthetic system (CIRCAS-3 SW), and biochemical analyses such as total soluble protein content (PC), total free amino acids (AA)

and reducing sugar (RS) content were estimated from leaf tissue after 60 days of sowing. Total soluble protein content (mg/g) was estimated by the Bradford protein-dye binding assay (Bradford, 1976), and total free amino acids (mg/g) were estimated by the ninhydrin method (Yemm *et al*., 1955). The dinitro salicylic acid method was used to estimate reducing sugar (mg/g) (Manickam and Sadasivam, 1996).

Soil nitrogen analysis

Soil samples were collected at the tillering, panicle initiation and grain filling stages of the crop, and soil N was analyzed by the micro-Kjeldahl method (Jackson, 1973).

Nitrogen use efficiency (NUE)

The agronomic nitrogen use efficiency of different varieties under organic and conventional management was calculated from the observations taken during the experiment. The formula for calculating agronomic NUE is below (Dobermann and Achim, 2005).

Agronomic NUE= $(Y_N - Y_0)/F_N$

F_N - Amount of (fertilizer) N applied (kg/ha), Y_N - Crop yield with applied N (kg/ha), Y_0 - Crop yield (kg/ha) in a control treatment with no N

Statistical analysis

Analysis of variance (ANOVA) was performed for all traits using GRAPES$_{1.0.0}$ (General R-shiny-based Analysis Platform Empowered by Statistics) developed by the Department of Agricultural Statistics, College of Agriculture, Vellayani, Kerala Agricultural University, Kerala, India based on R software (Gopinath *et al*., 2020). The difference between treatments was separated using least significant difference (LSD) tests at 5% probability. Correlation coefficient analysis (Pearson's linear correlation) using R software was performed between NUE and other shortlisted parameters, and their significance was tested.

Results and Discussion

Growth parameters

The effects of nutrient management on plant height and the number of tillers per hill at all stages of the crop are presented in Table 1. Irrespective of varieties, plant height and number of tillers per hill varied significantly in response to treatments. In the variety Jaiva, plant height at the tillering stage (84.24 cm) under organic management was statistically similar to that under conventional management (87.38 cm).The exception was seen in the number of tillers per hill for the varieties Uma and Ezhome 2 at the panicle initiation stage (21.14 and 14; 20 and 13.2), in which both conventional and organic treatments were statistically similar. A previous study by Ismael *et al*. (2021) depicted that N fertilizer urea in combination with manure improved plant height and the number of tillers and suggested that a combination of chemical fertilizers along with organic manure showed more efficient production than the sole application of fertilizers. In Nepal, a study conducted by Budhathoki *et al.* (2018) compared N fertilizer applications in rice, namely, farmers' fertilizer practices and nutrient expert practices. It was found that the nutrient expert's advice on the application of N significantly increased the plant height of rice. In a study conducted in China, when organic manure coupled with inorganic fertilizer was used in rice, it was found that 70% of chemical fertilizers along with poultry manure showed better growth characteristics, including rice root morphology (Iqbal *et al.,* 2019).Root biomass and root depth were significantly different in all varieties, and maximum root biomass and root depth were recorded in conventional management except in the case of variety Jyothi, in which maximum root biomass and root depth were seen in organic management (Table 2). The root depth of variety Uma under conventional and organic management was found to be statistically similar. Genotypes with higher NUE adapt themselves to the availability of soil N and enhance absorption by regulating different transporter genes encoding NO_3^- and NH_4^+ uptake, which also leads to variation in root morphology (Garnett *et al*., 2009). The plant will adapt by modifying root length density and root hair growth to enable better N transport and metabolism. These variations in root morphology vary within the species and are essential for acquiring nutrients with low mobility (NO_3^- and NH_4^+) in the soil (Otoole and Bland, 1987). This can be a reason for the variation in root morphology among rice varieties. In contrast to this finding, Fan *et al*. (2010) showed that increased N application increased root length and root biomass. However, Wang *et al.* (2005) noted that high N availability reduces root biomass.

Table 1: Effect of nutrient management on plant height (cm), number of tillers and soil N (kg/ha)

Plant height (cm)												
Treatments	**Tillering stage**				**Panicle initiation stage**				**Grain filling stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	87.38	117.42	81.40	68.00	129.18	156.9	104.18	104.64	132.56	136.54	105.48	92.18
Organic	84.24	103.66	66.40	59.30	119.20	113.6	83.70	88.44	114.08	131.70	87.78	84.02
Control	73.44	98.56	60.38	49.22	96.16	131.63	82.20	87.38	84.24	126.04	82.02	59.20
SEm(±)	1.64	3.25	1.65	0.96	1.90	4.43	1.27	1.80	1.16	0.74	0.83	0.68
CD (0.05)	5.06	10.00	5.07	2.96	5.85	13.66	3.92	5.53	3.56	2.29	2.57	2.11
Number of tillers												
Treatments	**Tillering stage**				**Panicle initiation stage**				**Grain filling stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	42.2	30.4	33	21.0	21.4	14.0	21.4	21.4	20.0	14.4	21.0	19.2
Organic	22	19.4	28	15.2	15.2	13.2	20.0	13.4	15.2	13.0	18.0	17.2
Control	14.2	11.0	17	11.0	11.4	9.0	14.0	12.0	12.4	9.2	14.0	13.2
SEm(±)	1.03	0.78	0.75	1.12	0.70	0.67	0.89	0.90	0.66	0.58	0.71	1.97
CD (0.05)	3.17	2.40	2.32	3.44	2.15	2.06	2.73	2.77	2.03	1.78	2.18	0.64
Soil N (kg ha^{-1})												
Treatments	**Tillering stage**				**Panicle initiation stage**				**Grain filling stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	255.72	338.45	213.15	250.36	225.52	300.66	288.93	225.91	388.53	363.31	328.08	275.59
Organic	351.23	255.39	250.53	250.48	250.85	351.24	250.88	263.18	391.58	288.30	288.30	326.08
Control	213.25	263.17	300.52	225.47	263.38	238.20	242.32	240.47	246.95	250.85	250.76	255.70
SEm(±)	2.43	1.85	3.15	2.12	4.2	2.36	2.89	3.76	4.98	2.40	3.34	2.87
CD (0.05)	7.49	5.69	9.69	6.52	12.94	7.28	8.93	11.6	15.35	7.40	10.30	8.83

*** J-Jaiva E-Ezhome 2 U- Uma Jy- Jyothi**

Table 2: Effect of nutrient management on root biomass (g) and root depth (cm)

Treatments	**Jaiva**		**Ezhome 2**		**Uma**		**Jyothi**	
	RB	**RD**	**RB**	**RD**	**RB**	**RD**	**RB**	**RD**
Conventional	72.32	43.3	103.32	39.70	38.62	35.34	15.52	41.80
Organic	29.52	34.0	65.60	29.52	28.64	33.10	18.60	46.34
Control	15.22	36.3	15.80	47.54	15.10	33.44	4.57	26.52
SEm(±)	0.43	0.28	5.40	0.45	0.45	0.42	0.65	0.32
CD (0.05)	1.34	0.87	16.63	1.4	1.39	1.30	1.99	0.97

***RB- Root biomass RD- Root depth**

Yield attributes

The productive tillers per hill were significantly higher under conventional management for varieties Jaiva (17) and Ezhome 2 (14.4) (Table 3). However, in varieties Uma and Jyothi, it was statistically at par with organic management. Conventional management was found to be significantly superior in the length of the panicle. The rice varieties Jyothi (3.53 g) and Uma (2.82 g) under conventional management showed maximum grain weight per panicle, and Jaiva (4.57 g) and Ezhome 2 (5.86 g) under conventional management were significantly similar to those under organic management (4.24 g and 4.54 g, respectively). Conventional management in all varieties resulted in a considerably higher number of filled grains per panicle during the experiment. The thousand-grainweight of all rice varieties, except Jyothi, was significantly higher under conventional management (Table 3). In variety Jyothi, this parameter was statistically at par under both conventional (30 g) and organic management (30.24 g). Conventional management showed maximum grain yield and straw yield in all varieties. Previous studies comparing conventional and organic management conditions also showed similar results. A significant yield gap was seen between conventional and organic management (Ponisio *et al*., 2015). This yield gap varies in accordance with the crop and was found to be higher for cereals (Seufert *et al*., 2012). Plants can absorb nitrogen in the form of urea faster than

Table 3: Effect of nutrient management on yield attributes

Varieties	Treatments	Number of Productive tillers per hill	Length of the panicle (cm)	Grain weight per panicle (g)	Filled grains per panicle	Thousand grain weight (g)	Grain yield per hill (g)	Straw yield per hill (g)
Jaiva	**Conventional**	17.0	33.14	4.57	194.6	22.24	78.10	39.08
	Organic	14.2	28.60	4.24	135.2	19.27	61.04	24.94
	Control	8.4	20.70	1.60	84.0	18.92	13.80	20.02
	SEm(±)	0.41	0.67	0.24	5.91	0.45	3.43	0.69
	CD(0.05)	1.26	2.06	0.74	18.22	1.37	10.56	2.12
Ezhome 2	**Conventional**	14.4	31.52	5.86	157.0	31.40	86.30	47.08
	Organic	12.4	29.82	4.54	174.0	27.44	63.11	22.98
	Control	9.2	26.40	4.97	135.2	26.52	42.10	10.36
	SEm(±)	0.54	0.37	0.09	2.85	0.22	1.97	0.46
	CD(0.05)	1.65	1.14	0.28	8.78	0.68	6.071	1.43
Uma	**Conventional**	18.4	24.54	2.82	147.2	24.80	53.03	36.32
	Organic	16.4	22.72	1.90	112.2	22.92	27.76	24.72
	Control	12.2	20.64	1.70	67.0	20.60	23.56	16.12
	SEm(±)	0.79	0.33	0.10	3.97	0.27	2.09	0.34
	CD(0.05)	2.43	1.02	0.31	12.22	0.85	6.425	1.04
Jyothi	**Conventional**	17.0	25.34	3.53	95.2	30.00	56.01	24.40
	Organic	16.0	22.72	2.61	75.4	30.24	40.21	17.04
	Control	12.4	22.06	2.44	48.2	28.60	27.82	7.60
	SEm(±)	0.67	0.37	0.15	4.13	0.21	1.12	0.35
	CD(0.05)	2.08	1.14	0.47	12.71	0.65	3.432	1.07

Table 4: Effect of nutrient management on physiological parameters

Photosynthetic rate (µmol $CO_2/m^2/s$)								
Treatments	**Vegetative stage**				**Reproductive stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	25.53	26.63	29.67	29.59	44.27	45.80	31.35	41.37
Organic	24.58	22.82	25.51	27.01	33.24	30.45	28.54	29.52
Control	25.46	21.82	25.28	25.38	29.05	29.58	29.42	28.11
SEm (±)	0.67	1.18	0.62	0.82	0.11	0.19	0.28	0.07
CD (0.05)	NS	3.63	1.91	2.52	0.34	0.60	0.86	0.22
Transpiration rate (m mole $H_2O/m^2/s$)								
Treatments	**Vegetative stage**				**Reproductive stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	3.78	3.17	3.72	2.65	8.37	13.00	12.91	13.29
Organic	3.48	2.79	3.12	3.58	12.84	12.06	17.90	19.44
Control	1.40	1.35	2.42	2.61	15.63	14.58	17.26	17.98
SEm (±)	0.28	0.11	0.19	0.21	0.36	0.15	0.19	0.2
CD (0.05)	0.88	0.35	0.61	0.67	1.09	0.47	0.61	0.62
Stomatal conductance (mmol/m^2/s)								
Treatments	**Vegetative stage**				**Reproductive stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	214.00	167.20	213.20	121.40	365.8	536.0	385.2	384.0
Organic	177.40	149.20	134.60	181.60	354.2	285.8	635.6	593.8
Control	71.60	62.80	236.20	172.60	455.4	376.2	427.6	655.8
SEm (±)	13.41	8.77	7.88	13.18	3.68	2.83	6.45	70.82
CD (0.05)	41.32	27.01	24.27	40.61	11.33	8.72	2.09	218.21
Water use efficiency (mmol CO_2/mol H_2O)								
Treatments	**Vegetative stage**				**Reproductive stage**			
	J	**E**	**U**	**Jy**	**J**	**E**	**U**	**Jy**
Conventional	13.91	7.70	8.38	15.07	5.44	3.36	3.19	1.93
Organic	7.06	7.97	8.19	10.57	2.43	2.74	2.73	1.55
Control	18.03	18.62	8.33	11.60	1.76	2.03	3.19	1.52
SEm (±)	2.20	1.03	0.84	1.8	0.01	0.03	0.01	0.01
CD (0.05)	6.76	3.18	NS	NS	0.04	0.08	0.03	0.03

organic manures (Bana *et al*., 2022; Xin *et al.,* 2022), and the easily available nitrogen content in organic fertilizers was found to be lower than that in inorganic fertilizers (Ruan *et al*., 2023). This may be a reason for the higher yield in conventional management in comparison to organic nutrient management. Some researchers suggest that the combination of organic and inorganic fertilization will help increase rice productivity and can be used for sustaining soil fertility (Haque *et al.,* 2019). N is an important element in controlling the number of ineffective tillers of *indica* rice and increasing the number of effective panicles (Budhar and Palaniappan, 1996). A previous study by Vanaja *et al.* (2013) compared conventional and organic nutrient management of rice varieties Jyothi, Uma, Athira and Culture MK 157 and found that conventional management produced more grain and straw yields than organic nutrient management. A similar yield reduction (20 to 30%) was also seen under the organic nutrient management of other crop varieties (Seufert *et al.*, 2012). This signifies the development of nitrogen use-efficient varieties for better performance under organic nutrient conditions.

Physiological parameters

In the vegetative stage, the rice genotypes Ezhome 2, Uma and Jyothi showed significantly higher photosynthetic rates (26.63 µmol CO2/m2/s, 29.67 µmol CO2/m2/s, 29.59 µmol CO2/m2/s, respectively) under conventional management (Table 4). The different nutrient management systems did not significantly affect the photosynthetic rate of the variety Jaiva. However, in the reproductive stage, all rice genotypes showed a significantly high photosynthetic rate under conventional management. In the vegetative stage, the transpiration rates of varieties Jaiva and Uma were statistically similar. The rice variety Jyothi exhibited a higher transpiration rate under organic management (3.58 mmole $H_2O/m^2/s$) than under conventional management (2.65 mmole $H_2O/m^2/s$). A similar trend was also observed in the reproductive stage. The stomatal conductance of the variety Jyothi at the vegetative (181.6 $mmol/m^2/s$) and reproductive (655.8 $mmol/m^2/s$) stages was higher in the organic management and control treatments than in the conventional management treatment. In this study, the rice varieties Uma and Jyothi did not show any significant difference in water use efficiency in their vegetative stage under different nutrient management practices. However, all rice varieties progressing through the reproductive stage showed a significant increase in water use efficiency under conventional management. Previous studies report the strong relation of photosynthesis to N supply and uptake. The photosynthetic rate, transpiration and stomatal conductance were found to be higher in the N treatment than in the control (Iqbal *et al*., 2019). Inorganic fertilizers helped to increase the photosynthetic rate at the early stages of crop growth, and organic fertilizers helped enhance photosynthetic ability throughout the growing period (Yang *et al*., 2015). Previous studies also reported that modification of stomatal conductance led to better nutrient absorption and higher yield (Kingori *et al*., 2016). It was found that ammonium fertilizer responded more to plants' photosynthetic machinery than N's nitrate form. These studies strongly prove that the state of N affects the photosynthetic machinery of plants (Torralbo *et al*., 2019). Various forms of N fertilizer may differentially regulate stomatal conductance.

Biochemical parameters

The protein content of the rice varieties Jaiva, Uma and Jyothi was significantly higher under conventional management, except in the case of Ezhome 2 (Table 5). The rice varieties Jaiva and Uma showed significantly higher amino acid contents under conventional management than under organic management, whereas the amino acid content of Jyothi was statistically similar. Reducing sugar was significantly higher under conventional management for varieties Jaiva, Ezhome and Uma. In Jyothi, the nutrient management practices did notshow any significant effect on reducing sugar content. Various studies showed that the protein content in the leaf varied according to the form of nutrients applied, and ammonium- and nitrate-fertilized plants were found to have higher leaf protein contents (Torralbo *et al*., 2019). These results support higher protein content in conventional than organic nutrient management. The form of N available in the soil, N uptake efficiency, photosynthesis, etc., are critical factors affecting the assimilation of amino acids in plants. These compounds vary according to the genotype of the crop because of the difference in the expression of genes encoding key enzymes required

Table 5: Effect of nutrient management on total soluble protein content, total free amino acids and reducing sugar content

Treatments	Protein content (mg/g)				Amino acids (mg/g)				Reducing sugar (mg/g)			
	J	E	U	Jy	J	E	U	Jy	J	E	U	Jy
Conventional	1.57	1.06	1.03	1.27	0.74	0.62	0.64	0.57	9.33	9.04	9.24	8.77
Organic	1.22	1.20	1.17	0.93	0.53	0.60	0.53	0.53	9.15	8.90	8.64	8.77
Control	0.95	1.03	0.92	0.78	0.50	0.52	0.52	0.47	8.96	8.59	8.59	8.73
SEm(±)	0.02	0.04	0.02	0.03	0.02	0.03	0.02	0.01	0.04	0.02	0.05	0.04
CD(0.05)	0.07	0.12	0.07	0.08	0.07	NS	0.07	0.04	0.12	0.06	0.17	NS

for amino acid synthesis (Decouard *et al.,* 2022).

Soil nitrogen analysis

At the tillering stage, higher soil N was found in Jaiva under organic management (351.23 kg/ha) (Table 1). The soil N status at the panicle initiation stage was significantly high under organic management for varieties Jaiva (250.85 kg/ha), Ezhome 2 (351.24 kg/ha) and Jyothi (263.18 kg/ha). At the grain filling stage of the crop, soil N was significantly higher in organic management for Jyothi (326.08 kg/ha) than in conventional management. Significant variation in soil N was observed based on the genotype. N uptake and N availability in the soil vary depending on the genetic variability of the crop (Han *et al*., 2015). This report substantiates the difference in soil N in different varieties even though the applied fertilizer dosage was the same under conventional and organic conditions. The long mineralization nature of organic manures compared with that of conventional management may be a reason for the high soil N in organic conditions. Previous studies have shown that the duration and availability of mineral nitrogen vary for manures and inorganic fertilizers. Mineralization of organic manures such as vermicompost and neem cake is slower than that of urea. Therefore, organic manures are more effective for providing a nitrogen supply for an extended period (Velmurugan and Swarnam, 2013). Inorganic fertilizers are readily available to the plant and quickly depleted from the soil. However, with organic manures, the initial quick release phase lasted for 10 to 20 days, followed by a sluggish phase for 30 to 40 days, maximal mineralization for 55 to 90 days, and then a decreased period for 120 days. Hence, organic manures ensure the long-term availability of nutrients in the soil, and the stage of application of organic fertilizers is important for better yield. It was also reported that if nutrients were readily available to the plant, plant absorption would be enhanced (Inthavong *et al.,* 2011).

Nitrogen use efficiency (NUE)

All the varieties under conventional management showed higher NUE than those under organic management conditions (Figure 1). The maximum NUE was seen in Jyothi (0.28) under conventional management. Variation in nitrogen use efficiency under organic and conventional management was found to be lower in the variety Jaiva (23.8%). Rice varieties Ezhome 2, Jyothi, and Uma showed differences of 53.3, 57.1 and 80 percent, respectively. As the first organic rice variety of Kerala Agricultural University (KAU), the performance of Jaiva was studied earlier and found to be superior among 65 genotypes under organic management (Manjunatha *et al.*, 2016). A previous report also showed that rice's agronomic NUE and N recovery efficiency were significantly lower under organic production (Huang *et al*., 2016). Varieties differ in terms of uptake and usage of the N fertilizer that is available. Huang *et al*. (2016) recommended that variety selection for organic farming might be made under low-input organic conditions to generate more N-efficient crops.

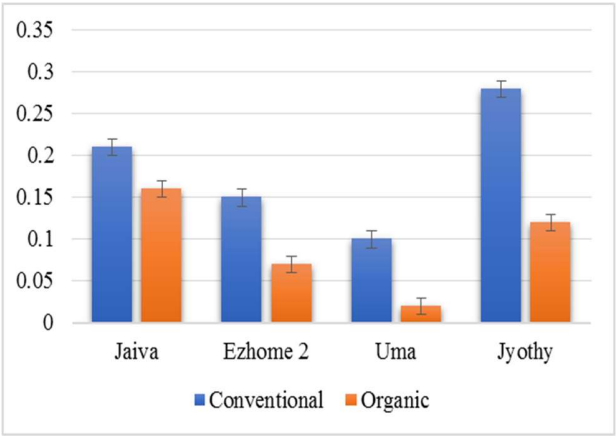

Figure 1: Effect of nutrient management on nitrogen use efficiency

Correlation analysis

The correlation coefficients among NUE and parameters such as grain yield, morphological parameters, soil N status, and some of the physiological and biochemical parameters under conventional and organic management conditions are shown in Figures 2a and 2b, respectively. The perusal of the data (Figure 2a) showed that under conventional management, root depth (0.81***), the photosynthetic rate at the panicle initiation stage (0.47*) and protein content (0.58**) had a significant positive correlation with NUE. Under organic management (Figure 2b), the soil nitrogen content at the tillering stage (0.66**), photosynthetic rate at the panicle initiation stage (0.74***), transpiration rate (0.49*), and stomatal conductance at the vegetative stage (0.74***) showed a significant positive correlation with NUE. Correlation studies in relation to NUE are very few. Some previous studies showed that grain yield per plant had a positive and significant correlation with panicle number per plant, full grain number per panicle, and thousand-grain weight and showed a negative correlation as the growth stage progressed, especially with thousand-grain weight (Saleh *et al*., 2020). The leaf area index, nitrogen uptake, agronomic efficiency, and recovery efficiency were also positively correlated with grain yield. In contrast, nitrogen uptake and leaf area index showed a significant negative correlation with internal N use efficiency (Chen *et al*., 2022). Ahmed *et al.* (2016) found a significant interaction between the N rate and grain yield of crops.

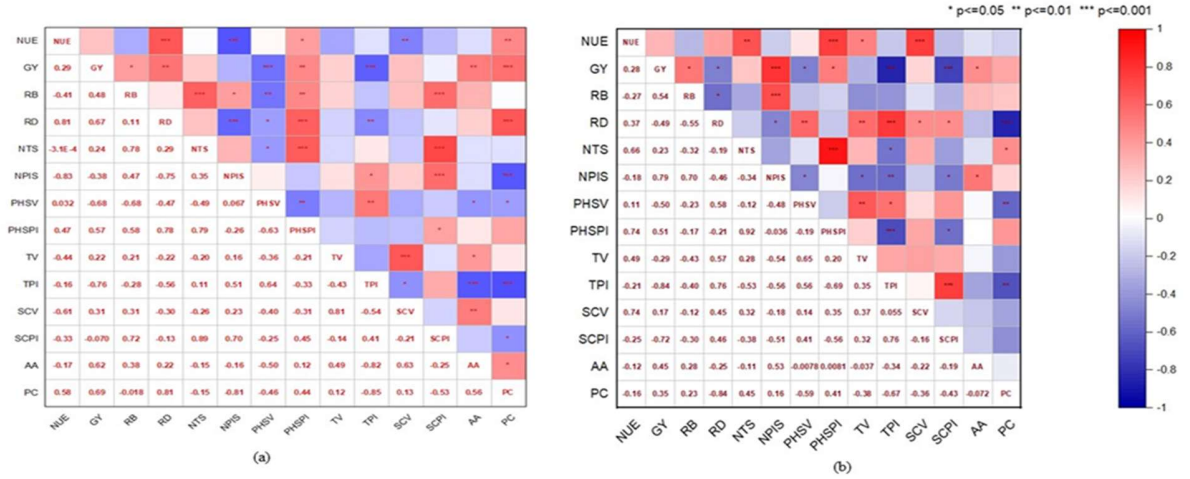

Figure 2: Correlation coefficient analysis of nitrogen use efficiency (NUE) and its contributing traits under (a) conventional management and (b) organic management

NUE - Nitrogen use efficiency, GY - Grain yield per hill, RB - Root biomass, RD - Root depth, NTS - Soil N at Tillering stage, NPIS - Soil N at panicle initiation stage, PHSV - Photosynthetic rate at vegetative stage, PHSPI - Photosynthetic rate at panicle initiation stage, TV - Transpiration rate at vegetative stage, TPI - Transpiration rate at panicle initiation stage, SCV - Stomatal conductance at vegetative stage, SCPI - Stomatal conductance at panicle initiation stage, AA – Amino acid content, PC - Protein content

Conclusion

The four rice varieties used in this study showed significantly better performance under conventional nutrient management in terms of growth, physiological, and yield parameters. Although the nitrogen use efficiency of each variety varied significantly with nutrient management practices, the variation was least in Jaiva (23.8%), which is the organic rice variety released by Kerala Agricultural University. The increased photosynthetic rate at the panicle initiation stage, transpiration rate, and stomatal conductance at the vegetative stage might have contributed to increased uptake of nitrogen, leading to increased NUE and productivity under organic management.

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Conflict of interest

The authors declare that they have no conflict of interest.

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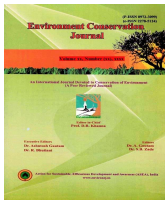

A randomized clinical trial of Shaman chikitsa versus Shaman chikitsa with vamana in vitiligo (Shwitra)

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ABSTRACT

Vitiligo is a chronic skin disorder due to melanocyte destruction in the epidermis. It is a social stigma. Therefore, it affects the patient psychologically as well. Various therapies have been evaluated in the management of vitiligo. Vamana is a helpful panchakarma to treat skin diseases, but it has still not been studied. To study the efficacy of Vamana, we randomly selected 30 patients with vitiligo and divided them into two groups of 15 patients in each group. For Group A, Vamana was administered, and oral Swayambhu Guggul was administered at 500 mg/day with cow urine and Savarnakar Lepa for local application. This treatment was given for six weeks. In Group -B, the same treatment was given without Vamana. The Vitiligo Area Severity Index (VASI) and overall assessment were used to assess the results. Group A decreased the score from 59.67 to 27.20 ± 18.28, and Group B decreased the score from 42.6 to 36.2 ± 7.58. The P value was statistically significant in Group A and nonsignificant in Group B. In the overall assessment, Group A showed statistically significant results. We concluded that Vamana with Shaman Chikitsa is more efficacious than Shaman Chikitsa alone, but more studies are required to ascertain whether vitiligo can be reversed completely by the combined treatment of Vamana and Shaman Chikitsa.

Introduction

Vitiligo has been known to humanity since ancient times. 'Celsus' first introduced the term 'vitiligo'. Kaposi demonstrated pigment granules in the epidermis of patients suffering from vitiligo. Brocq and Izzedine also contributed to understanding the pathogenesis of vitiligo (Bergqvist and Ezzedine, 2020). It is a disorder affecting melanocyte pigments in the skin. It appears as a dilution in the pigment of the affected skin areas. Vitiligo can be seen as a chalky white macule (Arora and Kumaran, 2017). The etiopathogenesis of this disorder is not entirely understood, but the autoimmune factor has been accepted as the cause. Other causes, such as genetic factors, melanocyte self-destruction, and oxidative stress, also play an essential role in the causation. Melanin pigment is found in the epidermis and is responsible for skin color. In this condition, the melanin pigment is absent, which causes white patches over the skin. In addition, $CD8^{+}$ T cells produce interferon-gamma (IFN γ), which destroys melanocytes(Rodrigues *et al.,* 2017). Social stigma has been reported with vitiligo across the world. In India, most vitiligo patients suffer from discrimination in their social life, leading to frustration, lack of confidence, and depression (Bergqvist and Ezzedine, 2020). Vitiligo is classified as segmental (SV) or nonsegmental vitiligo (NSV). The prevalence rate of vitiligo is 0.5 – 2% worldwide (4), but it is 8.8% in India. (Sarma *et al.,* 2020). A few studies have been carried out on managing vitiligo with Ayurvedic drugs. Sr. Donata, (Donata *et al.,* 1990) performed trials of

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Ayurvedic drugs in oral medication and external application for six months. The study claims that four out of ten patients had good relief. Another study (Narahari *et al.,* 2011) tried Virechana and local applications for vitiligo. Bharad S K et al.(Barad *et al.,* 2021) prepared Marichyadi Lepa in two different ways and tried it in vitiligo.

Bakuchi (Psoralea coryfolia) has been proven to be anti-vitiligo by H. Irshad et al. Moreover, D Ajay et al. (Dhanik *et al.,* 2011) used Shwitrahara Kashaya orally and Shwitrahara Lepa as a local application in 50 patients suffering from vitiligo and obtained promising results in their preliminary study.

In Charaka Samhita, Vitiligo is described as Kilas in the Kustha chikitsa chapter. Two terms, Shwitra and Kilas, are synonymously used in Ayurvedic texts. (*Charaka Samhita by Shukl, n.d.*2o16)' Tridosha is vitiated to cause Shwitra and Rasa, Rakta, Mamsa, and Meda Dhatu to be seen as involved (Barad *et al.,* 2021). There are two methods of treatment in Ayurveda: Panchakarma or Shodhan Chikitsa and only Ayurvedic medicines or Shaman Chikitsa. Panchakarma is advised as the mainstay in the treatment of vitiligo. However, there has yet to be a study carried out on Vamana Karma. Therefore, this study was carried out.

In this study, the conventional Ayurvedic drugs used in our hospital were administered to one group. In contrast, the same drugs were administered after Vamana Karma in the other group, assessing the results. The research question we tried to answer is "is Vamana Karma with Shaman Chikitsa more efficacious than Shaman Chikitsa alone in managing vitiligo? "

Our aim is to determine whether Vamana Karma with Shamana Chikitsa is more efficacious than Shamana Chikitsa alone in managing vitiligo. Therefore, the present study was carried out to study the efficacy of Vamana Karma and Shamana Chikitsa in managing vitiligo and to compare the efficacy of the combined effect of Vamana Karma and Shaman Chikitsa and Shaman Chikitsa alone in managing vitiligo.

Material and Methods

A randomized controlled clinical trial (RCT) was planned. A simple random sampling technique was used. No blinding was performed, as Vamana is a procedure. Therefore, this was an open study. A total of 30 patients suffering from vitiligo were included and divided into two groups (Figure 1) (Table 1).

Table 1: Table showing details of intervention

Procedure	Drug & dose	Duration in days/weeks
Deepana - Pachana	*Trikatu Churna* 3 gm twice a day	3
Snehapana	*Ghruta*	3-7
Abhyanga – Swedana	*Tila Taila* (*Abhyanga*)	2
Vamana Karma	*Madanphala pippali* 4 gm,, *Vacha* 2 gm, *Saindhav* 1 gm, *Madhu,* as per the requirement	1
Samsarjana Krama	Diet as per *Shuddhi*	3-7
Shamana chikitsa	*Svaymbhu Guggulu* 2tab thrice/day with *Go-mutra*	6 weeks
External application	*Savarnkar Lepa*	6 weeks
Svaymbhu Guggulu	Two tabs thrice/day with *Gomutra*	6 weeks
Savarnkar Lepa	External application once a day	Six weeks

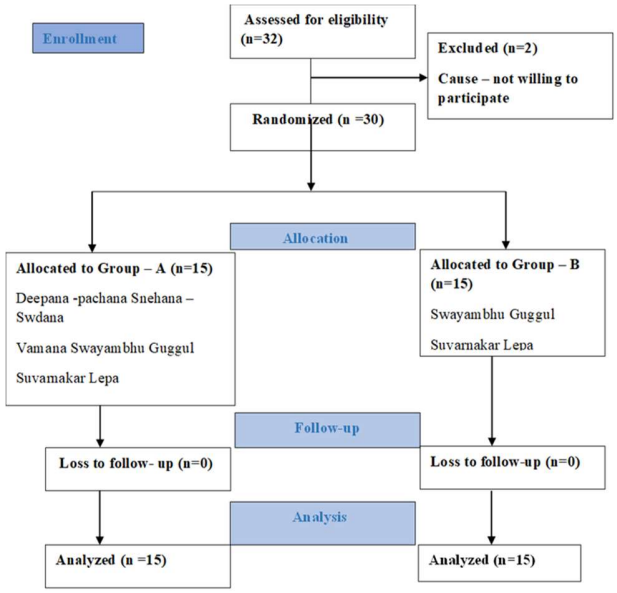

Figure 1: Randomized controlled trial (RCT) flow chart

Group A: Vamana group - 15 patients of Shwitra (Vitiligo) were treated with classical Vamana followed by internal administration of Svaymbhu Guggulu with Gomutra and external application of Savarnkar Lepa for six weeks.

Group B: Shamana group – In this group, 15 Shwitra (Vitiligo) patients were treated with

Svaymbhu Guggulu with Gomutra internally and Savarnkar *Lepa* for local application for six weeks. Classical Vamana Karma includes Deepana, Pachana, Snehapana, Abhyanga and Swedana, and the Vamana and Samsarjana Krama administration. We used the following inclusion criteria: patients showing classical signs and symptoms of Shwitra (segmental Vitiligo)**,** patients between the ages of 18 and 60 years, and patients with a chronic condition for less than five years. patients indicated for Vamana as per Ayurvedic classics. We used the following exclusion criteria: patients with all other depigmentary disorders, patients with serious cardiac, renal, and hepatic diseases, patients with systemic diseases such as hypertension, cardiac diseases, and diabetes, patches due to burning, chemical explosion, patients with known drug hypersensitivity, pregnant and lactating women, and patients with ongoing medications such as systemic corticosteroids, systemic or local photosensitizers or drugs that have been scientifically proven to cause hyperpigmentation on local application or systemic administration. The institutional ethics committee cleared the study (IEC No.69 Dated: 13/07/2016). The study was started after registration in the Clinical Trial Registry of India. (CTRI) Ref. CTRI/2018/ 04/013129, Registered on: 10/04/2018). Informed written consent was obtained on a printed consent form before enrollment in this study. This study was conducted in the OPD and IPD of Panchakarma, Akhandananda Ayurvedic College (Government) and Hospital, Ahmedabad, India, from 2016 to 2018. The materials, such as the drugs required for the local application, were obtained from the pharmacy of Akhandananda Ayurvedic College, Ahmedabad, and standardized in the Dravyaguna department, Akhandananda (Government) Ayurvedic College, Ahmedabad. Vamana was performed in the Panchakarma department, and the drugs and other materials required for Vamana were obtained from the Panchakarma department. (Table 2). Vamana involves Deepana – Pachana, Snehapana as a preparatory procedure, Vamana (drug-induced vomiting) as the primary procedure, and Dhoompana, dietary regimen as the postoperative procedure. It was performed per our institute's standard operating procedure (SOP). Svayambhu Guggul is a preparation mentioned in the 'Bhavprakash". (*Bhavprakash Nighantu 2017*.)

Table 2: Table showing drug name and proportion

Drug	Latin name	Proportion (In gram)
Swayambhu Guggul		
Bakuchi	*Psoralia corylifolia*	200
Shilajit	*Asphaltum punjabinum*	200
Swarnmakshik	*Copper pyrite*	200
Loha	*Iron sulfate*	400
Mundi	*Sphaeranthus indicus*	400
`Haritaki	*Terminalia chebula*	200
Amalaki	*Emblica officinalis*	200
Karanja	*Pongamia pinnata*	200
Khadir	*Acacia catechu*	200
Guduchi	*Tinospora cordifolia*	200
Trivrut	*Operculina terpenthum*	200
Danti	*Boliospermum montanum*	200
Musta	*Cyperus rotundus*	200
Vidang	*Embelia ribes*	200
Haridra	*Curcuma longa*	200
Kutaja	*Holarrhena antidysenterica*	200
Nimba	*Azadirachta indica*	200
Guggul	*Commiphora mukul*	400
Madhu	Honey	200
Suvarnakara Lepa		
Bakuchi	Psoralea corylifolia	1 part
Hartal	Yellow arsenic sulfide	1/4th part
Gomutra	Cow urine	As required

medicine was used orally at 250 mg in the morning and the same dose (total dose – 500 mg/day) in the evening after dinner with hot water. It is a Herbo-mineral preparation containing 19 constituents. Suvarnakar Lepa is a preparation mentioned in the text 'Ashtanghridayam' *(Ashtang Hridaya by Vagbhata,* 2019). It has three ingredients. It contains 'Hartal', a toxic substance (arsenic sulfide); therefore, it was used only after purification, as mentioned in the text. A fine powder of the above ingredients was prepared and given to the participants, and they explained the procedure of Lepa in the local language. Then, they were asked to add fresh cow urine or cow urine prepared from Gomutra Arka if fresh was unavailable. The patients were assessed after the

intervention. In both groups, the intervention was given for six weeks, and on the 7th week, the participants were called to assess the outcome measures. We used the Vitiligo Area Severity Index (VASI) score (Hamzavi *et al.,* 2004) for the assessment.

$$\text{Total Body VASI} = \sum[\text{all body sites}] \times [\text{residual depigmentation}] \div [\text{hand units}]$$

In this study, the measurement of VASI requires area in palm units. This is because many lesions are within a palm area. Therefore, it is difficult to note it in palm units. To make the calculation, we divided the palm into 4 parts: <¼ palm = 0.25%, ¼ to ½ palm = 0.5%, ½ to ¾ palm = 0.75%, and > ¾ to 1 palm = 1%. Measurements of the lesions were taken. All the parameters are mentioned in grades, so the VASI scores are given in 6 grades to make and compare calculations. The VASI score was noted in the study group, and the minimum score was calculated. For the overall assessment, we recorded repigmentation as unchanged (0%), 'mild' (> 25%), 'moderate' (> 50%), 'marked' (>75%), and complete remission (< 75%).

Results and Discussion

Of the 30 patients, more males (n= 19, 63.33%) were involved in the study than females (n= 11, 36.67%). Of the age groups, the 18 – 30 age group had a higher percentage than the others. The Hindu religion category consists of more patients. In the profession, students are at the top. More educated patients suffered from vitiligo in our study. The middle-class more than others. More rural residents are seen than urban residents. More married individuals were found than unmarried individuals in our study. Kapha – Pitta Prakriti individuals were more numerous than other Prakriti individuals. Among the Koshtha, Madhyam was seen to be more prevalent (Table 3). In Group 1, Vamana was performed. During Vamana, Madhyam Shuddhi was found in more individuals. In the skin color, white was seen more than others. In the area involved, the body parts hand and legs were commonly seen.

Table 3: Categories of Patient studied

VARIABLE	DATA		
	Gr.1 (n=15)	Gr.2 (n=15)	Total (n=30)
Sex			
Male	11 (57.89)	8 (42.11)	19 (63.33)
Female	4 (36.37)	7 (63.63)	11 (36.67)
Age (in years)			
18 – 30	7 (46.67)	8 (53.33)	15 (50.00)
31 -40	5 (71.42)	2 (28.58)	7 (23.33)
41 – 50	2 (33.34)	4 (66.66)	6 (20.00)
51 -60	1 (50.00)	1 (50.00)	2 (06.60)
Religion			
Hindu	13 (50.00)	13 (50.00)	26 (86.66)
Muslim	2 (50.50)	2 (50.00)	4 (13.33)
Occupation			
Students	4 (36.37)	7 (63.63)	11 (36.36)
Housework	5 (50.00)	5 (50.00)	10 (33.33)
Laborer	3 (60.00)	2 (40.00)	5 (16.66)
Business or job	3 (75.00)	1 (25.00)	4 (13.33)
Education level			
Less educated	6 (42.86)	8 (57.14)	14 (46.67)
Highly educated	9 (56.25)	7 (43.75)	16 (53.33)
Economic status			
Poor	3 (50.00)	3 (50.00)	6 (20.00)
Middle class/rich	12 (50.00)	12 50.00)	24 (79.99)
Habitat			
Urban	3 (42.86)	4 (57.14)	7 (23.24)
Rural	12 (52.17)	11 47.83)	23 (76.66)
Marital status			
Married	11 (57.9)	8 (42.10)	19 (63.33)
Unmarried	4 (36.37)	7 (63.63)	11 (36.66)
Prakriti			
Vata-Pitta	3 (37.5)	5 (62.5)	8 (26.66)
Vata -Kapha	5 (83.33)	1 (16.67)	6 (20.00)
Kapha – Pitta	7 (43.75)	9 (56.25)	16 (53.33)
Koshtha			
Krura	1 (25.00)	3 (75.00)	4 (13.33)
Mridu	3 (57.15)	7 (42.85)	10 33.33)
Madhyam	11 (68.75)	5 (31.25)	16 (53.33)
Shuddhi in Vamana			
Pravara	6 (40.00)	-	6 (40.00)
Madhyam	8 (53.55)	-	8 (53.55)
Avara	1 (6.66)	-	1 (6.66)
Color of patches with a score			
Normal skin (1)	0 (0)	0 (0)	0(0)
Red (2)	2 (33.33)	4 (66.67)	6 (20.00)
Whitish/reddish (3)	6 (60.00)	4 (40.00)	10 (33.33)
Red to white (4)	3 (50.00)	3 (50.00)	6 (20.00)
White (5)	4 (50.00)	4 (50.00)	8 (26.66)
Area involved			
Scalp	4 (80.00)	1 (20.00)	5 (16.66)
Face	3 (50.00)	3 (50.00)	6 (20.00)
Hands	9 (42.85)	12 57.15)	21 (70.00)
Legs	13 (54.16)	11 (45.89)	24 (80.00)
Buttocks	2 (50.00)	2 (50.00)	4 (13.33)
Trunk	2 (28.57)	5 (71.43)	7 (23.33)
Back	8 (66.66)	4	12 (40)

Effect on VASI

The VASI score decreased from 59.67 to 27.20 ± 18.28 in Group A and from 42.6 to 36.2 ± 7.58 in

Group B. The P value is statistically significant in Group A and nonsignificant in Group B (Table 4).

Overall Assessment

The overall assessment showed that 6 (40%) patient in Group A reported marked improvement, while 9 (60%) patients had moderate improvement. In group B, 8 (53.5%) reported moderate improvement, while 7 (46%) reported mild improvement (Table 5 and Figure 2).

Table 4: Table showing effect on VASI score

Score	Gr	Mean		Mean Difference	%	Diff SD±	Diff SE±	't'	'p'	S
		BT	AT							
VASI	A	59.67	27.20	32.47	53.42↓	18.28	4.721	6.878	<0.001	HS
	B	42.6	36.2	6.4	15.02↓	29.38	7.58	0.843	>0.05	IS

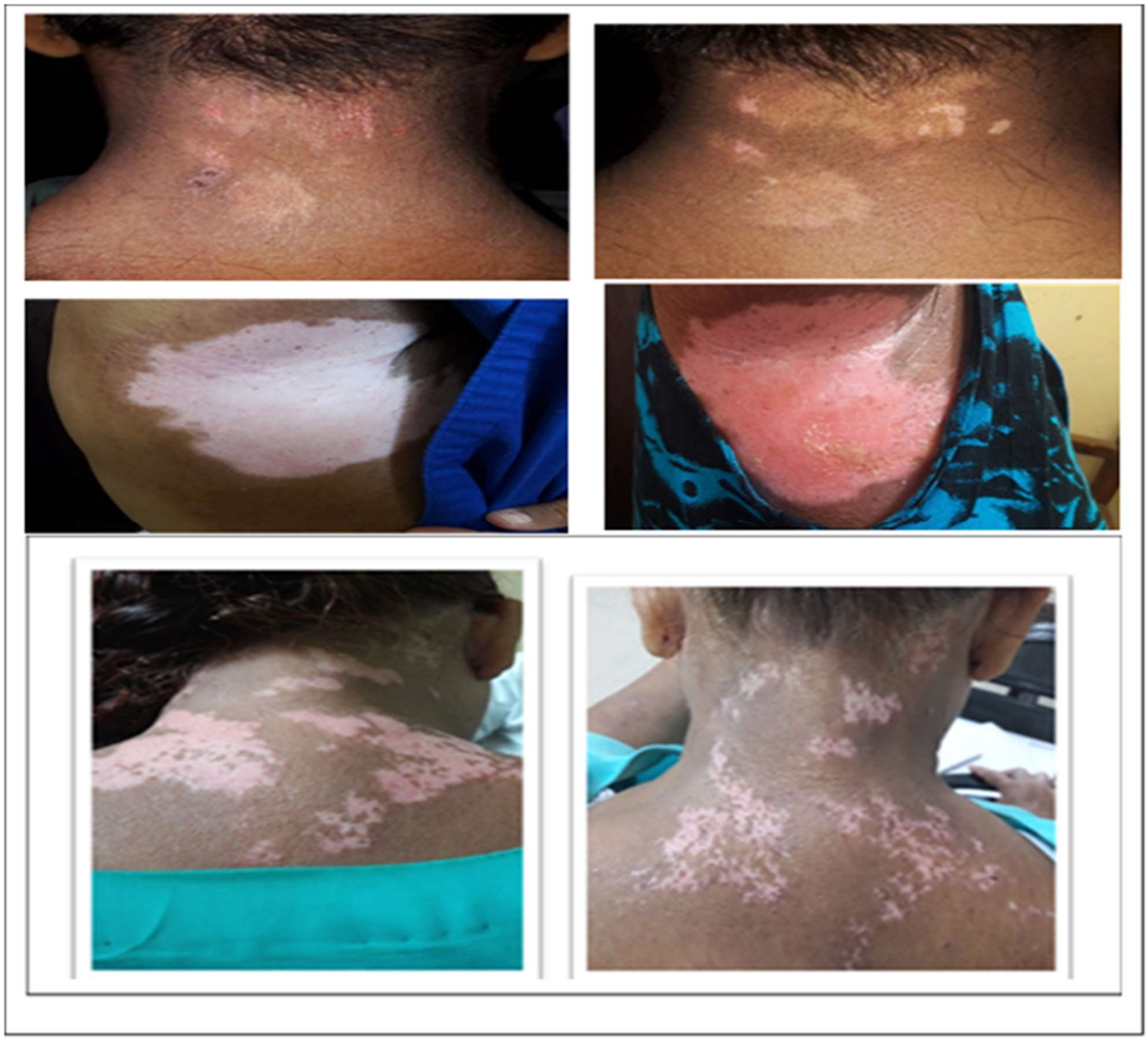

Figure 2: Figure showing before and after treatment pictures of patient

Table 5: Table showing overall assessment

Gradation	Group A (n=15)	Group B (n=15)
Complete remission	0	0
Marked improvement	6 (40)	0
Moderate Improvement	9 (60)	8 (53.53)
Mild improvement	0	7 (46.67)
Unchanged	0	0

This trial was aimed to determine whether Vamanakarma with Shaman Chikitsa is more efficacious than Shaman Chikitsa alone. The 30 randomly selected Vitiligo patients were divided into two groups: in one group, Vamana Karma was administered with Shamana Chikitsa, and in the other group, only Shaman Chikitsa was administered. The assessment criteria were the VASI scale and the overall assessment. The Vamana Group showed statistically significant results compared with Shaman Chikitsa alone. We found more males (19, 63.33%) than females (11, 36.67%), which is in contrast to the findings made by N Sama, S Chakraborty, S Poojary et al.(Sarma *et al.,* 2020) who found 0.86 and 0.93% in their study. We found 50% of patients in the age group 18-30 years (23%), 20% in 31-40 years, 41-50 years and 51-6 years, which was also found by N Sama, S Chakraborty, S Poojary et al.(Sarma *et al.,* 2020) We also found more Hindu patients (26%) than Muslims (4%), students (11%) than housework (10%), laborers (5%), and business or job workers (4%). vitiligo is seen with a varied presentation. The state of Gujarat has the highest prevalence of vitiligo (8.8%) in India. This study was carried out in Gujarat (Bergqvist and Ezzedine, 2020) Therefore, there are some contrasting findings compared to previous studies that apply to all of India. In the VASI score, Group A had statistically significant results, and overall assessment, Vaman Karma showed better results. It causes drastic changes in the epidermis and dermis of the skin. Normally, the epidermis consists of dead or about-to-die cells in large amounts. Vamana Karma may stimulate the rapid disposal of dead cells. It may also stimulate melanocyte production.The patients accepted Vamana Karma well, and we did not find any complications of Vamana. Vitiligo is very chronic and very refractory to any treatment. However, it should be noted that marked changes occurred in the vitiligo during Vamana Karma and the administration of medicines. The milky white appearance of the skin began to change into pink, red, or brown in some cases. The boundaries of the vitiligo patches stopped spreading further. However, the patches were sometimes very refractory and did not change. This study has many limitations. We could not perform a long follow-up to check the viability of these effects. It is essential to follow up at least for a year to see the changes in the vitiligo patches. A sample size of 30 is low. Vamana being a procedure, blinding was not possible.

Conclusion

The Vamana Karma group showed a better effect than palliative therapy (Shaman Chikitsa) alone in managing vitiligo. This study highlights the importance of Vamana Karma before Shaman Chikitsa. Therefore, Vamana Karma should be performed in Vitiligo. However, the results are primary, and longer follow-up needs to be conducted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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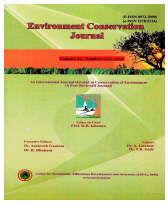

Performance of different rice-based cropping systems in the wet temperate zone of Himachal Pradesh

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ABSTRACT

A field experiment to diversify the rice–wheat system to increase employment and income was conducted during 2017-18 at the research farm of CSK Himachal Pradesh Krishi Vishwavidyalaya Palampur with eight cropping systems that were replicated four times. One-year results revealed that the highest rice grain yield (16477 kg/ha), net returns (₹ 219828/ha) and marginal returns (₹80946) were recorded from the rice – palak – cucumber sequence. Okra – radish – onion resulted in maximum food availability (55.16 kg/ha/day), whereas employment generation was maximum from rice – lettuce – potato + coriander (102.91%). In terms of carbohydrate yield (5146 kg/ha), protein yield (640.6 kg/ha) and energy equivalent (23919 MJ/ha), it was highest from the rice – wheat sequence; however, in the case of carbohydrate and energy equivalent, it was on par with rice – lettuce – potato + coriander.

Introduction

The Indian population is growing at a rate of 1.8% annually and is likely to surpass that of China within the next decade (Samir *et al.,* 2018). This is an inevitable concern that further raises other issues, such as food security, unemployment and poverty, in the country. Demand for food is increasing with population rise and is expected to increase by 75-100% globally by 2050 (Keating *et al.,* 2010; Tilman *et al.,* 2011), which will be even higher for India. Cereal-based mono- and double-cropping cannot meet the food and income demand of the rising population and thus requires land intensification with high-value crops. Crop diversification is a sustainable way to intensify land use, which not only increases income but also provides employment to youth and balanced food to farmers. System integration improves both food and nutritional security, enhances land and water productivity, and preserves ecosystems (Ayyappan *et al.,* 2009). Rice – wheat is a predominant cropping system in India, which covers approximately 10.5 Mha of the country (Sarkar, 2015). This system is highly exhaustive and unsustainable, causing resource depletion. India is a land with a diverse climate that is rainfall dominant and allows farmers to grow different crops. Growing short-duration vegetables after rice or replacing both cereal crops with vegetables has a positive effect on equivalent yield and employment. Diversification with high-value crops such as vegetables is also required to fulfill the motto of doubling farmers' income by encouraging the export of farm produce. Therefore, traditional rice–wheat cropping systems can be replaced by vegetable crops for more profitability and sustainability.

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Therefore, the present study was carried out to diversify the rice–wheat system to increase employment and income.

Material and Methods

Study area

A field experiment was conducted during 2017-18 under the All India Co-ordinated Research Project at the Bhadiarkhar research farm of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, which is situated at $32^0 6'$ N latitude and $76^0 3'$ E longitude and at an altitude of 1223.7 m above mean sea level. Farm soil was acidic in reaction with silty clay loam texture at the start of the experiment. The soil of the study area was medium in available nitrogen and potassium and high in available phosphorus. The research farm received 2851 mm of rainfall during the one-year crop cycle, the majority of which was received from June to September (Figure 1).

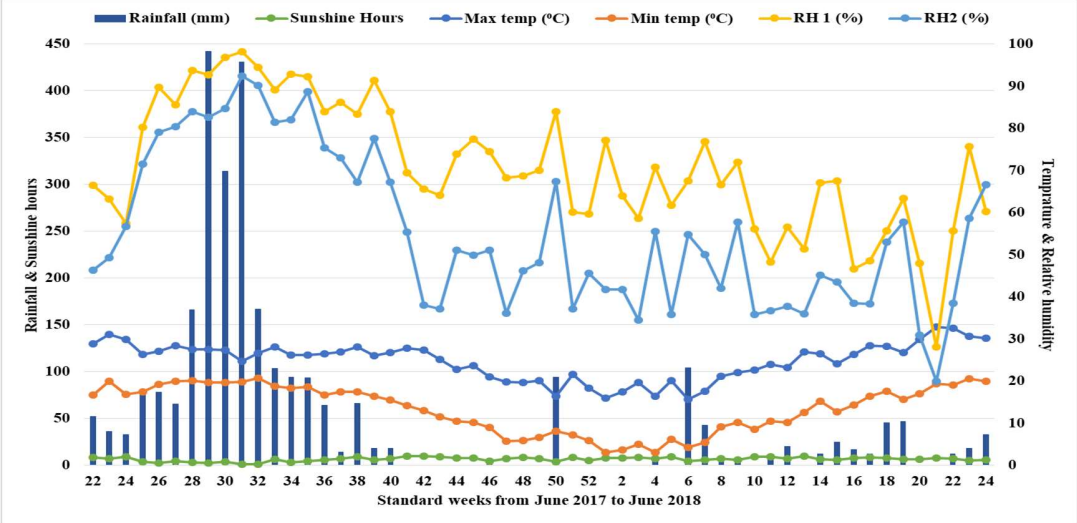

Figure 1: Mean weekly meteorological data from June 2017 to June 2018

Treatment details

Eight rice-based cropping systems, viz. T_1: Rice – Wheat, T_2: Rice – Pea – Summer squash, T_3: Okra – Radish – Onion, T_4: Turmeric – Pea – Summer squash, T_5: Rice – Lettuce – Potato + Coriander, T_6: Rice – Palak – Cucumber, T_7: Rice – Broccoli – Radish and T_8: Colocasia – Pea + Coriander were evaluated in a randomized block design with four replications.Economic yield obtained from different crop sequences has been expressed in terms of carbohydrate yield, protein yield and chemical energy to facilitate comparison among crop sequences based on their content in the economic part.

$$\text{Rice equivalent yield (kg/ha)} = \text{Economical yield of a crop (kg/ha)} \times \frac{\text{Price (₹/kg) of same crop}}{\text{Price (₹/kg) of rice}}$$

$$\text{Relative production efficiency (\%)} = \frac{\text{REYD} - \text{REYE}}{\text{REYD}} \times 100$$

where REYD is the rice equivalent yield under the diversified system and REYE is the rice equivalent yield of the existing system.

$$\text{Relative economic efficiency (\%)} = \frac{\text{NRD} - \text{NRE}}{\text{NRE}} \times 100$$

where NRD is the net returns obtained under a diversified system and NRE is the net returns of the existing system.

$$\text{Employment generation (\%)} = \frac{\text{MDD} - \text{MDE}}{\text{MDE}} \times 100$$

where MDD is the man-days required in the diversified system and MDE is the man-days required in the existing system.

The cost of cultivation of each crop was computed based on the prevailing market price of the inputs during the respective crop season.

Net returns were measured by deducting total returns from the cost of cultivation.

Results and Discussion

Economic yield and rice equivalent yield

The yields of most of the crops were reported to be lower than the reported yields in the region except for rice, wheat, onion and cucumber (Table 1) Yield of kharif crops viz. okra, turmeric and colocasia were very low because of adjacent flooded rice plots, which made conditions unfavorable for the growth of these crops. Rice yield was maximum for the rice – palak – cucumber 164.77 q/ha) sequence, followed by okra – radish – onion (140.25 q/ha). The lowest rice yield was recorded in the rice–wheat system. The equivalent yield of rice–wheat was reported to be 2.6–1.2 times less than that of other vegetable-intensive cropping systems because of the low cropping intensity and lower sale price of cereals in the market compared to other high-value crops, such as vegetables. Babu *et al.* (2016) and Choudhary *et al.* (2013) also reported higher yields in diversified systems than in existing traditional systems.

Table 1: Economic yield. Rice grain equivalent yield (RGEY), production efficiency and relative production efficiency of different cropping systems

Cropping sequence		Yield of crops (kg/ha)				RGEY (kg/ha)	Production efficiency	Relative production efficiency
		Kharif	Rabi	Summer	Intercrop			
T_1	Rice – Wheat	4072	2273	-	-	6259	21.44	
T_2	Rice – Pea – Summer squash	3598	2273	7292	-	13163	46.68	110.31
T_3	Okra – Radish – Onion	1098	5398	13636	-	14025	52.14	124.08
T_4	Turmeric – Pea – Summer squash	2794	1610	7008	-	13506	42.61	115.79
T_5	Rice – Lettuce – Potato + Coriander	3930	3409	8523	379	12547	45.96	100.46
T_6	Rice – Palak – Cucumber	3409	1420	11648	-	16477	63.62	163.25
T_7	Rice – Broccoli – Radish	2841	2367	3598	-	7599	29.46	21.41
T_8	Colocasia – Pea + Coriander	8144	1894		237	10630	40.26	69.84
	CD					1737	6.61	

Productivity and relative production efficiency

Productivity also followed a similar trend as rice grain equivalent yield. Significantly higher production efficiency was reported in the rice – palak – cucumber cropping system, whereas the rice – wheat cropping system recorded the lowest productivity. Production efficiency greatly depends upon the yield and market price of the crop (Dhiman, 2010). Similar results were also reported by Singh *et al.* (2007), where more productivity in diversified cropping systems was recorded. The relative production efficiency of rice – palak – cucumber was 163.25% higher than that of the existing rice – wheat system, which was followed by the okra – radish – onion sequence (124.08%). Overall, system productivity compared to the rice–wheat system varied from 163.25% to 21.41%.

Carbohydrate yield, protein yield and energy equivalent

Significantly higher carbohydrate yields (5146 kg/ha) and energy equivalents (23919 MJ/ha) were reported in the rice–wheat sequence (Table 2). This sequence was statistically at par with rice – lettuce – potato + coriander. The next highest carbohydrate yields were rice – pea – summer squash, rice – palak – cucumber, rice – broccoli – radish and turmeric – pea – summer squash. Significantly lower carbohydrate yield (1767 kg/ha) and energy equivalent (8120 MJ/ha) were reported in okra – radish – onion, which was on par with colocasia – pea+coriander (2034 kg/ha and 9765 MJ/ha, respectively). This was because of the higher carbohydrate content in rice, wheat and potato. Earlier similar results have been reported by Singh and Sharma (2002) in the case of carbohydrate yield in rice-based crop sequences. Sharma *et al.* (2008) reported similar results with higher energy equivalents of rice – wheat crop sequences than the rest of the rice-based sequences. The maximum protein yield (640.6 kg/ha) was recorded from the rice–wheat sequence. This sequence was followed by rice – lettuce – potato + coriander, rice – pea – summer squash, colocasia – pea + coriander, rice – palak – cucumber, turmeric – pea – summer squash and rice – broccoli – radish. A significantly lower protein yield (222.3 kg/ha). Sharma *et al.* (2008) reported similar results with higher energy equivalents of rice – wheat crop sequences than the rest of the rice-based sequences. The maximum protein yield (640.6 kg/ha) was recorded from the

rice–wheat sequence. This sequence was followed by rice – lettuce – potato + coriander, rice – pea – summer squash, colocasia – pea + coriander, rice – palak – cucumber, turmeric – pea – summer squash and rice – broccoli – radish. A significantly lower protein yield (222.3 kg/ha).

Table 2: Carbohydrates, protein yield, energy equivalent, man-days and employment generation in different cropping systems

Cropping sequence		Carbohydrate Yield (kg/ha)	Protein Yield (kg/ha)	Energy Equivalent (kg/ha)	Man-days per ha	Employment generation (%)
T_1	Rice – Wheat	5146	640.6	23919	172	
T_2	Rice – Pea – Summer squash	3369	470.0	15731	299	73.83721
T_3	Okra – Radish – Onion	1767	222.3	8120	322	87.2093
T_4	Turmeric – Pea – Summer squash	2433	326.9	12368	317	84.30233
T_5	Rice – Lettuce – Potato + Coriander	5049	515.2	22747	349	102.907
T_6	Rice – Palak – Cucumber	2947	330.7	13679	288	67.44186
T_7	Rice – Broccoli – Radish	2459	305.0	11246	281	63.37209
T_8	Colocasia – Pea + Coriander	2034	388.5	9765	257	49.4186
	SEm±	164	23.4	767		
	CD	481	68.9	2257		

was recorded in the okra – radish – onion crop sequence. Higher carbohydrate yield, protein yield and chemical energy equivalent in the rice – wheat sequence was because of more carbohydrates, protein and calories in cereals compared to vegetable crops.

Food availability and employment generation

Okra – radish – onion resulted in a significantly higher availability of food (55.16 kg/ha/day) throughout the year than the rest of the crop sequences (fig 2). This was due to the higher yield of onion. This sequence was followed by rice – palak – cucumber, rice – lettuce – potato + coriander and rice – pea - summer squash. The rice–wheat cropping sequence resulted in significantly lower food availability (18.94 kg/ha/day). All the systems generated more employment (table 2) compared to the rice – wheat system (172 man-days).

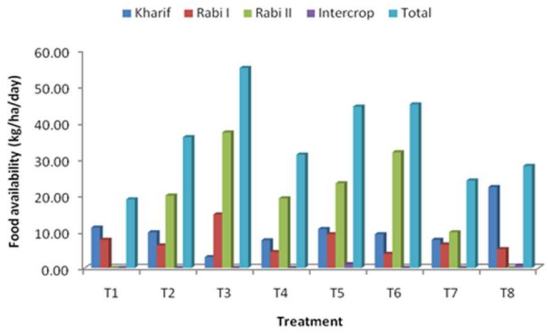

Figure 2: Effect of different treatments on food availability

The maximum employment generation was from rice – lettuce – potato + coriander, which generated 349 man-days. This was because of more intensification of land than other sequences. Similar observations were also recorded by *Chitale et al.* (2011) with diversified rice-based cropping systems. Relative employment generation shows that rice – lettuce – potato + coriander generated 102.91% more employment than the existing rice – wheat system.

Economic analysis

Table 3 and Figure 3 shows that the highest cost of cultivation was recorded in turmeric – pea – summer squash (₹ 168040/ha), followed by rice – lettuce – potato + coriander, okra – radish – onion and colocasia – pea + coriander sequences. This was due to the high seed rate of turmeric and potato as well as the labor-intensive nature of both crops. The rice–wheat sequence had the lowest cost of cultivation (Rs 82014/ha) among all the crop sequences. This was due to the less intensified system, which requires less labor for management. Significantly higher returns (gross and net returns) were observed from the rice – palak – cucumber crop sequence (₹ 344488/ha and ₹ 219828/ha, respectively), whereas the lowest returns were recorded from the rice – wheat and rice – broccoli – radish sequences. This may be attributed to the high yield and price of cucumber resulting in higher returns. Similar results were also reported by Prasad *et al.* (2013).

Table 3: Economic analysis of different cropping systems

Cropping sequence		Gross returns	Net returns	Relative economic efficiency (%)	Marginal Cost (MC)	Marginal Returns (MR)	MR:MC	Returns (₹/ha/day)
T_1	Rice – Wheat	163542	80163					274.53
T_2	Rice – Pea – Summer squash	285914	156903	95.73	45632	122372	2.68	556.39
T_3	Okra – Radish – Onion	282395	152392	90.10	46624	118853	2.55	566.51
T_4	Turmeric – Pea – Summer squash	275846	107806	34.48	84661	112304	1.33	340.08
T_5	Rice – Lettuce – Potato + Coriander	266004	84044	16.11	98581	102462	1.04	340.93
T_6	Rice – Palak – Cucumber	344488	219828	174.23	41281	180946	4.38	848.76
T_7	Rice – Broccoli – Radish	168196	45609	-43.10	39208	4654	0.12	176.78
T_8	Colocasia – Pea + Coriander	224091	94718	18.16	45994	60549	1.32	358.78
	SEm±	11999	11999					45.64
	CD	35295	35295					134.26

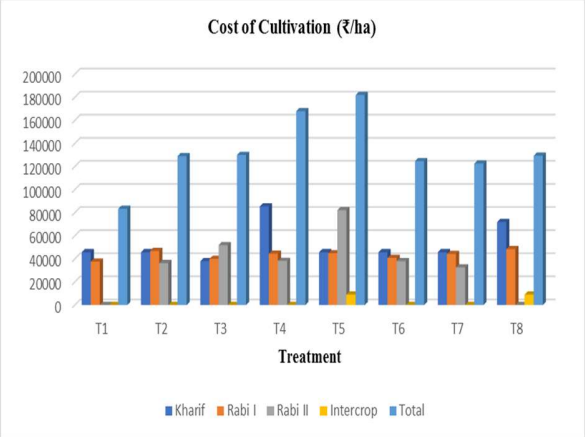

Figure 3: Cost of cultivation of different cropping systems

All cropping systems except rice – broccoli – radish were found to be advantageous over the rice – wheat system in terms of relative economic analysis. The highest economic efficiency was obtained in the rice – palak – cucumber sequence, which was 174.23% higher than that in the traditional rice – wheat system. This system was followed by the rice – pea – summer squash (95.73%) and okra – radish – onion sequences (90.10%). Similarly, marginal returns, MR:MC and returns (₹/ha/day) were also higher for the rice – palak – cucumber system (₹180946, 4.38 and 848.76 ₹/ha/day, respectively).

Conclusion

From the research, it can be concluded that in terms of rice grain equivalent yield, productivity, food availability, employment generation, profitability and returns, the diversified systems remained superior to the rice–wheat system. Therefore, farmers should opt for diversified cropping systems depending upon the available resources and market demand of crops.

Conflict of interest

The authors declare that they have no conflict of interest.

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Health status of college-going girls (female undergraduates) as an expression of anemia and BMI

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ABSTRACT

Anaemia and being underweight are two global public health issues that include the significant population of girls of adolescents, directly affecting one's working capacity and posing a great risk for future motherhood. In this study, a total of 798 college-going girls were observed with their height, weight and haemoglobin level over a period of five years. The mean age of the girls was 18 years, within the range of 17 years to 22 years. Anaemia poses a significant threat on a worldwide scale; in the present study, it was observed that 22% of the girls are severely anaemic, 34% are moderately anaemic, 17% are mildly anaemic, and only 26% are non-anaemia, which is significantly alarming about their health condition. On the other hand, we calculated their body mass index (BMI) with height and weight. It was found that only 36% of girls had a normal BMI, while 44% of the girls were underweight, which also raises concerns about their health issues for the near future. Although no direct relation can be drawn between the severity of anaemia and BMI, the parameters can help to express one's overall health status and can be used to improve health rights from adolescence.

Introduction

Anemia and being underweight are two global public health issues that not only affect one's working capacity but also raise concerns about health conditions in the near future. This is one of the most widespread blood disorders. RBCs carry hemoglobin, an iron-rich protein that attaches to oxygen in the lungs and takes it to tissues throughout the body. Anemia occurs when the body does not have enough red blood cells or the hemoglobin concentration is lower than expected.

Iron deficiency anemia is a highly prevalent and seemingly intractable problem, particularly among females of reproductive age in developing countries. Following early childhood, during the adolescent growth spurts, the risk of iron deficiency and anemia reappears for both boys and girls, after which it subsides for boys but remains for girls because of menstrual loss **(**Rahman, *et al.,*2023).

Anemia is usually widespread among all age groups in all states of India. College girls contribute a significant portion of the population. Reports state that approximately 80% of the female population has hemoglobin deficiency (Minchekar, 2017).

Anemia and being underweight are two significant problems that are nutritionally related to global public health problems. Adolescent girls are future mothers, and the future generation's health directly rests on their health condition. Studies have shown that anemic girls show a reduced physical and mental capacity and diminished concentration in studies, thus causing a significant threat to future safe motherhood (WHO, UNICEF, &UNU;2001).

"In the academic life of students, academic achievement has been considered an important factor and can be associated with self-confidence and self–esteem, which leads to better adjustment in school and society. Academic achievement upgrades self-improvement, self-actualization, and some degree of competitiveness" (Mehdi *et al.,*2014). A significantly positive relationship between hemoglobin and academic achievement was also found by Sungthong and Mo-suwan (2002). A decreased ability of the blood to carry oxygen to the body's tissues leads to lowered resistance to

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infection, poor cognitive development, fatigue and skin paleness, shortness of breath, fast, irregular heartbeat, low blood pressure, headache, poor memory, difficulty in thinking, cold hands and feet and reduced work productivity, thereby reducing one's work capacity. Therefore, to fight this burning problem in girls, the Iron^{+} initiative program was initiated for newly admitted girl students in the college.

The girls were advised to carry out blood tests, especially HB content, to know their anemic status, as hemoglobin concentration is widely used to measure anemia. Moreover, other parameters, such as height and weight, were considered to calculate their BMI values and age. The study aimed to determine the hemoglobin levels and body mass index (BMI) as an expression of the overall health status of college-going girls (female under graduates).

Material and Methods

Approximately 798 female students aged 17-22 years were estimated for their HB level, and the same was true for the BMI value. Hb count (Hb gm %) was determined by a standard Sahli's apparatus. All precautions that must be taken during blood experiments were taken. Only disposable pricking needles were used for every individual, and the form was filled out to obtain the girl students' height, weight and age.

Following WHO criteria were followed to diagnose anemia.

No anemia	Hb content	≥12 g/dl
Mild Anemia	Hb content	11–11.9 g/dl
Moderate anemia	Hb content	8–10.9 g/dl
Severe anemia	Hb content	<8 g/dl

Body mass index (BMI) was calculated as weight for height using the standard WHO formula:

Weight (kg)/size (m)]^2

BMI	Categories
Underweight	<18.5
Average weight	18.5–22.9
Overweight	23–24.9
Obesity	≥25

The weight was measured by standing in the center of the scale platform or weighing machine and remaining motionless until the measurement could be obtained—weight (kg). The height was measured by a wooden height measuring board that had a sliding head bar to the nearest 0.1 cm on standing straight without wearing shoes. Height: (Feet) (inches).

Results and Discussion

In this study, a total of 798 college girls were observed with their height, weight and hemoglobin level over a period of five years (2013-14 to 2017-18). The mean age of the girls was 18 years, with a range of 17 years to 22 years. With the help of height and weight, the body mass index of the girls was calculated by the formula

BMI=Weight (kg)/Height (Feet) (inches).

The girls' minimum BMI was 11.98 kg/m^{2,} and the maximum was 41.42 kg/m^2. The mean BMI was 19.96 kg/m^2 with a standard deviation of 4.47. Yearwise summary findings are presented in the tables and figures given below.

From Table 1, we can see that the maximum number of girls (65%) was ≥18 years of age. Moreover, each year, the percentage of girls over or similar to 18 years of age is greater than that of girls under age less than 18 years., i.e., a significant population of girls are adolescents (Figure 1).

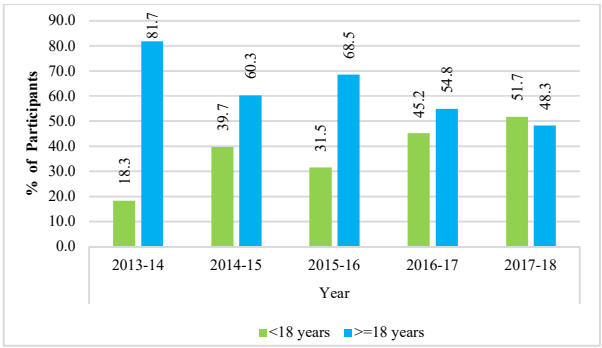

Figure 1: Distribution of age

In the sample of the years 2017-18, a maximum number of girls (31%) were observed with severe anemia. In 2016-17, almost equal numbers of girls (37%) were moderately anemic, while only 37.9%

were nonanaemic. In 2015-16, a similar percentage (26.8%) of the girls were severely anemic, whereas the rest were almost equally distributed among the remaining categories. In 2014-2015, a minimum percentage (13%) of the girls had severe anemia, and 20% had mild anemia, as the rest were almost equally distributed among the remaining categories. In 2013-2014, the lowest percentage (14%) of the girls had mild anemia, and only 19% were nonanaemic. The majority of girls (43%) had moderate anemia. Table 4 shows that 22% of the girls were severely anemic, 34% were moderately anemic, 17% were mildly anemic, and only 26% were nonanaemic (Table 2, Figure 2). From Table 3, we can observe that 44% of the girls are underweight, and 36% have a normal BMI. Only 7% were overweight, and 12% were in the category of obesity. An almost similar distribution pattern was observed through the years concerning body mass index (Figure 3). The results indicate that only 26% of students were found to be Non-Anemic, 17% were Mild Anemic, 34% were Moderately Anemic, and 22% girl students were severely anemic (Table 2), which might result in lower scores on the examination as they become

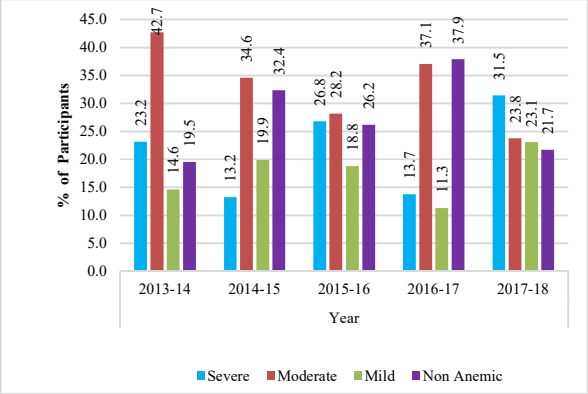

Figure 2: Categorization of anemia level

Table 1: Distribution of age

Age (years)	Year					Total
	2013-14	2014-15	2015-16	2016-17	2017-18	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
<18	45 (18.2)	54 (39.7)	47 (31.5)	56 (45.1)	74 (51.7)	276 (34.59)
>=18	201 (81.7)	82 (60.2)	102 (68.4)	68 (54.8)	69 (48.2)	522 (65.41)
Total	246 (100)	136 (100)	149 (100)	124 (100)	143 (100)	798 (100)

Table 2: Categorization of anemia level

Anemia (g/DL)	Year					Total
	2013-14	2014-15	2015-16	2016-17	2017-18	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Severe (<8)	57 (23.1)	18 (13.2)	40 (26.8)	17 (13.7)	45 (31.4)	177(22.2)
Moderate (8-10.9)	105 (42.6)	47 (34.5)	42 (28.1)	46 (37.0)	34 (23.7)	274 (34.3)
Mild (11-11.9)	36 (14.6)	27 (19.8)	28 (18.7)	14 (11.2)	33 (23.0)	138 (17.3)
Non Anemic (>=12)	48 (19.5)	44 (32.3)	39 (26.1)	47 (37.9)	31 (21.6)	209 (26.2)
Total	246 (100)	136 (100)	149 (100)	124 (100)	143 (100)	798 (100)

Table 3: Categorization of Body Mass Index

Body Mass Index (kg/m2)	Year					Total
	2013-14	2014-15	2015-16	2016-17	2017-18	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Underweight (<18.5)	110 (44.7)	48 (35.2)	71 (47.6)	69 (55.6)	55 (38.4)	353 (44.2)
Normal (18.5-22.9)	86 (34.9)	63 (46.3)	47 (31.5)	42 (33.8)	50 (34.9)	288 (36.1)
Overweight (23-24.9)	21 (8.53)	7 (5.14)	14 (9.39)	6 (4.83)	15 (10.4)	63 (7.9)
Obese (>=25)	29 (11.7)	18 (13.2)	17 (11.4)	7 (5.64)	23 (16.0)	94 (11.8)
Total	246 (100)	136 (100)	149 (100)	124 (100)	143 (100)	798 (100)

exhausted early and could not attend classes with much concentration and energy.

In academic life, academic achievement is an essential factor. Sungthong and Mo-suwan (2002) found an appreciably positive relationship between hemoglobin and academic achievement. "The optimal hemoglobin concentration needed to meet physiologic needs varies by age, sex, etc. The most shared sources of anemia are nutritional deficiencies, particularly iron deficiency, although deficiencies in folate, vitamins B12 and A are also important causes; hemoglobinopathies; and infectious diseases, such as malaria, tuberculosis, HIV and parasitic infections" (https://www.who.int health-topics /anaemia#tab=tab_1.)

All the above observations found a need to create more awareness about eating habits among girl students, as they have a habit of eating junk food rather than nutritional food. Moreover, each year, the percentage of girls over or equal to 18 years of age is more than the percentage of girls aged less than 18 years. A significant population of girls were adolescents (Table 1).

Prevention of anemia is effective when the strategy is focused right from adolescence for their future reproductive life. Very few studies have focused on anemic adolescent girls (Rawat *et al.,* 2001; Kaur *et al,*2006; Kakkar *et al.,* 2011; Gupta *et al.,*2012; Deshpande *et al.,* 2013). The study's main objective was to determine an individual's health status in a correlation between the level of Hb and BMI in a healthy body. Previous studies have also reported a higher prevalence of anemia in underweight individuals (Pandey and Singh, 2013) (Sarathaet *al.,* 2010). This may be attributed to being malnourished, predisposing to iron depletion and increasing the risk of anemia. There is a significant association between nutritional status and anemia, as anemia is more prevalent among underweight students than expected and overweight students. However, the severity of anemia is not associated with body mass index (Khan *et al.,* 2018). From Table 5 above, we can observe that only 36% had a normal BMI. In comparison, 7% were overweight, 12% were in the category of obesity, and 44% of the girls were underweight; an almost similar distribution pattern was observed through the years concerning body mass index. A percentage of girls, only 36%, have an average BMI, and 44% are underweight, which raises concerns about their health status with respect to various diseases. BMI for age was the main predictor of anemia. Anemia is significantly associated with low BMI for age. Adolescent girls with a low BMI were 3.2 times more likely to be anemic than those with a high BMI. Comparable findings were also reported in Bonga Town; those with low BMI were 2.54 times more likely to develop anemia than those with high BMI (Bano *et al.,* 2012).

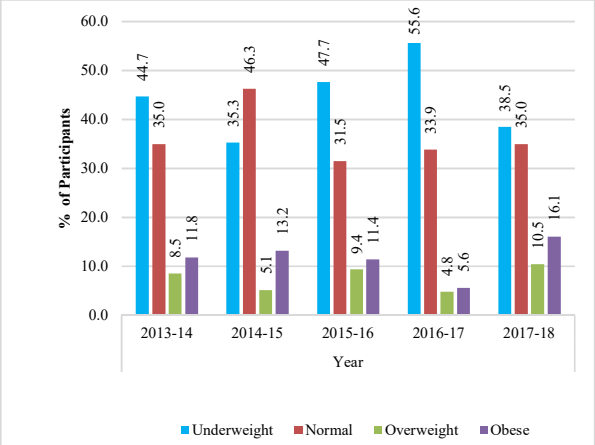

Figure 3: Categorization of Body Mass Index

Conclusion

A positive correlation between being underweight and anemia has been established, especially in iron deficiency anemia. According to the WHO, nutrition-related problems are the leading causes of anemia in developing countries. Although no association can be drawn between anemia and BMI but can help to express one's overall health status, the occurrence of different risks can be prevented by using BMI as an effective tool not only during adolescence but also during pregnancy, childbirth and further. Anemia can be prevented to some extent by having a diet that includes iron-rich food and a variety of vitamins and minerals consisting of iron, folate, vitamin B-12, and vitamin C. (Ref Mayoclinic). The results will be better if good nutritional food habits are maintained. There is a need to create awareness of healthy eating habits and avoid junk food among them to improve their lifestyle and practice deworming programs along with Hb detection. Along with creating awareness about the importance of maintaining their BMI and

hemoglobin regularly, not only for energy to concentrate on studies during their college life but also to prevent health risks in the near future and to do well in life.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Seasonal variations in ostracod species in two freshwater lakes in Yavatmal District (Maharashtra) India

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ABSTRACT

The diversity and density of ostracods (zooplankton) in two freshwater lakes in the Yavatmal district of Maharashtra, India, were studied from March 2021 to February 2022 to determine seasonal variations. Plankton net (64µ pore size) was used to collect the samples and analysis was performed using standard keys. In all, 11 species from 8 genera belonging to 3 families of Ostracoda were identified from Mama Lake and Singhada Lake of the Yavatmal district of Maharashtra, India. The overall population of Ostracoda is greater in Mama Lake than in Singhada Lake. Additionally, the species diversity was higher in the summer season and lowest in winter.

Introduction

Ostracods are tiny crustaceans that belong to the class Crustacea. They exist in all aquatic conditions and have a wide range of salinity tolerances. Only the benthonic species are preserved in the fossil record. They serve as helpful indices in geochronology, correlation, hydrocarbon exploration, paleogeographic reconstructions and interpretations. Ostracods are a key component of secondary energy transfer in the aquatic food web between autotrophs and heterotrophs, as observed by Deivanai *et al.* (2004). Freshwater ostracods are valuable biological indicators, as observed by Schneider *et al.* (2016). Ostracods are extremely sensitive to changes in their environment. Therefore, changes in species diversity or community composition can offer crucial indicators of environmental changes. Fisheries and reservoir health for the general public may be significantly impacted by a reservoir's zooplankton species composition, distribution diversity, and relative abundance, as suggested by Mustapha (2009). Ostracods are bivalve microcrustaceans that are one of the most diverse species of living crustaceans and are found practically everywhere in water, as observed by Sontakke and Mokashe (2014). Patil (2018) studied the abundance and diversity of zooplankton at Nandurmadhmeshwar Dam of Nasik district in Maharashtra and recorded 16 species belonging to 4 different groups, of which rotifers contributed 7 species, Cladocera reported 5 species, Copepodawith 3 species and Ostracoda by 1 species. Deshmukh *et al.* (2019) conducted a study on the correlation between abiotic factors and zooplankton diversity in wetlands and reported that phytoplankton significantly contribute to the development of zooplankton and fish diversity. The

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work of Khaire (2020) recounted monthly variations in zooplankton dynamics and their correlations with some physicochemical characteristics of Sina Dam and reported 17 genera from four major groups of zooplankton. Patel and Laharia (2021) reported the presence of 5 species from 5 genera of Ostracoda from freshwater perennial ponds in Wani city.

The percent composition and seasonal change in freshwater ostracods in various regions of India have been the subject of very little research. The current paper focuses on a comparative study of the diversity of ostracods in two freshwater lakes, Mama and Singhada, in the Yavatmal district of Maharashtra, India. This result helps to understand the current status of the ostracod fauna of freshwater lakes in India.

Material and Methods

Samples were collected from two different lakes, Mama and Singhada, from the Yavatmal district of Maharashtra between March 2021 and February - 2022. Mama Lake (34 km from Wani) is present near Mukutban. It is at 19°48' North latitude and 78°51' East longitude and at 249 meters above sea level. Singhada Lake is situated near Wani Tahsil of Yavatmal District. It is at 20°02' North latitude and 78°57' East longitude and 215 meters above sea level. Analysis was performed using standard keys of Edmondson, (1992), Meisch, (2000) and Altaff, (2004). Various diversity indices, such as the Shannon–Wiener index and richness index, were calculated.

The Shannon diversity index was calculated by the formula below:

$$\mathbf{H} = -\sum_{i=1}^{s}[(\mathbf{pi})\mathbf{x}\,\mathbf{ln}(\mathbf{pi})]$$

where H- Shannon diversity index
pi - proportion of individuals of i^{th}species in a whole community
ln- natural logarithm

Simpson's diversity index was calculated by the following formula:

$$\mathbf{D} = \sum_{i=1}^{s}\frac{\mathbf{ni}(\mathbf{ni}-\mathbf{1})}{\mathbf{N}(\mathbf{N}-\mathbf{1})}$$

where
D- Simpson's diversity index
ni - number of individuals in i^{th} species
N- Total number of individuals

However, the species richness was calculated by the formula given below:

Margalef Richness Index in Biodiversity = (S - 1)/Log (n)

where
S = Total Number of Species
n = Total Number of Individuals in the Sample

Results and Discussion

In all, 11 species from 8 genera belonging to 3 families of Ostracoda were identified from Mama Lake and Singhada Lake of the Yavatmal district of Maharashtra, India (Table 1). In the present study, 11 species from 8 genera of Ostracoda were observed, of which *Candonafaveolata* was observed to be the dominant species in both lakes. The highest diversity of ostracods was recorded in the summer season in Mama Lake (H – 1.609) and Singhada Lake (H – 1.606), while the lowest was observed in the winter season (H – 1.604) in Mama Lake and (H – 1.583). Simpson's index (D) was found to be very low, as all the diversity indices shown in Table 3 were below 0.5 in both lakes. The highest species richness was observed in the summer season, and the lowest was observed in the winter season in both lakes. (table-3). Antal *et al.* (2020) also recorded higher values of ostracod populations in summer and lower values in winter in Lake Mansar and Lake Surinsar. Most freshwater communities contain significant amounts of ostracods. The availability of suitable food for aquatic species affects the population of zooplankton. The nearby macrophytes and flora have a special impact on the abundance of ostracods. Ostracod populations may increase in the summer because of the abundance of food, which comes in the form of debris and organic materials due to the high rate of decomposition and suitable temperature. Mama Lake has a lower average species richness than Singhada Lake. According to Mukherjee (1997), a wider food chain indicates a higher species richness. The Simpson index of Mama Lake was higher than that of Singhada Lake. Poorer diversity in Mama Lake is indicated by higher Simpson index scores. Kulkarni *et al*. (2011) reported a similar discovery in Dharamtar, India. The ANOVA results (Table 4) showed that among

Table 1: Seasonal number of Ostracoda observed during 2021-2022 in two lakes in the Yavatmal district

Family	Genus/Species	Individuals observed in Mama Lake			Individuals observed in Singhada Lake		
		Summer	Rainy	Winter	Summer	Rainy	Winter
Cyprididae	*Chlamydotheca speciosa*	8	6	5	5	4	2
	Candonafaveolata	28	23	21	19	15	10
	Candonaparvula	19	16	14	8	6	9
	Candonajeaneli	15	11	10	3	2	0
	ParaCandonaeuplectella	5	4	4	3	2	1
Cytheridae	*Bicornucytherebisanensis*	7	5	3	5	3	1
Cypridae	*Cyprinotuspellucidus*	25	22	20	15	11	4
	Cypricercuspassaica	9	6	4	3	2	0
	Cyclocyprisforbesi	7	5	4	2	1	1
	Physocypriagibbara	5	4	3	2	0	1
	Rabilimisseptentrionalis	3	2	0	1	0	0
	Total	**131**	**104**	**88**	**66**	**46**	**29**

Table 2: Seasonal percentage of Ostracoda in the lakes studied during 2021-2022

Lakes	Seasons	individuals observed	Percent
Mama	Summer	131	41%
	Rainy	104	32%
	Winter	88	27%
	Total	**323**	
Singhada	Summer	66	47%
	Rainy	46	33%
	Winter	29	21%
	Total	**141**	

Table 3: Species diversity indices of Ostracoda in the lakes studied during 2021-2022.

Lakes	Indices	Summer	Rainy	Winter
Mama	Richness (S)	2.23	2.15	2.05
	Shanon-Weiner (H)	1.609	1.608	1.604
	Simpson (D)	0.200	0.201	0.202
Singhada	Richness (S)	2.97	2.61	2.39
	Shanon-Weiner (H)	1.606	1.598	1.583
	Simpson (D)	0.205	0.201	0.210

Table 4: ANOVA between ostracod species and between seasons in Mama Lake

Source of Variation	*SS*	*df*	*MS*	*F*	*P value*	*F crit*
Between Species	1808.8485	10	180.884848	215.495	4.18E-18	2.347878
Between Seasons	85.8788	2	42.9393939	51.155	1.37E-08	3.492828
Error	16.7879	20	0.83939394			
Total	1911.5152	32				

***Significance at the 0.05 level**

Table 5: ANOVA between ostracod species and between seasons in Singhada Lake

Source of Variation	*SS*	*Df*	*MS*	*F*	*P value*	*F crit*
Between Species	623.8788	10	62.38788	17.257334	9.83E-08	2.347878
Between Seasons	62.3636	2	31.18182	8.6253143	0.001991	3.492828
Error	72.3030	20	3.61515			
Total	758.5455	32				

***Significance at the 0.05 level**

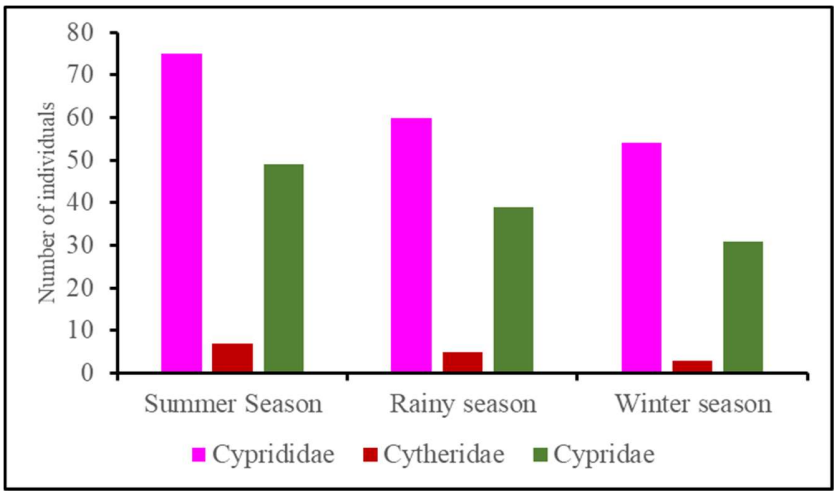

Figure 1: Seasonal variation in families of Ortracods studied in Mama Lake during 2021-2022

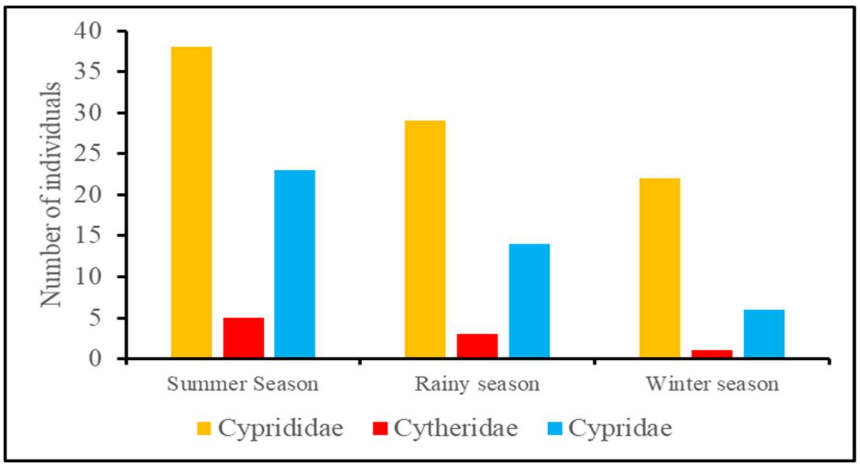

Figure 2: Seasonal variation in families of Ortracoda studied in Singhada Lake during 2021-2022

Ostracods, there was a significant difference at Mama Lake, as the obtained 'f' value of 215.495 was greater than the 'f' critical value of 2.347, whereas among different seasons, it was also found to be significant, as the obtained 'f' value of 51.155 was greater than the 'f' critical value of 3.492. The ANOVA results (Table 5) showed that among Ostracods, there was a significant difference at Singhada Lake, as the obtained 'f' value of 17.257 was greater than the 'f' critical value of 2.347, whereas among different seasons, it was also found to be significant, as the obtained 'f' value of 8.625 was greater than the 'f' critical value of 3.492. In comparison to Singhada Lake, Mama Lake has greater Shannon–Wiener index values. The diversity increases as the value increases. There is less conflict between species when there is a situation with higher diversity, as observed by Colinvaux (1973).

Conclusion

From the comparative study of Mama Lake and Singhada Lake, it can be concluded that Singhada Lake has high diversity compared to Mama Lake. The study also revealed that there was variation in Ostracod diversity and abundance between the two lakes, which might be due to some differences in abiotic conditions.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Analysis of noise pollution level in and around SIDCUL area in District Haridwar (Uttarakhand) India

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ABSTRACT

The primary objective of this study is to evaluate the issue of noise pollution in the vicinity of the industrial zone of Haridwar city and its correlation with the health of the people in and around the SIDCUL (State Industrial Development Corporation of Uttarakhand Limited). The study revealed that noise pollution levels were above the CPCB Standard for the sound level for industrial zones and residential zones. During the period from January to December 2018, noise levels were observed and monitored in both the industrial zone (Site-I SIDCUL) and the residential zone (Site-II Siwalik Nagar). The average noise levels varied throughout the year. At Site-I (Industrial Zone), during the daytime in July, the maximum average noise level recorded was 89.5 dB, while in April; the minimum average noise level was 81.1 dB. During nighttime, in January, the maximum average noise level reached 84.1 dB, and in May, the minimum average noise level was 76.6 dB. In Site-II Siwalik Nagar (Residential Zone), the daytime noise levels were a maximum average noise level of 61.1 dB in May and a minimum average noise level of 58.8 dB in September. During nighttime, the noise level reached a maximum average of 47.2 dB in October and a minimum average of 44.5 dB in May. Overall, the study revealed that noise levels were generally higher in both zones, except for the average nighttime noise level at Site II (residential zone), which was below the prescribed standard limit for noise.

Introduction

Noise pollution is an unwanted, unpleasant and unexpected sound level and is derived from the Latin word nausea. Anthropogenic activities such as urbanization, industrialization, transportation, other development activities and many types of festivals are key factors that produce different levels of noise pollution in different areas of society. It is very harmful for humans and very hazardous to all biotic components. Nonbiotic components are also affected by noise pollution (Pawar and Joshi 2005). Noise pollution is a pervasive environmental issue that has negative impacts on individuals and the balance of ecosystems. It refers to the presence of excessive or disturbing sounds in the environment that disrupt natural harmony and cause adverse effects on human health, wildlife, and overall quality of life. With the rapid growth of urbanization and industrialization, noise pollution has become a pressing concern in many cities and regions worldwide. From the relentless buzz of traffic to the clamor of industrial machinery, noise pollution infiltrates various aspects of daily life, leading to a range of physical and psychological consequences. Prolonged exposure to high noise levels has been

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linked to hearing impairment, sleep disturbances, stress, and cognitive impairments. Additionally, wildlife and natural habitats suffer from disruptions in mating, foraging, and communication patterns. Efforts to combat noise pollution encompass a broad spectrum of measures, including urban planning, technological advancements, and awareness campaigns. Striking a balance between development and environmental sustainability becomes crucial to mitigate the adverse effects of noise pollution and foster healthier and more harmonious living conditions for present and future generations.

Noise is one of the hazardous problems in urban and semiurban zones in different parts of the world and is the result of urbanization as well as industrialization or anthropogenic activities by humans (Gangwar *et al.,* 2006). Currently, the many types of vehicles, such as heavy vehicles and modified bikes, create considerable noise in the atmosphere. Traffic noise creates a number of problems, such as chronic effects and sleeping disorders. A high level of noise pollution may also damage hearing capacity at the temporary level as well as the permanent level (Pachpande *et al.,* 2005). Obviously, noise pollution has a very negative effect on living things as well as nonliving things. Failing to take appropriate measures to manage and diminish noise levels may result in the worsening of the problem due to ongoing urbanization and industrialization. This could lead to an irreparable situation. The rising number of complaints filed with the police and administration highlights the growing problems related to noise pollution as a law and order concern. Moreover, there have been reports indicating that noise pollution during pregnancy can give rise to a variety of complications for newborns (Vidya Sagar and Rao 2006).

Material and Methods

To conduct this study, two locations for each zone (Viz. residential and Industrial zone) were selected in Haridwar city. Siwalik Nagar was selected for the residential zone. Both sites are located within the periphery of 5 km. The study on ambient noise monitoring was conducted from January to December 2018. Noise levels were measured for 18 hours of study between 0600- 2400 hrs with the help of a sound level meter. Ambient sound levels were compared with those of the standards prescribed in Environmental Protection Rules, 1986 (Tripathy 2008) and standards of CPCB (Kudesia and Tiwari 2018).

Health survey in the local community:

An extensive health survey was carried out in the different localities in and around the SIDCUL, Haridwar, to determine the health problems among the local people residing in the concerned area. A survey was carried out during the study period at specific locations, encompassing 600 participants. The survey included various segments of the community, such as local residents, shopkeepers, hawkers, and autorickshaw drivers. Participants were requested to report any health-related issues they experienced, including hearing problems, headaches, stress, sleeping disorders, heart and blood pressure problems, and other diseases. Furthermore, data concerning the respondents' age, gender, occupation, income, place of residence, and dietary habits were collected for later analysis. The results (January to December 2018) of the health survey in and around SIDCUL, Haridwar, are summarized in Figure 1.

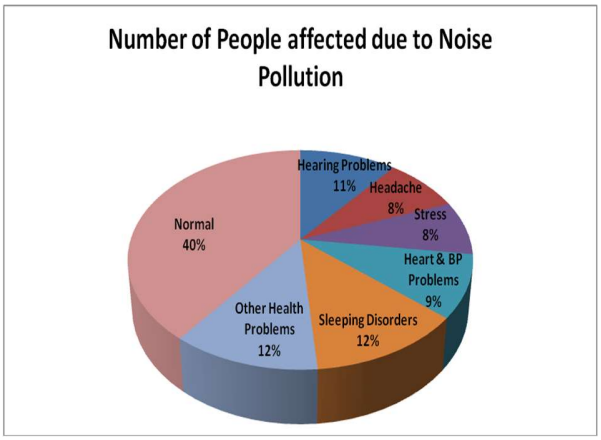

Figure 1: Percentage of noise pollution-oriented diseases among the people in the study area during 2018

Results and Discussion

In the current study, it was observed that the noise levels in both the industrial zone and residential zone exceeded the standard prescribed limits for noise. The study involved monitoring noise levels in these two distinct zones within Haridwar city from January to December 2018. During the period from

January to December 2018, noise levels were observed and monitored in both the industrial zone (Site-I SIDCUL) and the residential zone (Site-II Siwalik Nagar). The average noise levels varied throughout the year. At Site-I (Industrial Zone), during the daytime in July, the maximum average noise level recorded was 89.5 dB, while in April, the minimum average noise level was 81.1 dB. During nighttime, in January, the maximum average noise level reached 84.1 dB, and in May, the minimum average noise level was 76.6 dB. In Site-II Siwalik Nagar (Residential Zone), the daytime noise levels were a maximum average noise level of 61.1 dB in May and a minimum average noise level of 58.8 dB in September. During nighttime, the noise level reached a maximum average of 47.2 dB in October and a minimum average of 44.5 dB in May. Overall, the study revealed that noise levels were generally higher in both zones, except for the average

Table 1: Monthly average noise level (in dB) at two selected sites from Jan to Dec 2018

Months	SITE-I(SIDCUL)		SITE-II (Siwalik Nagar)	
	(Day Time)	**(Night Time)**	**(Day Time)**	**(Night Time)**
January	88.2 (75.2-92.7)	84.1 (80.7-87.5)	59.4 (50.6-65.7)	45.7 (40.5-50.9)
February	89.3 (74.6-93.5)	83.2 (80.3-86.2)	59.0 (51.3-64.4)	46.2 (41.2-51.3)
March	87.9 (74.5-101.2)	81.4 (76.4-86.4)	59.4 (50.3-65.6)	45.8 (41.2-50.4)
April	81.1 (76.4-103.4)	79.4 (73.4-85.4)	59.2 (49.3-65.4)	45.9 (41.4-50.4)
May	88.4 (77.8-99.4)	76.6 (71.8-81.4)	61.1 (44.3-68.9)	44.5 (39.3-49.8)
June	89.2 (77.4-98.4)	80.7 (80.1-81.3)	59.6 (51.3-64.5)	46.6 (42.1-51.2)
July	89.5 (71.4-98.4)	78.7 (76.1-81.4)	58.7 (50.1-65.1)	46.2 (41.2-51.3)
August	86.1 (71.2-95.3)	80.3 (78.2-82.4)	59.2 (48.2-65.5)	44.7 (38.1-51.3)
September	89.3 (78.1-101.4)	77.0 (71.6-82.5)	58.8 (50.1-64.4)	46.4 (41.2-51.6)
October	88.0 (74.3-98.6)	81.7 (80.1-83.4)	57.5 (43.5-64.6)	47.2 (42.3-52.1)
November	88.5 (76.4-99.2)	83.2 (78.2-88.3)	59.8 (51.2-65.1)	46.6 (41.2-52.1)
December	87.9 (76.3-100.3)	82.5 (78.8-86.3)	58.5 (50.1-64.1)	45.5 (40.2-50.8)

nighttime noise level at Site II (residential zone). Similar findings have been observed by different researchers in different parts of the world. Singh *et al.* (2011) conducted a study evaluating the ambient noise levels in the city of Bareilly, Uttar Pradesh. They have a positive correlation of noise pollution with human health problems. Vidya sagar and Rao (2006) have also studied noise pollution with special reference to hospitals, residential zones, etc., in the city of Visakhapatnam. Sharma *et al.* (2015) assessed the noise pollution in some industrial, commercial, residential and silence zones within Jagiroad town in Assam. He also found a positive correlation between noise pollution and human health problems related to noise.

Correlation between noise pollution and human health:

During the present study, the observation indicated that noise pollution contributes to health problems related to hearing problems, headache, stress, sleeping disorders, heart and BP problems and other diseases among society. It was also observed that health problems related to noise pollution have

increased. Among the total respondents, 11% had hearing problems, 8% had headache, 8% had stress problems, 12% had sleeping disorders, 9% had heart and BP problems, and 12% had other diseases.

Table 2: Standards for noise level in different zones [Source: Uttarakhand Pollution Control Board]

Noise Level Zone	(Limits in dB)	
	(Day time)	(Night time)
Industrial zone	75	70
Commercial zone	65	55
Residential zone	55	45
Silence Zone	50	40

related to water pollution and air pollution in the year of the study period. In all total respondents of the study period, 60% suffered from various diseases related to noise pollution as well as industrial pollution. Most of the people were disturbed by noise pollution as well as traffic noise, and approximately 60% of the people suffered from various problems related to noise pollution, such as high blood pressure (HBP), stress problems, headache, sleeping disorders, and hearing problems. Pathak *et al.* (2008) described noise pollution in Varanasi city with special reference to health problems related to higher noise levels. He also found a negative effect of noise pollution on human health in the area of concern of Varanasi city of India. Mangalekar *et al.* (2012) reported the noise level of Kolhapur City in Maharashtra, India, and found a high level of noise than the prescribed limit of noise by the Central Pollution Control Board. According to the survey conducted by Pachpande *et al.* (2005), approximately 84% of teachers and 92% of students experienced a reduction in their hearing capacity as a result of regular exposure to noise pollution from highway traffic. Sharma *et al.* (2010) reported a positive correlation between traffic noise and various health issues in individuals working in different workplaces in Haridwar City. The health problems identified included headaches, high blood pressure, and stress. In his study, Deka (2000) assessed the average noise level in Guwahati City, Assam, which was found to be 83 dB in the commercial zone and 68 dB in the residential zone. These levels were 27.7% and 23.6% higher than the standard noise limits for commercial and residential areas, respectively. In the present study, it can be confirmed that the primary cause of the increased noise levels in and around SIDCUL, Haridwar, is the growing number of traffic vehicles. Therefore, it is imperative to raise awareness among the public, including the management of SIDCUL officials, to effectively mitigate and prevent the long-term health risks associated with noise pollution. In conclusion, the examination of noise pollution levels in and around SIDCUL, Haridwar, Uttarakhand, India, has brought attention to the significant impact of industrialization and urbanization on both the environment and human well-being. The study uncovered that noise levels in both the industrial and residential zones surpassed the standard prescribed limits, indicating a growing concern for public health and overall quality of life. The results strongly suggest that the increase in traffic vehicles significantly contributes to the elevated noise levels in the area. Such heightened noise pollution poses potential long-term health risks for residents and workers in the region, leading to hearing problems, headaches, stress, and other related health issues, which necessitate immediate attention and remedial actions.

Conclusion

Considering the implications for human health and the ecosystem, there is an urgent requirement for raising awareness among all stakeholders, including the public, authorities, and management of SIDCUL. Collaborative efforts should be initiated to implement effective strategies for noise reduction while adhering to the prescribed noise standards. Regulating noise levels, adopting noise control technologies, and promoting sustainable urban planning are critical steps toward mitigating noise pollution in and around SIDCUL. By addressing this pressing issue, we can foster a healthier and more conducive living and working environment for current and future generations. Moreover, such measures will play a pivotal role in preserving the ecological balance and ensuring the overall well-being of the community.

Conflict of interest

The authors declare that they have no conflicts of interest.

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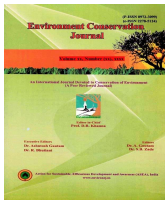

Analysis of the growth profile, biochemical composition and nutrient removal efficacy of *Spirulina* sp. NCIM 5143

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ABSTRACT

In the present manuscript, the growth profile of the microalgae *Spirulina* sp. NCIM 5143 was studied. Screening was performed on four commercial media, i.e., blue–green-11 (BG-11), Bold's basal medium (BBM), algal culture medium (ACM), Zarrouk's medium (ZM), and different concentrations (20%, 40%, 60%, 80%, and 100%) of unsterilized dairy effluent (UDE). Characterization of biomass was performed to assess its biochemical composition through various assays. Elemental composition and bioactive compound analysis were accomplished by inductively coupled plasma-atomic emission spectroscopy (ICP–AES) and gas chromatography–mass spectrometry (GC–MS), respectively. The results revealed that maximum values of most of the parameters, i.e., optical density (0.21), chlorophyll (2.00 mg/l), proteins (119.17 mg/l), and wet (4.06 g/l) and dry biomass weight (0.28 g/l), were found on ZM. For UDE, maximum growth parameters and the highest nutrient removal efficiency were obtained at 100% concentration. Biochemical analysis revealed that total Kjeldahl nitrogen (7.14±0.49%), crude protein (48.23± 3.34%), total antioxidant activity (3.07±0.03 mg AAE/g), and total phenols (8.88±1.93 mg GAE/g) were present in the biomass. Elemental and GC–MS analysis detected essential micronutrients and many bioactive compounds, respectively. Hence, this study proved that *Spirulina* sp. NCIM 5143 has the potential for the management of waste dairy effluent. This study also showed its cost-effectiveness, as the dairy effluent analyzed is used without any kind of sterilization. In addition, its biomass is rich in several essential elements, antioxidants, and bioactive compounds of therapeutic and nutraceutical importance.

Introduction

The dairy industry is the food industry of prime importance and produces an enormous volume of waste causing environmental contamination. Dairy industry effluent has a characteristic unpleasant odor that is offensive in nature, has high BOD (40–48,000 mg/l) and COD (80–95,000 mg/l) contents (Kushwaha *et al.,* 2011) with varying pH ranges (Kothari *et al.,* 2012), and contains ample amounts of nitrogen and phosphates, i.e., 14–830 mg/l and 9–280 mg/l, respectively (Gavala *et al.,* 1999). Dairy effluent is primarily alkaline; however, it turns acidic due to the fermentation of sugar present in milk in the form of lactose to lactic acid. Putrefaction of milk protein casein generates heavy and blackish sludge. Untreated dairy effluents are generally released into nearby water bodies, causing environmental pollution. Dairy effluent decomposes rapidly and reduces the dissolved oxygen levels of the receiving water bodies, causing anaerobic conditions and producing a foul smell. These water bodies further become breeding grounds for mosquitoes, flies, and other vectors harboring malaria, dengue fever, yellow fever, and chikungunya. Physical and chemical techniques are

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being employed for the management of these effluents. However, these traditional methods are not only expensive but also produce a great amount of sludge and are not as efficient (Yuan *et al.,* 2011). These constraints limit their use and prompted scientists to search for an alternate approach that is economical, efficient, and eco-friendly.

In recent years, microalgae have received much attention in the treatment and recycling of waste effluents. Microalgae can perform photosynthesis and can adapt to various environments. They have the astounding capability to thrive in nutrient-rich environments and consume them for sustaining and bioabsorbing heavy metals from effluents in their biomass. Mixotrophic cultivation of microalgae on dairy effluent has been reported to be successful, as this waste contains copious amounts of sugars and organic carbon (Girard *et al.,* 2014). All these characteristics make them remarkable means for sustainable and inexpensive waste effluent treatment. A microalgal sp. with enormous bioremediation potential is *Spirulina*. It is a blue–green microalga with a photoautotrophic mode of nutrition and requires mainly nutrients such as nitrate, urea, and ammonium salts for sustaining growth (Ariede *et al.,* 2017). *Spirulina* sp. employed for the treatment of dairy waste removed 80%, 72%, 61%, 56%, 71%, 56%, 77%, 54%, and 59% of nitrates, phosphorus, sulfate, total hardness, alkalinity, chloride, COD, TDS, calcium and magnesium hardness, respectively (Ahmed, 2014). Moreover, biomass harvested after effluent treatment is rich in many value-added products. *Spirulina* contains 70% proteins, 15 to 30% carbohydrates, and 3-9% lipids in its biomass along with other essential β-carotene, vitamins, and phycocyanin pigments (Andrade *et al*., 2019).

Several synthetic media have been used for microalgae cultivation, but the constraint associated with the use of these commercial media is that costs exceed the final products (Li *et al*., 2007). Therefore, it is imperative to search for alternative lower-cost substrates for microalgae cultivation. Dairy waste contains a sufficient amount of nitrogen and phosphates, which makes it an ideal medium for algal cultivation. The growth and biomass composition of *Spirulina* sp. depends upon several factors, including pH, salinity, temperature, and bicarbonate ions.

Although there are some studies on the characterization of microalgae, they are still fewer than the vast number of prevailing species of microalgae. Therefore, the current study was executed to analyze the growth profile, biochemical composition, and nutrient removal efficacy of *Spirulina* sp. NCIM 5143. To meet this end, the growth profile of *Spirulina* sp. NCIM 5143 was studied on four commercial media, viz. BG-11, ZM, BBM, and ACM and different concentrations of UDE. The biochemical composition of microalgal biomass was determined through various assays, and micronutrient composition and bioactive compounds were detected through inductively coupled plasma-atomic emission spectroscopy (ICP–AES) and gas chromatography–mass spectrometry (GC–MS) analysis.

Material and Methods

Collection of mother culture

The standard microalgal strain *Spirulina* sp. NCIM 5143 was procured from the National Collection of Industrial Microorganisms (NCIM) Laboratory, Pune, India.

Collection of dairy effluent

The dairy effluent sample was collected from a local dairy near gate no. 3, PAU, Ludhiana in plastic cans, sealed aseptically, and stored at -20°C until further analysis.

Morphology of *Spirulina* sp. NCIM 5143

The morphology of microalgal cells was observed and photographed at 40X under an Olympus 528293 microscope (Magnus Icon Freedom Model) using a Debro 5.1 Megapixel digital camera and Toup view software program.

Cultivation of microalgae in commercial media

Triplicate 250 ml Erlenmeyer flasks containing 100 ml of four different commercial media, i.e., BG-11 (Moghazy *et al*., 2019), BBM (Sorokina *et al.*, 2020), ACM (Dar and Phutela, 2020), and ZM (Rajasekaran *et al*., 2016), were sterilized by autoclaving at 121 °C at 15 psi for 15 minutes. Inoculum was added at 10% inoculum. Flasks were maintained at 28±2 °C under light conditions of 54 µmol photons m^{-2} s^{-1} using compact fluorescent lamps maintaining a photoperiod cycle of 16:8 for 30 days. Growth was evaluated mainly in terms of

change in optical density (at 750 nm) on every 3rd day, pigment concentration, i.e., chlorophyll (mg/l) on every 5th day, and dry biomass weight (g/l), carbohydrates, lipids, and protein content at the end of the 30-day growth period. Commercial media in which the highest values of all growth parameters were obtained, called respective growth media, were selected for further experimental analysis with UDE.

Cultivation of microalgae in dairy effluent

Triplicate Erlenmeyer flasks (250 ml) containing different concentrations of UDE, i.e., 20%, 40%, 60%, 80%, and 100%, were supplemented with the respective growth media to make a final volume of 100 ml. Inoculum was added at 10% inoculum. Flasks were maintained at 28±2 °C under light conditions of 54 µmol photons m^{-2} s^{-1} using compact fluorescent lamps maintaining a photoperiod cycle of 16:8 for 30 days. After completion of the incubation period, flasks were tested for various growth parameters of microalgae and reduction in various physicochemical parameters of unsterilized dairy effluent (UDE). A control consisting of only UDE without inoculum was run simultaneously.

Growth kinetic study

Microalgal growth kinetics were studied on commercial media as well as the concentration of UDE, whereby the highest rate of all the growth parameters was obtained using a modified nonlinear logistic equation (Dar, 2017) as given below:

$$\mathbf{Y} = \frac{\mathbf{A}}{[\mathbf{1} + \mathbf{exp}\left\{\mathbf{4}\left(\frac{\boldsymbol{\mu}}{\mathbf{A}}\right)(\boldsymbol{\lambda} - \mathbf{t}) + \mathbf{2}\right\}]}$$

where A is defined as the asymptote value (biomass g/l), µ is the growth rate (day^{-1}) and λ is the lag time (days). The fitting of the data in the model was done by using the MS Solver of Excel 2007.

Analytical methods

Physicochemical parameters

Standard protocols of APHA (2005) were followed for analyzing physicochemical parameters, i.e., dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS), before and after treatment with microalgae.

The nutrient removal efficacy of microalgae was calculated by the following formula (Ramsundar *et al*., 2017):

$$\textbf{Percent removal}\ (\%) = (\mathbf{IC} - \mathbf{FC})/\mathbf{IC} \times \mathbf{100}$$

where IC = initial concentration and FC = final concentration

Determination of total nitrogen and crude protein

The standard total Kjeldahl method (TKN) was used to determine the nitrogen content in a semiautomatic N-analyzer (Pelican Kelplus-KES06LR, Classic DX). Crude protein was calculated based on the expression: N × 6.25 (AOAC, 1990).

Determination of phosphorus

The method of Jackson (1967) was followed for the estimation of phosphorus content.

Determination of total phenols

Total phenols were estimated as per Taga *et al*. (1984).

Determination of total antioxidant activity

For estimation of total antioxidant activity, the procedure of Prieto *et al*. (1999) was followed.

Determination of DPPH radical scavenging activity

The standard protocol was used to estimate the DPPH radical scavenging activity. The percent scavenging effect (%) was calculated using the equation:

$$\textbf{Scavenging effect (\%)} = (1-(A_{sample}-A_{blank})/A_{blank}) \times 100$$

Determination of pigment content

Chlorophyll was analyzed by the protocol developed by El-Baky *et al.* (2008). The equations given by Lichtenthaler (1987) were used to calculate chlorophyll pigment.

$$\textbf{Chl a} + \textbf{b} = 7.05 * \textbf{A661.6} + 18.09 * \textbf{A644.8}$$
$$\textbf{Chl a} = 11.24 * \textbf{A661.6} - 2.04 * \textbf{A644.8}$$
$$\textbf{Chl b} = 20.13 * \textbf{A644.8} - 4.19 * \textbf{A661.6}$$

Determination of lipids
The sulfo-phospho-vanillin (SPV) assay is used to determine the lipid content in microalgal biomass (Mishra *et al*., 2014).

Determination of carbohydrates
The protocol devised by Dubios *et al.* (1956) was adopted for the estimation of carbohydrate content in microalgal samples.

Determination of proteins
The procedure given by Lowry *et al.* (1951) was employed for estimating total soluble proteins.

Elemental analysis by ICP–AES
The elemental composition of *Spirulina* sp. NCIM 5143 was determined through inductively coupled plasma-atomic emission spectroscopy (ICP–AES) (iCAP 6000 SERIES, ICP Spectrometer, Icap 6300 Duo, Thermo Electron Corporation, UK). The wet digestion method was used to digest the sample for further analysis (Hseu, 2004).

GC–MS analysis
The processing of samples for GC–MS analysis was performed according to the procedure of Krishnakumar *et al*. (2013). Lyophilized microalgal biomass was extracted with 50 mL of 80% methanol for 2 hours and filtered, and then the residue was again digested for 2 hours with methanol. The same extraction process was repeated 3 times. Then, sample extracts were vacuum evaporated at 45 °C, filtered, dissolved in 80% methanol, and stored in amber glass bottles for analysis by GC–MS (Thermo Trace 1300 GC coupled with Thermo TSQ 800 Triple Quadrupole MS) equipped with a splitless injector. The column was a BP 5MS with dimensions of 30 m×0.25×0.25 µm employing helium as a carrier gas with a flow rate of 1.0 ml/min. Initially, the oven temperature was programmed at 50 °C for 4.0 min, reprogrammed to 250 °C at a rate of 5 °C/min for 1.0 min, then again programmed to 280 °C at a rate of 15 °C/min and held for 18 min at 280 °C, with an injector temperature of 260 °C. Mass spectra (range=m/z 40–650) of the injected sample (1 µl) were collected. A comparative search of different mass spectra was carried out in the National Institute of Standards and Technology (NIST) library (2.0) for the identification of compounds detected in the analysis.

Statistical analysis
Experiments were completed in triplicate. Values are depicted as the mean±standard deviation. Values superscripted by different letters in tables represent the significant difference between the values based on Tukey's HSD Multiple Range test using IBM SPSS Statistics 22.

Results and Discussion

Morphology of *Spirulina* sp. NCIM 5143
Spirulina sp. NCIM 5143 cells were viewed under a microscope to determine their cellular morphology. Under 40 X, cells were large and filamentous. They were long unicellular nonheterocystous filamentous cells that grew in the form of a tightly coiled right- or left-handed helix (Plate I).

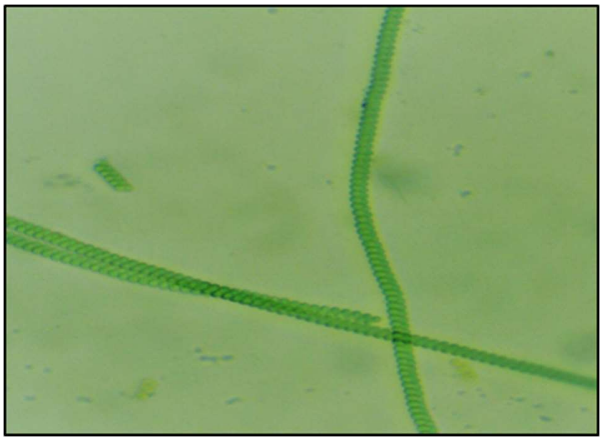

Plate I: *Spirulina* sp. NCIM 5143 cells under the Olympus 528293 microscope

Growth profile of *Spirulina* sp. NCIM 5143 on commercial media
Four commercial growth media were screened to find the best media for *Spirulina* sp. NCIM 5143, favoring its maximal growth. The growth and productivity of microalgal biomass are largely determined by the nutritional composition of the culture media (Madkour *et al*., 2012). The highest optical density (0.21) was recorded on ZM, followed by BBM, BG-11, and ACM, which showed absorbances of 0.20, 0.19, and 0.18, respectively. The lag phase of a very short duration was observed in all four commercial growth media and had a long exponential phase and pursued this

phase until the completion of the experimental period, i.e., 30 days. No stationary or death phase was detected (Fig. 1). This may be because a longer time is required for the decay phase to commence (Kodihalli *et al.,* 2018), but in this study, the cultivation time was only 30 days. The concentration of microalgal biomass can be determined by its optical density. The studied wavelength of 750 nm was selected for absorbance measurements because this wavelength measures in accordance with the light scattered and no light will absorb by the pigments present in the given sample (Yap *et al.,* 2018). Chlorophyll content followed a similar trend as optical density (Fig. 2). The highest chlorophyll content (2.00 mg/l) was recorded on ZM, followed by BG-11 (1.66 mg/l), BBM (1.54 mg/l), and ACM (1.21 mg/l). The chlorophyll content increased in the exponential phase and continued until the end of the experimental period. Hence, the most favorable commercial media for *Spirulina* sp. NCIM 5143 according to the current study is ZM. Similarly, Pandey *et al.* (2010) also reported that the most favorable media for *Spirulina maxima* is ZM. Other studies reported that the best growth medium for *Spirulina* (*Arthrospira fusiformis*) was LCMA medium compared to ZM. Madkour *et al*. (2012) found chlorophyll contents of 0.0701 ± 0.0089 µg/l and 0.0685 ± 0.0024 µg/l in *Spirulina platensis* grown on ZM and reduced cost media, respectively. Hence, the screening experiment revealed ZM as the most suitable medium for *Spirulina* sp. NCIM 5143.

Growth profile of *Spirulina* sp. NCIM 5143 on UDE

Cultivation of *Spirulina* sp. NCIM 5143 was carried out on different concentrations of UDE supplemented with ZM (Plate II). The results showed that the optical density and chlorophyll content increased from 20 to 100%. Absorbance was higher at all concentrations of UDE than in the control (0.23). The maximum optical density (1.77) was observed at 100% UDE, followed by 80% UDE (1.68), 60% UDE (1.61), 40% UDE (1.51), and 20% UDE (1.40). The microalgal strain showed a small or no prominent lag phase. However, the exponential phase lasted up to the 30th day (Fig 1). Maximum chlorophyll (4.59) was observed at 100% UDE, followed by 80% UDE (4.51), 60% UDE (4.11), 40% UDE (4.02), and 20% UDE (3.54), which were significantly higher than the control (2.34) (Fig. 2). The rationale behind the increase in microalgal growth with increasing concentrations of UDE might be because of the availability of essential nutrients required for sustaining microalgal growth because at lower concentrations, nutrient levels are not sufficient to support growth. Kothari *et al.* (2012) observed that the algal strain *Chlorella pyrenoidosa* showed the highest growth at a 75% concentration of dairy wastewater. The employment of UDE as algal culture media serves a dual purpose. First, it provides an inexpensive and readily available culture medium for microalgal cultivation. Second, dairy effluent is a waste product of the dairy industry that has no use and is generally discarded without any treatment. Hence, it prevents environmental pollution.

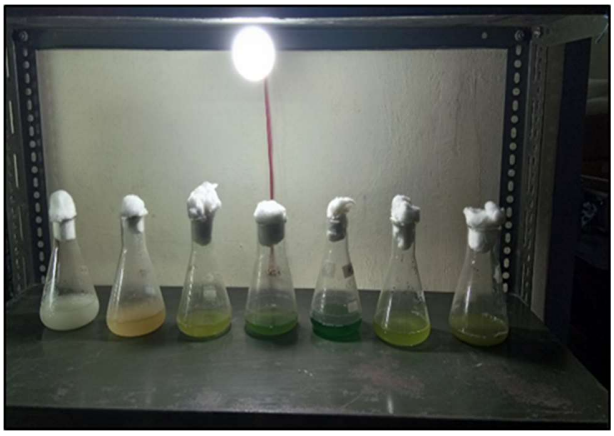

Plate II: Microalgae cultivation on dairy effluent

Biochemical constitution of *Spirulina* sp. NCIM 5143 on ZM

Proteins, lipids, carbohydrates, dry and wet biomass of *Spirulina* sp. NCIM 5143 growing on four culture media and different concentrations of UDE was done to predict the effect of nutrients present in UDE to support the biochemical composition of microalgae (Fig 1 & 2). The highest wet biomass (4.06 g/l) and dry biomass (0.28 g/l) were observed in ZM, while the highest protein (119.17 mg/l), carbohydrate (74.90 mg/l), and lipid contents (29.87 mg/l) were found in ZM, BBM, and BG-11 (Table 1). These observations again support the fact that ZM supports the maximum growth of *Spirulina*

sp. NCIM 5143. As ZM (2.50 g/l) is rich in nitrogen, the highest proteins were found in algal biomass growing in ZM. Bajwa *et al*. (2017) also observed a similar trend in different parameters, viz. biomass yield, chlorophyll content, total carbohydrate, protein, and lipid production for four microalgae strains (*Chlorococcum aquaticum, Scenedesmus obliquus, Nannochloropsis oculata, Chlorella pyrenoidosa*) grown on five media (BG-11, BBM, Modified HS CHU#10, Modified Hoagland Medium, Half strength CH#10 medium). The microalgal strains in the current study showed different growth rates and different values of various biochemical parameters in different media. This is because in their natural cultural conditions and habitats, different species of microalgae show varied physiological needs (Falkowski, 1984). Our results are in agreement with those of Michael *et al*. (2019), where the highest protein (65.00 ± 0.26%) and highest lipid content (6.84 ± 0.05%) were observed in ZM, while the carbohydrate content (15.29 ± 0.41%) was higher in LCMA medium than in ZM media. Carbohydrates present in *Spirulina* are cellulose and sugar-free, which confers them the property of easy digestibility and is ideal for diabetic and obese patients (Braga *et al*., 2018). The lipids in *Spirulina* are enriched in PUFAs such as DHA, EPA, and ALA and are free from cholesterol, which is favorable in diseases such as atherosclerosis, obesity, and blood pressure (Anvara and Nowruzib, 2014).

Biochemical constitution of *Spirulina* sp. NCIM 5143 on UDE

In UDE, the highest wet biomass (2.80 g/l), dry biomass (0.15 g/l), protein (162.33 mg/l), carbohydrate (49.72 mg/l), and lipid content (99.80 mg/l) were observed at the 100% DE concentration (Table 1).

Table 1: Biochemical profile of *Spirulina* sp. NCIM 5143 on commercial media and dairy effluent (DE)

Growth Media	Wet biomass (g/l)	Dry biomass (g/l)	Protein (mg/l)	Carbohydrate (mg/l)	Lipid (mg/l)
BG-11	1.23^{h}	0.11^{bcd}	90.63^{h}	34.89^{e}	29.87^{f}
ACM	3.77^{b}	0.12^{bc}	87.42^{i}	45.54^{c}	17.20^{h}
BBM	2.40^{d}	0.10^{cde}	98.30^{g}	74.90^{a}	11.52^{i}
ZM	4.06^{a}	0.28^{a}	119.17^{d}	22.33^{h}	19.69^{g}
0% UDE	4.03	0.31	118.96	22.19	18.73
20% UDE	1.82^{g}	0.02^{g}	98.68^{f}	16.70^{i}	48.24^{e}
40% UDE	1.92^{f}	0.04^{fg}	115.99^{e}	33.24^{g}	50.73^{d}
60% UDE	2.69^{c}	0.06^{efg}	145.42^{c}	34.58^{f}	63.16^{c}
80% UDE	2.70^{c}	0.07^{def}	153.99^{b}	36.33^{d}	82.70^{b}
100% UDE	2.80^{c}	0.15^{b}	162.33^{a}	49.72^{b}	99.80^{a}

DE= Dairy effluent. Values superscripted by different letters in the column differ significantly (P≤0.05) from each other

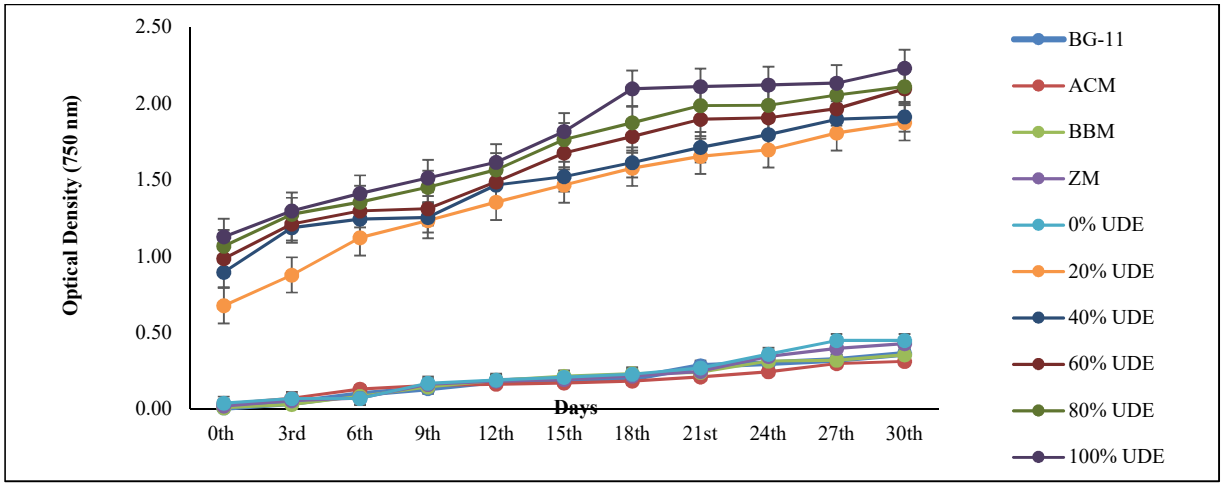

Figure 1: Growth profile of *Spirulina* sp. NCIM 5143

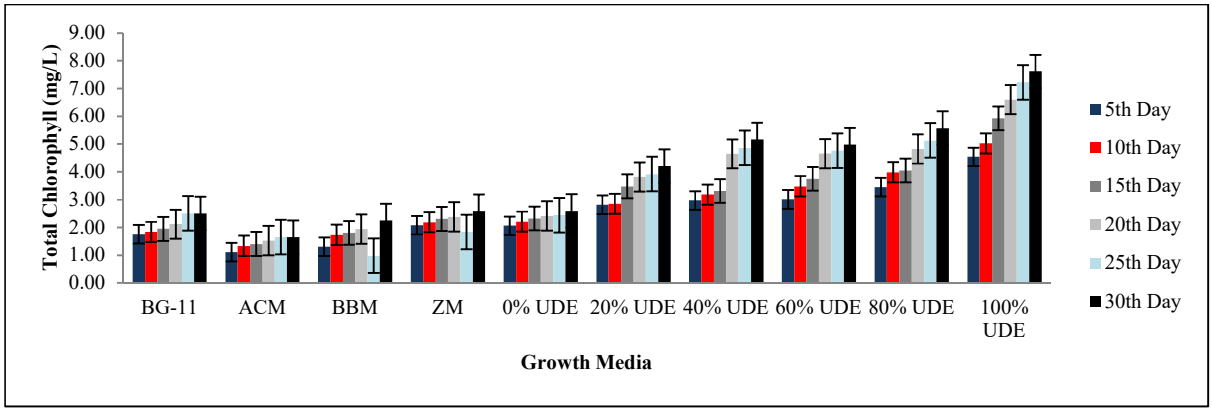

Figure 2: Total chlorophyll of *Spirulina* sp. NCIM 5143

It was observed in this study that all the parameters, i.e., optical density, chlorophyll, carbohydrates, proteins, and lipids, were higher in UDE than in commercial media. Salla *et al.* (2016) found that mixotrophic growth of *Spirulina platensis* grown on ZM supplemented with whey residues and lactose showed increased production of biomass and carbohydrates. In another study, Girard *et al.* (2014) observed that *Scenedesmus obliquus* under mixotrophic culture (in standard media supplemented with 40% whey) showed higher specific growth and biomass compared to standard media only. Similar results are obtained in our study, whereby all the growth parameters are higher in the mixotrophic culture of *Spirulina* sp. NCIM 5143 on dairy effluent compared to any of the four commercial media. This may be because compared to heterotrophic and autotrophic cultures, mixotrophic cultures are less affected by photoinhibition and have the additional advantage of assimilating both substrates and performing photosynthesis because both carbon sources maintain fixation and the organic carbon source maintains the acetyl-CoA pool (Mohan *et al.*, 2015).

Growth kinetic study

A study of microalgal growth kinetics by a nonlinear logistic model showed that the value of asymptote A, which determines the biomass production potential, was 0.65 at a growth rate per day (μ (day^{-1})) of 0.02 with a lag phase of 4.26 days in ZM media. In the case of UDE, the value of asymptote A was 2.02 with a growth rate per day (μ (day^{-1}) of 0.25 with no lag phase at 100% DE. The absence of a lag phase in 100% UDE indicated the microalga's potential to adapt to the new environment immediately and hence increase its nutrient removal ability (Daneshavar *et al.*, 2018). Similarly, Cardoso *et al.* (2020) also observed that *Spirulina* sp. LEB 18 showed no lag phase when grown in wastewater from aquaculture containing 25% and 50% ZM.

Nutrient removal efficacy of *Spirulina* sp. NCIM 5143

The high-level nutrient profile of UDE made it an ideal medium for algal growth. Dairy effluent does not contain any pathogens or toxic components but has vast amounts of oils, oxygen-demanding waste, and total suspended matter contributing to its polluting nature. Whey constitutes nutrients (55%) present in milk and is a useful waste generated by the milk industry with a considerable amount of organic content capable of supporting the mixotrophic and heterotrophic growth of microalgae (Sales *et al.*, 2017). In this study, the physicochemical parameters of UDE were analyzed to determine the nutrient removal efficiency of *Spirulina* sp. NCIM 5143. The results showed that *Spirulina* sp. NCIM 5143 cultivated on different concentrations of UDE at lab scale conditions reduced these undesirable characteristics to a greater extent. UDE was procured in the morning immediately after milk processing operations. Microalgae require organic carbon, nitrates, and phosphates for growth. The organic carbon and energy needs of microalgae are fulfilled by consuming the dissolved oxygen in wastewater. This organic carbon is used in the form of BOD and COD (Sarfraz *et al.*, 2021). Changes in various

physicochemical parameters of UDE after cultivation of *Spirulina* sp. NCIM 5143 are described below (Table 2).UDE had an off-white color with a temperature of 42 °C, pH of 6.5, and an offensive odor. The decomposition of lactose present in milk into lactic acid under aerobic conditions contributed to the pH in the acidic range (Joseph, 1995). *Spirulina* sp. NCIM 5143 showed a maximum percent reduction in various physicochemical parameters at 100% UDE (Table 2). pH gives the measure of H^+ or OH^- ion activity of the solution by determining the acidity, alkalinity, or neutrality. All the concentrations of UDE showed significantly higher pH values than the control (6.50). The highest percent increase in the pH of the UDE after treatment with *Spirulina* sp. NCIM 5143 was 55.30% (10.11) in 100% DE, which was found to be significantly higher than the percent increase in pH at 80% UDE (51.77%) (9.88), 60% UDE (36.71%) (8.90), 40% UDE (21.97%) (7.94) and 20% UDE (10.25%) (7.21). Enhanced carbonates due to the photosynthetic activity of the microalgae may be the reason for increased pH. During the light period, microalgae use inorganic carbon sources autotrophically for the photosynthesis process, increasing the pH content (Kumar *et al*., 2014).BOD is the measure of the quantity of oxygen required by the microbial population to oxidize the organic matter in a waste (Bhutiani and Ahamad 2108; Bhutiani *et al*., 2021). For BOD, the provision of a standard nutrient supply and pH conditions is mandatory. Low oxygen solubility and strong-strength wastes are diluted to assure that demand does not exceed available oxygen (Verma and Singh, 2017). No dissolved oxygen was detected in the control, while the BOD was 11,000 mg/l. The results of statistical analysis showed that the highest percent reduction in BOD was 45.45% (6,000 mg/l) at 100% UDE, which was on par with the percent reduction at 80% UDE (45.45%) (6,000 mg/l). This was followed by 60% UDE (27.27%) (8,000 mg/l), and a minimum percent reduction was obtained at 40% and 20% UDE (9.09%) (6,000 mg/l) (Table 2). The ability of microorganisms present in wastewater to oxidize organic matter into CO_2 and water is defined as the BOD. The major objective of wastewater treatment is BOD removal. The reduction in BOD was due to a decrease in dissolved organic compounds and their derivatives (Kotteswari *et al.,* 2012). Fats, nutrients, lactose, detergents, protein, and inorganic salts in UDE may be responsible for high BOD and COD (Porwal *et al*., 2015).BOD alone does not give precise information about the organic matter content of wastewater because various toxins present in wastewater affect the validity of BOD tests (Hendricsk and David, 2007; Ahamad et al., 2023). Hence, a better estimate of organic matter is confirmed by COD. The COD content in the control experiment was 11,500 mg/l. The high amount of organic content in UDE is another reason for the higher COD value because COD accounts for the amount of nonbiodegradable organic matter present in effluents (Malaviya and Rathore, 2001). The reduction in COD was also maximum at 100% UDE (56.52%), followed by 80% UDE (47.82%) and 60% UDE (47.82%) (Table 2).

Table 2: Percent reduction in physicochemical parameters of dairy effluent after treatment with *Spirulina* sp. NCIM 5143

Growth media	pH	Biological Oxygen Demand (mg/l)	Chemical Oxygen Demand (mg/l)	Total Solids (mg/l)	Total Dissolved Solids (mg/l)	Total Suspended Solids (mg/l)	Total Phosphorus mg/l)	Total Kjeldahl Nitrogen (mg/l)	Crude Protein content (mg/l)
Control	6.50	11,000.00	11,500.00	24,190.00	23,600.00	1,000.00	10.88	195.55	1319.96
20% DE	7.21 (10.75) ^{e}	10,000.00 (9.09) ^{c}	10,000.00 (13.04) ^{c}	0.14 (99.99) ^{a}	17.94 (99.92) ^{b}	0.35 (99.96) ^{a}	6.40 (32.82) ^{d}	131.34 (32.83) ^{d}	0.08 (32.83) ^{d}
40% DE	7.94 (21.97) ^{d}	10,000.00 (9.09) ^{c}	10,000.00 (13.04) ^{c}	0.10 (99.99) ^{a}	2.34 (99.99) ^{a}	0.20 (99.98) ^{a}	6.60 (41.77) ^{c}	113.83 (41.79) ^{c}	0.07 (41.78) ^{c}
60% DE	8.90 (36.71) ^{c}	8,000.00 (27.27) ^{b}	6,000.00 (47.82) ^{b}	0.06 (99.99) ^{a}	2.06 (99.99) ^{a}	0.20 (99.98) ^{a}	6.70 (50.77) ^{b}	96.25 (50.78) ^{b}	0.06 (50.77) ^{b}
80% DE	9.88 (51.77) ^{b}	6,000.00 (45.45) ^{a}	6,000.00 (47.82) ^{b}	0.66 (99.99) ^{a}	0.04 (99.99) ^{a}	0.22 (99.98) ^{a}	7.40 (59.69) ^{a}	78.80 (59.70) ^{a}	0.05 (59.70) ^{a}
100% DE	10.11 (55.30) ^{a}	6,000.00 (45.45) ^{a}	5,000.00 (56.52) ^{a}	0.14 (99.99) ^{a}	0.78 (99.99) ^{a}	0.17 (99.98) ^{a}	7.90 (59.69) ^{a}	78.80 (59.70) ^{a}	0.05 (59.70) ^{a}

DE=Dairy effluent. Values in parentheses show percent reduction. Values superscripted by different letters in the column differ significantly (P≤0.05) from each other

In addition to CO_2, the inherent potential of microalgae to metabolize organic compounds as a source of energy might be responsible for the reduction in COD, which is an indirect means of organic components in the effluent (Hu *et al.,* 2012).The TS content is due to the prevalence of compounds such as carbonates, bicarbonates, chlorides, sulfate, phosphate, nitrate, calcium, magnesium, sodium, potassium, manganese, and organic matter (Sahana and Shirnalli, 2018; Bhutiani et al., 2018; Bojago et al., 2023). TS in the control was 24,190.00 mg/l. The maximum percent reduction of total solids (99.99%) was at par at 20% (0.14 mg/l), 40% (0.10 mg/l), 60% (0.06 mg/l) and 80% UDE (0.66 mg/l), and 100% UDE (0.14 mg/l) (Table 2).TSS quality checks the wastewater after treatment in the plant and is defined as the dry weight of particles trapped by a filter (Chikwe and Onojake, 2016). UDE is characterized by higher levels of TDS, which is contributed by a higher concentration of biodegradable organic matter (Kotteswari *et al.,* 2012). The concentration of TSS was 1,000.00 mg/l in the control. The maximum reduction in TSS was at 100% UDE (99.98%) (0.17 mg/l) (Table 2). The reason might be the transformation of the TSS present in UDE into dissolved materials that are assimilated by the algae (Rao *et al.,* 2011).The total dissolved solid content of UDE (control) was 23,600.00 mg/l. The percent reduction in TDS (99.98%) was highest and similar at all concentrations, i.e., 100% (0.78 mg/l), 80% (0.04 mg/l), 60% (2.06 mg/l) and 40% UDE (2.34 mg/l), except for 20% UDE (99.92%) (17.94 mg/l), which was significantly lower than the others (Table 2). The presence of sodium (Na^+) and chloride (Cl^-) ions is attributed to the employment of a great number of cleaners (alkaline) in the dairy plant (Demirel *et al.,* 2005). *Spirulina* sp. growth in mixotrophic culture is supported by the availability of glucose, acetate, or glycerol, which are sources of organic carbon (Cardoso *et al*., 2020). The high TDS percent reduction in the present study reflects that the utilization of specific salts by *Spirulina* sp. NCIM 5143 is required to meet its metabolic needs. Phosphorus deficiency greatly affects the ability to grow, chlorophyll synthesis, and cellular metabolism because the Calvin cycle and many phosphorylation syntheses are dependent on it (Liang *et al.,* 2013). On the other hand, excess phosphorus causes less growth and leaf necrosis and reduces zinc availability (Loneragan and Webb, 1993). The maximum percent reduction in total phosphorus was 59.69% (7.90 mg/l) at 100% UDE and 80% UDE (59.69%) (7.40 mg/l) compared to the control (10.88 mg/l) (Table 2). Algae use phosphorus for sustaining their growth and development, synthesizing their biomass and phospholipids, adenosine triphosphate (ATP) molecules, intracellular polyphosphate compounds, and nucleic acids, which are assimilated as inorganic orthophosphate, in the form of $H_2PO_4^-$ or HPO_4^{2-} (Ding *et al.,* 2015), which might be the most appropriate reason for phosphorus reduction. Removal of phosphorus from industrial effluent is a complex process. Orthophosphates are the preferred form for assimilation and for growth and synthesis of nucleic acids and several value-added compounds, such as astaxanthin and PUFAs.The preferred forms of nitrogen used by plants are ammonium (NH_4^+) and nitrate (NO_3^-). Nitrogen deficiency affects the productivity and growth of plants, whereas surplus nitrogen causes groundwater pollution and is harmful to humans (Akao *et al*., 2021). The total Kjeldahl nitrogen (TKN) and crude protein content were 195.55 and 1319.96 mg/l, respectively, in the control experiment. Total Kjeldahl nitrogen and crude protein content showed the highest percent reduction at 100 and 80% UDE (59.70%) and the lowest at 20% UDE (32.83%) (Table 2). Amino acid and protein synthesis requires nitrogen (Sialve *et al*., 2009). High rates of nitrogen removal were observed in the study because of the presence of the enzymes nitrate and nitrite reductase, which reduce nitrate ions (NO^-_3) to nitrite ions (NO^-_2) and then to ammonium ions (NH_4^+) (Salama *et al*., 2017). Cardoso *et al*. (2020) observed 72.11% nitrate and nitrite (79.28%) nitrate and 72.72% TDS removal rates by *Spirulina* sp. LEB 18. Therefore, both nitrogen and phosphorus elements in water and soil should be within permissible limits because both the excess and deficiency of these elements negatively affect living organisms. Sahana and Shirnalli (2018) reported significant reduction efficiency in various physicochemical parameters (pH, total solids, chemical oxygen demand, nitrate, phosphate) in 40% of untreated effluent and 100% of treated effluent after treatment with *Chlorella*

MA-6 microalgae. Previously, Choi *et al.* (2016) reported that *Chlorella vulgaris* after 10 days of treatment reduced the biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), total nitrogen (TN), and total phosphorus (TP) contents in dairy wastewater by 85.61%, 80.62%, 29.10%, 85.47%, and 65.96%, respectively.

Biochemical analysis of *Spirulina* sp. biomass

Total Kjeldahl Nitrogen (TKN) and crude protein (CP)

The results of the biochemical characterization of *Spirulina* sp. NCIM 5143 by various assays showed that TKN and CP were 7.14±0.49% and 48.23±3.34%, respectively. The protein content in *Spirulina,* as reported by Sharoba (2014), was 62.84%, which was higher than that obtained in this study. The protein content of microalgae varies widely based on the species, environmental conditions, and protocols used for measurement (Maehre *et al*., 2018). Exceptionally high levels of protein content in *Spirulina sp.* are not common even in microbes except bacteria such as *Cellulomonas,* which is reported to have 80% dry-weight protein content (Kameshwari *et al*., 2020). However, it also has high levels of nucleic acid content, which can cause many detrimental health conditions, such as gout. In *Spirulina,* even after the presence of high levels of proteins, the nucleic acid content is less than 5% of the dry weight (Anvara and Nowruzib, 2014). As seen from the above results, the protein content determined by the total Kjeldahl method (48.23±3.34%) was much higher than that obtained from Lowry's method (119.17 mg/l). A possible explanation for this could be that the Kjeldahl method determines the proteins in the sample based on the amount of nitrogen detected in the sample by simply multiplying the nitrogen content by a conversion factor (6.25), and all nitrogen in food samples is protein bound. Varied relative nitrogen content is observed between amino acids, and accordingly, different food proteins have a variable composition of amino acids. Moreover, nitrogen is present in several compounds, such as nitrate, ammonia, urea, nucleic acids, free amino acids, chlorophylls, and alkaloids, called nonprotein nitrogen, and variable relative contents contain nitrogen. Many workers have also reported that this conversion factor (6.25) overestimates the total protein count (Maehre *et al*., 2018**)**.

Total phenols

Total phenols in *Spirulina* sp. NCIM 5143 was 8.88 ±1.93 mg GAE/g. Different researchers reported varied values of phenolic compounds in *Spirulina,* e.g., El-Baky *et al*. (2009) reported that total phenols in *Spirulina maxima* were 12.94 ± 0.93 mg GAE/g, while phenolic compounds reported by Sehghiri *et al*. (2019) in *Arthrospira platensis* were 4.19 ± 0.21 mg GAE/g. According to Rechner *et al.* (2001), phenylalanine amino acid is a precursor for the enzyme ammonia lyase, which converts it into trans-cinnamic acid, then to cumaric acid, and caffeic acid, and ultimately several chemical reactions finally convert these into phenols and flavonoids in green algae. Phenolic compounds from algae have been reported to ward off oxidative damage to DNA, proteins, and lipids, which have a crucial role in cancer and brain dysfunctions such as diseases (Droge, 2002). Li *et al*. (2007) reported phenolic components of 23 microalgae strains to differ from 2.12 to 39.87 mg GAE g^{-1}, 0.01 to 9.80 mg GAE g^{-1}, and 0.95 to 10.68 mg GAE g^{-1} in hexane, ethyl acetate, and water fractions, respectively.

Total antioxidant activity and DPPH radical scavenging activity

The total antioxidant activity of *Spirulina* sp. NCIM 5143 was 3.07±0.03 mg AAE/g. In this study, good DPPH radical scavenging activity was recorded in *Spirulina* sp. NCIM 5143. The DPPH radical scavenging activity showed maximum % inhibition (75.07±0.09%) at the highest concentration (1000 µg/ml), followed by 500 µg/ml (41.20±0.30%) and 250 µg/ml (20.95±0.11%). This was lower than the antioxidant activity of the standard (ascorbic acid) at three different concentrations, i.e., 250, 500, and 1000 µg/ml (80.00±0.12, 90.00±0.45 and 109.88±0.11, respectively). Abd El-Baky *et al*. (2007) found that antioxidants inhibit the lipid peroxidation reaction by free radical scavenging activity, and their effect on DPPH activity was due to their ability to donate a hydrogen atom. Antioxidants inhibiting lipid peroxidation are generally validated by the free radical scavenging mechanism. In this study, methanolic extracts of *Spirulina sp.* NCIM 5143

containing phenols halted the extension of the chain reaction in the lipid oxidation reaction via the donation of a hydrogen atom to free radicals.

Phytochemical analysis

Phytochemical detection assays showed that quinones, tannins, saponins, terpenoids, and steroids were present in *Spirulina* sp. NCIM 5143. The results of the present study for phytochemical screening are in agreement with a study reported by Mane and Chakraborty (2018) that phytochemicals such as alkaloids, terpenoids, steroids, saponins, phenols, flavonoids, tannins, coumarins, and quinines are present in *Spirulina platensis.* Seghiri *et al*. (2019) studied the characterization of *Arthrospira platensis* and showed that the protein, carbohydrate, mineral, crude fiber, lipid, ash, flavonoid, and phenolic contents were 76.65 ± 0.15%, 6.46 ± 0.32%, 20.91 ± 0.88%, 4.07 ± 1.42%, 2.45 ± 0.82%, 14.56 ± 0.74, 15.60 ± 2.74 mg RE/g dw, and 4.19 ± 0.21 mg GAE/g dw, respectively. Moreover, they further reported that higher antioxidant activity (23 mg TE/g dw) was observed in methanolic extracts of algae, and these algae are safe for consumption as human food.

ICP–AES

Micronutrient analysis of *Spirulina* sp. NCIM 5143 showed that essential elements such as calcium (21.33 mg/kg), magnesium (28.96 mg/kg), iron (7.34 mg/kg), phosphorus (86.30 mg/kg), boron (1.16 mg/kg), copper (0.16 mg/kg), manganese (0.74 mg/kg), and zinc (0.64 mg/kg) were present. These elements have a variety of functions in various important metabolic activities. Micronutrients such as magnesium, calcium, and iron, which are metallic in nature and found in *Spirulina* sp. NCIM 5143, are required for the regulation of protein and chlorophyll synthesis, osmotic regulation, and nitrogen assimilation in microalgae (Beltrán-Rocha *et al*., 2017). Wuang *et al.* (2016) previously reported that *Spirulina platensis* growing on wastewater contained nitrogen (7.8%), phosphorus (0.8%), potassium (1.6%), and calcium (0.4%) in its biomass. Likewise, our results agree with those of Liestianty *et al*. (2019), who reported that *Spirulina* sp. contains essential micronutrients such as potassium (K), phosphorus (P), calcium (Ca), zinc (Zn), sodium (Na), manganese (Mn), magnesium (Mg) and iron (Fe), as analyzed by ICP–OES. These elements are of utmost importance for human nutrition. Maintenance of tissues, formation of bone and teeth, role as cofactors and coenzymes, body function regulation, and several other biochemical and physiological activities of the body are some of the important functions of micronutrients. For the maintenance of health throughout a lifetime, these micronutrients are essential (Gernand *et al*., 2016). Calcium, sodium, magnesium, phosphorus, and potassium are the five prime minerals in the human body, while all other remaining inorganic elements are called trace elements, including iodine (I), molybdenum (Mo), copper (Cu), chlorine (Cl), zinc (Zn), manganese (Mn), selenium (Se), sulfur (S), iron (Fe) and cobalt (Co). Bones and teeth contain approximately 99% calcium and constitute up to 920 g to 1200 g of the total body weight (Berdanier*et al*., 2013). Sodium, chlorine, sulfur, magnesium, and potassium are the major nutrients that constitute 0.85% of the total body weight (Awuchi and Godswill, 2020).

Heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni) were completely absent in *Spirulina* sp. NCIM 5143. The WHO has imposed certain specific limits for heavy metal concentrations in plants (Table 3). The maximum permissible limits for cadmium, chromium, nickel, and lead in plants are 0.005, 0.01, 0.05, and 0.05 ppm, respectively. The concentrations of heavy metals in *Spirulina* sp. NCIM 5143 were well below the permissible limits of the WHO, which ensures its safety for consumption purposes. Rebolloso-Fuentes *et al*. (2001) observed that calcium, potassium, sodium, magnesium, zinc, iron, manganese, copper, nickel and cobalt were present at concentrations of 972, 533, 659, 316, 103, 136, 3.4, 35, 0.22 and <0.1 mg, respectively, while heavy metals such as cadmium and lead were absent per 100 g dry biomass weight of marine microalgae *Nannochloropsis* spp.

GC–MS analysis

GC–MS is a high-value and authentic technique for the identification of bioactive compounds in a given sample. GC–MS analysis of methanolic extracts of *Spirulina* sp. NCIM 5143 detected several bioactive compounds (Fig. 3) (Table 4). A total of twelve

compounds of therapeutic and nutraceutical value were present in the biomass. The major compounds were as follows: phytol (26.15%), hexadecanoic acid, methyl ester (18.82%), trans-13-octadecenoic acid, methyl ester (22.31%), and 9,12-octadecadienoic acid, methyl ester (20.75%). Phytol was present as a major compound (26.15%) at a retention time (RT) of 38.87. Phytol is a component of various pharmaceuticals used for prophylaxis, prevention, and treatment of hypercholesterolemia, for maintaining normal levels of cholesterol in serum, obesity, insulin resistance, diabetes, atherosclerosis, and related cardiovascular diseases (Olofsson, 2011). Many plants used in Chinese medicines traditionally contain phytol, which has low toxicity, is

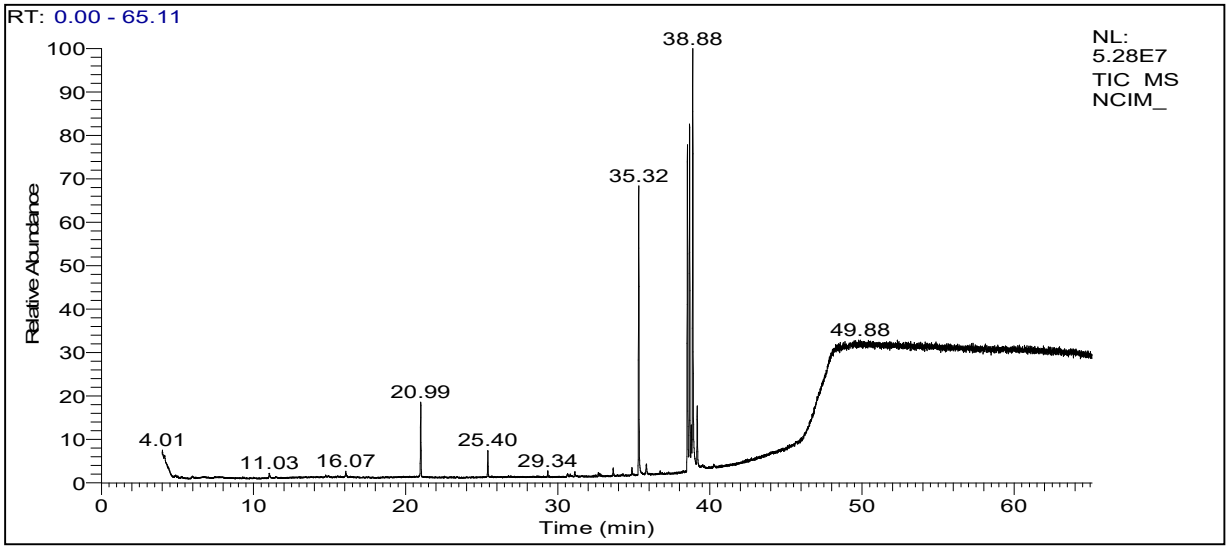

Figure 3: Peaks showing various bioactive compounds in Spirulina sp. NCIM 5143 as revealed in the GC–MS profile

Table 3: Maximum permissible limits for heavy metals in plants and soil

Element	Symbol	WHO Limits (ppm)
Cadmium	Cd	0.005
Chromium	Cr	0.01
Copper	Cu	1.00
Iron	Fe	0.30
Manganese	Mn	0.01
Nickel	Ni	0.05
Lead	Pb	0.05
Zinc	Zn	5.00

Source: Musa *et al*., 2016.

inexpensive, and is used in pharmaceuticals (Yu *et al*., 2009). The fatty acid compound 9,12-octadecadienoic acid, methyl ester (20.75%), found at RT 38.52, has been reported to have an anticancer effect (Yu *et al.,* 2005). Trans-13-octadecenoic acid, methyl ester (22.31%) at RT 38.66 has anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene-D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, and flavor properties (Krishnamoorthy and Subramaniam, 2014). Similarly, at an RT of 35.32, hexadecanoic acid, methyl ester (18.82%) was detected, which was reported to have antibacterial and antifungal properties (Chandrasekaran *et al.,* 2011), anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicidal, insectifuge, antihistaminic, antieczemic, antiacne, alpha-reductase inhibitor, antiandrogenic, anti arthritic, and anticoronary properties (Krishna moorthy and Subramaniam, 2014). Organosil oxane compounds such as Cyclohexasiloxane dodeca

methyl,Cycloheptasiloxanetetradecamethyl, Cyclo detected in methanolic extracts of *Spirulina* sp. NCIM 5143. Cyclohexasiloxanedodecamethyl has antimicrobial, antifouling immunomodulatory, and antitumor activities, and cyclo heptasil oxane tetradecamethyl has skin-conditioning agent, fragrance, and antimicrobial properties (Chaudhary and Tripathy, 2015). Jubie and Dhanaba (2012) discovered eight compounds in the GC–MS

octasil oxane, and hexadecamethyl were also analysis of *Spirulina platensis*. Among them, stearic acid, gamma-linolenic acid, linoleic acid,heptadecanoic acid, and oleic acid were the five major compounds. Soltani *et al.* (2005) reported many fatty acids and volatile components, such as phytol, fucosterol, neophytadiene, or palmitic, palmitoleic, and oleic acids, from liquid extracts from cyanobacteria by GC–MS and HPLC-DAD.

Table 4: Bioactive compound analysis by GC–MS

SN	Compound name	Molecular Formula	Area (%)	Retention time (RT)
1.	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	4.87	20.99
2.	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	1.33	25.41
3.	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	0.03	29.34
4.	Z-3-Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	0.13	33.64
5.	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$	0.07	34.87
6.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.82	35.32
7.	Dibutyl phthalate	$C_{16}H_{22}O_4$	0.24	35.82
8.	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	20.75	38.52
9.	Trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	22.31	38.66
10.	6-Octadecenoic acid, methyl ester, (Z)	$C_{19}H_{36}O_2$	1.85	38.78
11.	Phytol	$C_{20}H_{40}O$	26.15	38.87
12.	Methyl stearate	$C_{19}H_{38}O_2$	3.46	39.17

The main mandate of performing GC–MS analysis of *Spirulina* sp. NCIM 5143 is to explore its biochemical constitution, which further has application as a functional food. GC–MS analysis of *Spirulina* sp. NCIM 5143 revealed the presence of phytol (26.15%), hexadecanoic acid, methyl ester (18.82%), trans-13-octadecenoic acid, methyl ester (22.31%), and 9,12-octadecadienoic acid, methyl ester (20.75%) as major bioactive compounds. Each of these compounds has an important biological function. A functional food contains compounds that have biological and physiological importance, and it also confers several health advantages. The compounds present in such foods that provide health benefits have been generally termed bioactive compounds. Bioactive compounds are of great significance because they are endowed with antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, and antitumor activities. Therefore, they protect humans from damaging free radicals and reactive oxygen species (ROS) (Banwo *et al.*, 2021). Microalgal biomass is also enriched in these bioactive compounds, as previously reported by many workers. The compounds produced by microalgae possess myofibroblast differentiation-inducing, anti-proliferative, angiotensin I-converting enzyme inhibitory, antioxidant, antimicrobial, anti-chymotrypsin, anti-parasitic, anti-trypsin, anti-elastase, and hepatic fibrosis inhibitory activities (Saha and Murray, 2018).

Conclusion

Microalgae are endowed with the remarkable property of treating waste effluent, thus recycling water sources along with biomass enriched with many valuable compounds, such as phenols, antioxidants, proteins, lipids, carbohydrates, and phytocompounds. The present study evaluates the nutrient removal efficiency of *Spirulina* sp. NCIM 5143 along with its biochemical characterization. The results showed that *Spirulina* sp. NCIM 5143 was able to use pollutants present in dairy effluent to meet its growth requirements. This showed that wastewater treatment by microalgae can be employed either as a secondary or tertiary treatment step. In addition, biomass harvested after treatment is enriched in many valuable compounds for human health. The absence of heavy metals in microalgal

biomass ensures their safety for human consumption purposes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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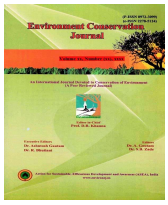

Pharmacological and ethnobotanical studies of angiosperms from Shamli region of district Meerut, Uttar Pradesh, India

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ABSTRACT

Plants are essential to our life as they provide us with food and, through photosynthesis, release oxygen into the atmosphere. Historical accounts about plants demonstrate their many benefits to humans and other living things. Rapid population growth and industrialization have damaged agricultural and forest flora. Raising awareness of the value of plants for sustainable development is necessary. This study focuses on the medicinal benefit of the phyto-diversity of angiosperms from the Shamli region of Uttar Pradesh and their medicinal value for mankind. Shamli is located near the Ganga River, on the eastern side of the Yamuna River at an elevation of 248 meters above sea level. This region's ideal temperature is between 13.8°C and 33.2°C. The vegetation of Shamli is enhanced by a variety of trees, shrubs, and plants. In addition to documenting fifty plant species that belong to several angiosperm genera and families, such as *Abutilon indicum, Aegle marmelos, Azadirachta Indica, Moringa oliefera, and Calotropis gigantea*, a critical study of the area's plant life has been conducted. The engagement with the locals of the Shamli region recorded the ethnomedicinal significance of the collected plants.

Introduction

The term Ethnobotany was coined by the American botanist, John Harshburger (Harshburger, 1896). Ethnobotany is developing and progressing beyond simple documentation to producing complex, durable drugs. Humans have used wild plants for food, medicine, fuel, and a variety of other practical purposes throughout history (Haq *et al.,* 2023; Mirzaman *et al.,* 2023). People that reside in less developed locations depend heavily on the collection and use of these wild botanical resources for their livelihoods (Waheed *et al.,* 2023). Geographical and cultural variables have a significant influence on plant consumption patterns, which shapes human interactions with flora into a combination of behavior and wisdom (Morell-Hart *et al.,* 2019). However, the disappearance of

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ancestors' traditions and conversion of forests into other types of land use pose a serious threat to the priceless wealth of traditional knowledge (Haq *et al.,* 2022). In the wake of future advancement, there is a chance that this looming loss will vanish permanently (Arshad *et al.,* 2023). Therefore, it becomes imperative to carefully record and preserves the ancient botanical knowledge associated with these species (Haq *et al.,* 2023a).

The Greek terms angeion, which means "vessel," and sperma, which means "seed," are the source of the word "angiosperm," which together imply "enclosed seeds," referring to seeds that are carried inside fruits. *Magnoliophyta, Anthophyta,* and *Angiospermae* are other names for angiosperms, or blooming plants. With over 2,500 species spread across 350 families, they represent the most varied group in the plant kingdom (Kenrick, 1999; Simpson, 2006). They are found in nearly every type of habitat on the planet, including freshwater habitats, marine estuaries, high alpine peaks, and deserts. The number of flowering plant species that are now recognized, characterized, and accepted has been estimated by Christenhusz and Byng (2016) to be 95,383, of which 2,10,008 are eudicots and 74,273 are monocots. Put another way, about 96% of the species of angiosperms that are currently known belong to the monocotyledon (monocots) or eudicotyledon (eudicots) groups, with the remaining 4% of angiosperms being classified as magnoliids, or non-eudicots, a group of plants that are thought to exhibit primitive characteristics. Generally speaking, these can be separated into paleoherbs and woody magnoliids (www.bsienvis.nic.in/Database/Angiosperms_of_India_26171.aspx).

India's diverse physical environment, including variations in latitude, altitude, climate, and geology, contribute significantly to the country's great biological variety. Hooker (1904) said that Relating to the great degree of plant diversity in the country, "The Flora of British India is more varied than that of any other country of equal area in the eastern hemisphere, if not on the globe," Even though India only makes up 2.4% of the world's landmass, the nation is home to 55,048 taxa that are divided among 21,984 Angiosperms, 82 Gymnosperms, 1314 Pteridophytes, 2800 Bryophytes, 2989 Lichens, 15602 Fungi, 9008 Algae, and 1269 Microbes. These taxa account for approximately 11.4% of the entire world's currently known species (Anonymous, 2022). Additionally, approximately 25% of India's vascular plants are endemic. About 10% of India's blooming plant species are thought to be threatened (Nayar and Sastry, 1987–1990; Singh *et al.,* 2015; Lakshminarasimhan and Paul, 2023).

The World Health Organization (WHO) estimates that up to 80% of people on the planet receive their primary medical treatment from traditional medicine. The utilization of medicinal plants to treat a variety of ailments and the advancement of indigenous medicine have significant positive economic effects (Azaizeh *et al.,* 2003). In rural and tribal India, medicinal plants have long held a significant place in the sociocultural, spiritual, and medical spheres. One of the richest, oldest, and most varied traditional medical systems is found in India. There is a long history of using plants to treat illnesses. Preparing medicinal plants that are readily available locally is still a crucial aspect of providing healthcare to people, particularly for those who live in rural regions without access to modern medical facilities or cannot afford the expensive synthetic pharmaceuticals. India's woods are a treasure trove of priceless medicinal plants that have been used for human health care ever since people discovered the preventative and therapeutic qualities of plants.

Native medicinal herbs are affordable to synthesize, easily accessible, biocompatible, and safe to use (Cavero and Calvo, 2015; Damor *et al.,* 2023). Worldwide, plants constitute the source of 25% of prescription drugs (Tribess *et al.,* 2015). There were 2,68,600 angiosperm plants in the globe overall, of which 18,386 (6.84% of the world's total) were found in India, according to B.S.I., West Bengal's Plant Statistics of India. Indian indigenous people use a wide range of herbal remedies to treat a wide range of illnesses. There are regional variations in the plant parts utilized, drug formulation, and drug delivery (Verma *et al.,* 2015). As the "cradle of flowering plants," Northeast India is home to more than 130 species of primeval angiosperms (Takhtajan, 1969). An estimated 3000 species of angiosperms are thought to have therapeutic potential; of these, roughly 1300 species are widely employed in various traditional medical systems, including Allopathy, Siddha, and Ayurveda. This region contains a

significant number of primitive angiosperm genera, including *Magnolia, Mangelietia, Tetracentron, Alnus, Aspidocarya, Betula, Decaisnea, Euptelea, Exbucklandia, Haematocarpus, Holboellia, Houttuynia,* and *Pycnarrhena* (Malhotra and Hajra, 1977). According to Shankar (2020), the Indian subcontinent is home to 34 species of magnolia, of which 9 are introduced or hybridized species.

In India, around 3000 plant species are recognized to offer health benefits such as anti-diabetic and antioxidant properties (Chhetri *et al.,* 2005; Chauhan *et al.,* 2010; Debbarma *et al.,* 2017).

Scientists worldwide have investigated the therapeutic characteristics of these plant species because of their composition, pharmacologic activity, minimum noxiousness, and commercial feasibility (Semwal *et al.,* 2010; Tewari *et al.,* 2014). Because contemporary health care facilities are expensive and inaccessible, Native Americans from low-income backgrounds rely heavily on traditional medicine. Ayurvedic clinical competence in rasayana tries to ward against disease and mitigate the signs of aging. These substances are said to be powerful antioxidants, nourishing nutrients, and rejuvenators (Chaturvedi, 2012).

Notably, in the current study area, there is a dearth of research examining the ethnomedicinal potential of native plants. Therefore, the purpose of this study was to methodically compile and record traditional ethnobotanical knowledge on a variety of plants from around the world.The present study focused on the collection of some angiosperms and the study of their medicinal importance in specific diseases like diabetes, blood pressure, colds and coughs, diarrhoea, dysentery, cholera, pneumonia, leprosy, bronchitis, sore throats, etc.

Material and Methods

Study area

Uttar Pradesh is the most populous and fourth-largest state in India. Situated in the north-central region of the country, it shares borders with the states of Uttarakhand, Nepal, Bihar, Jharkhand, Chhattisgarh, and Madhya Pradesh to the east, and Rajasthan, Haryana, and the national capital territory of Delhi to the west. On January 26, 1950, the state of Uttar Pradesh was renamed as the "Northern State" when India became a republic. The capital of the state is Lucknow. Of the 75 districts of Uttar Pradesh, 26 are located solely in Western Uttar Pradesh.

One of Uttar Pradesh's districts, Shamli is comprises of three tehsils, five blocks, and 134 villages under the jurisdiction of Shamli tehsil (Figure 1). This region features strong humus content, clayey soil that is rich in fertility, and a high water-retaining capacity. Alluvial soil is found in the majority of the district, which is the Gangetic plain, and is formed from silt that the Yamuna and Ganga rivers have deposited. It is located in the nation's North Central area. Many different types of medicinal plants can be found in the northern area of India due to its distinct topography and environmentally marginal conditions. The people who live in northern India continue to honor the traditional medical practices that are ingrained in their culture. These traditional techniques have been used to treat complex disorders for more than three millennia (Kumar *et al.,* 2015).

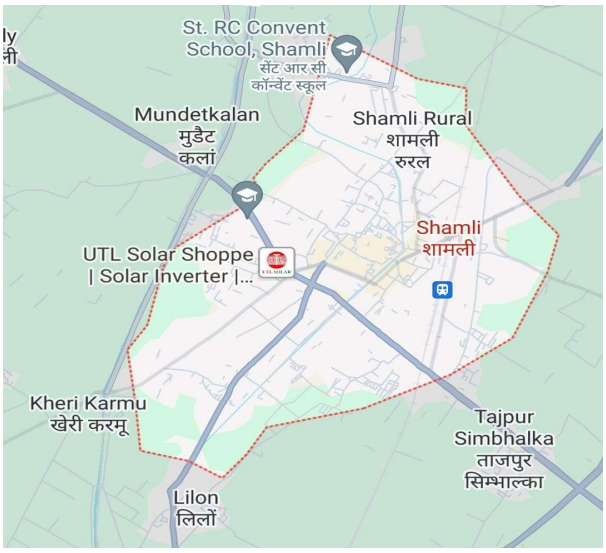


Figure 1: Map showing sampling sites

Sampling and Identification

Beginning with a collection of plants from Shamli (Uttar Pradesh), the work was carried out in the Laboratory of Department of Botany, Keral Verma Subharti College of Science at Swami Vivekanand Subharti University, Meerut (UP). In order to gather information, a survey was conducted in various villages within the Shamli district of Uttar Pradesh between December 2022 and May 2023.

These villages included Bhaju, Kurmali, Choonsa, Adampur, Kairana, Un Rural, Babri, Banat, Bantikhera, Jalalabad, Salahkhedi, Karmukhedi, Sikka, Silawar, and Lilaun. Additionally, Vaidyas and herbal practitioners were consulted regarding the medicinal significance of the collected plants. While many of them felt uncomfortable sharing information, a few mentioned the plant names in their native tongues as well as the natural compounds that were utilized to treat particular diseases. By gathering samples from the area, these plant names from the local dialect were confirmed. With the aid of online taxonomic literature, these collected plant specimens were identified (eFloras 2008).The region's documented ethnomedical data is also cross-referenced with primary source data, including books and herbal pharmaceutical firms. Additional details about the therapeutic applications of plants and their parts were gathered using questionnaires, which were then shared with senior villagers and the local Vaidya. The plants from this survey were compared to other surveys of historically utilized plants.

Results and Discussion

In Table 1, ethnomedical plants are listed alphabetically by botanical name, family, and the term or portion of the plant that is being used.

This study demonstrates the ethnomedicinal significance of 45 local and wild plant species found in the Shamli region, which is still uncharted scientific territory with few records of additional local and wild plants as well as ethnomedicinal plants (Figure 2). The plant species under study are either regularly grown or occur naturally in the surrounding area. According to Table 2, the main family with the most plants is the *Asteraceae* (4 plant species), which is followed by the *Fabaceae, Malvaceae,* and *Annonaceae* (3 plant species each), and finally the *Myrtaceae, Solanaceae, Annonaceae, Lamiaceae, Rutaceae,* and other families (2 plant species each).

The survey also reveals that leaves are the most common plant portion used for therapeutic purposes. Nearly all plants under study have leaves that are extremely therapeutic and frequently used to cure various illnesses. The next most useful plant element after leaves is fruit, which is followed by some species' bark. Many plants are utilized in their complete form to treat a variety of illnesses. In addition, a variety of plant parts, including seeds, stems, roots, aerial roots, rhizomes, buds, flowers, calyxes, and others, are employed in the treatment of various illnesses. The current study demonstrates the great ethnomedicinal worth of the native plants in the Shamli region, which can be further explored and examined to record their medicinal value and gain a wealth of knowledge. There are many medicinally valuable plants in the examined area, and the study documents the ethnomedicinal value of these plants. While some of the locals are still ignorant of the health benefits offered by the plants, many of them rely on the medicinal qualities of diverse plants. Many diseases have been observed to respond quite well to the plants. Many plants have been shown to be highly effective in treating various human diseases. For example, a number of plants, including *Abelmoschus esculentes, Aloevera, Annona squamosa, Azadirachta indica, Memordica charantia, Psidium guajava, Syzygium cumini, Tinospora cordifolia, Morus alba,* and *Catharanthus roseus*, have been shown to help lower the level of diabetes.

Aegle marmelos, Aloe vera, Azadirachta indica, and *Curcuma longa* are examples of plants that have both antimicrobial and anti-inflammatory properties. *Cassia fistula, Eclipta prostate, Lagerstroemia speciosa, Moringa oleifera,* and numerous other species have antimicrobial properties. Another health concern is dysentery, which can be addressed with a variety of plant species such as *Abutilon indicum, Solanum nigrum, Muraya koeingii,* and various plant parts of *Achyranthes aspera, Calotropis gigantea, Carrisa carandas, Euphorbia hirta,* and *Prosopis cineraria*. Certain species, such as *Cassia fistula, Cannabis sativa, and Argemone Mexicana*, have anti-cancer qualities. Plants that help with venomous reptile bites include *Achyranthes aspera*. Aside from these, many plant parts can be consumed or applied as a tonic, paste, or in any other form to treat a wide range of additional illnesses and allergies. Numerous plants can help with a wide range of illnesses, allergies, and conditions. They are said to be more successful than allopathic medicine in healing the body organically. In the village and surrounding area, traditional medical knowledge about numerous plants and their diverse sections is still in use and highly respected (Table 1 and Figures 2-6).

Table 1: List of Ethno-medicinal plants being used

SN	Botanical Name	Name's of family	Vernacular Names	Plant part used	Ethno-pharmacological application
1	*Abelmoschus esculentus*	Malvaceae	Bhindi	Fruits	Used in diabetes, chopped fruits soaked in water overnight and aqueous extract taken in morning
2	*Abutilon indicum*	Malvaceae	Kanghi	Whole plant	Extract of fresh leaves mixed with a tea spoon honey is taken during Dysentery. Fresh roots are crushed and consumed with milk in Weakness.
3	*Achyranthes aspera*	Amaran-thaceae	Chirchita	Whole plant	Plant is crushed and given in case of pneumonia, also helpful in asthma, cough and piles. Spikes or seed paste is used to cure snake and venomous reptile bites.
4	*Aegle marmelos Correa*	Rutaceae	Bel	Leaves, fruits	It has antiviral and antimicrobial properties. Leaves give extreme benefit during ulcers, digestive disorders and tuberculosis.
5	*Aloe vera*	Asphode-laceae	Gwarpatha, Gheekwar	Leaves	Pulp used for various skin infection and acne. Helps in lowering sugar level. It has antimicrobial properties.
6	*Annona squamosa*	Annonaceae	Sitaphal	Leaves, seed	Helps in diabetic conditions, improves immunity and eyesight. Seed oil acts as an antioxidant.
7	*Argemone mexicana*	Papaveraceae	Peeli kateri	Stem, leaves	Aqueous extracts have anticancerous and antifungal properties.
8	*Azadirachta indica*	Meliaceae	Neem	Bark, leaves	Paste of bark is used in healing skin injuries. Extraction of leaves and roots is helpful in treating skin problems and jaundice.
9	*Calotropis gigantea*	Asclepiadaceae	Safed Akara	Leaves, Crushed roots	Used to treat asthma, cold and cough, diarrhoea, fever, indigestion, leprosy, leukoderma, and rheumatism.
10	*Cannabis sativa*	Cannabinaceae	Bhang	Buds, leaves, flowers	Used in asthma, cancer, cystitis, diarrhea, dysentery, diuretic, epilepsy and fever.
11	*Capsicum annuum*	Solanaceae	chilli	Leaves, Fruit	Used in the treatment of diabetes, sore throat, arithritis. It has analgesic effect and relieves sore throat.
12	*Carissa carandas(linn.)*	Apocynaceae	Karonda	Fruit and leaves	Leaves decoction is used in cough, cold, asthma and various skin disease.it also cures diarrhea and chronic constipation
13	*Cassia fistula*	Fabaceae	Amaltas	Leaves	It has antitumor, antimicrobial properties. Pulp is effective in easing bowel. Plant juice helps with various skin disorders.
14	*Catharanthus roseus*	Apocynaceae	Sadabahar	Leaves, fflower	Aqueous extract and fresh leaves are chewed daily empty stomach in morning. Raw flowers are chewed in treating diabetes.
15	*Croton bonplandianum*	Euphorbiaceae	Jangli jamal ghota	Stem, leaves	Leaf decoction is used in dandruff removal. Juice of stem is used in eye infection.
16	*Curcuma longa*	Zingeberaceae	Haldi	Rhizome	Anti- inflammatory, acts as anticoagulant have antimicrobial and nephron protective properties.
17	*Eclipta prostrata*	Asteraceae	Bhringraj	Whole plant	Fresh leaves juice is used in treating fever, skin disorders and joint pains. Shows antimicrobial and antifungal properties and helps in hair growth when paste is applied to scalp.
18	*Euphorbia hirta*	Euphorbiaceae	Dudhiya	Leaves	Leaves juice is taken to treat bronchitis and cough.
19	*Ficus benghalensis*	Moraceae	Bargad	Aerial roots	Tips of fresh prop roots chewed in morning empty stomach with water.
20	*Ficus relegiosa*	Moraceae	Pipal	Bark, leaves	Bark has antibacterial, antiprotozoal properties. Leaves are used in the treatment of different infections and diseases.
21	*Hibiscus rosa sinensis*	malvaceae	gudhal	Leaves, calyx	Acts as sedative, antiseptic and astringent etc. emollient leaves boiled calyx is helpful in nauseous conditions.
22	*Lagerstroemia speciosa*	Lythraceae	Pride of India, Jarul	Leaves, fruit, bark	Bark extracts have antimicrobial effects, Leaves extract shows antioxidant activity and decreases blood glucose level.
23	*Mangifera indica*	Anacardiaceae	Aam	Bark, leaves, seeds	Seeds and kernels are used for vaginal and uterus problem. Bark is used during jaundice and coughing. It is even used during diabetes.

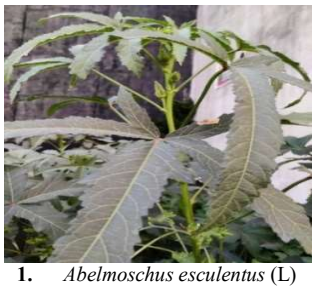
24	*Memordica charantia*	Cucurbitaceae	Karela	Fruits	Extremely helpful in diabetic condition due to its bitter taste.
25	*Mimosa pudica*	Mimosideae	Chhui-mui	Seeds	One teaspoon of powdered seeds are taken with milk/water twice a day
26	*Moringa oliefera Lam.*	Moringaceae	Shahjan	Leaves, Pods	It has anti-inflammatory, antimicrobial properties. Leaves are used in treating various skin related problems, asthma and conjunctivitis.
27	*Morus alba*	Moraceae	Shahtoot	Leaves, Fruits	Leaves have ant diabetic and antioxidant effect.
28	*Muraya koeingii Sprenge*	Rutaceae	Curry Patta	Leaves	Leaves are crushed and taken during piles, dysentery etc.
29	*Nyctanthus arbor-tristis*	Oleaceae	Harsingar	Leaves, fruits	Dried fruits are taken during cough. Fresh Leaf extract is used to treat ringworm and for other inflammatory problems.
30	*Ocimum tenuiflorum*	Lamiaceae	Tulsi	Leaves	It acts as mosquito repellant, anti-arithritic, antimicrobial etc. Raw Leaves are chewed. It also helps in DNA repair when consumed and eliminates toxic compounds from the body.
31	*Origanum majorana*	Lamiaceae	Marva	Leaves	Extract used as essential oil to treat toothache, soothes muscular pain. It also acts as antidote and shows antiseptic properties.
32	*Phyllanthus emblica*	Phyllanthaceae	Amla	Fruits, Leaves	Used as tonic and as a source of vitamin C. also helps to cure diarrhea, inflammation and jaundice.
33	*Polyathia longifelia*	Annonaceae	Ashok	Seed, bark and flowers	Cytotoxic function, antiulcer, hypoglycemic and hypotensive effect.
34	*Prosopis cineraria*	Fabaceae	Shami	Bark, flowers, pods	Bark extracts have antibacterial properties. It is also beneficial in bronchitis, cough and asthma. Leaves extract reduces blood sugar level.
35	*Psidium guajava*	Myrtaceae	Amrood	Leaves, fruits	Leaf extracts helps in diabetes, diarrhea. Pulp of the fruit helps in increasing the platelet count during dengue.
36	*Punica granatum*	Punicaceae	Anaar	Fruit, bark	Fruits act as an important astringent. Fruit juice is useful in leprosy, fever and dyspepsia.
37	*Solanum nigrum l.*	Solanaceae	Makoi	Whole plant	Used in fever, stomach problems, ulcers, skin diseases, dysentery. Fruits are used for asthma and cough.
38	*Sonchus asper*	Asteraceae	Prickly sowthistle	Leaves	It has anti-fungal properties and cure many skin related problems.
39	*Sonchus oleraceus*	Asteraceae	Common sowthistle/ peeli dudhi	Leaves	Used in treatment of inflammatory diseases and gastrointestinal tract disorders. Leaves are used in salads.
40	*Stellaria media*	Caryophyllaceae	Chickweed	Whole plant	Whole plant is crushed and applied during skin inflammation and allergy. Leaves are taken as tea to relieve pain.
41	*Syzygium cumini*	Myrtaceae	Jamun	Fruits, seeds and leaves	Seed powder consumed to cure diabetes. It purifies blood. Fruit removes bad smell from mouth and is ant diabetic.
42	*Terminalia arjuna*	Combretaceae	Arjun	Bark, Leaves	Used as tonic during heart failure, anemia. Used in treatment of fractures, Ulcers and shows antioxidant, antimicrobial effects
43	*Tinospora cordifolia*	Menispermaceae	Giloy	Leaves	It helps in improvement of immunity, also beneficial in detoxifying skin and releases toxins from body, boon for a diabetic patient.
44	*Trifolium repens*	Fabaceae	White clover	Whole plant	Used in treating cold and cough and leucorrhoea. Flower Mixture is used as Eyewash.
45	*Xanthium Strumarium*	Asteraceae	bhurunt	Seed, root, fruit	Seeds are useful to get relief during headaches whereas roots are helpful during tumor treatment. It's fruits are used for treating constipation.

The importance of ethnobiological knowledge in leading the way toward new avenues for scientific research on ecology and conservation monitoring has received considerable attention in resource management (Berkes *et al.,* 2000; Huntington, 2000). Through their "people and plants" project, international organizations such as the World Wildlife Fund (WWF) and UNESCO have provided assistance for research on ethnobotanical knowledge and the integration of local populations' viewpoints and practices in resource management (Cunningham, 2014).

Table 2: Distribution of Plant species with reference to family

SN	Name of Family	Number of Species	S. No.	Name of Family	Number of Species
1.	Asteraceae	4	16.	Moraceae	3
2.	Ascelpiadaceae	1	17.	Moringaceae	1
3.	Meliaceae	1	18.	Mimosideae	1
4.	Fabaceae	3	19.	Cucurbitaceae	1
5.	Menispermiaceae	1	20.	Anacardiaceae	1
6.	Combretaceae	1	21.	Lythraceae	1
7.	Myrtaceae	2	22.	Malvaceae	3
8.	Caryophyllaceae	1	23.	Euphorbiaceae	2
9.	Solanaceae	2	24.	Zingeberaceae	1
10.	Punicaceae	1	25.	Apocynaceae	2
11.	Annonaceae	2	26.	Cannabinaceae	1
12.	Phyllanthaceae	1	27.	Papaveraceae	1
13.	Lameaceae	2	28.	Asphodelaceae	1
14.	Oleaceae	1	29.	Amaranthaceae	1
15.	Rutaceae	2	-	-	

Figure 2: Photos of collected plants



1. *Abelmoschus esculentus* (L)

2. *Abutilon indicum* (L)

3. *Achyranthes aspera* (L)

4. *Aegle marmelos* (L) Correa

5. *Aloe vera* (L) Burm.F.

6. *Annona squamosa* (L)

7. *Argemone Mexicana* (L)

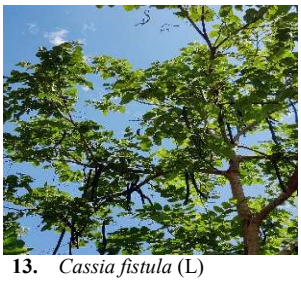
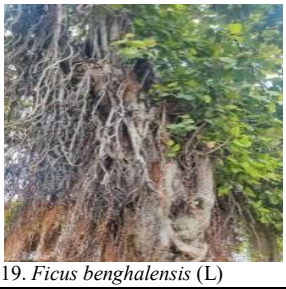
8. *Azadirachta indica* (L)

9. *Calotropis gigantean* (L)

10. *Cannabis sativa* (L)

11. *Capsicum annum* (L)

12. *Carissa carandas* (L)

 **13.** *Cassia fistula* (L)	 14. *Catharanthus roseus* (L)	 15. *Croton bonplandianum* (L)	 16. *Curcuma longa* (L)
 17. *Eclipta prostrate* (L)	 18. *Euphorbia hirta* (L)	 19. *Ficus benghalensis* (L)	 20. *Ficus relegiosa* (L)
 21. *Hibiscus rosa sinensis* (L)	 22. *Lagerstroemia speciosa* (L)	 23. *Mangifera indica* (L)	 24. *Memordica charantia* (L)
 25. *Mimosa pudica* (L)	 26. *Moringa oliefera* (L)	 27. *Morus alba* (L)	 28. *Muraya koeingii* (L)
 29. *Nyctanthus arboritis* (L)	 30. *Ocimum tenuiflorum* (L)	 31. *Origanum majorana* (L)	 32. *Phyllanthus emblica* (L)


33. *Polyathia longifelia* (L)


34. *Prosopsis cineraria* (L)


35. *Psidium guajava* (L)


36. *Punica granatum* (L)


37. *Solanum nigrum*


38. *Sonchus asper*


39. *Sonchus oleraceus*


40. *Stellaria media*


41. *Syzygium cumini*


42. *Terminalia arjuna*


43. *Tinospora cordifolia*


44. *Trifolium repens*


45. *Xanthium oxydentalis*

The incorporation of local-use patterns and the institutional and social environment that determines human-nature relationships into biological and ecological studies has led to a greater understanding of the interplay between social and ecological dynamics (Kumar *et al.,* 2011). Many common weeds have medicinal properties, and for over 3,000 years, traditional medical systems have cultivated them for both culinary and medicinal uses (Kareti *et al.,* 2023; Arya *et al.,* 2022; Mukherjee *et al.,* 2006).Chhetri *et al.* (2005) explore the antidiabetic plants of Sikkim state and found that about 37 species belonging to 28 families of plants were utilized as antidote for diabetes. Kumar *et al.* (2011) studied the ethnomedicinal and ecological status of Garhwal Himalaya and reported a total of 57 species of plants including 24% tree species, 17.5% shrub species, and 57.90% herb species. Vibha *et al.* (2019) wrote a review on ethnobotanical applications of two species (*Acampe praemorsa* (Roxb.) Blatt. & McCann)] of *Orchidaceae* families of angiosperms. The authors concluded that the selected plant species can be used as antidote for cancer and bacterial infections. In Mastuj tehsil of Chitral, Pakistan, Dastagir *et al.*

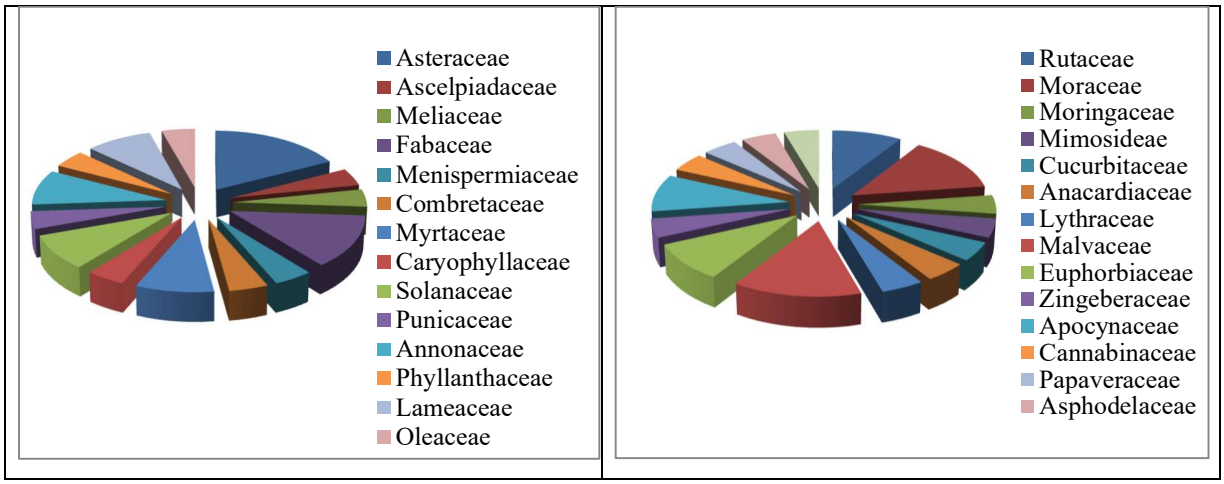


Figure 3: Graphical representation of distribution of Plant species with reference to family

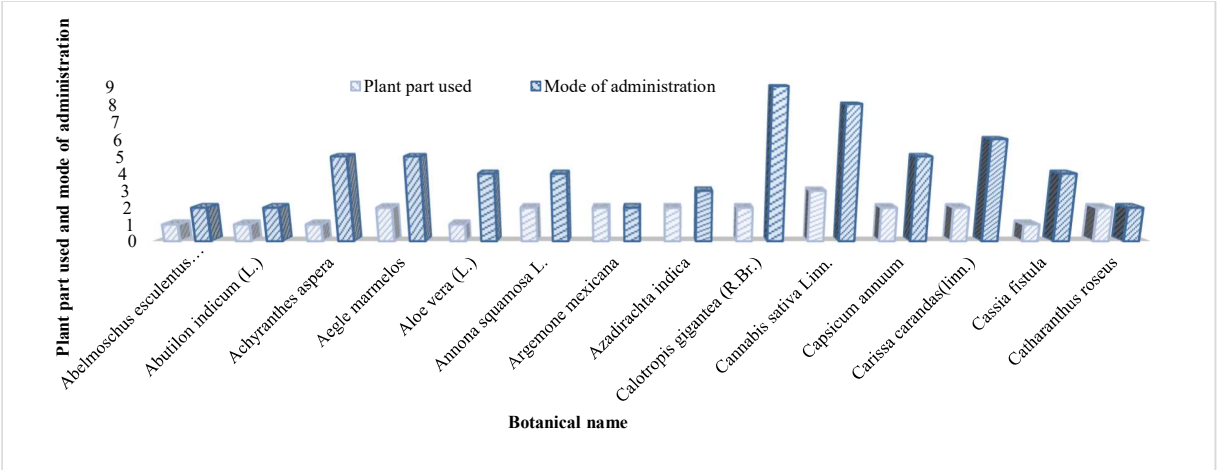


Figure 4: Ethno-medicinal plants and their mode of administration

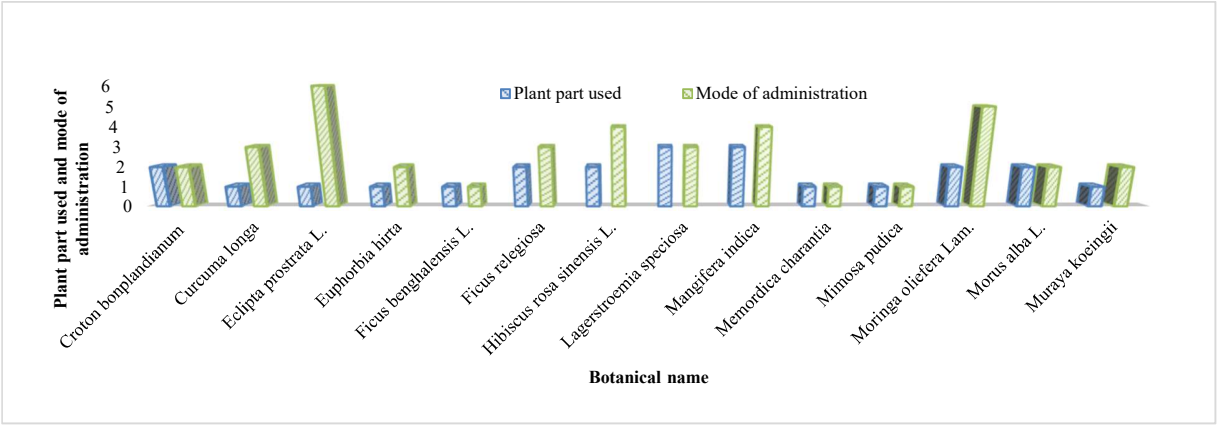


Figure 5: Ethno-medicinal plants and their mode of administration

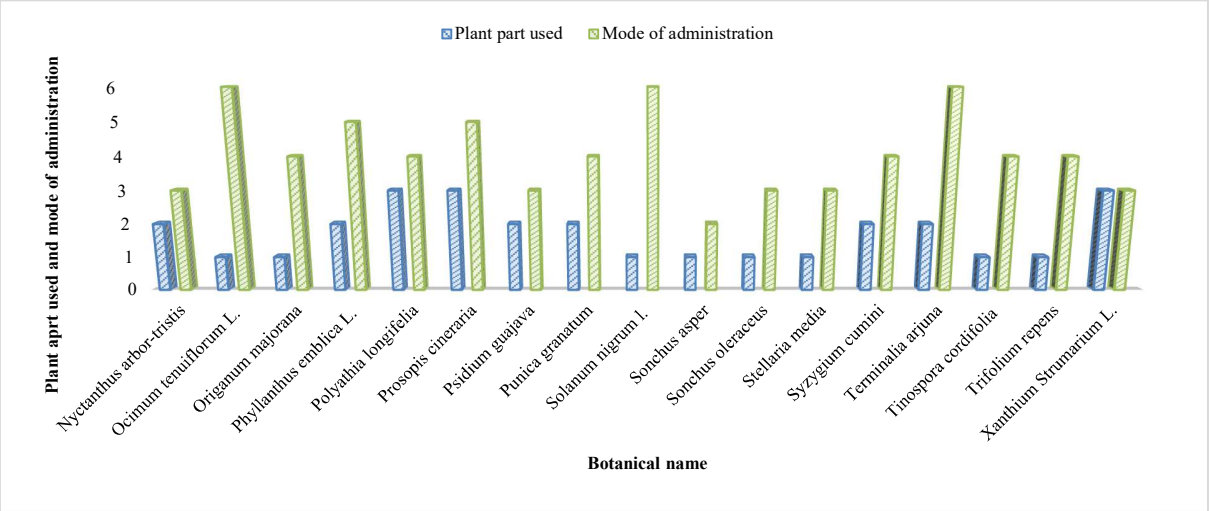


Figure 6: Ethno-medicinal plants and their mode of administration

(2022) investigated the variety of medicinally significant plants and identified 44 plant species across 25 families. Of them, twenty-four families are classified as angiosperms. Over 85 percent of plant species were used as vermifuges and anthelmintics, and to cure a wide range of ailments, including rheumatism, narcosis, dyspepsia, malaria, bronchitis, vomiting, oedema, backache, dysentery, eczema, purgative, typhoid, and rheumatism. Mirzaman *et al.* (2023) perform the ethnobotanical study at Muzaffarabad of Pakistan and reported 68 plant species belonging to 36 families were used by local inhabitants for various purposes and among them angiosperm were in leading number. Out of the total plant species found, most of them (about 57) were medically important. Ortiz-Mendoza *et al.* (2023) investigate the potential of the some plants of subfamily Nepetoideae (Lamiaceae) for the treatment of inflammatory diseases and reported that 308 species of selected subfamily were medically important and can be used for various inflammatory diseases.

Conclusion

There are 45 plant species in all that are known to be utilized for various ethnomedicinal purposes. These species are divided into 29 families. The parts usually employed for therapy were the stem, leaves, bark, and flowers. Their extracts and tonic are still utilized in many ayurvedic treatments; they can even be eaten and applied raw to wounds to speed up healing. The majority of the plants had the ability to treat diabetes, and several also showed distinct pharmacological benefits and qualities such as antibacterial, antifungal, and anti-inflammatory. Every plant has the capacity to treat a wide range of illnesses, but because locals are unaware of the benefits of medicinal plants, it appears impossible for them to learn about them. It is important to promote awareness and research among locals and other inhabitants regarding the ethnomedicinal potential of adjacent plants. The residents of this area should take cooperative efforts for the rehabilitation of disappearing plants in order to maintain the growth of these priceless plant resources. Such ethnomedicinal plants need to be documented in order to be used in the future. The management and preservation of the wealth of medicinal plants in the Shamli region will benefit from this survey. Enhancing traditional knowledge about wild plants and documenting them is a crucial part of raising local awareness of the ethnomedicinal benefits of various plants, enabling people to embrace ayurveda and cultivate these kinds of plants on a large scale. Even the conservation of wild flora will benefit from this awareness. The locals employ this kind of research to treat a variety of illnesses naturally by utilizing various plant parts. The current study demonstrates the ethnomedicinal usefulness of many plant species components that are native to the region.

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Conflict of interest

The authors declare that they have no conflict of interest.

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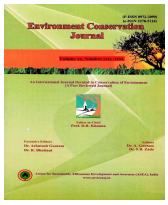

Efficiency of spent mushroom (*Agaricus Bisporus*) waste biomass for the biosorption of basic fuchsin dye from aqueous solution

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ABSTRACT

The dumping of wastewater containing the dyes is harmful to the health of aquatic living beings. The colour in water bodies reduces the penetration of light and thereby reduces the concentration of dissolved oxygen (DO) of water bodies. The decreased value of DO is also harmful to aquatic organism. Therefore treatment of wastewater containing dyes becomes essential. Mushrooms have proven to be highly efficient and economical for removing pollutants through bioabsorption. Therefore, in the present study an attempt has been made to study the efficiency of Spent Mushroom Waste (SMW) *viz. Agaricus bisporus* as biosorbent for the biosorption of Basic Fuchsin Dye (BFD) from aqueous solution. The effects of certain factors such as the dose of adsorbent, temperature, exposure time, and pH were studied on the dye degradation by a given biomass of SMW. The results of the present study revealed that the optimum value of temperature, contact time, adsorbent dose, pH, was 7, 20 minutes, 20 mg, and 30°C respectively. The biosorption efficiency of the used SMW ranged from good to excellent. The results of the present study revealed that the SMW of *Agaricus bisporus* is an economically and environmentally sound adsorbent and can be used for the degradation of dyes from water based solutions. Further investigation is required to enhance the adsorption rate of SMW of *Agaricus bisporus*.

Introduction

Water is the most important natural source and therefore its treatment is essential for the survival of humanity on this planet (Bhutiani and Ahamad, 2018; Bhutiani *et al.,* 2021). The color in the effluents enhances the toxic effects of effluents. Usually effluents containing color due to the presence of dyes long with other pollutants is treated using various physicochemical processes. But all these physicochemical processes are less efficient in dyes removal, expensive, and not flexible as per the nature of dye. Various types of effluents especially industrial effluent is complex in nature and possesses diverse sorts of organic dyes and pollutants (Mahmooda, 2014; Feng *et al.,* 2022; Islam *et al.,* 2023). Most of the synthetic dyes are classified into cationic (basic), anionic (direct, acid, and reactive) and nonionic (disperse) based on the presence of functional groups. Due to their high soluble nature it is very difficult to remove the dyes from effluents and therefore their dumping in the aquatic bodies can cause mutations and can produce secondary products which are toxic in nature to

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aquatic life either in one way or in other (Farhan Hanafi and Sapawe, 2020).Biosorption is defined as "the elimination of undesired substances from solution by biotic material (living or dead) and or their derivatives, which form complexes using ligands or serviceable clusters of ions present on the cell surface" (Volesky, 2001). It is a property of both alive and lifeless organisms (and their components); and is believed to be a favorable biological method for the elimination (and retrieval) of metals, dyes, radionuclides and organic pollutants for countless years due to their several properties such as (i) its simplicity; (ii) operation analogous to conventional ion-exchange technique; (iii) apparent efficiency; and (iv) easy availability of different biosorbents (Gadd, 2009; Chukki and Shanthakumar, 2016). Qin *et al.* (2023) studied the adsorption efficiency of mushroom along with the impacts of present minerals of its efficiency and concluded that the present minerals such as sodium, potassium, calcium, and magnesium enhance its cadmium adsorption potential from aqueous solutions. AbuQamar *et al.* (2023) studied the potential of algae's for the remediation of emerging pollutants from aqueous solutions and concluded that mycoremediation is a suitable technology but there is a need of process optimization and pilot scale studies. Chaurasia *et al.* (2023) wrote review on the mycoremediation techniques applied for heavy metal, dyes, pesticides, insecticides, herbicides, and pharmaceutical wastes remediation from wastewater mushrooms were used even before man understood the nature of other organisms. Mushroom cultivation started in the ancient times for their nutritional value and flavor (Quimio and Royse, 1990; Baysal *et al.,* 2014; Chakraborty *et al.,* 2016). Mushrooms have rich nutritional value with high content of proteins, vitamins, minerals, fibers, trace elements and low calories and cholesterol (Wani *et al.,* 2010; Waktola and Temesgen, 2018). Besides nutritive value, mushrooms also possess some medicinal properties (Thakur and Singh 2013; Meng *et al.,* 2016). The mushrooms (fungi) have

Material and Methods

Spent mushroom waste (SMW)

The spent mushroom waste (SMW) of *Agaricus bisporus* was present in laboratory at Department of Botany, KVSCOS, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. The procedure described by Yan and Wang (2013) is used to prepare the absorbent from SMW (Figure 3). been proven to be highly efficient and economical for the removal of pollutants even from dilute aqueous solutions through biosorption because of their filamentous morphology and high proportion of cell wall. The groups of fungi that have mainly been used in biosorption of heavy metals, dyes etc. comprise filamentous fungi (Ayimbila and Keawsompong, 2023).Spent mushroom waste (SMW) is the waste product of mushroom industry generated during different processes of mushroom production. Approximately 5 times of SMW is generated during the manufacturing of unit quantity of mushrooms (Del Campo *et al.,* 2018). The disposal of this huge quantity of waste causes environmental pollution. SMW is highly rich in protein, chitin, chitosan, cellulose, and hemicelluloses having certain important serviceable clusters such as amide, carbonyl, and hydroxyl. All these functional groups provide dynamic binding locations for contaminants in the effluent treatment process. Literature suggests that unprocessed SMW can be used for the degradation of dyes but it has a partial ability to remove acidic/basic dyes in environments (Savoie *et al.,* 1996; Vos *et al.,* 2017). The process of wastewater treatment requires environmental friendly, low cost, and efficient adsorbents. The efficiency of the dye uptake by a given microbial biomass is subject to a variety of factors including temperature, pH, biosorbent concentration and initial concentration of the dye in the reaction system (Ahmed and Ebrahin 2020; Aragaw and Bogale, 2021). The advantages of biosorbent in comparison other adsorbents are given in figure 1 and the factors affecting the adsorption process are given in figure 2.The Basic Fuchsin ($C_{20}H_{20}$ N_3Cl) is also a triphenylmethane dye. It is a mixture of three dyes pararosaniline, rosaniline, and magenta II and is also known as magenta II. Literature suggests that for the degradation of basic fuchsin dye very few biosorbents have been employed (Yamil *et al.,* 2020). Therefore the present study was designed to study the efficiency of spent mushroom waste (SMW) biomass of *Agaricus bisporus* for the degradation of basic fuchsin dye.

Effects of different factors such as SMW dosages, contact time, temperature, and pH on the biosorption process was also studied.

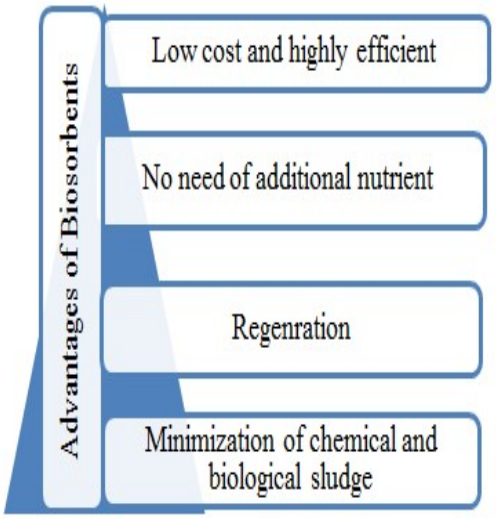

Figure 1: Showing the advantages of Biosorbent (Source: Okoro *et al.*, 2022)

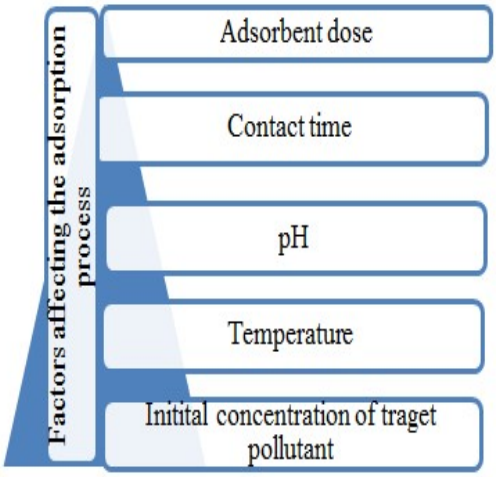

Figure 2: Showing the factors affecting the adsorption process (Source: Okoro *et al.*, 2022)

Preparation of Basic Fuchsin Solutions of different concentrations

One gram of analytical grade Basic Fuchsin dye ($C_{20}H_{20}N_3 \cdot HCl$) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was dissolved in distilled water and then diluted to one liter with the help of distilled water. The final concentration of the stock dye solutions was 1 g/L.

The basic fuchsin dye was used to assess the ability of the SMW biomass to adsorb dyes. The stock solutions of the dyes were prepared and then a solution containing 500 mg/l of dyes in representative solutions was prepared.

Experimental setup

A total of 12 sets of flask of were prepared for each selected adsorbent dose. Among which 3 were used as control while the rest of the flask were designated as experimental. Each flask contains 100ml sample of 500 mg/l solution of Basic Fuchsin dye.

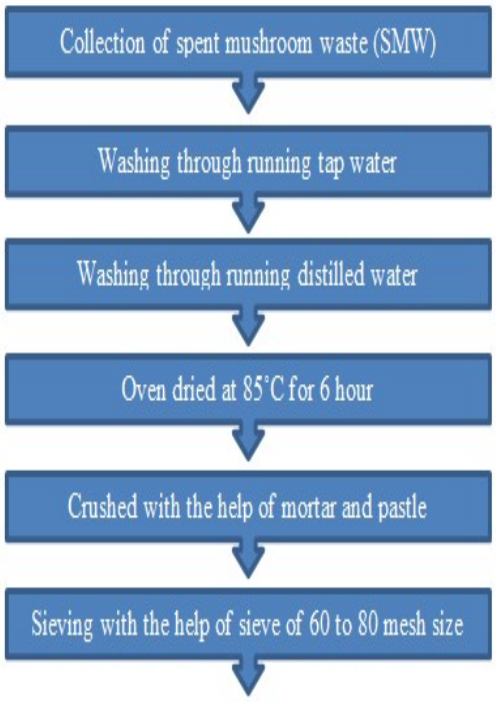

Figure 3: Showing the process of absorbent preparation from SMW (process adopted from Yan and Wang, 2013)

Effect of different adsorbent dose, pH, and temperature at the efficiency of SMW for basic Fuchsin dye reduction: 100 ml of 500 mg/l solution of basic Fuchsin dye solution were taken in each of a set of 12 flasks of 250 ml capacity. To these flasks, different pH (5.5, 7.0 and 8.5) of the dye solutions were adjusted with HCl (0.1N) and NaOH (0.1N) solution using an ESICO pH meter (ESICO). To these flasks, dead SMW biomasses were added as under. For each selected dose (10,

20, 30 mg/l), 3 flask were prepared for the first selected pH (5.5) for three different selected temperature (20, 25, 30˚C) and required time period (10, 20, 30 minutes). In the similar way flask were prepared for second selected pH (7.0) and third selected pH (8.5). In this way 9 experimental flasks were prepared for each dose.

Running of Experimental

All these flasks were then placed on a rotary shaker at 110rpm for the required time period. The rpm and time was selected based on the available literature (Yan and Wang, 2013; Dardouri and Sghaier, 2017). The fungal biomass was separated by filtering the mixture through a nylon sieve and the unadsorbed dye (that remaining in the solution) was estimated in supernatant using a *uv-vis*spectrophotometer (EI India, Model SL-3375) at 618 nm and 550 nm wave lengths. The adsorbed quantity (Q) and adsorption efficiency (E) was calculated as follows:

$$\mathbf{Q\ (mg/g) = \{(Ci - Cf) \times V\} / m}$$

$$\mathbf{E\ (\%) = \{(Ci - Cf)/ Ci\} \times 100}$$

where,
E=reduction in percentage
Q = dye uptake (mg dye/g biosorbent)
V = the liquid sample volume (ml)
Ci = the initial concentration of the dye in solution (mg/l)
Cf = the final concentration of the dye in solution (mg/l)
m = the amount of added biosorbent on the dry wt. basis (g)

The biosorption efficiency of particular biomass was interpreted as under:
1. 0–10 Very poor
2. 10–20 Poor
3. 20–40 Moderate
4. 40–60 Good
5. 60–80 Very good
6. 80–100 Excellent

Results and Discussion

Effects of various variables such as dose of adsorbent, pH, temperature, and contact time were studied in the present study. Initial concentration of the Basic Fuchsin dye was taken as 500mg/l. All the results are given in table 1. Maximum adsorption percentage efficiency of Basic Fuchsin Dye by Spent Mushroom (*Agaricus Bisporus*) at different chosen factors such as (a) Dose (b) Time (c) pH value and (d) Temperature is given in fig. 4.

Effect of different biomass dose

Three different doses 10, 20, and 30mg of SMW were taken for the study. On increasing the adsorbent dose from 10 mg to 30 mg, the adsorbent efficiency first increased and then decreased. Different efficiencies were observed at different doses of SMW at different pH, temperature, and contact time. Highest efficiency was observed at 20mg/l adsorbent dose and 30˚C at a contact time of 20 minutes at a pH of 7.0. Lowest efficiency was observed at 10mg/l adsorbent dose and 35˚C at a contact time of 30 minutes at a pH of 5.5. The adsorption percentage increased with the increasing dose but after certain dose it starts reducing. This may be due to availability of more active sites and sorption surface area (Mall *et al.,* 2005; Hameed, 2009; Tian *et al.,* 2011; El Haddad, 2016; Ali *et al.,* 2020). At the increased adsorbent dose, the decrease in removal efficiency may be due agglomeration of adsorbent molecule at active sites (Malekbala *et al.,* 2012; Ahmed and Ebrahin, 2020). The dose below which the adsorption percentage increased and at that dose the percentage reduction is highest and starts reducing is termed at optimum dose. In the present study, the optimum dose is 20mg. Hameed (2009) used the spent tea leaf for the reduction of basic dye concentration from aqueous solution and observed the reduction from 44 to 96%. Mall *et al.* (2005) studied the adsorption potential of bagasse fly ash for the removal of malachite green dye from aqueous and observed the similar reduction efficiency. Ali *et al.* (2020) found the highest efficiency of activated charcoal for the removal of Malachite Green Dye at 27mg/l and 45minutes. The spent mushroom waste was observed to have uptake capacity of 950.0 mg/g to 3920.0 mg/g for Basic Fuchsin dye.

Effect of variation in contact time

Contact time is an important factor for the treatment of wastewater using adsorption process (Doğan *et al.,* 2005). Three different contact times (10, 20, 30 minutes) were chosen based on

Table1: Efficiency of dead biomass of spent mushroom waste (*Agaricus bisporus*) for the reduction of basic fuchsin dye

Initial concentration of dye = 500 (mg/l)																										
Adsorbent dose = 10 (mg/l)																										
pH	**5.5**									**7**									**8.5**							
Temperature	**25**			**30**			**35**			**25**			**30**			**35**			**25**			**30**			**35**		
Contact time	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**
Final concentration of dye (mg/l)	210	189	203	189	149	183	212	195	220	146	134	140	119	96	113	148	139	146	198	177	187	145	108	154	202	184	197
Extent of dye biosorption (mg/l)	290	311	297	311	351	317	288	305	280	354	366	360	381	404	387	352	361	354	302	323	313	355	392	346	298	316	303
% Adsorption	58	62.2	59.4	62.2	70.2	63.4	57.6	61	56	70.8	73.2	72	76.2	80.8	77.4	70.4	72.2	70.8	60.4	64.6	62.6	71	78.4	69.2	59.6	63.2	60.6
Q Value	2900	3110	2970	3110	3510	3170	2880	3050	2800	3540	3660	3600	3810	4040	3870	3520	3610	3540	3020	3230	3130	3550	3920	3460	2980	3160	3030
Adsorbent dose = 20 (mg/l)																										
pH	**5.5**									**7**									**8.5**							
Temperature	**25**			**30**			**35**			**25**			**30**			**35**			**25**			**30**			**35**		
Contact time	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**
Final concentration of dye (mg/l)	200	184	197	175	107	168	203	189	211	136	122	130	108	87	105	142	127	135	187	169	175	132	109	129	192	175	187
Extent of dye biosorption (mg/l)	300	316	303	325	393	332	297	311	289	364	378	370	392	413	395	358	373	365	313	331	325	368	391	371	308	325	313
% Adsorption	60	63.2	60.6	65	78.6	66.4	59.4	62.2	57.8	72.8	75.6	74	78.4	82.6	79	71.6	74.6	73	62.6	66.2	65	73.6	78.2	74.2	61.6	65	62.6
Q Value	1500	1580	1515	1625	1965	1660	1485	1555	1445	1820	1890	1850	1960	2065	1975	1790	1865	1825	1565	1655	1625	1840	1955	1855	1540	1625	1565
Adsorbent dose = 30 (mg/l)																										
pH	**5.5**									**7**									**8.5**							
Temperature	**25**			**30**			**35**			**25**			**30**			**35**			**25**			**30**			**35**		
Contact time	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**
Final concentration of dye (mg/l)	204	192	202	168	112	174	209	201	215	145	128	132	121	106	117	152	133	144	195	183	196	156	110	151	199	188	210
Extent of dye biosorption (mg/l)	296	308	298	332	388	326	291	299	285	355	372	368	379	394	383	348	367	356	305	317	304	344	390	349	301	312	290
% Adsorption	59.2	61.6	59.6	66.4	77.6	65.2	58.2	59.8	57	71	74.4	73.6	75.8	78.8	76.6	69.6	73.4	71.2	61	63.4	60.8	68.8	78	69.8	60.2	62.4	58
Q Value	987	1027	993	1107	1293	1087	970	997	950	1183	1240	1227	1263	1313	1277	1160	1223	1187	1017	1057	1013	1147	1300	1163	1003	1040	967

literature study. Batch experiments were carried out to study the effect of contact time. The lowest adsorption 56.0% was observed at 30minutes contact time and 35˚C at 10mg/l adsorbent concentration at a pH value of 5.5. The highest adsorption 82.6% was observed at 20minutes contact time and 30˚C at 20mg/l adsorbent concentration at a pH value of 7.0. Gradual increase in adsorption revealed the fact that adsorption increases with the increment in time despite of the dye concentration. Even high dose of dye can adsorb in prolonged time/ long exposure of time (Tong *et al.,* 2018; Ihsanullah *et al.,* 2020). Finally, the contact time of 20 min and dose on 20 mg is determined as the optimum contact time for further investigations. Keeping all the variables fixed, the adsorption efficiency was observed high in the starting of experiment due to availability of large number of active sites and the reduction efficiency decreased at a particular contact time due to repulsion forces between the adsorbed and free molecules (Malekbala *et al.,* 2012; Yan and Wang, 2013) and agglomeration process (Mall *et al.,* 2005). After 20 minutes of contact period, reduction efficiency decreased on increasing contact time and was found negligible after 90minutes. According to Batana *et al.* (2022) the maximum biosorption of *Fusarium oxysporum* was determined to be about 79%. Doğan *et al.* (2007) observed the adsorption equilibrium at 4 hour contact time during the reduction of two different dyes (methyl violet and methylene blue) using sepiolite bagasse fly ash as an adsorbent. Similar results were observed by Mall *et al.* (2006) in case of removal of Orange-G and Methyl Violet dyes using bagasse fly ash bagasse fly ash as an adsorbent. El Haddad (2016) observed the highest adsorption rate at 40 minute contact time after that the rate starts decreasing and complete equilibrium was attained at 60 minute. Yildirim *et al.* (2020) also observed the similar results while studying the reduction in the concentration of heavy metals with the help of fungal extract.

Effect of variation in temperature

Variation in adsorption rate was studied at different temperatures ranging from 25 to 35˚C. The results were depicted in table 1. As far as temperature is considered high adsorption 70.2 to 82.6% was observed at 30˚C, which is far ahead of 60% absorption at 20˚C. Adsorption percentage decreased on increasing temperature may be due to exothermic nature of adsorption process (Yan and Wang, 2013). Yan and Wang (2013) observed the maximum adsorption efficiency at 30 ˚C and concluded the requirement of lower temperature for adsorption process. Ali *et al.* (2020) studied the sorption rate at different temperatures ranging from 0 to 60˚C and observed the highest sorption rate at 45 ˚C.

Effect of different pH

The pH value of the solution greatly influences the adsorption percentage especially the initial pH (El Haddad *et al.,* 2012; Kooli *et al.,* 2015; El Haddad, 2016). The adsorption rate depends on the presence of functional groups at the surface of adsorbent whose dissociation depends on the pH of solution (Ho and McKay, 2000). The adsorbent surface may attract both H^+ and OH^- based on the charge of own surface (Kocaoba *et al.,* 2007; Malik *et al.,* 2007; Nethaji *et al.,* 2010; Alhujaily *et al.,* 2018). Effect of different pH (5.5, 7.0. 8.5) value was also studied on the adsorption of basic fuchsin dye from aqueous solution using SMW. The required pH was obtained by adding the 0.1M HCl and 0.1M NaOH solution. Highest adsorption 82.6% took place at pH 7.0 on 20minute contact time, 30˚C temperature and 20mg/l adsorbent dose while the lowest adsorption 57.0% took place at pH 5.5 on 30minute contact time, 35˚C temperature and 30mg/l adsorbent dose. Therefore we can interpret that neutral to slightly basic pH favour the biosorption through dye adsorption phenomenon if pH is considered as sole parameter. The initial pH of the dye solution strongly affected the chemistry of both the dye molecules and fungal biomass in an aqueous solution. El Haddad (2016) observed the highest adsorption rate at a pH of 9.2 using calcined mussel shell material (CMS). The author concluded that the negative charge on the surface of CMS is responsible for the highest adsorption rate at a pH of 9.2 and decreasing rate at acidic value of pH. At a low pH (in acidic media), occurred greatly between cationic dyes (MB, MG) ions and positively charged groups on the surface of the mushrooms. On the contrary, with increasing pH, electrostatic attractions between cationic dye ions and negatively charged sites on mushrooms' surface were enhanced both cationic dyes

adsorption (Tong *et al.,* 2018; Liu and Lee, 2021). These results indicate that the adsorption of BF is more influenced by acidic nature of pH on the surface of spent waste mushrooms. Tian *et al.* (2011) observed the highest adsorption rate at strong acid pH in case of Congo red dye reduction from aqueous solution. Ali *et al.* (2011) observed the highest adsorption rate in acidic medium. The author observed the decrease in adsorption rate at a pH greater than 5.

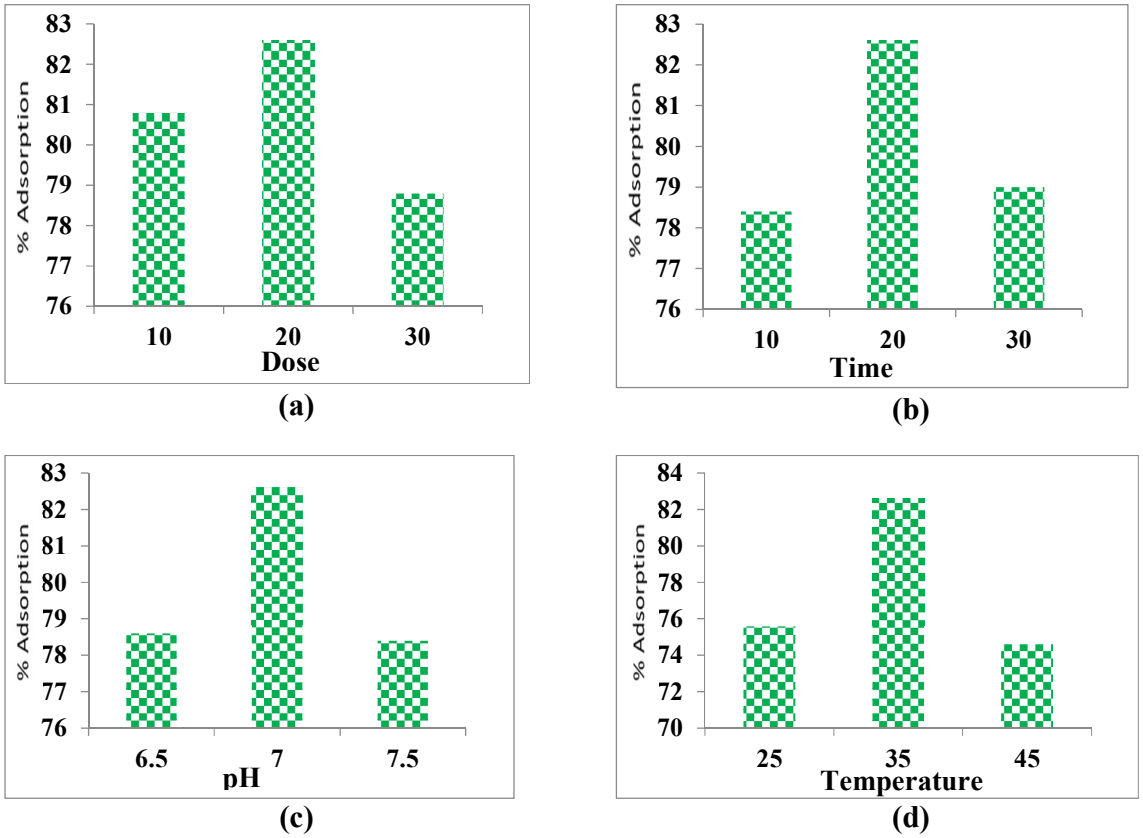

Figure 4: Maximum adsorption percentage efficiency of Basic Fuchsin Dye by Spent Mushroom (*Agaricus Bisporus*) at different chosen factors such as (a) Dose (b) Time (c) pH value and (d) Temperature.

Conclusion

Although various good adsorbents were reported in the literature for the removal of dyes from the aqueous solutions but due to their high cost, harmful impacts on environment, and regeneration ability, now the whole world is focusing on biosorbent. Therefore, in the present, an attempt has been made to study the efficiency of Spent Mushroom Waste (SMW) *viz. Agaricus bisporus* as biosorbent for the biosorption of Basic Fuchsin Dye (BFD) from aqueous solutions. The effects of the dosages of SMW, temperature, contact time, and pH were studied on the adsorption efficiency of SMW of *Agaricus bisporus*.

Batch experiments results obtained revealed that the mushrooms can also be used for the reduction of dyes from aqueous solutions in place of high cost adsorbents. The biosorption efficiency of the used SMW ranged from good to excellent. The efficiency of the mushrooms can also be improved using the further optimization process.

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Conflict of interest

The authors declare that they have no conflict of interest.

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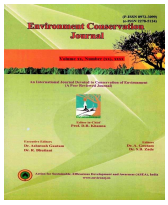

Historical summary of terminologies in community ecology

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ABSTRACT

This article highlights the importance of terminology in ecology as a fundamental tool for clear and objective communication among scientists, as well as for standardizing study methods and techniques used by ecologists. Terminology refers to a set of terms, concepts, and definitions that are established and accepted by professionals in the field, enabling precise descriptions of species, ecosystems, and ecological processes. However, it is important to note that terminology in ecology is constantly evolving, and it is essential for ecological professionals to stay up to date with changes to contribute to the advancement of science. Understanding the historical process of ecological terminologies is also necessary to comprehend their meanings and how their interactions can affect the understanding of ecology itself. In this sense, we have provided a historical contextualization of several important concepts in community ecology, such as biodiversity, trophic levels, ecological niches, ecological guilds, and functional groups. We have also made comparisons and differentiations between these concepts throughout the history of these terms.

Introduction

Ecology is a science that seeks to understand the interactions between living beings and the environment in which they live. Due to the complexity of this science, one of the fundamental tools for communication and understanding in ecology is terminology. Terminology refers to the technical and specialized language used by ecological professionals to describe and classify natural phenomena (Kempton, 1982). The importance of terminology in ecology is related to the need to establish clear and objective communication among scientists. Terminology allows concepts and information to be transmitted precisely and unambiguously, avoiding misunderstandings and errors. Furthermore, terminology is essential for standardizing the study methods and techniques used by ecologists, contributing to comparative research and the advancement of scientific knowledge (Mayr, 1976). Therefore, terminology is composed of a set of terms, concepts, and definitions that are established and accepted by professionals in the field. These terms are used to describe characteristics of species, ecosystems, and ecological processes, such as ecological succession, biodiversity, and the food chain. It is important to note that terminology in ecology is constantly evolving, influenced by new discoveries and scientific advances. Therefore, it is essential that ecologists stay up-to-date and attentive to changes in terminology to communicate effectively and contribute to the development of science (Pickett & Ostfeld, 1995). In conclusion, terminology is an essential tool for ecology, allowing clear and precise communication among scientists and contributing to the standardization of study methods and the advancement of scientific knowledge. It is crucial that ecological professionals are familiar with terminology and keep up with its evolution to contribute significantly to the advancement of science. In this sense, summarizing the historical process that terminologies in this science have gone through is necessary to understand their meaning over the years and publications and how the interaction of

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these terminologies can affect the understanding of ecology itself.

1. Biodiversity

Biodiversity, also known as biological diversity, is a fundamental concept in ecology. It can be summarized as the sum of all biotic variations from the genetic level to the scale of ecosystems (Purvis & Hector, 2000). In 1992, the Second Convention on Biological Diversity of the United Nations proposed a broader concept of biodiversity, which includes the variability of living organisms of all origins. This encompasses intra- and interspecific variation, diversity of terrestrial and aquatic ecosystems, and the ecological complexes of which they are a part (Brazil, Ministry of Environment - MMA, 2000). In this context, the term refers to the quantification of the diversity, quantity, or multitude of species within a limited geographic area, encompassing genetic and phenotypic variations, distinct taxonomic classification, and endemism (Tilman, 2001). Therefore, biodiversity is a multidimensional concept that cannot be reduced to a single index, as it would be unable to represent the variety of life forms in the environment (Barbault, 1995; Purvis & Hector, 2000).

The understanding that different organisms interact with each other and with the environment is not new. This observation has permeated society since the dawn of civilizations (Mayr, 1998). Raymond F. Dasmann first used the term "biological diversity" in ecology in his 1968 book, "A Different Kind of Country." However, it was not until the 1980s that the term became prevalent in the scientific community, largely due to the efforts of Thomas Lovejoy. Lovejoy used the term in the preface of a collection of works titled "Conservation Biology: An Evolutionary-Ecological Perspective," edited by Michael E. Soulé and Bruce A. Wilcox in 1980 (Dasmann, 1968; Soulé & Wilcox, 1980). The term "biodiversity" as a replacement for "biological diversity" was first proposed by Professor Walter G. Rosen of the National Research Council/National Academy of Sciences in the United States (NRC/NAS) in 1985. Rosen suggested the use of "biodiversity" while organizing a forum on the topic, titled the National Forum on BioDiversity (Wilson, 1997; Sarkar, 2002; Noss, 2006). This forum was subsequently published in the first issue of the journal Conservation Biology as a scientific dissemination vehicle on issues related to biodiversity conservation (Lewis, 2007).

The term "biodiversity" was first officially published in 1988 in the book "Biodiversity," edited by Edward O. Wilson. This book presented the results of the National Forum on BioDiversity and included articles authored by 60 leading experts on the subject, including Wilson himself, Paul R. Ehrlich, Norman Myers, David Ehrenfeld, Robert E. Jenkins, Thomas E. Lovejoy, Lester R. Brown, Michael Soulé, and James Lovelock. However, despite the publication of this book, the concept of biodiversity remained ambiguous, and categorizing biodiversity in nature remained a challenging task.

In his 1992 book "The Diversity of Life," Edward O. Wilson emphasized the importance of species as the fundamental unit of biodiversity. Wilson believed that the concept of species was essential for studying biodiversity, as it provided a natural unit for the comparison and evaluation of research findings. Without species, ecosystems could only be analyzed using imprecise and changeable descriptions of their constituent organisms. Thus, species played a crucial role in the study of biodiversity, enabling a more accurate analysis of ecosystems and their components (Wilson, 1992, p. 48). In the 1960s, to early 1970s, researchers George E. Hutchinson and Robert H. MacArthur began studying biotic interactions, especially interspecific competition between different species, creating significant momentum for studies on community structure. In the 1980s, in addition to competition, spatial (abiotic and geographic data) and temporal (seasonal, etc.) variations became important topics in community analysis (Barbault, 1995). In the late 1980s, research began to seek a better understanding of species interactions, considering the relationship between distribution and environmental variations. It is understood that biological diversity represents the balance of biogeochemical processes, evolutionary history, and the extrinsic portion of changes in abiotic factors (Brown & Heske, 1990; Naeem & Wright, 2003). In the early 1990s, studies on the relationship between biodiversity and ecosystem functioning using combinatorial analyses aimed at

manipulating taxonomic diversity for the representation of functional sets gained prominence (Naeem & Wright, 2003). By the end of the same decade, ecologists increasingly used studies of ecosystem structure and function with an emphasis on the use of nonphylogenetic classifications of organisms (Gitay & Noble, 1997). According to Lévêque (1999), biodiversity specifically refers to three interconnected levels of biological hierarchy: (a) species diversity, which involves identifying and inventorying species as the simplest way to describe the biological diversity of a geographic area; (b) genetic diversity, which encompasses the set of genetic information contained within all living beings, corresponding to the variability of genes and genotypes among species and within each species; and (c) ecological diversity, in which ecosystems are composed of complexes of species (or biocenosis) and their physical environment. Numerous types of natural ecosystems can be distinguished, such as tropical forests, coral reefs, mangroves, savannas, tundras, etc. In his book, he also emphasizes:

"Biodiversity is not a simple catalog of genes, species, and environments. It must be perceived as a dynamic and interactive set of the different levels of biological hierarchy. According to current theories of evolution, it is thanks to the existence of genetic diversity within species that they can adapt to changes in the environment that have always marked the history of the Earth. Reciprocally, the genetic diversity of a species evolves in response to these changes in the environment as well as to mutations. The same is true of plant and animal communities, which constitute ecosystems and respond through qualitative and quantitative changes to fluctuations in the environment in which they live. This dynamic of biological systems and ecological conditions to which they are confronted explains why species evolve and diversify and why ecosystems host richer or poorer floras and faunas, depending on their history. In this regard, biological diversity is a modern version of the sciences of evolution, which synthesizes recent advances in molecular biology and ecology..." (Lévêque, 1999, 18-19).

The traditional approaches used to quantify biological diversity can be broadly grouped into two categories: quantification based on several species and indices of species diversity that take into account both diversity and relative abundance. The first group includes three diversity measures proposed by Whittaker in 1970: (i) alpha diversity, which represents the number of species found in a habitat or sample unit within a region and is a measure of local species richness; and (ii) beta diversity, which measures community heterogeneity in a given territory and quantifies differences in species composition among ecological communities. Beta diversity is the result of two distinct processes, species turnover and gain or loss of species. (iii) Gamma diversity represents the total number of species in a region or the regional set of species, generally covering large extents of ecosystems (Anderson *et al.,* 2011).

These measures are not able to elucidate the processes present in communities that can lead to diversity. Currently, there are diversity measures that take into account important - but usually ignored - information about species. The most notable are measures that incorporate the relationships between species and those that take into account the functional characteristics of organisms present in the community (McGill *et al.,* 2006). Ecological communities are composed of different species that may compete or interact with each other. These communities are the result of various ecological processes, such as competition (Hutchinson 1959, Leibold 1998) and environmental filters (Weiher & Keddy 1995, Chase 2003), as well as evolutionary processes that have occurred over time (Tofts & Silvertown 2000, Ackerly 2003). Therefore, the composition and dynamics of ecological communities are influenced by both ecological and evolutionary factors.

2. Trophic levels

The scientific community became increasingly concerned about the Earth's ability to support life and its expansion with the publications of Thomas Malthus. In the early 19th century, this concern led to the development of a mathematical basis for the study of populations by biologist Raymond Pearl (1920), mathematician Alfred James Lotka (1925), and physicist/mathematician Vito Volterra (1926). This mathematical framework enabled researchers to investigate predator–prey interactions, competitive relationships between species, and population control through experiments. During the

early 20th century, European botanists initiated the study of plant communities, including their composition, structure, and distribution. Meanwhile, in the United States, research has focused on understanding the development of these communities, known as succession. These studies expanded the understanding of plant-animal interactions, acknowledging the importance of all biotic components in shaping ecological communities. In 1920, August Thienemann introduced the concept of trophic levels, which describes the transfer of energy through a series of organisms from green plants (producers) to various levels of animals (consumers). This concept was further developed by English ecologist Charles Sutherland Elton in 1927, who introduced the concept of ecological niches and pyramids of numbers. Birge and Juday, two American biologists, built upon Elton's work in the 1930s by measuring the energy reserves of lakes and developing the idea of primary production, which refers to the proportion of energy generated or fixed by photosynthesis.

A new concept gained strength in the early 20th century, stoichiometry, which means "The application of the laws of conservation of matter and definite proportions for understanding the rates and products of chemical reactions of a group of reactants," according to Elser & Hamilton (2007). Lotka and other authors began to analyze the stoichiometric ratios of essential chemical elements between organisms and the abiotic environment. Alfred Redfield, for instance, focused on the relationship between the availability of chemical elements in the oceans and the elemental composition of marine plankton (Redfield, 1934).

In 1986, American William A. Reiners initiated the discussion between the energy and matter flow approach and proposed the use of the stoichiometry of living beings and their mechanical structures as a way to interconnect matter and energy in ecosystems. The abundance of chemical elements in organisms, especially C, N, and P, provides a perspective on the ecosystem and the stocks and flows of matter and energy in the environment (Sterner & Elser 2002). Thus, it is possible to establish a communication network between levels of organization because it becomes feasible to calculate the elemental composition and estimate the flows of chemical elements in a enormous variety of biological entities, from organelles and cells to ecosystems and the entire biosphere (Elser *et al.,* 2000, Sterner & Elser 2002). The trophic-dynamic concept of ecology was developed by Raymond Laurel Lindeman in 1942, providing detailed information on energy flow in ecosystems. This approach was later expanded upon by Americans Eugene and Howard Odum and Australian John Derrick Ovington, who integrated quantitative data into their research. As new techniques such as radioisotopes, microcalorimetry, computing, and applied mathematics became available, studies on energy flow, nutrient cycling, and stoichiometry were stimulated, allowing for a better understanding of the structure and functioning of ecosystems.

Studies on trophic interactions have been developed for this purpose, particularly the organization of food webs (Dunne 2005, Montoya *et al.,* 2006, Giacomini 2007). A food web is a representation of the feeding relationships between predators and prey in an ecological community (Pimm 1982, Cohen 1978). Trophic interactions are essential components for understanding population dynamics and, consequently, the emerging patterns of coexistence and diversity in ecosystems (Tokeshi 1999, Chesson 2000, Giacomini 2007). However, the patterns of interaction in communities are much more complex and diverse than was assumed in the 1970s and 1980s (Brown *et al.,* 2001, Woodward & Hildrew 2002), and as a result, a large number of attributes are needed to understand community structure (Cattin *et al.,* 2004, Williams & Martinez 2008, Vermaat *et al.,* 2009).

3. Ecological niche

Joseph Grinnell introduced the concept of the ecological niche in 1917, which he defined as "the smallest unit of distribution within which each species is maintained due to its instinctive and structural limitations." This approach emphasized environmental factors while ignoring the potential effects of other species on the niche. For Grinnell, the niche was a characteristic of the environment, not the species itself. Therefore, the Grinnellian niche can be defined by noninteractive variables (cenopoietic) and broad-scale environmental conditions, which are relevant to understanding ecological and geographical properties on a large

scale. This concept was further elaborated by subsequent authors, such as James *et al.,* (1984) and Austin (2002), leading to a better understanding of the niche and its role in ecological processes. Following this, Charles Sutherland Elton (1927) provided one of the most widely used definitions, describing the ecological niche as the role an organism plays within an ecosystem, without considering the location of individuals. Elton's approach is primarily based on trophic relationships, defining niches based on predator–prey interactions, where resources consist of living organisms for higher trophic levels. As a result, the niche is defined by an organism's place in the biotic environment, its interactions with food resources, and its competitors or predators. The distinction between Grinnell's and Elton's ideas generally lies in their concepts of niches, attributing a primarily abiotic character to the former and a biotic character to the latter. Thus, Grinnell's definition was similar to "habitat," and Elton's was similar to "functional niche." Colwell (1992) groups these two definitions under what he calls the environmental niche.

George Evelyn Hutchinson introduced a broader definition of the niche concept in 1944, which considers it as the combination of all environmental factors that affect the survival and reproduction of a species. Accordingly, the niche can be regarded as a hypervolume in the n-dimensional space that encompasses all the factors that shape the distribution and abundance of a species. Hutchinson also postulated that, by the principle of competitive exclusion, two similar coexisting species necessarily occupy different niches. Hutchinson changed the notion that the niche is an attribute of the environment, considering it as a characteristic of the species and delimited by the combination of factors that allow it to persist in the environment. However, his concept does not elucidate questions such as: (1) some regions of the niche must be better than others in terms of the species' survival probabilities; (2) not all variables that affect an organism can be represented linearly; and (3) it does not consider a temporal dimension, i.e., his model refers to a single moment in time (Pulliam 2000). In the mid-1960s, researchers Robert MacArthur, Richard Levins, and Eric Pianka, among others, began to develop a new definition of niche, creating what is known as the modern niche theory. The focus of the niche was shifted to consider a diversity of environmental conditions that enable a species to survive, including the distribution of resource use by the species. In this sense, theoretical models were developed to investigate how many species (and how similar) can coexist in a given community under the premise that competition for resources is the mechanism that determines the ecology of populations. This representation allows the evaluation of some niche properties, such as their amplitude or overlap. This idea was heavily criticized because it lacked an adequate null hypothesis and statistical rigor. Additionally, several ecologists argued that competition was not necessarily the main process driving ecological dynamics.

Therefore, the definition of ecological niche considers the relationship of species with the environment, but how this configuration is structured differs from author to author; below are some examples:

"The position or status of an organism within its community and ecosystem resulting from its structural adaptations, physiological responses, and specific behavior (by inheritance and/or learning)." (Odum, 1959). "Ecological niche is the total sum of biotic and abiotic resource use by an organism in its ecosystem." (Campbell, 1996)

"The relationship of the individual or population to all aspects of its environment - and thus the ecological role of species within the community." (Ricklefs, 2003). Hubbell proposed the "Neutral Theory of Biodiversity and Biogeography" in 2001, which suggests that niche differences are not essential. He argued that the principle of competitive exclusion often takes a long time, so other processes such as dispersal and random ecological drift become dominant, along with certain population characteristics (birth, mortality, and reproduction).

Chase and Leibold put forth a new interpretation of the ecological niche concept in 2003. They proposed that the niche can be defined as the combined set of environmental factors that permit a species to meet its basic needs, such that the local population's birth rate equals or exceeds its death

rate, taking into account the impact of individuals on the environment.

In 2004, Eugene Odum proposed: The ecological niche is a term with a greater scope that includes not only the physical space occupied by an organism but also its functional role in the community (such as its trophic position) and its position in the environmental gradients of temperature, humidity, pH, soil and other conditions of existence Consequently, the ecological niche of an organism depends not only on where it lives but also on what it does (how it transforms energy, behaves, responds to its physical and biotic environment and modifies it) and how it is limited by other species. By analogy, it can be said that the niche is its "profession," biologically speaking (Odum 2004, p.375).

The modern concept of ecological niche is considered to be the ecological relationships, resource availability, and conditions for an individual or species. However, the niche is not rigid; there is a tolerance in niches, meaning that an individual can live in a spectrum of temperature, pH, or resource availability. These parameters are said to be dimensions of a niche; thus, a niche can have n dimensions.

Hubbell has recently presented a compelling challenge to the conventional niche concept by introducing a neutral theory of diversity. In this theory, diversity is defined in terms of species distribution and abundance, and it posits that all species occupy the same niche, with individuals having equal fitness regardless of species (Hubbell, 2001). According to neutral theory, community dynamics are random and independent of species composition. This perspective stands in stark contrast to the Darwinian approach, which emphasizes competition as the driving force behind community assembly (Leigh, 2007).

In their theory of island biogeography, MacArthur and Wilson explained large-scale distribution patterns by considering fluctuations in colonization and extinction rates, following a probability distribution (MacArthur and Wilson, 1963; Wilson and MacArthur, 1967). Interestingly, MacArthur did not appear to explore the potential link between biogeography theory and niche theory.

Furthermore, the competitive exclusion principle, which suggests that one species will exclude another through competition, has been challenged by spatial ecology studies. These studies have demonstrated that limited dispersal can indefinitely delay the exclusion of one species by another, even without any trade-offs (Hurtt and Pacala, 1995). Hubbell found support for his intuitions in these works (Hubbell, 2001), as he believed that competitive exclusion lacked sufficient empirical evidence. He consolidated neutralist models in his influential monograph, "The Unified Neutral Theory of Biodiversity and Biogeography" (Hubbell, 2001), which gained widespread popularity (Leigh, 2007) and generated considerable controversy.

The concept of symmetry, also known as equivalence, can be perplexing in discussions surrounding niche and neutrality. Symmetry can be defined at various levels, such as the intraspecific or interspecific level (Kimura, Hubbell). Importantly, asymmetry at one level can coexist with symmetry at another level.

4. Functional groups

Despite the fact that each species has a distinct evolutionary trajectory shaped by its interaction with the environment, it is widely acknowledged that there is a certain degree of functional redundancy among species in regard to their role in ecosystem processes. This means that there are species that perform similar functions within the ecosystem. The idea of formalizing groups that structure the environment has emerged repeatedly in the history of ecological concepts (Barbault 1995). The classification of plants based on the functionality and physiology of species has been found since Theophrastus, approximately 300 BC (Barbault 1995, Gitay & Noble 1997). In the descriptions of pollinator studies, Kölreuter in 1761 and Sprengel in 1793 suggested that organisms with similar behavior and similar interactions on flowers could be grouped. This represented an important step in studies of pollination syndromes, with Darwin and other scholars in 1862 developing the perspective that combinations of floral characteristics could reflect the type of associated pollinator (Fenster *et al.,* 2004).

In 1934, Raunkiaer indicated that groupings of plant life forms were useful in analyses of the influence of climate on plant composition and dynamics. This practice took into account the

morphological foundation found in specimens, but as important aspects of vegetation are not expressed solely by morphological references, it became necessary to recognize additional characteristics related to habitat and plant ecology (Barbault 1995). The ideas presented above have led to inconsistency in the terminology used to describe them, resulting in the emergence of numerous terms that express the same concept. For instance, growth forms, life forms, and strategies are terms that have been used interchangeably to convey these ideas (Semenova & Van der Maarel, 2000). The modern development of the concept began with suggestions raised in the 1960s by ecologists who, following Hutchinson and MacArthur, adopted the consensus on the organization of communities into assemblies of species defined by their functional basis (Barbault 1995, Gitay & Noble 1997).

In 1974, ecologist Kenneth W. Cummins recognized the need to define "functional groups" of organisms based on their ecological processes rather than relying solely on traditional taxonomic classifications. Other biologists, such as Korner (1993) and Hobbs *et al.,* (1995), have similarly defined functional groups as associations of individuals with similar roles or functions. Gilbert (1980) proposed a framework that demonstrated how the diversity in neotropical ecosystems is organized through chemical mosaics and mutualism, emphasizing the importance of functional groups. For instance, he identified groups of species, such as hummingbirds, bats, moths, and bees, that perform critical functions such as pollination, as well as birds and bats that aid in seed dispersal and ants that protect plants from predators. These species belong to different functional groups and play distinct roles in shaping their environment.

Presently, a more straightforward description can be formulated wherein a functional group comprises organisms that share comparable sets of functional attributes, co-occur together, and exhibit similar responses to external factors and/or impacts on ecosystem processes (de Bello *et al.,*, 2010).

5. Ecological guilds

The term "ecological guild" was first introduced in 1903 by German Andreas Franz Whilheim Schimper, who translated the German word "Genossenschaften" into English as "ecological guild," referring to the distribution of plants with the same life form. The term "guild" had already been used by geographers and botanists (Schimper 1903, Clements 1905). In 1904, Grinnell used the term guild to define the concept of subdivision of the habitat in which an organism lives, including all the components necessary for the survival of the species.

From there, various authors began using this terminology for different meanings, such as groups of invasive species, functional groups, and species occupying the same environment (Wilson 1999). In 1927, Charles Sutherland Elton suggested that animal communities would be structured in groups with similarities in terms of survival ability or food acquisition, coining the term "ecological guild." This idea gained more structure in the 1950s when the emphasis of ecological studies was on interspecific competition as a process of community structure. Following Elton's ideas, George Evelyn Hutchison (1957) considered a guild as a group of species that share maximum overlap in their multidimensional niche characteristics, understanding that species could act similarly in the ecosystem, being ecologically similar in their functions.

In 1967, Richard Bruce Root created the definition of "ecological guilds" to be used today:

"A guild is defined as a group of species that exploit a class of environmental resources in a similar way. This term groups species that present significant overlaps in their niche requirements, regardless of their taxonomic position. (...) just as for the genus in taxonomy, the boundaries that circumscribe the membership of any guild are necessarily somewhat arbitrary. To be considered a member of the guild of foliage-gleaning birds of oak woods, the major fraction of a bird's diet must consist of arthropods gleaned from the foliage zone of the oaks. As a result, birds that only occasionally use the foliage zone are excluded, even though they exert some influence on the food resource supply of the guild."

In summary, the term ecological guild refers to groups of species that derive their subsistence from the same types of resources and use the same strategies in the occupation of their niches (Terborgh & Robinson 1986), indicating that the ecological relationships between the species of the

guild are shaped by competition for limited resources. Ecologists Hutchinson and MacArthur (1959) described guilds as coevolved entities and "arenas of intense interspecific competition." However, Root's definition was not the only one presented. Considering the potential taxonomic limitation imposed by the term "in a similar way," James MacMahon and his collaborators 1981 proposed the removal of this term from the definition of guilds (MacMahon *et al.,* 1981). Thus, the concept of a guild came to make sense only in relation to the individual in the environment, encompassing larger and more diverse groups of species that can use the resource in different ways and for different purposes.

In his review of competition studies, Schoener (1974) noted that the degree of niche overlap between species is dependent on the abundance of resources. Specifically, when resources are plentiful, there tends to be less co-occurrence and greater overlap in resource use among species compared to situations where resources are scarce.

In 1983, Ralph C. MacNally proposed the inclusion of taxonomic criteria. The taxonomic criterion could alleviate a priori judgments of how species relate, usually assumed in a competitive context. The taxonomic limitation, but not necessarily as a criterion, was indeed present in most guild studies, both for practicality and lack of information and for the general validity of the premise on greater niche similarity among phylogenetically closer species.

In 1996, John E. Fauth and colleagues proposed subdividing the concept of the guild into its global and local components, maintaining the term guild with its broader definition, as the set of all species that exploit the same type of resource similarly, without the need for co-occurrence, and adding the term "local guild" for a subset of species in the guild that co-occur in the same community.

In 1999, John Bastow Wilson suggested subdividing ecological guilds into "alpha guilds" (use of resources within a community) and "beta guilds" (distribution according to environmental conditions). Both categories are subdivided into four classes that depend on the criteria commonly used for groupings; thus, it is possible to incorporate the different senses for the term guild, functional groups, and other taxocenoses.

Theoretically, the guild is independent of the phylogenetic relationship between species, but guild members are often closely related species; they probably share traits and life history adaptations similar to resources and habitats (Blondel 2003). The concept of guilds emphasizes the importance of a resource that can be exploited "in a similar way," which is more readily observable in animals than in plants and difficult to categorize and quantify (Simberloff & Dayan 1991, De Kroon & Olff 1995). MacMahon *et al.,* (1981) noted that the identity of the resource used is less important than the fact that it was used, as users of the same resource belong to the same guild. Guilds are typically distinguished based on differences in morphological characteristics that are closely associated with feeding techniques and often linked to character displacement in phylogeny (Blondel 2003). Moreover, the same species may belong to different guilds over the course of its life due to ontogenetic changes in resource use (Simberloff & Dayan 1991, Gerking 1994).

Guilds provide a convenient way to separate complex communities into manageable ecological units and offer an alternative perspective on community composition that is different from richness-based metrics or taxonomic identity, as they focus on life strategies. Guilds are also useful for comparative studies across different communities, even when there is no direct overlap in species composition (Hawkins & MacMahon 1989, Terborgh & Robinson 1986, Wilson 1999). The application of the concept of guilds is particularly valuable in ecological studies because guilds group organisms that have significant overlap in niche requirements and share resources (Jaksic 1981, Pianka 1980). The use of classification models in ecological guilds of animals, especially insects, is uncommon because it requires obtaining various ecological information about the animal under study, as well as the correct taxonomic characterization of the animals. When these conditions are met, it is possible to use the information through variables with several categories. For example, in a category related to the type of termite nests, the inserted categories would be arboreal nests, epigeal nests, or subterranean nests. When all the information is compiled, we can use it for clustering analysis, in which guilds are

revealed by the distance between clusters. Objective methods for describing clusters can also be used (Farias & Jaksic 2006). In some cases, guild models are not appropriate, such as the application of models in communities where there are animal species that differ in ecology during different developmental stages and/or in different sexes (Hawkins & MacMahon 1989). Communities that contain species that alternate resource consumption in different seasons of the year are not suitable for this type of study (Jaksic 1981).

6. Ecological guilds or functional groups?

The correct use of terms and concepts is particularly important in the development of research that uses, for example, modeling land use and responses to environmental changes (Wilson 1999, Blaum *et al.,* 2011, Blondel 2003). In the past, ecological groups were the basis of indices initially used to quantify functional diversity (Petchey *et al.,* 2004, McGill *et al.,* 2006), as well as the recognition of bioindicator groups (Stork & Samways 1995, Dufrêne & Legendre 1997).

Simberloff & Dayan (1991) conducted a review of the concepts of ecological guilds and functional groups, and they concluded that these terms are often used interchangeably by researchers. They found that the majority of researchers prefer the term "guild" due to its metaphorical reference to professions, which they considered more elegant.

We know that in zoology, the term "guild" was first used by Root (1967), and the parallel term "functional groups" was first used by Cummins (1974). Both terminologies refer to fundamental principles of collective attributes of groups of species, with a group being formed by species that exploit resources in similar ways (the guild) and ecosystem processes that require resource exploitation by species (the functional group).

In the example given by Gilbert (1980) in the subtitle above (Functional groups), we can argue that the species involved in each of the functions, such as seed dispersers and pollinators, belong to the same functional group. However, they can also be considered part of the same guild because they share the same resources, such as fruits or nectar.

It is important to note that the guild concept focuses solely on resource acquisition relationships among guild associates, whereas the functional group concept encompasses a broad range of ecosystem functions, such as biochemical cycles, resource acquisition, invasion or fire resistance, water absorption, resource storage, defense against herbivory, pollination, seed dispersal, or any physical processes, such as ecosystem engineering, disturbance, and bioturbation (Blondel 2003). A group of species can be classified as either a guild or a functional group depending on the research question being addressed. The term "guilds" is used to identify species that share and utilize resources in a similar way (Root 1967). On the other hand, functional groups emphasize how resources are processed by species, performing the same ecosystem function or playing a similar role.

The guild concept emphasizes the species that utilize resources, whereas the functional group approach emphasizes the resource that is mediated by the members of a functional group (Blondel 2003). In addition, while the guild deals with species at the community scale and addresses existing competition, functional groups deal with species at the ecosystem scale and address functional similarities in a given context (Blondel 2003). Another characteristic that differentiates them is the consequence of removing species from the environment. In guilds, it results in system alteration, while in functional groups, functional redundancy between species does not alter ecological functions (Blondel 2003).

Individuals within a guild commonly utilize the same resources and may develop partnerships through participation in the same ecosystem process. On the other hand, functional groups are composed of partners who are inherently involved in the same ecosystem process. While guilds and functional groups are often viewed as two sides of the same coin, representing structure and function, respectively, the use of resources does not always impact the execution or provision of ecosystem services (Loreau *et al.,*, 2001). Theoretically, a functional group can contain more than one guild, while a guild cannot group more than one functional group (Silva *et al.,* 2018).

Wilson (1999) presents a classification of alpha and beta guilds, with alpha referring to resources and beta to environmental characteristics. This structure is quite similar to Blondel's (2003), as the alpha guild corresponds to the guild (sensu lato) and the beta guild corresponds to the functional group.

Another purpose is that the niche theory-based guild approach offers a promising avenue for studying interspecific competition and related phenomena in ecological communities. However, it is paradoxical that current ecological dictionaries still adhere to the original Rootian concept when defining guilds. To address this paradox, Blaum *et al.,* (2011) propose a new ecological term, "functional effect group/type," which categorizes species based on their similar environmental effects, as initially suggested by Diaz and Cabido (2001). Notably, the introduction of this new niche theory does not impact the proposed definition of the term guild. By incorporating the functional effect group/type term within the niche theory framework, researchers can enhance their understanding of species interactions and ecological implications.

Conclusion

In conclusion, this study underscores the critical role of terminology in ecology as the cornerstone of effective scientific communication and method standardization within the field. The evolution of ecological terminology highlights the dynamic nature of ecological science, emphasizing the need for continuous learning and adaptation among ecological professionals. By delving into the

historical context of key ecological concepts, such as biodiversity, trophic levels, ecological niches, ecological guilds, and functional groups, this research not only illuminates their evolution but also enables valuable comparisons and distinctions across different periods. Acknowledging and comprehending these historical processes are paramount, as they enrich our understanding of ecological terminologies and their intricate interconnections, ultimately enhancing the depth and accuracy of ecological research. As the realm of ecology advances, staying cognizant of these historical nuances is indispensable, ensuring that scientists remain adept in their communication and interpretation of ecological phenomena, thereby contributing significantly to the progress of ecological science as a whole

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Conflict of interest

The authors declare that they have no conflict of interest.

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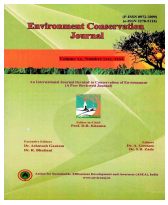

Biotic stress alleviating strategies in chickpea

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ABSTRACT

The third-most important food legume in terms of economic importance worldwide is the chickpea (*Cicer arietinum L.*). Its potential production is frequently constrained by numerous biotic stressors, such as the nematodes, insects *Ascochyta blight, fusarsium* wilt, and botrytis grey mould are the three major fungal diseases that cause significant economic losses, while *Helicoverpa armigera, Aphis craccivora, cowpea weevil* are the three major pre-harvest pest of chickpea. Several biological, chemical, cultural and, agronomical practices are used to control biotic stress, apart from that few modern biotechnological approaches also developed for high yielding and biotic stress resistant varieties. This paper aims to elaborate about different biotic stresses that affect Chickpea plant, their management strategies including traditional chemicals and adaptation of transgenic varieties with their limitations and also enlightened newer ray of hope *i.e.*, plant growth promoting rhizobacteria that holds the ability to combat against biotic stress by mitigating stress ethylene level.

Introduction

Chickpea(*Cicer arientinum L.)* is one of the most significant leguminous cool-season food crops, widely grown in the Asian Pacific region.Chickpea has large levels of all the essential amino acids, except for the Sulphur (Methionine) containing amino acids (Jukanti *et al*., 2012).The main storage carbohydrate is starch, which is followed by dietary fibre, oligosaccharides, and simple sugars like glucose and sucrose. With the addition of other pulses and cereals, chickpeas may have favorable effects on various serious human ailments, including as cardiovascular disease (CVD), type 2 diabetes, and digestive disorders, several cancers too (Lukus *et al.*, 2020).Chickpeas are a significant pulse crop with a wide range of possible nutritional and health advantages. However, yields are frequently modest and unpredictable (Verma *et al*.,2021). A variety of biotic and abiotic stress factors have a negative impact on yield, which is the main cause of yield variability *i.e*., 20-35% by weeds, 50-100% by disease and 10-90% by insect pest (Rana *et al*., 2016).Major biotic stresses, such as fungi, bacteria, and viruses, insect pests, nematodes, and parasitic weeds, have an impact on chickpea output worldwide. Due to wilt disease, the chickpea crop is seriously damaged in India, Myanmar, Nepal,Iran and Pakistan (Davies *et al*., 2007). *Fusarium* wilt, a fatal fungal disease, has a detrimental effect on chickpea productivity.Causal organism of fusarium wilt is *Fusarium oxysporum. F. sp. ciceris,* a most common disease in India (Dhawale and Dhale, 2021). Superior cultivars with improved resilience have been able to combat several of the main biotic stresses on chickpeas.There are still a few biotic stresses, nevertheless, for which no resistance has been found. This review focuses on the major biotic stresses affecting chickpea productivity as well as

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modern strategies to manage them.

Biotic stress:

When living things, such as weeds, insect pests, disease-causing agents, nematodes, allelopathic compound and pathogen harm plants it results in biotic stress. During various phases of plant growth, fungi and viruses are most prevalent and significant groupings affecting all areas of the plant (Mahmoud, 2021; Pande *et al.,* 2006).

Chickpea diseases:

Fungal diseases, followed by viral and bacterial illnesses, are the most important disease that contribute to a general decrease in the annual yield of chickpeas. On chickpea 67 fungus, 22 viruses, 3 bacteria, and 80 nematodes have been observed (Kukreja *et al*., 2018), but only small number of these result in economically significant disorders. Diseases like *Fusarium* wilt (*Fusarium oxysporum f. sp. ciceri*), *Ascochyta blight* (*Ascochyta rabiei*), *Botyris grey mould* (BGM) (*Botrytis cinerea*) *etc.* are the major diseases of chickpeas (Rasool *et al*., 2015)

Fusarium wilt* (*Fusarium oxysporum f. sp. ciceri):

Using 10 chickpea lines as distinguishing factors,revealed the occurrence of four physiological races of *F. oxysporum* which are cicers in India (Ramanamma *et al*., 2020). Later, two further races (0-1) from Spain and another (race 6) from California were identified on the basis of variations in responsiveness on various host, Race 1 was subsequently split into two races, namely Race 1A (from India) and Race 1B/C (from Spain). Syria,Turkey, and United States (California) have also reported races 1B/C.Many soils borne organisms belonging to the genus *Fusarium* were widespread worldwide and were referred to as plant pathogens. Wilted plant caused by *Fusarium* sp.,*Rhizoctonia sp*. and *F. oxysporum* which are Cicers pathogenic strains of chickpea are currently accepted worldwide as the causal agent (Jendoubi *et al*., 2017, Nikam *et al*., 2007).

Pathogen:

A globally distributed fungus called *Fusarium* can be found in soil not only in tropical and temperate climate condition but also occurs in regions including the polar and other enviroments. *Fusarium* species are among the most persistent species of soil borne fungal pathogen. It produces conidia, chlamydospore, micro and macro conidiospore.

Symptomatology :

The disease known as chickpea wilt was seen in seedling and growing phase of the plant (Lodhi *et al*., 2006). Petioles and rachis droop are symptoms seen as the condition progresses. Base to upward yellowing, leaf dryness and browing in plant vascular bundles, wilting were noticed.The pathogen produces enzyme that break down cell walls and obstruct the plants transport system. This is followed by discoloration of the roots vascular system. Later, the plant begins to yellow, wilt, develop necrosis, and eventually die.In soil plant waste, the pathogen *Fusarium oxysporium* can live and spread through seed. The fungus was revealed to be present in the seed hilum as chlamydospore-like structures.During the study of the pathogen distribution in seed, Basaiah *et al*. (2006) discovered that it was concentrated in the cotyledons and axis, this may be studied via disease cycle (Figure 1). The main source of infection is either mycelia or chlamydospore. While the fungus conidia are short-lived, its chlamydospores can survive until the next harvest season. Even in perfectly healthy plants growing next to infected ones, the pathogen can survive for a long time in the roots and stem (Haobing *et al*., 2015).

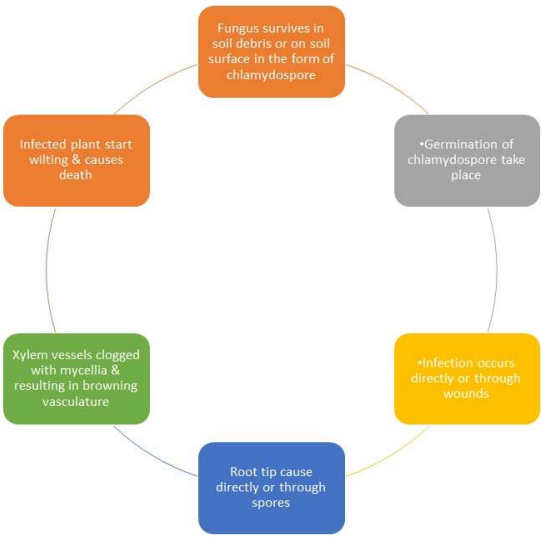

Figure 1: Disease cycle of *F. oxysporum f.sp. ciceris*

Disease management:

Agronomicalculturalpractices*:*

It is not possible to control the illness by crop rotation since the fungus is soil-borne and seed-

borne and can live in soil for extended periods of time even without a host. The use of pathogen-free seeds and avoiding affected fields are thus preventive measures (Gurjar *et al*., 2011). *Fusarium* wilt has also been successfully controlled by bio-control agents that offer an environmental friendly method of eradicating the ailment, such as a non-pathogenic *Bascillus sp.*(Kamali *et al*., 2019) and *Pseudomonas fluorescens* (Pandey *et al*., 2022; Fravel *et al*., 2003). However, the most efficient and environmental friendly way to treat the illness to use wilt resistant chickpea cultivars when they are available (Biratu, 2017).

Usage of chemicals:

In 2000 and 2001, the global use of pesticides exceeded 5.0 billion pounds. Disease control for plants has relied heavily on the use of insecticides (Whipps and Gerhardson, 2007). There was a 1.8% rise in production when output per hectare increased. However, this hasn't always been the case for instance, if farmers use pesticides recklessly or ignorantly, some of the pesticides may contaminate soil and/or groundwater stay in the environment for a long time, and be hazardous to farmers (Dasgupta *et al*., 2007)

Biotechnology tools:

Agriculture and food systems could undergo a transformation, thanks to nanoscale science and nanotechnologies. It has ushered forth a new era of Agronanotechnology. Nanoparticles possess been tested as antifungal medications against fungi as "magic bullets" that are nutrients, fertilizers, fungicides, herbicides, or targeting particular plants to transfer their energy for the intended effectsin plants (Pramanik *et al*., 2021). Different types of nanomaterials like copper (Cu), Gold (Au), Silver (Ag), Zinc (Zn), Titanium (Ti) may one day benefit Nano-agrotechnology by acting as a means of delivering nutrients. The seeds with the required compounds during germination capable of shielding them from the disease since it encourages growth, it will not contain any harmful, restricting, or unfavorable the plant's impact.

***Ascochytablight*:**

The most damaging form of chickpea disease, *Ascochyta blight*, is brought on by the fungus *Ascochyta rabiei*, which only lives on chickpeas. There are many reports of serious losses caused by blight, which reports of yield reductions of up to 100 %.The cause of ascochyta blight in chickpea, *Ascochyta rabiei* can found as both a teleomorph and an anamorph. *A.rabiei* ananamorph, is distinguished by the development of spherical pycnidia, which are black fruiting structures that resemble pears. A large number of hyaline unicellular and spores that have two cells, pycnidiospores, or conidia, grew on brief conidiophores incorporated within a limp that is mucous (Liu *et al*., 2016).

Symptomatology:

All of the plants aerial components are susceptible to developing *Ascochyta blight* symptoms. Brown lesions form at the stem base of newly emerging seedlings due to seed-borne illness. Following that the lesions expand in bulk and encircle the stem, breaking it and plants demise. Many pycnidia appear on the necrotic wound.These plants tend to cluster in patches but they can spread quickly (Figure 2). Depending on threat, plants are attacked at any stage of growth. Younger leaves are infected by *Conidia* and *Ascospore*, which produces tiny, necrotic areas drenched in water that quickly become larger and coalesce. When symptoms spread quickly to all aerial components, such as leaves, petioles, flowers, pods, branches and stem, tissues quickly collapse and the affected organism dies. Infected pod frequently causes seed infection and infection during the pod maturation stage often result inshriveled and infected seed (Foresto *et al*., 2023).

Figure 2*: Ascochyta blight* symptoms on chickpea. a) aerial part infection. b) infected pod

Disease cycle:

A.Rabiei undergoes periods in its life cycle that are both teleomorphic (sexual) and anamorphic (asexual). When both compatible mating types are present on crop debris from an *Ascochyta blight* infection during the winter the teleomorph develops (Singh *et al*., 2022). After successful mating, a *pseudothecium*, a sexual fruiting organism that is initially encased in host tissue is produced, have extensively discussed the condition of *pseudothecium* development on artificially infested chickpea straw in field conditions. Each ascus contains eight, two-celled ascospores, under moist condition mature pseudothecia discharged ascospores into the air (Figure 3). Meiotic recombination creates new pathogen varieties during sexual reproduction, and ascospores aid in the pathogen long distance dissemination (Bayraktar *et al*., 2007; Valetti *et al*., 2021).

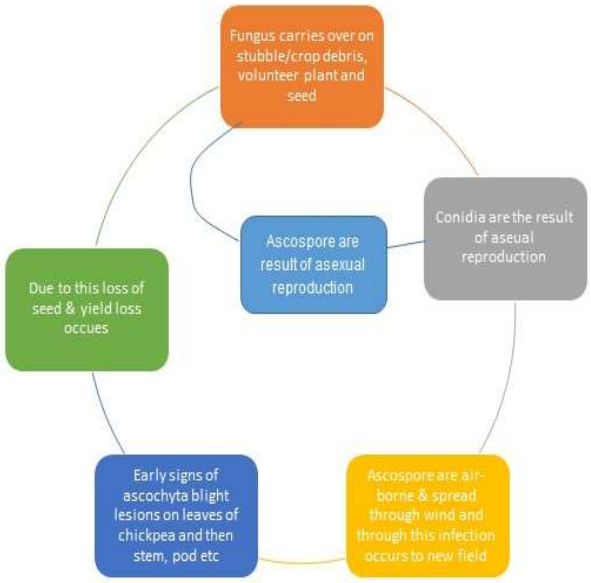

Figure 3: Disease cycle of *Ascochytarabiei*

Disease management:

Agronomicalculturalpractices:

Increased chickpea yields are necessary to feed the world's population, which is constantly expanding. Disease management strategies are therefore crucial. It is feasible to manage disease using variety of well-thought-out tactics (Manjunatha *et al*., 2022). Only chickpeas are susceptible to *A.rabiei*so culture practices like rotating with non-host crops and only growing chickpeas after a gap of three to four years will enable the control of this disease (Gurjar *et al*., 2011). Similar to this, using disease-free seeds and removing infected plant detritus can help lower inoculum level and prevent the spread of severe epidemics (Gan *et al*., 2006).

Modern Biotechnology tools:

Marker-Assisted Selection (MAS) seeks to choose a genetic feature of interest such as productivity, disease resistance, etc. indirectly. Plant breeding uses MAS to increase disease resistance and quality improvement (Kukreja *et al*., 2018). MAS have demonstrated to be effective in selecting for qualities that are challenging to measure. The utilization of molecular markers related to quantitative trait loci (QTLs) that provide resistance has been discovered. DNA based markers also promoted the adoption of uncommon disease resistance sources. These markers shorten the time required to generate resistant cultivars and improve the range of sources for the pyramiding of resistant genes.

Botrytis grey mould (BGM):

The second most significant disease to affect chickpeas is Botrytis Grey Mould (BGM), which is brought on by *Botrytis cinere* has potential to completely destroy chickpea particularly in winter rainfall and high humidity (Pande *et al*., 2006; Nene *et al.,* 2012). The timing of the disease emergence in relation to crop development and the severity of the disease-both of which are significantly influenced by the weather and the pathogen inoculum level.

Pathogen:

Micheli created the genus botrytis in 1929, and since then it has gained widespread recognition as a family of fungi that can cause economically and potentially significant plant diseases. This is especially true for form that cluster together since species forms tend to be concentrated in the temperature around 25°C and they can be found on a range of crop plants at 30º latitude (Manjunatha *et al*., 2019).

Symptomology:

The disease can affect all of the chickpea plant aerial components, with growth tips and flowers being the most vulnerable (Pande *et al*., 2006). BGM symptoms typically emerge after crop canopy closure (Knight and Siddique, 2002). BGM frequently first manifests as stem lesions that have been wet. That start at ground level and spread

along the stem, infecting other stem (Knights and Siddique, 2002). The fungus survives on infected seed, as a saprophyte on decaying plant debris and as soil-borne sclerotia. The disease is often established in new areas by sowing infected seeds. Masses of spore can be produced on infected plants. These fungal spores can be carried from plant to plant by air currents and spread the disease rapidly (Figure 4).

Figure 4: Disease cycle of Botrytis Grey Mould in chickpea

Management of disease

Agronomicalculturalpractices:

Utilizing pathogen-free seed can lower the disease's seed transfer rate. Minimize plant densities, taller cultivars, and altered sowing dates can all help to lower the amount of BGM in chickpeas (Pande *et al*., 2006). Late seeding slows vegetative development, which lessens the likelihood of illness. However,this may also result in a decline in grain yield. Greater crop aeration is made possible by wider row spacing. Lowered relative humidity, leaf wetness, and canopy, which in turn lowers the likelihood of disease (Pande *et al*., 2006), linseed intercropping and wider plant spacing in paired rows (Nene *et al*., 2012) have been shown to improve grain yield while reducing illness.

Useofchemicals:

Fungicide seed treatments, such as iprodione, mancozeb, thiabendazole, triadimefon, triadimenol, vinclozolin, thiram, benomyl, carbendazim, or captan, are successful in lowering seed infection (Pande *et al*. 2002). As soon as the disease first manifests, foliar treatments applied at regular intervals can control especially when combined with a seed-dressing (Pande *et al*., 2006).

Modern Biotechnology tools:

The creation of dependable and effective regeneration and transformation systems is crucial for gene technology to be successful in delivering novel features like BGM resistance in chickpea. Furthermore, cloned and characterized genes are particularly significant in expressing genes with antifungal metabolites. Several antifungal proteins, such as the hydrolytic fungal cell wall disintegrating chitinolytic enzymes prevents *B. cinerea* from growing as a fungus inside of leaf tissue (Kumar *et al*., 2018).

Root rot:

Dry root rot in chickpeas is brought on by *Macrophomina phaseolina* (*Rhizoctonia bataticola*). Australia, Ethiopia, Iran, Pakistan, Bangladesh, Nepal, and a number of other nations have reported it as a severe issue (Singh *et al*., 2022). The disease typically manifests at the flowering and podding stages and is more severe in sandy soils. Just the top of the plant has drooping petioles and leaflets. Tap roots lose their lateral roots and develop black and rotten, among other symptoms (Figure 5). On the tap roots, a whitish mycelium can occasionally be seen clearly. Dead roots are fragile and exhibit bark tearing. When touched, the root's tip is readily broken. When the collar section is cut vertically or with the arid of handles on the exposed woody root parts, little sclerotia can be observed. Both seeds and soil can spread the illness. Several members of the Leguminosae family become good hosts for pathogens. Lack of soil moisture is advantageous for the development of illness (Singh *et al*., 2007).

Management practices

Agronomical practices:

The severity of the disease is lowered by deep ploughs and the removal of infected host detritus from the soil. Conditions of moisture stress should be avoided. To avoid the hot weather when the illness is mature, early maturing types should be sown in the right time frame (Kaul *et al*., 2007).

Figure 5: Root rot disease

Insect pest attack on chickpea

Around the world, it is known that 60 different insect species eat chickpea. Among these, leaf miners and pod borers (*Helicover paspp*.) are the most significant insect pests. The Bruchid weevil (*Liriomyz acicerina*), cutworms (*Agrotis spp*., *etc*.), armyworms (*Spodoptera spp*.) and cowpea aphids (*Aphiscrac civora*) are among the leaf eating pests (Sharma *et al*., 2015).

Pre-harvest pest

Pod borers:*Helicoverpa armigera* (Lepidoptera: Noctuidae)

There are several different crops that are severely economically damaged by *Helicoverpa* species, which are widely distributed geographically. Major crop losses could result from the larvae feeding directly on the seed pod, which results in seed abortion and damage. Major crop losses could result from the larvae feeding directly on the seed pod, which results in seed abortion and damage. *Helicoverpa* favours chickpe as above lupins, canola, Indian mustard, and linseed, just like he does with field peas and faba beans (Grundy *et al*., 2004).The adult moths are grey to brownish in appearance and migrate over great distances in search of host plants.

Black cutworm- *Agroti sipsilon* (Lepidoptera: Noctuidae):

In North India, black cutworm is a pest of chickpea and other crops. During the rainy season, it causes significant harm in places that are inundated; however, in the summer, it may move to a hilly area. The species has different generations every year depending on the weather. On chickpea plants and soil clods, eggs are laid. Up to 2250 eggs can be laid by each female.The entire life cycle, including the egg (3 to 6 days), larvae, and pupae, lasts approximately 60 to 120 days. There are typically six to seven larval instars found.

Practices to minimize infestations:

Deep soil ploughing, hand-picking of large-sized larvae/pupae, shaking of plants to remove insects, weeding, mulching with grass, and sowing at the right time are cultural practices to avoid or reduce lepidopteran insect pest infestations (Dahiya *et al*., 1999), preserving the distance between individual plants, and applying fertilizer.Even though cultural customs they are generally time-consuming and cost-effective to produce a good harvest, but their implementation falls behind contemporary farming.

Since a few decades ago, the majority of the diseases and insect pests of chickpea have been largely controlled by chemical pesticides. Chemical pesticides' primary benefit is their ability to manage infestations, even when applied at an advanced stage of infestation. However, due to its persistent and widespread use, insects are subjected to strong selection pressure, which caused resistance to develop many chemical pesticides. There have been reports of resistance in *H. armigera* against several pesticides, including as carbamates, organophosphates and pyrethroids (Ahmad *et al*., 2001).

Role of PGPR in controlling biotic stress

Stratigies to control fusarium wilt in Chickpea

1. By using biocontrol agent

The most widely used and environmentally benign way of controlling *Fusarium oxysporum* is biological control (Anjajah *et al*., 2003). Rhizobacteria that promote plant development (PGPR) can be used to combat the wilt pathogen (Schmidt *et al*., 2004). These rhizobacteria produce the siderophores pyrolnintrin, phenazin, and phloroglucinol, which suppress and inhibit *Fusarium oxysporum* (Fridlender *et al*., 1993). *Burkholderia, Bacillus, Pseudomonas, Trichoderma* and others exhibit notable inhibition, making them effective biocontrol agents for chickpea wilt (Wani *et al*., 2007). By boosting -1, 3-glucanase enzyme activity and thus suppressing the pathogen growth, *Trichoderma harzianum* and *Bacillus subtillis* block and suppress the disease (Anjajah *et al*., 2003; Moradi *et al*., 2012).

2. By using plant extract ;

The antifungal activity of four plant species' aqueous extracts, including *Azadaracta indica In-vitro* research revealed the presence of *A. Juss., Datura metel* L. var. *quinque cuspida Torr.,Ocimum sanctum* L., and *Parthenium hysterophorus* L. At 100% concentration, *Azadirachta indica* leaf extract fully prevented pathogen spore germination (Singh and Chand 2004). Three weed species *Capparis decidua, Lantana camara* and *Tridax procumbens* have extracts that exhibit antifungal properties when applied to *Fusarium oxysporum* (Kumar *et al*., 2021.) *Datura stramonium* acetone extracts have reportedly been shown to have antifungal effect against a number of fungus, including *Fusarium oxysporum*.

3. By using agronomic practices

Crops that are planted early typically experience greater illness. According to several research, delaying planting and maintaining low temperatures during flowering are both beneficial for disease prevention (Chandra *et al*., 1974). Planting of seed at proper depth can also minimizes the disease incidence. Pigeon pea wilt incidence was significantly reduced by intercropping with sorghum in the first year (down to 55%), and thereafter it steadied at around 20–30%. Chick peas mixed with wheat and berseem produce measurable disease control (Basha *et al*., 2017).

PGPR mediated enzyme production

According to Cappuccino and Sherman (1992), many PGPR produce a variety of cell-degrading enzymes, including amylase, cellulase, pectinase and protease to degrade cell wall of dangerous bacteria, which thus lowers the Stress caused by living things. In addition, the fungus pathogen is diminished by the component of the cell wall is destroyed by the enzymes, for instance, chitinase, 1,3-glucanase, and proteases are made by PGPR.

Phytohormonal modulation:

Several phytohormones, such as cytokinin, gibberellin, abscisic acid, salicylic acid, jasmonic acid, brassinosteroids, auxin, and ethylene, play a crucial role in regulating various plant physiological activities (Orozco-Mosqueda *et al*., 2023). It is noteworthy that numerous plant growth-promoting bacteria (PGPB) possess the ability to synthesize or degrade some of these phytohormones, including cytokinin, gibberellin, salicylic acid, auxin, and ethylene (Delcarmenorozco-mosqueda *et al*., 2023)

Cytokinin:

The effects of cytokinins extend to various plant cell types, influencing functions such as seed germination, apical dominance, root elongation, xylem and chloroplast differentiation, transition to reproductive growth phase, flower and fruit development, leaf senescence, nutritional signaling, and interactions with plant pathogens. It is worth noting that cytokinins play a significant role in promoting plant cell division while simultaneously inhibiting senescence. Interestingly, the ratio of cytokinins to auxins in plants growing in their natural environment determines the degree of shoot and root formation, with higher cytokinin to auxin ratios promoting shoot formation (Maxton *et al*., 2018a).

Salicyclic acid *:*

Plants treated with PGPB frequently develop systemic, broad-spectrum resistance to a variety of phytopathogenic bacteria and fungi. Before plants engage with phytopathogens, this induced systemic resistance (ISR) primes plant defenses so that plants are more resistant to disease attack in the future. The ISR is frequently connected involves an increase in plant cell lignification and an uptick in the expression of Reactive oxygen species (ROS)-reducing enzymes such peroxidase, catalase, superoxide dismutase.

Auxin *:*

Auxin produced by PGPB (plant growth promoting bacteria) is traditionally found to be the key method by which bacteria promote plant development. Moreover, the majority of scientific literature focuses on acid (IAA), one of multiple auxins having biological action, hence the terms IAA and auxin are usually used synonymously. IAA stimulates cell division, cell expansion, root bacterial colonisation, differentiation of vascular tissues, and defense against pathogens, elongation of stems and roots, and loosening of root cell walls, among other plant growth features (Maxton 2017a).

PGPR mediated enhanced nutrient availability:

The primary purpose of siderophores, which are metal-chelating agents, is to draw insoluble ferric iron from various habitats (sideros, which means iron, and phores, which means carrier) (Nagoba and

Vedpathak, 2011). In general, it was discovered that the majority of facultatively anaerobic and aerobic bacteria create siderophore in the absence of iron ions (Neilands, 1995). There are three main categories into which siderophores can be divided: hydroxa-mates, catecholates (phenolates), and carboxylates. Because of the insoluble iron form (Fe3+), iron has a limiting effect on plant growth. PGPR known as *Pseudomonas putida* develops symbiotic interactions with plants. Phosphorus is essential for ATP generation and the phosphorylation of photosynthetic proteins and enzymes, two processes involved in plant growth (Zer and Ohad, 2003). *P. putida* increases chlorophyll content in the leaf also enhances antioxidant enzymes activities in chickpea leaves under phosphorus deficiency. The mobility of nutrients including P, Fe, Zn, and Mn is increased by the root exudation of organic acids (Zhang *et al*. 1997). Citric acid, which is widely found among root exudates, mobilises P in soils primarily by ligand exchange, dissolution, and occupation of P sorption sites (Fox *et al*., 1990; Gerke, 1995).

Nitrogen fixation

One well-known and important critical ingredient for plant growth and development is nitrogen. The global nitrogen cycle, however, contaminates groundwater and raises the danger of chemical leaks. Chemical fertilizer manufacturing is a very energy-intensive process that relies heavily on fossil fuels. High input farming methods that produce high yields have led to environmental issues and resource deterioration. Therefore, during the past few decades, the application of PGPR for environmentally friendly and sustainable agriculture has significantly increased in different parts of the world (Figueiredo *et al*., 2008). Increased and expanded use of PGPR for bio-fertilization would decrease and reduce the demand for chemical fertilizers and the unfavorable consequences they have on the environment (Maxton *et al*., 2017c).

Phosphorus availibility

One of the major and important elements that restrict plant growth, along with nitrogen, is phosphorus (Podile and Kishore, 2006). Even in phosphorus-rich soil, the majority of the phosphorus is insoluble and so unavailable to the plants. This issue can be solved using phosphate solubilizing bacteria (PSB), which are widespread in the rhizosphere (Vessey, 2003).In addition to solubilizing inorganic phosphate and converting insoluble phosphates into soluble monobasic and dibasic ions, PSB secretes organic acids and phosphatases that release soil phosphorus that would otherwise remain fixed and make it available to plants (Richardson, 2001)

Nutrient uptake

Mainly sixteen basic elements are needed for living plants to survive. Carbon, hydrogen, and oxygen are three of the sixteen elements that are predominantly obtained from air and water. The other thirteen are typically taken up by plant roots. Each of these fundamental components plays at least one distinct role in the development of plants. As a part of a method to ensuring appropriate plant nutrition and minimizing the harmful impacts of fertilizers on the environment, PGPR has been promised. In order to reduce the demand for fertilizers and minimize the buildup of nitrates and phosphates in agricultural soils, PGPR may boost nutrient uptake from soils. As known, phosphorus and nitrogen are the principal nutrients limiting plant growth and an essential macronutrient needed for plant growth (Podile and Kishore, 2006). In addition, some PGPR encourage the development of roots, which is accomplished by the creation of phytohormones like indole acetic acid (Kloepper *et al*., 2007)

Conclusion

In this manuscript, we identified that the main obstacles to increasing the production of chickpea crops in India are biotic stressors. To address the problem of the nation's nutritional security, it is vital to reduce the negative effects of these pressures on the pulse crops; productivity and production. Future of chickpea crop is bright because it is also basic food crop. It is a crop that uses few inputs and is adapted to use less water. We have highlighted several biotic stresses that can affect chickpea production, including *Fusarium wilt, Ascochyta blight, Botrytis grey mould, Pod borers* and *Black cutworm*. While several management strategies, including cultural and chemical control measures have been suggested for mitigating the impact of these biotic stresses, *fusarium* wilt is the major disease in chickpea crop and it can be control by using various different strategies such as bicontrol agent, agronomical

practices and also using plant extract. The use of resistant varieties has also shown to be an effective approach. Plant breeders have developed several chickpea varieties that are resistant to one or more of these biotic stresses. Therefore, planting resistant chickpea varieties can be an important component of an integrated pest management strategy for controlling biotic stresses in chickpea. By using resistant varieties in conjunction with other management strategies, farmers can improve the resilience of chickpea crops to biotic stresses and achieve higher yields and better quality produce. Apart from this, PGPR mediated strategy is an emerging ray of hope in this scenario that supports plant yield by mitigating stress ethylene level. It is recommended and urged to use PGPR as a method for bioremediation and biocontrol. PGPR offers everything of the best. Potential to serve as a bio-fertilizer that might be effective an ecology that is improved by improvements in productivity. The successful adoption of resistant chickpea varieties coupled with PGPR utilization by farmers will lead to reduced use of chemical pesticides, thus promoting sustainable agriculture and reducing environmental impacts.

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Conflict of interest

The authors declare that they have no conflict of interest.

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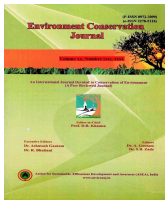

Herbal treatment as an alternative to antibiotics for bovine mastitis in the system of obtaining environmentally safe milk

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ABSTRACT

Antibiotics are known as the first option for treating any disease. While, the difficulty in terms of antimicrobial resistance and antibiotic residue as well as antibiotic impact is application on health of the public, results in numerous limitations on unregulated antibiotic treatment worldwide within the dairy industry. Scientists looked into new healing strategies that could be used to replace antibiotic use in mastitis disease treatment. Bovine mastitis causing a direct impact on food safety issues and the farm's profitability. This pathology's treatments and preventions are specially performed using antimicrobials, However, this disease's pathogens' increasing antimicrobial resistance may have an impact on the customary drug's effectiveness. Moreover, the environment and the presence of antimicrobial residues in milk are a probable danger in terms of human health. As a result, the utilization of plant extracts could become a hopeful alternative for bovine mastitis prevention. Antibacterial properties are included in numerous plants. Plants extracts are usually considered secure for animals, humans, and the environment. This analysis contains the common issues that came across in the customary Mastitis Treatment, including the potential uses of plant extracts as substitutes for the control of these pathogens, as well as the constraints of using these plant derivatives.

Introduction

Milk is as old as mammals. Milk is the most many-sided product in the food industry. It gives us balanced nutrition from birth until the time when we can eat solid feeds. It is supposed that ancient people discovered domesticating animals for the production of milk for human consumption. They domesticated animals like animals such as cows, buffaloes, goats, sheep, and camels. Some of these creatures are still in use for milk production in numerous regions of the world. Beyond milk, financial relevance, and milkproducts are essential in the human diet because a source of micro and macronutrients (Akin, 2018), imparting proteins, potassium, calcium, vitamin D, vitamin B12, riboflavins, fatty acids, as well as phosphorus (Keast *et al.,* 2013), (O'Neil *et al.,* 2018), (Quann *et al.,* 2015).The aforementioned items are consumed straight away. Accompanied by excellent skeletal well-being, mainly concerning kids as well as teenagers, and a reduced risk of hypertension, coronary artery disease (CAD), and non-insulin-dependent diabetes mellitus (NIDDM) in adults.To get milk of superior quality, perfect health of the udder is not solely necessary only in support of the dairy producer, However, it is also applicable to the entire chain of manufacturing for dairy products (Hogeveen *et al.,* 2011). Moreover, enhancing directed heightened public knowledge and government oversight focus towards the security and integrity of food troubles emphasize the importance of the sector of dairy production taking proactive measureswith the purpose of denoting and laying off new food safety concerns that may arise representing a negative influence on the

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perception of dairy products (Boor *et al.,* 2001). Pathogens of udder control can minimize foodborne diseases and offer nutritious food to consumers that is safer and of superior quality (Bajpai *et al.,* 2013). Because of public health concerns reasons, antibiotic residues, and microbial resistance allied with the dairy swarm, Agriculture animals must alertly investigate the use of antimicrobials and implement exemplary stewardship procedures structured to decrease the danger of creating recently discovered bacteria that are resistant to antibiotics that could be passed to humans from animals (Foutz*et al.,* 2018).The financial growth pertaining to the dairy industry prompts research into increasing food safety and environmental production that is long-term. As a result of this standpoint,plant extract utilization could be a promising artificial drug substitute. The purpose of this review contains to go over the utilization of a collection of extracts from plants asa substitute for mastitis dairy cattle control, with an emphasis in terms offuture tendencies and potential implications for the application of suchproducts in the veterinary field. Bovine mastitis results inyearlyfinancial losses of approximately $35 billion worldwide and in the United States of America is $2 billion (Mubarack HM *et al.,* 2011). Mastitis is known asthet cost-effective diseaseinthe dairy industry, affecting both highly developed and developing countries. Mastitis has an impact on milk quality, and its consequences extend beyond the dairy farm. Consumer concerns about milk quality, antimicrobial resistance, antimicrobial residues, and animal welfare necessitate the development of appropriate policies for effective prevention and mastitis control. Because of the permanent damage that mastitis can cause to the mammary secretory tissues, the loss of production of milk is not limited to the course of the disease but may continue throughout the animal's life.

Antibiotic treatment disadvantages in mastitis

Several studies in recent years have found that mastitis pathogens are developing antimicrobial resistance and that conventional treatments have low cure rates (Freitas *et al.,*2018). Bacterial resistance mechanisms are very versatile, and they are influenced by whether a population has resistant variants, whether there are differences in reproductive successoccurringwhen antibiotics are present, and whether the variation is inherited. According tosuch cases, the composition of the population will shift in support of resistant strains over time (Baquero, 2011). During the lactation period when antimicrobials are administered, As a result of the high danger of residues of drugs, milk must be discarded for an extended period, posing a possible danger to human well-being. These leftovers may be harmful to antimicrobial-sensitive consumers, encourage bacterial immunity, and impede the production of derived products of milk (Blowey and Edmondson, 2010). Long-acting antibiotics can be used to treat the cows, however, milk from these types of cows cannot be sold until the residues of the drug have passed through the system ofthe cows. If the levels of antibiotic residuals in milk are high, milk should be siphoned off and discarded. The UK government convened a meeting on the global situation of antibiotic resistance in 2014, as well as a subsequent impact assessment's performance. To keep antibiotics effective both humans and animals can benefit from this therapeutic measure., both veterinary and human medicine sectors must limit the development of resistance and take responsibility. The current study sought to investigate the occurrence of mastitis resistant to medication and antibiotic resistance patterns in dairy cows. Humans have been exposed to Staphylococcus aureus resistant to methicillin (MRSA) strains by animals, but this transmission's epidemiology is unknown (Juhász-Kaszanyitzky *et al.,* 2007). Regardless of the results of in vitro tests, MRSA should be taken into account as resistant topenicillin, cephems, cephalosporins, as well as other beta-lactam antibiotics like ampicillin-sulbactam, ticarcillin-clavulanic acid, piperacillin-tazobactam, amoxicillin-clavulanic acid, and carbapenems. (CLSI, 1997). Antimicrobial agents that are most commonly used such as aminoglycosides, tetracycline macrolides, chloramphenicol, and fluoroquinolones are also frequently resistant to these organisms (Clinical and Laboratory Standards Institute, 2006), (Türkyılmaz *et al.,* 2010). Antimicrobial therapy is commonly used to prevent and treat mastitis. Unfortunately, despite the most effective antimicrobial therapies available, bacteriological treatment failures are quite common, especially in the case of antimicrobial resistance and mastitis which is thought to be among the causes of a low rate of cure (Barkema *et*

al., 2006). Antimicrobial resistance bacteria are a public health risk. Several strains of *Staphylococcus aureus* isolated from mastitis patients are resistant to a variety of antimicrobials, including penicillin-G, ciprofloxacin, streptomycin, gentamicin, ampicillin, and oxytetracycline (Kumar *et al.,* 2011).

Plant extracts used as alternative treatments for bovine mastitis

Some plants, also known as medicinal plants, may be used for therapeutic purposes. Chemical compounds are found in these plants that have beneficial biological properties for the benefit of animal and human health (Paz *et al.,* 2018). Lately,promising experimental findings by the utilization derived from botanical sources with medicinal properties havegiven the stimulus for bioactive substance research and the possibility of new products. All plants generate primary metabolites, Amino acids, fatty acids, carbohydrates, and organic acids are examples, which are required to survive (Tian *et al.,* 2018). Bioactive compounds, in contrast, occur in lower amount and usually necessary to the defenses of plants (for example, causing bitter formation, substances that are detergent, toxic, or pungent) guarding them against the majority of Herbivores, microorganisms, and plants that compete for resources are all examples of herbivores directly. These metabolites were potentially derived through plant extracts, which are preparations with varying consistency as well as compositions (GouveaFD, *et al.,* 2017),that seek to extract as well aspurification of bioactive compounds by botanical species matter (Tan *et al.,* 2013).

Maceration, digestion, percolation, infusion, and decoction, Soxhlet hot uninterrupted retrieval, fermentation extraction with a water-alcohol solution, microwave assisted solvent extraction, supercritical fluid based extraction, counter-current and ultrasonic extraction, as well as distillation methods are all used to obtain those extracts (Pandey and Tripathi, 2014). among the older methods based onorganic solvent extraction is the hot continuous extraction process, where the sample comes inat a distance from the solvent comparatively moreheat level (Ghaderi and Ebrahimi, 2015). Solvents permeate within the solid materials ofthe nt during the process and make the compounds more soluble with comparable polarities (Pandey and Tripathi, 2014), the elimination process will follow. Also, extraction with the assistance of ultrasonic waves employs dissolving agents and is superior to the more conventional proceduresbecause of its high effectiveness, lower solvent volume requirements, andshort period of extraction. (Macías-Sánchez *et al.,* 2009). Supercritical fluid extraction, alternatively, is a widely used procedure that makes use of supercritical fluids (above-critical-point temperature and pressure) and offers benefits such as increased selectivity and lower temperatures, staying away from Solvents and thermal degradation that remain. The most significant disadvantage of fluid that is supercritical is the more advanced equipment cost needed for extraction when in comparison to conventional extraction of dissolving agents (Yen *et al.,* 2015).To address the issue of bacterial resistance caused by the continued antibiotic usage in dairy herds, exploration into complementary therapiesis increasing. The specified types of therapies, which primarily make utilization of extracts of plants, are effective In vitro control of pathogens that cause mastitis. Natural occurrence, feweradverse effects (Khe*et al.,* 2018) as well as a lack of resistance that develops following extended (Montironi*et al.,* 2016).These compounds have a significant advantage over conventional drugs. Indeed, numerous Several studies have confirmed the effectiveness as a result of these plant derivatives. For example, Extracts of *Azadirachta indica* leaves exhibitthe properties of bactericidal power.It is now underway to have substantial influences on gram-positive and also on gram-negative pathogens as well as additional bacterial species responsible for a variety of illness affecting both humans and animals(Maragathavalli*et al.,* 2012).

Some major losses due to mastitis

Milk must be discarded during treatment days and waiting periods due to the treatment of a clinical case. In general, milk is assumed to have been discarded for six days: three days of treatment and 3 days of rest(Huijps*et al.,* 2008). The treatment's costis also a significant factor to consider. The cost of treatment is divided into two parts: veterinarian fees and drug costs.These two costs vary by country. Labor costs calculation is difficult.Farm to

Figure 1: Neem leaves

farm, labor opportunity costs may vary. farm to farm. If labor is external, the time spenton labor costs preventing mastitis is simple to compute (hourly wage). In contrast, if labororiginated with the farmer, It is worth noting that mastitis may cause Farmers should not to spend as much time on administrative tasks. (Halasa *et al.,* 2007).Poor milk quality, premature cow culling,oranimals with a shorter productive life.The losses can be either temporary or permanent.The disease is clinically or subclinically classified based on the specific form of clinical symptoms. Clinical mastitis is distinguished byvisible symptoms like milk clots, and Teats with hardness and swelling (Blowey and Edmondson, 2010) recognized through a visual examination and manual exploration (Sadek*et al.,* 2016).There are no externally visible changes. in a subclinical infection (Blowey and Edmondson, 2010) and Auxiliary examinations, for instance, the California Mastitis Test (CMT) or somatic cell count examination in the laboratory are used to make the diagnosis (Sadek*et al.,* 2016).Mastitis has an impact on milk quality goes outside of the dairy farm. Consumer concerns about residues of antimicrobials, resistance to antimicrobial agents, animal welfare, and milk quality necessitate the development of appropriate policies for effective mastitis prevention and treatment. Because of the permanent damage that mastitis can cause to the mammary secretory tissues, the loss of production of milk is not limited to the disease progression but may continue throughout the animal's life. Histological analyses were and continue to be widely used for determining mastitis pathogen-induced damage to secretory tissue in the bovine mammary gland (Benites *et al.,* 2002). An inflammatory response was detected in 96.9% of the samples. (edema, damage to mammary epithelial cells, and Infiltration of polymorphonuclear neutrophils), procedure for tissue repair, or both in dairy cow's mammary parenchyma from which microorganisms were isolated. At the same time, there were no histological changes in mammary glands with no evidence of microorganisms. These findings clearly show that the presence of microorganisms is linked to tissue harm. All at once, it should be noted that Because of pain and decreased movement, inflammation can cause a decrease in appetite and food intake which will hurt milk production. Many methods have been developed to compute the losses of production caused by Dairy cow mastitis. Neither of the methods are ideal because it is impossible to know how much milk a cow would have produced if lactation had not been interrupted by mastitis. Every technique has an inherent prejudice that, in the majority of cases, tends to understate the actual loss of milk production.Nonetheless, scientists agree that milk yield losses are the primary economic impact of subclinical mastitis (Schepers and Dijkhuizen, 1991).It was discovered that the sole item in all previous papers' estimates examined that was concerned with the Mastitis economics was a change in milk production.Estimates of milk yield loss continue to be a source of concern because they are most likely influenced by age, cow breed and type, udder's morphological characteristics s, and lactation stage, Before mastitis, milk yield was higher, organisms that cause mastitis, grade of inflammation, Early or late post-occurrence diagnosis, treatment type, feeding procedures, season, Mastitis recurrence during the same lactation or earlier, model of comparison (control group), and Some mastitis causal agents, for example, have been demonstrated to have a greater impact on the yield of milk than others. In general, the more inflammation there is, the less milk is produced (Petrovski *et al.,* 2006). Using the performance of an infected quarter as a comparison to the performance of the uninfected quarter on the opposite side, it is generally acknowledged that the contralateral udder quarters produce roughly the same amount of milk when both are uninfected. Simultaneously, this is scientific proof that quarter are s that mastitis-freemay make up for mastitis-infected quarters by boosting milk production. If

there is compensation occurs, the actual milk loss due to mastitis will be overestimated.(Petrovski*et al.,* 2006)

Transmission

Contact with the milking machine regularly, as well as contaminated materials or hands, transmission from mouth to udder among calves. The mastitis-causing bacteria strain will remain dormant in the calf's oral cavity until it is transmitted elsewhere, In a muddy, wet condition, with Inadequate milking techniques and hygiene.Mastitis is a multifactorial disease caused by the interaction betweenseveral parameters including the host, pathogens, management, as well as the environment. Mastitis is caused through a diverse range of etiological agents, incorporating a significant multitide of microorganisms that cause udder inflammation. Bacterial pathogens are the most dangerous infectious agents to the breast gland. They are frequently contagious and widespread in an environment of dairy animals and thus raise the rate of occurrence of intramammary infections. Infection is caused by either contaminated environments or infected udders. Pathogens are spread primarily through contaminated quarters and soiled udders, milking machines that have become contaminated, flies, washing clothes milker's hands, tea cups, and surgical instruments. Furthermore, lactation stage,lactations number, udder trauma, loose teat sphincters, teat, and teat canal, lesions on teat skin of each mammary gland's immunological status, the amount of contamination in the environment management conditions are among the factors that influence (Alemu *et al.,* 2013) the occurrence of mastitis in dairy animals.

Etiology

Mastitis etiological agents are classified divided into two: environmental and contagious. Contagious microorganisms typically reside in theteat skin or udderduring milking, they are transferred to the teat., where they multiply and as well as spread throughout mammary glands. Environmental agents, in contrast, survive in the environment of the cow and make their way into the udder via transportby teat canal for instance throughout milking, through the activity of capillaries, antibiotic administration of a tube, or teat cannula insertion) or teat canal indirect penetration quickly after the completion of milking (Scott *et al.,* 2011). While there maybe a few differences in how these microbes are classified. Transmissible pathogenic agents include *Staphylococcus aureus*,*Mycoplasma spp., Corynebacterium bovis*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae*, while environmental pathogens *Citrobacter spp*. is one of them, *Enterobacter spp*., *Klebsiella spp*., *Escherichia coli*, *Pasteurella spp*., (Blowey and Edmondson, 2010) Because of its potential to deliver a diverse set of pathogenic attributes that aid in bacterial intrusion. *Staphylococcus aureus* remains among the prevailing causative organism. (Saei, 2012), (Marques *et al*., 2017). Bovine intramammary infections can manifest as subclinical, acute, chronic, or toxic. resulting in significant monetary losses (Käppeli *et al*., 2019). *Staphylococcus aureus* is frequently regarded as the most significant microorganism linked to mastitis. This pathogen's intramammary infections are difficult to treat. Because They are vulnerable to recurrence and chronicity. (Peton*et al*.,2014). Mastitis can be caused by a traumatic or toxic event., but A microbiological infection is usually to blame according to The International Dairy Federation (ID A) (1987). In addition, over 150 different microorganisms that cause disease have been identified as mastitis-causing agents in dairy cows.Bacteria, fungi, and yeasts could all be involved,However, bacteria play by far the most important role. (Quinn *et al*., 2002).Although it is unknown what causes 20 to 35% of clinical mastitis cases.,(Wellenberg *et al.,* 2002) It is assumed bovine mastitis is primarily caused by bacteria. It can be either contagious or environmental.

Detection:California mastitis test (CMT):

CMT is anuncomplicated and reliable test for subclinical mastitis that can be applied universally. Its design was aimedat test milk from specificudder quarters as well as composite milk samples. To obtain reliable readings, recently collected milk and not refrigeratedcan be examined for a maximum of 12 hours, andThe CMT can test refrigerated milk for as long as 36 hours. The test helps to determine the level of infection occurring in every udder quarter as opposed to a total udder outcome andthe outcome onlyirrespective of the cell count is elevated or reduced is indicated. (CMT) is a straightforward indicator of milk's somatic cell

count (SCC). It works by introducing a reagent into the milk sample that disrupts the cell membrane of somatic cells. It is a simple but very effective technique that can be used by any member of the farm staff to provide an immediate result.

Somatic cell count:

Somatic Cell Count (SCC) is a significant milk quality determinant.Leukocytes constitute themost somatic cells (white blood cells) that are becoming increasingly common in milkas a defensivereaction against a mastitis-causing pathogen,in addition to a few glandular cellswhen an infection arises, these milk-secreting cells sloughed off from the udder's internal tissue.

Table 2: Scoring chart of california mastitis test

The number of leukocytes per milliliter	The appearance of the test	Score of CMT
Below 200,000	In the liquid mixture, there is no precipitation in the mixture.	Negative
150,000 to 500,000	The light precipitation dissipates with the movement of the paddle.	Slight
400,000 to 1,500,000	It distinct precipitate but does not blend with the movement of the paddle.	1
800,000 to 5,000,000	The formation of a gel is distinct.	2
Over 5,000,000	The formation of a strong gel that sticks to the paddle. It has a distinguishable central peak.	3

Flow cytometry (FC):

A methodofdeterminingcells' chemical and physical properties as they move in a state of suspension. past a sensing point. This method was recently created to quantifymilk somatic cell counts and it is especially useful in the detection of subclinical mastitis (Tian, 2005), (Holm, 2004)

Culture method:

Mastitis is most accurately diagnosed by identifying and isolatingany pathogenic microorganisms found in milk. This could be accomplished through Cultural methods, as well as a variety of additional determining tests.To obtain accurate results while avoiding contamination and, as a result, bias, it is critical to work safely as well as ina precise manneras much as possible given the circumstances (Quinn *et al*., 2002).

Vaccination

Mastitis vaccines are available, but they cannot prevent recurrent infections. There are several commercially available mastitis vaccines, however, neither of them provides adequate protection while also being cost-effective(Sharun*et al.,* 2021*)*.As a result of the multiple etiologies, no effective vaccine against all possible pathogens is available; however, various vaccines against bacterial pathogens have been attempted with mixed success. The insufficient protective potential may be due to a variety of variables, as well asthose pertaining to bovines. For instance health and age category, environment, or invading microorganism like elevated prevalence of pathogens that cause mastitisin spite of genetic differences within mastitis-causing genotypes from the same kind, as well as variations in individual animal immune responses based on genetic and environmental variables(Côté-Gravel and Malouin, 2019), (Merrill *et al*., 2019), (Scholte, 2019).

Control and prevention

The phrase "prevention is better than cure" perfectly describes the condition of mastitis. Mastitis can be reduced through better animal husbandry and better hygiene methods of the handling of animals (Kumar *et al.,* 2010*)* The majority of cases of bovine mastitis caused by udder damage, which then comeswith a microbial infection, these are avoidable, even if they occur by chance, treatment should be prompt as well as consistent. Pathogens enter primarily via open teat canals and animals with a high yield and a soft opening and teat canal closure is delayed during milking or milk dripping from teats as a result of delayed milking, which may bethe microbial invasion source bysoil, polluted water, or litter. All of these issues are manageable through good shed cleanliness and administration.The implementation of antiseptics after milking process and at the teat entrance reduces the possibility of microbial entry and is regarded as a successful management strategy fordisease avoidance (Olde *et al*., 2012). Bovine mastitis is always reduced when disinfectants are used on a consistent and timely basis in paddocks and the shed. Milk samples and regular milk screening always reducethe out of infected animals. Color and consistency observations, two milk-straining methods that are commonly used, increase the likelihood of detecting mastitis early. Breeding, control of flies,

proper nutrition, improved milking sanitation, prevention of cross-sucking among young children, use of procedures for disinfecting teats after milking, routinely inspection of milking equipment, and use of milking sequence, and bedding material enhancement are all measures aimed at preventing new mastitis cases (Shkreta *et al.,* 2004), (Calzolari *et al.* 1997*),*(Fontaine *et al*., 2002), (Chang *et al*., 2008), (Nielsen, 2009), (Yin et *al.,* 2009), (Vliegher *et al*., 2012).

Conclusion

A worldwide trend towards greater environmental sensitivity in farm animal management is currently being driven by increased Concerns about public health and consumer acceptance. Because pathogens have high levels of antibiotic resistance, alternative treatments for bovine mastitis are urgently needed. Plant derivatives hold great promise as a source of new antimicrobial agents., demonstrating efficacy against resistant microorganisms in vitro and, in some instances, in vivo, and are thought to be less harmful to plants, animals, and the environment. Bacteria have devised complex mechanisms to avoid antibiotic attacksand survive, which is a procedure that is accelerated most likely by heightened utilization of antimicrobials. Insusceptible to antibiotics has arisen as one of the most serious dangers to public health twenty-first century. To design novel anti-threat strategies, knowledge of the ways in which bacteriawhich bacteria developresistance to antibiotics is required. As a result, Antimicrobial drug development efforts andresearch resistance mechanisms must be ongoing to reduce the issue. Mastitis is a problematic condition that is currently one of the most serious conditions that cause damage in the dairy business. The financial losses caused by the circumstances are irreparable, as a result of the late misdiagnosis of the primary agent responsible for the etiology. Diagnosis failure is primarily due to disease complicity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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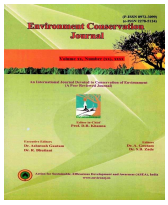

Application of natural preservatives and sweeteners in fruit products to reduce health risks - a review

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ABSTRACT

The costs of food deterioration in terms of both money and health are rising. Fungi, bacteria, yeast, insects, and rodent contamination of food supplies continue to be a major public health concern. Chemical preservatives are effective but can be potentially fatal to human health in certain cases. As potent food preservatives, essential oils made from plants are a great alternative to synthetic preservatives. They also possess a variety of anti-inflammatory, antibacterial, and antioxidant effects. The use of artificial sweeteners in food products, which raises safety questions and health issues while also having reduced nutritional value, is another problem in the food industry. Because natural sweeteners are linked to a healthy lifestyle and have superior nutritional qualities, consumers today prefer them. This article goes through the issues with artificial sweeteners and preservatives and goes into great length about the many different essential oils and natural sweeteners that are much safer and healthier alternatives.

Introduction

Preservatives are compounds, either natural or artificial, that are added to processed foods by preventing, delaying, or halting their fermentation, acidification, microbial contamination, and decomposition, which extend their shelf lives while maintaining their quality and safety. Today, chemical preservatives are more frequently used than natural preservatives. Some of them could have serious side effects, and a few of them are poisonous. Artificial preservatives such as nitrates, benzoates, sulfites, sorbates, parabens, formaldehyde, BHT (butylated hydroxytoluene), and BHA (butylated hydroxyanisole) have been associated with serious health hazards such as cancer, neurological damage, allergic responses, asthma, hyperactivity, and hypersensitivity, according to research. Many natural preservatives with antioxidant, antibacterial, and anti-enzymatic properties are obtained from plants, animals, microbes, and minerals. The extracts of neem, rosemary, basil, and clove are viable substitutes for their synthetic counterparts.

The contamination of food items with various microorganisms, including bacteria, fungi, viruses, parasites, etc., poses a significant challenge for the food industry. Through the production of various toxins during pre- and postharvest processing, these microbes degrade food products. As one of the most potent and thoroughly researched dietary pollutants of microbial origin, mycotoxins pose a serious health risk to people. Because synthetic chemicals are bioincompatible, nonbiodegradable,

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and unsustainable for the ecosystem, using them as food preservatives is currently a serious problem. Due to their ecofriendliness and largely accepted safety status, plant-based antimicrobials, particularly essential oils, have generated increasing interest as a potential replacement for synthetic preservatives (Maurya *et al.*, 2021).

Essential oils (EOs) are materials that have been intensely concentrated and are taken from the leaves, stems, flowers, seeds, roots, fruit rinds, resins, or bark of fragrant and medicinal plants (Hanif *et al.*, 2019). Due to their capacity to inhibit the growth of food-borne pathogens and preserve food products, these bioactive chemicals are appropriate for use in active packaging. Essential oils are currently used in active food packaging in the form of films and coatings that are applied to many food groups, including fruits, vegetables, fish, meat, milk, and dairy products, as well as bread and baked goods (Sharma *et al.*, 2021).

Due to consumers' increased attention to their health over the past ten years, the demand for zero-calorie and naturally produced sweeteners has increased significantly. Alternative sweeteners have been used to enhance food flavor, reduce blood sugar levels and draw consumers for a long time. They were initially adopted because of the high sugar-to-food ratio, which favored obesity in the general population and led to its widespread occurrence in infants and children. Saccharine, a low-calorie artificial sweetener, was thus made accessible in the 1980s. Since this sweetener was so well liked, others soon followed, the most common of which were cyclamates, aspartame, and acesulfame K. Over the years, there have been several disputes and debates surrounding sweeteners, including claims of liver and bladder toxicity, carcinogenicity, fetal deformities, and other risks (Saraiva *et al.,* 2020).

Natural sweeteners are produced by nature without added chemicals or fancy machinery (Neacsu and Madar, 2014). Neera (coconut), honey, mollases and stevia can be used as alternative sources of natural sweeteners because they do not increase blood sugar levels.

Detrimental effects of artificial preservatives:

When nitrate-based food preservatives are consumed, the nitrates transform into nitrites, which can react with hemoglobin to produce methaemoglobin, which can cause unconsciousness and even death, especially in young children. When proteins and nitrites react in the stomach, nitrosamines are produced, which are compounds that can cause cancer. Researchers have discovered a direct link between dietary nitrate levels and the number of Alzheimer's, Parkinson's, and type 2 diabetes fatalities. Foods containing monosodium glutamate may result in headaches, sweating, skin redness, nausea, and weakness after consumption (MSG). These include sodium nitrate, nitrosamines, potassium nitrate and more.

Sulfite-based food preservatives have the potential to exacerbate asthma and trigger life-threatening allergic responses. Along with methyl-isothiazoline and methyl-chloro-isothiazolinone, dangerous paraben compounds are frequently used. These are strong irritants and allergens that have been linked to potential neurological impairment in rats. The use of these harmful substances by expectant women may have a negative impact on the fetus's brain development. They are all powerful irritants of the skin, eyes, and lungs, including formaldehyde, DMDM hydantoin, diazolidinyl urea, and imidazolidinyl urea. These kinds of poisons can harm sperm DNA when exposed to high doses. According to research, hundreds of children's foods and beverages include food additives that can lead to tantrums and other disruptive behaviors. These include sodium metabisulfite, sodium sulfite, sodium bisulfite, potassium sulfite, *etc.*

Different chemical preservatives, such as sulfur dioxide, sulfites, sodium nitrite, sodium benzoate, benzoates, sorbates, formaldehyde, imidazoles, pyrrolidines, and thiocyanates, have significantly decreased the microbiological contamination of food goods (Gutiérrez-del-Río *et al*., 2018). These chemical preservatives have caused negative concern in consumers because of their long-term degradation cycles, environmental toxicology, insect revival, and potential for teratogenesis and human and animal cancer (Basak and Guha, 2018; Falleh *et al*., 2020).

Secondary metabolites:

There are five main classes of secondary metabolites: terpenoids and steroids, fatty acid-

derived substances and polyketides, alkaloids, nonribosomal polypeptides, and enzyme cofactors.

1. *Terpenoids and steroids*: Terpenoids and steroids represent a sizable class of chemicals produced synthetically from isopentenyl diphosphate. Over 35,000 terpenoid and steroid molecules have currently been discovered. Steroids are modified terpenoids that are biosynthesized from the triterpene lanosterol and have a common tetracyclic carbon skeleton, in contrast to terpenoids, which can have a broad variety of unrelated structures.
2. *Alkaloids:* There are approximately 12,000 known alkaloids, and all of them have basic amine groups in their basic structures, which are biosynthesized from amino acids.
3. *Fatty acid-derived substances and polyketides*: It is estimated that 10,000 different molecules can be biosynthesized from basic acyl precursors such propionyl CoA, acetyl-CoA, and methylmalonyl CoA.
4. *Nonribosomal polypeptides*: Without direct RNA transcription, these amino acid-derived molecules are biologically produced by a multifunctional enzyme complex.
5. *Enzyme cofactors*: Cofactors for enzymes are nonprotein, low-molecular enzyme components (Thirumurugan *et al*., 2018)

Essential oils: EOs are significant secondary metabolic products that have been derived from the leaves, bark, flowers, buds, seeds, roots, stems, and fruits of several fragrant plants. The name "essential oil" comes from the word "essence," which denotes the presence of flavor and scent. It has been established that EOs contain a number of structurally related phenyl propanoids, terpenoids, and low molecular weight lipophilic short-chain aliphatic hydrocarbons. By utilizing hydrocarbons and oxygenated molecules such aldehydes, ketones, esters, oxides, and alcohols, a variety of aromatic plants also actively engage in the creation of EOs (Baldim *et al*., 2019).

A typical extraction method, hydrodistillation, is used to extract oils from plant materials (Silvestre *et al*., 2019). The bioactive components of EOs can produce a fragrance or flavor and are very volatile, producing a strong odor (Smith *et al*., 2005). Among the plant species from which EOs have been isolated are members of the families Asteraceae, Lamiaceae, Cyperaceae, Zingerberaceae, Piperaceae, Apiaceae, Myrtaceae, Solanaceae, Apocynaceae, and Lauraceae.

Essential oils have a limited solubility in water, which is denser than oils but has a high solubility in ether, alcohol, and fixed oils (Dhifi *et al*., 2016; Filly *et al*., 2016). At room temperature, essential oils are typically colorless, liquid and have a distinct aroma. Measurements of the refractive indices and the high optical activity of these volatile liquids can be used to identify them (Dhifi *et al*., 2016).

These aromatic plant extracts contain organic compounds such as carbon, hydrogen, and oxygen, as well as, in certain cases, sulfate and nitrogen derivatives. Essential oils have a modest level of activity in their atomic structure due to the attraction of functional groups by carbon and hydrogen atoms (Moghaddam and Mehdizadeh, 2017). These aromatic liquids are versatile and can take on a number of forms since they include many functional groups, including aldehydes, alcohols, ethers, ketones, acids, amines, sulfides, epoxides, and others (Baser, 2007).

Diverse sources of essential oils:

Based on their fragrance constituents, essential oils comprise a wide range of mixes that can be distinguished. Essential oils come in a variety of forms, such as those from *Azadirachta indica* (neem), *Lavandula angustifolia* (lavender), *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Cinnamomum zeylanicum* (cinnamon), *Syzygium aromaticum* (clove), *Citrus limonum* (lemon), and *Melaleuca alterni* (Bhavaniramya *et al*., 2019). These volatile substances are responsible for regulating microbial development and food preservation. Neem essential oil, for instance, is a volatile combination that is produced from the tree's seed kernels. It smells strongly of sulfur and garlic (Bodiba and Szuman, 2018).

1. **Neem** EO: Neem essential oil considerably increased the antibacterial activity in poly(ethylene terephthalate) polyester fabric, according to a study by Ali *et al.* (2016). Neem has numerous secondary metabolites that can be discovered in different areas of the tree. Azadirachtin, azadirone, gedunin, meliacarpin, nimbin, salannin, and vilasinin groups, among others, were shown to be important pesticides and/or therapeutic principles. Neem crude extracts are shown to be more potent than pure

azadirachtin, indicating that the neem extract contains many additional chemicals with potentiating properties even at low concentrations (Hatti *et al.*, 2014).

2. **Lavender** EO: Steam distillation is used to extract lavender essential oil from the *Lavandula angustifolia* plant. This type of oil contains a number of chemical components, such as B-ocimene, l-fenchone, viridiflorol, camphor, and linalyl acetate (Bhavaniramya *et al*., 2019). Lavender essential oil was employed in starch-furcellaran-gelatin (S/F/G) films to assess their antioxidant, antibacterial, and physical properties in a study by Jamróz, Juszczak, and Kucharek (2018). The ability of packed foods to resist microbial growth and antioxidant damage was simultaneously dramatically enhanced.
3. **Eucalyptus** EO: In vitro and in a real food system, eucalyptus essential oil has the potential to be employed as an antibacterial agent against yeasts that cause food spoilage. The growth of yeast (*S. cerevisiae* SPA) in fresh fruit juices was successfully prevented by using eucalyptus essential oil in conjunction with thermal treatment. There is a possibility to use eucalyptus oil as an antibacterial agent in the preservation of beverages (Tyagi *et al*., 2014).
4. **Tulsi** EO: The storage stability of the product was enhanced via the incorporation of 20% *O. sanctum* leaf extracts in the mango leather at refrigerated temperatures (Jabez *et al*., 2015). Major metabolites in tulsi are eugenol, rosmerinic acid, apigenin and carnosic acid**.** Numerous qualities, including antimicrobial, antifungal, antibacterial, antiviral, antimalarial, anesthetic, antiprotozoal, and anthelmintic agents, are present in *O. sanctum*. Additionally, it contains antidiabetic, antifertility, anti-inflammatory, and antistress properties (Monga *et al*., 2017).
5. **Lemon grass** EO: With some restrictions, *Cymbopogon citratus* essential oil has the potential to be a powerful component in food and chemical preservation. CCEO has properties that extend shelf life due to its physiochemical properties, low mammalian toxicity and quick breakdown in water and soil, which may make it possible to use CCEO or its isolatable fractions in food processing and preservation. Citral, myrcene, geraniol, neral, citronellal, and limonene are some of the secondary metabolites found in *C. citratus.* Lemongrass oil's antibacterial and antioxidant properties can prevent foodborne infections and spoilage organisms from growing and subsequently degrading the food product (Ekpenyong and Akpan, 2015).
6. **Tasmanian pepper EO:** The Tasmanian pepper leaf, which is an Australian plant that is a member of the Winteraceae family, is distinguished by its high concentration of sesquiterpene and monoterpene essential oils (Smyth *et al*., 2012). The primary bioactive ingredient in Tasmanian pepper leaf essential oil, polygodial, is thought to have antibacterial and antifungal properties (Sultanbawa *et al*., 2016).
7. **Lemon myrtle EO**: The Myrtaceae family plant known as lemon myrtle includes citral (82–91%) as its main bioactive ingredient, which has potent antibacterial properties. This essential oil has shown in vitro antimicrobial activity against many yeasts, including the most weakly acid-resistant strain, *Z. bailii* (Pengelly, 2003).
8. **Cinnamon EO**: Numerous antioxidants can be found in cinnamon bark (Dragland *et al*., 2003). Researchers have discovered that the main components of cinnamon are eugenol and cinnamaldehyde, which exhibit antibacterial effects against bacteria, including *Salmonella enterica*, *E. coli*, and *Listeria monocytogenes,* and fungi, such as *Laetiporus sulphurous*, *Coriolus versicolor*, *Eurotium spp*., *Penicillium*, and *Aspergillus spp*. (Zhang *et al.*, 2018).
9. **Coriander EO**: *Coriandrum sativum L*., a member of the Umbelliferae/Apiaceae family, is a useful spice and medicinal plant. This plant's leaves and seeds are frequently used as seasoning, flavoring agents and preservatives in a variety of food preparations and for medicinal purposes. *C. sativum* essential oil and extracts can provide antifungal, antibacterial, and antioxidative actions due to distinct chemical components, such as linalool, camphor, and

cymene (Kačániová *et al.,* 2020). As a result, they can play a crucial role in preserving the shelf life of foods by preventing their deterioration (Pandey *et al*., 2022).

10. **Oregano EO**: Due to its antimicrobial, antidiabetic and antifungal qualities, oregano essential oil (OEO) is widely recognized. Thymol and carvacrol, which have been shown to have antibacterial, antioxidant, and distinctive odor-producing properties, are the two key components that are present. They can slow down the process of scavenging free radicals and lipid peroxidation in fatty diets (Leyva-López *et al*., 2017).

Due to their potential to stop the growth of food-borne viruses, yeast, mold, bacteria, and fungi and preserve food goods, essential oils are appropriate for use in active packaging and food processing and preservation. Essential oils are currently used in active food packaging in the form of films and coatings that are applied to many food groups, including fruits, vegetables, fish, meat, milk, and dairy products, as well as bread and baked goods. The final packaging material microstructure is influenced by the structural arrangement of the vital oil components. Food components, such as moisture, have a significant role in the migration of active compounds from biodegradable materials to food. This migration might speed up the emission of phenolic compounds from active food packaging materials. Essential oils increase the antioxidant activity of packaging materials by acting as oxygen scavengers and allowing the diffusion of active ingredients into coated food products. Essential oils boost the antibacterial properties of packing materials, protecting food from hazardous microbes because they are abundant in bioactive compounds (Sharma *et al*., 2021).

Adverse effects of artificial Sugars:

Carbohydrates account for 40–80% of the total energy intake among macronutrients. Both free and nonfree sugars can be found in foods; nonfree sugars are naturally present within the cell structure, such as sugar in fruits and vegetables, starchy carbohydrates in grains, and lactose in dairy products. Free sugars are those that are present outside of the cell structure. In contrast, free sugars such as disaccharides and monosaccharides (such as glucose and fructose) are frequently added to food and do not occur naturally. On the other hand, consuming too much energy is linked to the buildup of body fat (Onaolapo *et al*., 2020). More precisely, an excessive intake of free sugars increases the chance of developing other harmful health disorders, such as diabetes and cardiovascular illnesses, and reduces micronutrient density (Hagger *et al*., 2017).

The biggest drawback of refined common sugar derived from sugarcane juice is that it lacks extra advantageous components (such as bioactive molecules) that could improve its nutritional value. Brown sugar, molasses, and noncentrifugal cane sugars are byproducts from the refinement of sugarcane juice. Many bioactive compounds, including phenolic acids and flavonoid glycosides, were found in these byproducts (Singh *et al*., 2015). Because phenols and flavonoids have significant dietary effects, additional scientists have since proposed switching to noncentrifugal sugars instead of refined sugars (Cervera-Chiner *et al*., 2021; Lee *et al*., 2018). For the same reason, people are becoming increasingly interested in natural sweetening alternatives.

Alternatives to artificial sugars:

Currently, natural sweeteners can take the place of both sucrose and artificial sweeteners. Consumers may find natural food products more enticing because they see them as healthier alternatives, according to current market trends. The existing pattern suggests that customers are open to experimenting with natural sucrose substitutes (Mora and Dando, 2021). For instance, consumers perceive beverages sweetened with stevia more favorably than they do SSBs in general (Olivo, 2019). Therefore, the use of natural sweeteners may present a novel and sizable financial potential for many businesses. Additionally, the beneficial impacts of natural sweeteners include improved metabolic health, reduced weight gain, and lowered blood sugar levels.

Other benefits include:

1. Honey and agave nectar have low glycemic indices, which may be beneficial for people on low glycemic index diets.
2. Low fructose levels, such as those in maple syrup.
3. It contains nutrients and health-promoting macromolecules (such vitamins, phytohormones, and minerals) (Valle *et al*., 2020).

1. **Honey**: The most popular natural sweetener consumed worldwide is honey. Its usual chemical make-up includes minerals, vitamins, proteins, organic and amino acids, enzymes, and a number of bioactive chemicals. It also contains 60-85% carbs and 12-23% water, which contains compounds such as phenols and flavonoids (Machado *et al*., 2018). In contrast to other natural sweeteners, honey has antioxidant and antibacterial effects. It has been demonstrated that honey can delay or prevent food spoilage because of its oxidative effects.
2. **Molasses**: The term "molasses" is used to describe concentrated sugarcane or sugar beet juice. This sweetener is produced during the crystallization of sucrose, during which leftover syrups acquire crystallization inhibitors (Palmonari *et al*., 2020). The estimated components of molasses are 17–25% water, 30–40% sucrose, 4-9% glucose, and 5-12% fructose, vitamins and amino acids. Additionally, molasses has intriguing food processing qualities, such as the ability to hide undesirable flavors. Molasses has humectant and colligative qualities that lower water activity and increase the shelf life of baked goods (Mordenti *et al*., 2021).
3. **Maple syrup**: *Acer saccharum* Marsh, the most prevalent species of Canadian maple tree, is used to make maple syrup, a natural sweetener (Garcia *et al*., 2020). The antioxidant, antimutagenic, and antiproliferative characteristics of maple syrup in relation to human cancer are due to the presence of phenolic chemicals. Maple syrup is advantageous for type 2 diabetes professionals because it contains carbohydrate hydrolyzing enzymes such α-glucosidase, which has been found to have inhibitory activity against glucose absorption in the gut (Wan *et al*., 2012). Additionally, this sweetener's ethyl acetate-based extracts may be used to treat Alzheimer's disease. In addition, acetate-based extracts also exhibit anti-inflammatory effects.
4. **Coconut sugar**: Natural sweetener coconut sugar contains many carbs. Approximately 15% of the sugar from the inflorescence of coconut palm (*Cocos nucifera* L) is sucrose (Muriel *et al*., 2019). The sugar has a lower glycemic index (GI), ranging from 35 to 42, and a faster rate of digestion. A strong source of vitamins C, B1, B2, B3, and B6, the sugar has approximately 4 kcal per gram. Additionally, it exhibits a low glass transition temperature that is often linked to its fructose, glucose, and sucrose components (Srikaeo and Thongta, 2015 and Asghar *et al*., 2020). The sap from unopened spadices typically has a pH value of 7.0 to 7.3 and is strong in phenolic content and antioxidant activity (Asghar *et al*., 2020).
5. **Agave nectar**: Agave fructan is hydrolyzed to produce agave nectar, often known as agave syrup. The agave core stores nectar, which is its primary carbohydrate resource in the form of fructans (P'erez-Lopez and Simpson, 2020). Nearly 95% of the total soluble solids (TSS) in agave nectar are fructose concentrations, with 5% of glucose and sucrose. In comparison to other sweeteners (honey), syrup has a low glycemic index (17-27) due to the high fructose concentration and fewer calories. Because of this, agave nectar is used as an alternative to regular refined sugar and is suitable for obesity and the prevention of diseases such as diabetes. (Mejia *et al*., 2017 and Ozuna *et al*., 2020), against intestinal infections and the stimulation of the immune system (Catry *et al*., 2018). Additionally, agave syrup has prebiotic effects that encourage the growth of colonic bacteria, making it a good raw material for nutraceutical products.
6. **Date syrup**: The primary product from dates, one of the most common fruit trees (*Phoenix dactylifera* L.) in the Middle East, is date syrup. Date fruit has a high carbohydrate content (70–80% w/w), dietary fiber (8.7%), amino acids, proteins (1.8%), vitamins, salts, and minerals. Its primary physicochemical components are 16% moisture content and 79.5% total sugar, of which 94% is inverted sugar made up of glucose and fructose. The syrup has a high viscosity (17P at 20°C) and 4.1% coloring matter because of the complicated nonsugar molecular mixture. Additionally, the inverted sugar molecules affect the product's acidic elements (pH 3.8), which strengthens its ability to fend against germs (Ghnimi *et al*., 2017). Date syrup has high nutritional profiles, i.e., a

high content of unsaturated fatty acids (such as oleic, linoleic, palmitoleic, and linolenic acids) and a combination of 15 minerals, including potassium, iron, magnesium, and calcium. Additionally, the syrup contains fluorine and selenium, which provide effective tooth decay protection and immune system stimulation. According to Ibrahim *et al*. (2020), it includes at least six vitamins, including B1 thiamine, B2 riboflavin, nicotinic acid, A, and C.

7. **Stevia**: Due to its potential use as a sucrose substitute and wide range of applications as a natural sweetener in commercial food products, rebaudiana has attracted scientific and industrial interest (Bursa'c Kovacevic *et al*., 2018). Known for its great sweetness and possible use in pharmaceutical and therapeutic products, the perennial herb species *Stevia rebaudiana* is native to South America (Lemus-Mondaca *et al*., 2015). Stevia's sweetness is brought on by molecules called diterpene glycosides, notably stevioside and rebaudioside. Stevia's commercial significance in the food industry is increased by its potent sweetness, low calorie count, and cardiotonic, anticancer, and anti-inflammatory characteristics (Mathur *et al*., 2017).
8. **Monk fruit**: Ninety percent or less of the world's extract production comes from the *Siraita gosvernori* species, which is grown in the Chinese region of Guangxi. Monk fruit has historically been used as a natural sweetener and medication for the treatment of pharyngitis (Swiąder *et al*., 2019). It is currently offered commercially as table sweeteners and is typically offered in conjunction with *S. rebaudiana* and erythritol (Soejarto *et al*., 2019). Mogrosides, a class of terpene glycoside chemicals, are what give monk fruit its sweetness. Mogroside V, one of the five mogroside kinds in monk fruit, is the one with the highest concentrations and the highest sweetness intensity (256-378 times that of regular sugar) (Swiader *et al*., 2019). According to the antidiabetic and anticancer actions, mogrosides from *S. gosvernori* also have bioactive qualities (Liu *et al*., 2018). According to Liu (2018), mogroside V specifically causes apoptosis and cell cycle arrest in pancreatic tumor cells and is linked to free radical scavenging activities (Pandey and Chauhan, 2019). By boosting insulin secretion, decreasing lipid peroxidation, and lowering glucosidase activity, mogrosides also cause a hypoglycemic response (Gong *et al*., 2020).
9. **Yacon syrup**: Yacon (*Smallanthus sonchifolius*) is a perennial plant that is indigenous to South America's Andes. This plant's tubers can be used to make juice (or syrup) that can be used as a sugar replacement. Approximately 60% of their dry mass is made up of fructo-oligosaccharides (FOSs) and inulin (Kamp *et al*., 2019). Since human digestive enzymes cannot hydrolyze FOSs and they are not metabolized in the gastrointestinal tract, they are employed as low-calorie sweeteners (Yan *et al*., 2019). Chlorogenic acid, a phenolic and bioactive substance with therapeutic effects as well as antioxidant, antibacterial, anti-inflammatory, and hepatoprotective properties, was found in Yacon syrup. Importantly, the phenolic components in yacon syrup may aid in the prevention of chronic diseases, such as various cancers and cardiovascular diseases (Yan *et al*., 2019). Yacon syrup has also been advocated for diabetes patients as a different natural sweetener. Its extracts have shown inhibitory effects against the enzymes α-amylase and α-glucosidase, preventing the absorption of glucose and lowering postprandial hyperglycemia as a result (Russo *et al*., 2015).
10. **Palm sugar**: The sap of many palm species, including the sugar palm (*Arenga pinnata*), the nipa palm (*Nypa fruticans* Wurmb), and the palmyra palm (*Borassus flabellifer*), is used to make palm sugar, a common natural substitute in Asian nations (Lee *et al*., 2018). Palm sugar has been utilized in a variety of items, including drinks, desserts, and sweet soy sauce (Saputro *et al*., 2019). Lee *et al*. (2018) recently investigated the physicochemical characteristics and chemical content of palmyra palm granulated sugar. They showed a pH value of 6.90 and an overall Aw value between 0.30 and 0.48, which is ideal for long periods of storage. Although almost 91% sugar and approximately 5.6% reduced sugars made up the majority of the examined samples, several minerals (potassium, salt, and iron) were also

found. Additionally, considerable amounts of vitamins E, C, and D were discovered.

Future scope of research:

The future of research on natural preservatives and sweeteners has good potential, as they can improve the food chain, be used in the composition of processed food products, change packaging materials, and be used as coating materials, and natural sweeteners have many health benefits.

Conclusion

Chemicals used as artificial preservatives can be harmful to your health. The negative consequences of these substances in food, cosmetics, and drugs are becoming better known. Due to their nontoxic nature and several health advantages, natural preservatives are superior to their synthetic counterparts. Artificial preservatives to be replaced with better options such as essential oils. People should choose products with natural preservatives and carefully read food, cosmetic, and pharmaceutical labels to achieve and maintain good health. Another harmful additive in processed food is artificial sweeteners. This sugar lacks nutritional value, and an excessive intake of free sugars increases the chance of developing other harmful health disorders, such as diabetes and cardiovascular illnesses, and reduces micronutrient density and fat build-up in the body. Natural sweeteners such as honey, maple syrup, palm sugar, coconut sugar, and stevia also have favorable impacts on intake, including improving metabolic health, avoiding weight gain, and lowering blood sugar. Thus, it is time to start avoiding processed food products with artificial preservatives and artificial sweeteners to maintain a good healthy lifestyle.

Conflict of interest

The authors declare that they have no conflict of interest.

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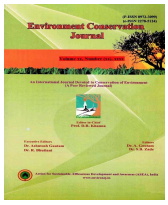

Technology driven livestock farming for food security and sustainability

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ABSTRACT

Advent of industrialization increased the human population significantly and it expanded very rapidly from nineteen sixties. Introduction of mechanization, chemical fertilization and genetic selection in agriculture increased the food production, reduced pestilence and thus improved life expectancy. However, in doing so the natural resources were over utilized, degraded and polluted. The greenhouse gas emissions from anthropogenic activities increased several folds that resulted into global warming, the consequences of which are being observed in the form of floods, draughts, cloud bursts, melting of glaciers, rising of sea level and loss of species. The soil fertility & water table is decreasing, resistance to pesticides, drugs, antibiotics is increasing and immergence & reemergence of diseases are common. Since the world population by 2050 is anticipated to touch 9 billion that means an increase of 30%. Obviously, the demand for food to feed such a huge population would require 70% increase in the food. With limited resources, depleted soil, polluted atmosphere, disturbed ecosystems and exhausted natural resources, the challenges for food security have amplified. Urbanization, improved incomes and dietary changes will increase the demand for food of animal origin in coming years. Globally animal products provide 67% of the protein and the requirement for meat and milk by 2050 is expected to increase by 73% and 58% respectively. Therefore, to ensure food and nutritional security in coming years, livestock production has to be augmented efficiently, smartly and sustainably. As such, precision, smart livestock farming is inevitable that must integrate all the techniques, skills, knowledge and innovations to produce safe, sufficient, affordable, accessible and sustainable animal food with minimum environmental impacts. With the advancement in robotics, biosensors, artificial intelligence, internet of things and information technology, the farming practices should now be technology driven, smart, need based, automated, productive and integrated.

Introduction

Technology as we understand is an album of techniques, methods, skills, and processes in the production of goods and in present case the production of safe, sufficient, nutritious, affordable, accessible and sustainable food for human consumption. Technology required for food security depends on country, culture, literacy, physical environment, climate, infrastructure, economic conditions and governance. Various technologies are involved in increasing the food production that include land preparation, management of soil and water, production of seed, management of farms and livestock, control of diseases, harvesting and post-harvest management like storage, processing, packaging, marketing and distribution. Mankind has been endowed with natural resources like land, water, soil, forest, climate, biodiversity that provides food security,

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nutritional security and of course ensures good health. Agriculture includes arable farming, horticulture, forestry and livestock that are committed to produce food, fiber, forest products, horticultural crops, and their related services, using natural resources for sustaining life. With the rise of sedentary human civilization, the era of agriculture dawned some thousands of years before (Stephens *et al.*, 2019). The gathering and eating of wild-grains began at least 105,000 years ago. Animal agriculture, the livestock has a history of domestication of sheep between 13,000 and 11,000 years ago (Herren, 2012), followed by cattle and pigs some 10,500 years ago (McTavish *et al.*, 2013). Bocquet-Appel (2011) stated that hunting and gathering alone could not have sustained increasing human population but the progress in agriculture enabled the humans grow several folds. With the British Agricultural Revolution in 17th century, the global population rose significantly and since 1900, the mechanization, use of chemical fertilizers, pesticides and selective breeding in agriculture in developed nations increased production of food several folds to feed growing population. The increased food production lead to ecological, environmental, political and economic concerns like water pollution, depletion of resources, climate change, degradation of soil and loss of species diversity. These issues were acknowledged globally and concept of sustainable agriculture was incubated and sustainable development goals (SDGs) were launched by global forum UNO. Sustainability does not mean to reduce production but increase the production with judicial use of resources and their conservation. In order to have sustainable food production, we need to adopt modern technologies, stress tolerant, climate resilient, smart, efficient cultivars/ breeds, along with use of automation, robotics, IOT, and information technologies to push the production curve up to feed ever increasing human population.

Food and nutritional security scenario

More than 11% of global population (811 million) regularly go to bed hungry. Some 193 million people suffer acute food insecurity. About 5,70,000 people are facing catastrophe, starvation and death in 4 countries (FAO, 2022). The main drivers of this global hunger on top are conflicts/wars followed by economic shocks and weather extremes. Moderate to severe food insecurity has been climbing slowly for last few years and 30% of the world population is affected. India that stands 2nd largest populated country has 17% of world population and is home to 194 million people who go to the bed hungry every day. India stands at place 101 in a list of 116 countries of global hunger index with hunger score of 27.5 (Fig. 1; Pampori, 2022). India has the highest burden of neonatal deaths, 45% U5M because of under-nutrition. India's children are amongst the most malnourished in the world, with stunted- 35.5%, wasted- 19.3% and underweight – 32.1% (NFHS-5) as against global average of 22%, 6.7% and 12.6% respectively (Fig.2; Sila Deb, 2022). The malnutrition in children has serious implications of reduced birth weight, lowered resistance to infections, increased neonatal mortality, poor learning and cognitive abilities, poor school performance, reduced work productivity which ultimately results into human capital & economic loss of a country (Pampori, 2021).

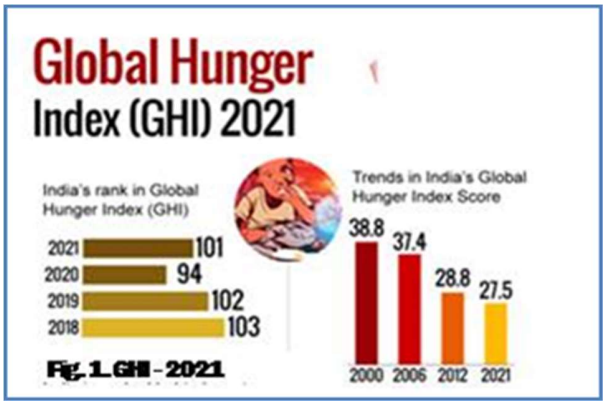


Fig. 1. GHI - 2021

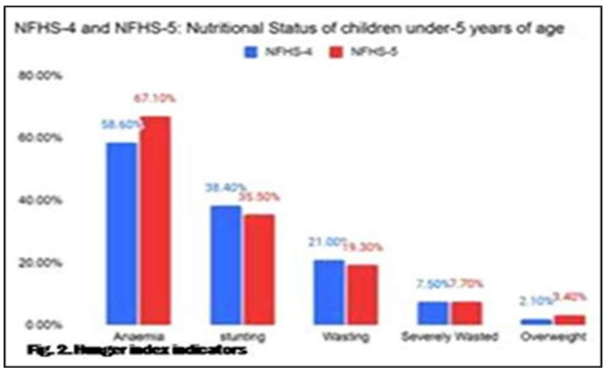


Fig. 2. Hunger index indicators

Livestock in food and nutrition security

Livestock is an important component of State economy and it contributes 40% of global

agricultural GDP and provides over 33% of the world's protein intake and 7% of global kilocalorie consumption. Livestock provides food and nutritional security through meat, milk and eggs. These animal products have high quality proteins with essential amino acids, besides they provide fats and fatty acids, minerals, vitamins, small quantities of carbohydrates and other bioactive components. The demand for food of animal origin is increasing with urbanization, improved incomes and changing dietary preferences. The per capita meat consumption is 42.1 kg/year, a global average, however it goes as high as 82.9 kg/year in developed countries and 31.1 kg/year in developing countries. Milk on the other hand has global per capita consumption of 108 kg per person per year that is below the FAO recommended 200 kg/capita/year consumption. Milk is consumed either as liquid or cheese or milk powder or cream. Malnutrition is common in some poor countries that do not sustain the minimum required levels of meat and milk. The demand for meat and milk in 2050 is expected to swell up by 73 and 58 percent, respectively, from their levels in 2010 because of population growth. About 9 billion human population is expected by 2050 and providing food security to such a huge population will be a great challenge for agriculturalists. Therefore, the priority for livestock producers will be augmenting production, processing and marketing of safe and affordable meat and milk.

Growth in livestock sector in independent India

India has achieved a rapid growth in protein of animal origin through white, red, blue and silver revolution after independence. There has been 287% increase in milk production, 1120% increase in egg production, 147% increase in meat production, 22000% increase in culture fish and 358% increase in capture fish production from nineteen eighties to 2020 in India (Pampori, 2021). The average meat consumption in India stands at 4.5 kg/capita/year as against ICMR recommendations of 11 kg/capita/year, milk consumption of 154 kg/capita/ year and 100 eggs /capita/year against recommended 287 ml of milk and 180 eggs/capita/year. The fish consumption in India stands at 4-5 kg/capita/year as against global per capita fish consumption of above 20 kilograms a year (FAO, 2016). Despite huge increase in production of food of animal origin, the Indian State still has home for hungry people (14% of population). The requirements of protein are being fulfilled by livestock products, however, the availability and accessibility to the livestock products is limited, that leads to hunger and malnutrition.

Strengths & challenges in livestock sector in India

India has 2.3% of global land and 17% of global population and 11.70% of world livestock population (Islam *et al.*, 2016). India has rich animal genetic resources and wide diversity of 50, 17, 34 and 44 breeds of cattle, buffaloes, goats and sheep respectively (NBAGR; http://14.139.252.116 /agris/breed.aspx). However, India is a home to high proportion of low yielding indigenous livestock having 51 % of the cattle non-descript with average milk production of 550 kg per lactation, first calving at 4 years and calving interval of 20-24 months. Similarly, 36% buffaloes are non-descript with very low genetic potential (Prakash, 2021). The poor animal management and care further deteriorate the production, only 30 % of the breedable animals are bred through AI & 70 % through natural service with AI success rate is only 40%. India has 86% of farmers with small livestock holdings of 2-5 animals. Above all, lack of strict and effective breeding policy for domestic livestock in India has remained a big lacuna in livestock sector. In-fact Indian dairy sector is characterized more by 'Production By Masses' than 'Mass Production'. 20% of total milk is handled by the organised sector and 20% equally shared by Co-operatives and Private dairy organizations (Fig.3; Vora & Vishwanath, 2020). 46 % of milk produced is retained by the households or sold to non-producers in the rural areas whereas 54% of the milk in the country is surplus for domestic marketing (Prakash, 2021). Similarly, meat sector in India is not an industry but serves as a supplementary and complementary enterprise with a huge gap in demand and production of meat. Meat purpose breeding is negligible in India that doesn't give boost to meat production. 1% of the total meat produced in the country is used for processing and 99% marketed fresh and hot carcasses. The quality of meat is poor as animals used for meat are spent animals and sold through

local retail shops with no basic facilities for different operations and little awareness about meat hygiene besides inadequate infrastructure. There is dearth of abattoirs and processing plants, besides abattoirs lack basic amenities for hygienic slaughter and proper utilization and disposal of byproducts.

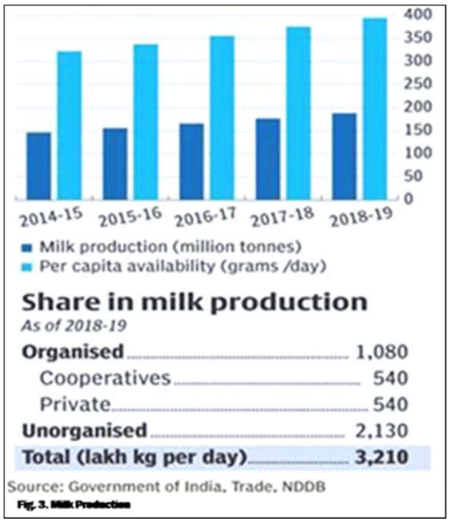


Fig. 3. Milk Production

Technology interventions in food security

In order to have food & nutritional security of large human population, livestock sector in India needs special attention of researchers, industry and policy makers so that production of food of animal origin is augmented significantly, efficiently, sustainably and made available, accessible and affordable through use of technologies and innovative practices – smart livestock farming. The various technologies available need to be roped-up, integrated and applied in livestock sector to have nutritional security. Use of digital technology, big data analysis, robotics and block-chain technology can be a game changer in the livestock sector. Some of the technological interventions in livestock sector to augment production are briefly discussed in present article under various headings.

Biotechnological interventions

Biotechnology is primarily the use of techniques and tools in animals, plants or microorganisms or their parts to produce or modify the products or to improve their characteristics for upkeeping the demands of human beings. Biotechnology has influenced many fields like agriculture, livestock and veterinary, industry, food sciences, pharmaceuticals and medicine. Biotechnology has become an effective and a vital tool for the development of a country world over. This technology requires regulatory policies to avoid threat to biodiversity, human health, the environment and ethical issues (Nguyen & Ly, 2018; Chekoland Gebreyohannes, 2018).

Animal biotechnology provides new and quick opportunities for genetic improvement in the production of farm animals. It can be used in promoting growth, increasing growth rates, improving nutrient intake efficiency, augmenting milk production, fecundity, hair, and fiber, reducing environmental impacts, imparting disease resistance and augmenting reproductive performance (Nguyen & Ly, 2018) (Fig. 4).

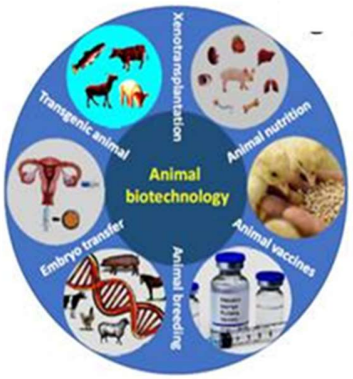


Fig. 4. Spectrum of animal biotechnology

Artificial insemination, *embryo transfer technology, cryopreservation, transgression, transgenesis, gene editing, embryo splitting, cloning* are some of the biological techniques employed in livestock for augmenting production. Artificial insemination and embryo transfers have remained two important techniques in animal breeding to improve the genetics of production traits. Artificial insemination technology supports development of high merit progenies in terms of production, reducing the number of breeding males, minimizing the venereal disease transmission and provides gender choices. While AI exploits male genetics, embryo transfer technology exploits female genetics also with production of high genetic merit embryos and transferring them in surrogates. (Wheeler, 2013; Murray and Maga, 2016). *Semen sexing* technology has provided an opportunity to produce progeny of the desired sex, either male or female. DNA polymorphism evaluation through restriction

fragment length polymorphisms (RFLP) and randomly amplified polymorphic DNA (RAPD) are reliable techniques to estimate the genetic uniqueness of populations that can be exploited for increasing genetic makeup of livestock for production traits, disease resistance, etc. (Yadav *et al.*, 2017).

Molecular-assisted breeding, a new molecular technology will change the traditional approach of phenotyping to genotyping. The genomes of domesticated livestock viz. chicken, pig, cow, sheep, and horse etc. have been completely sequenced (Bai *et al.*, 2012) and employment of molecular techniques will assist in selecting animals for desired traits.

Genomics and Marker-Assisted Selection (MAS) techniques are used in discovery and identification of DNA sequences associated with important animal traits referred to as molecular markers and has applications in trait improvement, heritability determination, and product traceability.

Molecular marker-assisted introgression (MAI) technique enables and guides livestock breeders in selecting individuals that are expressing the introgressed gene. Marker-assisted selection can be used to augment beneficial traits in livestock like controlled growth rate, disease resistance, heat and cold tolerance, lower cholesterol in eggs, and an increased lean-to-fat ratio in pigs. The use of molecular markers reduces the time incurred in selection and identification of the desired individual in contrast to conventional method of several backcrossing cycles. In livestock trait improvement, molecular markers are now being used for important traits like growth, meat and wool quality, milk quantity and quality, and disease resistance. Use of molecular markers by regulatory bodies serves as an important tool in ascertaining product quality and food safety. It is also a useful technique in identification and tracking livestock parentage and its products from field to the slaughter house and from carcass to the kitchen.

Somatic cell nuclear transfer (SCNT) technology has revolutionized the field of reproduction and reproductive biotechnology. The technology has been employed in producing clones of high merit animals and has also greatly facilitated the transgenic modification of animals. Cloning technology through SCNT will help livestock farmers to replicate animals of high genetic value as a seed-stock. The SCNT technology can be used to assess the interactions of genotype v/s environment efficiently. Transgenesis has opened an effective window of using transgenic farm animals as biological factories to produce high value products or pharmaceuticals or hormones in their milk and is known by "Gene Farming". Transgenic technology involves inserting of a desirable gene or set of genes from one animal species to another to get them expressed in transgenic animal in their production system and many genes for hormones, pharmaceutical peptides have been constructed and engineered to get expressed in the milk of mice, rabbits, sheep, goats, swine, and cattle. Similarly, micro-organisms have been used to produce hormones or pharmaceuticals in bulk through this transgenic recombinant technology, the best example is of *E. coli* that has been transgenically modified with the genes from a cow to produce bovine somatotropin hormone (BST), injections of which in dairy cattle enhance milk production. C1 inhibitor and antithrombin, produced through gene farming are now commercialized (Bosze and Hiripi, 2012). Transgene for lysine biosynthesis into a pig genome, or lysostaphin in cows have been expressed to eliminate supply of lysine in pig ration or resistance to *Staphylococcus aureus* in cow to reduce mastitis.

Research in omics like genomics, transcriptomics, proteomics, glycomics, metabolomics, pharmacogenomics, toxicogenomics and ionomics are seen as the future important target areas in future drug designing or personalized medicine. The omics research would become exceptional tool in understanding diseases and developing new drugs (Dunisławska *et al.*, 2017). Omics can be implemented in improving traits of production and abiotic stress tolerance, enhancing resistance to pests and diseases and improved product quality. Omics can be helpful in the understanding patho-physiology of livestock diseases, thus providing opportunity to improve the livestock health and production. Technology driven livestock farming will be taking advantage of the developments in molecular biology, biotechnology or bioengineering to speed and scale up the development of highly productive and healthy animals. *CRISPR-Cas*

technology offers great opportunity to reap the rapid changes in economic traits of any living organism including livestock. It presents a window to modify existing characteristics or incorporate novel traits into an organism within a short period of time. Genes that negatively regulate the economic traits can be silenced/ knocked down using CRISPR technology with considerable success rates. Increasing muscle mass in animals has been achieved by introducing CRISPR-based mutations in myostatin genes (Firdous, 2021).

The advanced biotechnological tools and techniques are effectively used in pathogen detection and characterization in infected hosts that facilitate early detection of diseases and their effective control. *The recombinant DNA* technology, is being now employed in the production of effective vaccines and the diagnosis of infectious diseases and their prevention (Nascimento and Leite, 2012). In recent years, recombinant DNA technology has been used widely in the food, dairy, and brewing industries through modification of genetic material of bacteria, yeasts, and moulds. Recombinant technology and protein engineering has been used to develop novel enzymes with modified structures for thermal stability, substrate specificity, or the ability to work under extreme conditions (Gurung *et al.*, 2013). Metabolic engineering, an important biotechnological tool, is in offering to produce valuable metabolites or natural secondary compounds in large amounts that are otherwise difficult to extract and purify from their natural sources. The technology is available for the production of antibiotics, vaccines, vitamins, enzymes, and other useful bioactive products that improve the animal health and consequently their production (Tang and Zhao, 2009).

AI, IoT, ML, Robotics & ANN

Artificial intelligence (AI), internet of things (IoT) and machine learning (ML) have been inviting the attention of agriculturalists and livestock managers to make a breakthrough in livestock management. The technology involves connecting of biological and environmental information of livestock obtained by IoT sensors, storing the data in cloud, analysing, diagnosing and changing it into simple and valuable information through artificial intelligence, making informed decisions and subsequently relayed to the farmers or automatically controlled system for effective implementation (Fig. 5). Sensors record the physiological parameters of every single animal, AI diagnoses possibility of disease and accordingly informs the farmers, and farmers can take timely necessary care of the livestock. This helps to improve production efficiency and reduce physical labour and labour cost. It can be a best innovative intervention in transforming every field of livestock. It will equip the farmer with forecasting skills of the highest grade for prompt prediction of

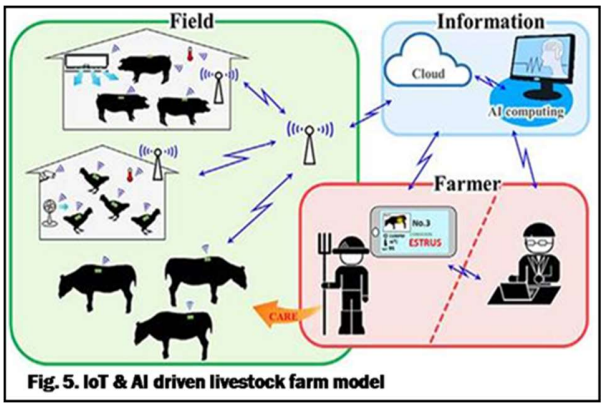


Fig. 5. IoT & AI driven livestock farm model

disease outbreaks, timing of various livestock practices - "Smart cow-house". It will enable farmers to improve the quality, quantity of livestock products and ensure their timely marketing. Through IoT & AI technology, farmers can formulate rations depending upon the production, functional state, weather, environment, behaviour of livestock and available resources. IoT-AI-ML technology can be mobilized for improving farm economy through prediction of customer preferences, precise prediction of shelf life of products to reduce the post-harvest losses. Blockchain technology is one more sensitive technology that ensures and guarantees traceability of animal products from farm to fork, thus providing a dynamic system of disease outbreaks monitoring and consequently preventing the related economic losses and food-related health pandemics. Use of robotic milking machines, automatic calf feeders or brushes for added cow comfort in dairy farms can make farming smart, efficient, more profitable, sustainable and green. For small farmers, use of robotics, drones, sensors and radio imaging technology may be very helpful in Indian conditions. Brainwired is an Agritech startup based

out of India, which has developed a livestock health monitoring, and tracking system named "WeSTOCK". This digital system uses the AI & IoT technology to record all the activities of individual animals, body temperature, behaviour besides environmental physical parameters and then through computing, predicts reproductive cycles, disease or sickness. Remote sensing technology, global positioning system (GPS), geographic information system (GIS), satellites can now be integrated and used in assessing the needs of livestock thus can help in efficient & sustainable utilization of resources, reducing the input costs with efficient production. The automated milking system was developed and commercialized in 1992 and De Koning (2010) reported that the automated milking system can manage larger herds with less physical labour and labour cost. Mastitis can be detected using AI & IoT technology with milking machines and in-line monitoring for automatic milking system can reduce the chances of mixing of contaminated milk with whole lot of safe milk thus minimising the economic loss (Hassan *et al.*, 2009; Steeneveld *et al*., 2010). Farmers can now provide animals with feeding mixtures and amounts tailored specifically to their needs or at least tailored to a herd or group using automated feeder systems. While employing the AI-IoT-ML technology we need to put continuous efforts to refine the exactness of measurements, requirements as well as focusing on physiological and biological considerations. The employment of AI-IoT-ML technology demands a closer coordination and a methodological linkage among livestock /animal, engineering and computer sciences. Similarly, use of automated cleaning systems in smart farms will help to remove waste and runoff from animal sheds, be it a pen or stall, and move it to a pile. The cleaning technology will reduce disease occurrence and shall create a cleaner environment for the animal besides minimising the contact of farmers with the waste.

Innovative interventions in reducing post-harvest losses and animal waste to wealth

i. Use of Apeel - A natural ingredients obtained from plants help in maintaining the quality of agricultural produce and greatly reduce food, water and energy waste during its sojourn from farm to kitchen. Since consumers are now more health conscious so the chemical preservatives are not liked, therefore, safe, organic alternatives are to be introduced in preserving food products.

ii. Cold sterilization - Maintenance of cold chain to prevent the post-harvest losses will be an effective measure to increase farm income, reduce environmental issues and sustain natural resources. Presently available cold storage capacity in India is less than the half of the required, only 29.7 million tons cold storage capacity as against 61.7 million tons (Pampori, 2021). Similarly, the transportation system in India is not much effective and needs more efficient, automated and programmed. Studies have shown that farmers have low price realisation and there is a huge wastage in the supply chain due to fragmentation, poor storages, inefficient information flow (Pingali *et.al*., 2019).

iii. Waste to wealth/ Circular bioeconomy - Circular bio-economy minimizes the leaks of energy and materials from the system by re-cycling them in production with alternate uses of food waste. The projections of 30% increase in human population by 2030 with more liking for processed foods will result into production of large amounts of agro-industrial by-products which could become an environmental burden. Therefore, the technologies of feed processing and manufacturing that can utilize the increased share of by-products or waste in livestock feeding will be promoted and needed. The livestock intensification that can be the only way to provide food security to increasing human population has resulted into production of huge amounts of animal manure. Animal manure on other side is associated with production of green house gases and source of parasites and pathogens which could lead to environmental issues and water pollution. Thus the introduction of appropriate management technologies that could mitigate the health and environmental risks associated with the overproduction of organic wastes will be welcome step. Composting and vermicomposting have become the two best-known environmentally appropriate technologies for the recycling of manures under aerobic conditions (Bernal *et al*., 2009; Domínguez and Edwards. 2010), by transforming them into safer and more stabilised products (compost and vermicompost) that has huge application in agriculture to increase soil fertility and hence production. Technologies like

biogas fermenters are being employed in recycling and recovering nutrients and energy from animal waste thus reduce environmental pollution. Anaerobic digestion reduces the risk of water pollution from manure slurries and also improves human/farm cohabitation in rural regions by reducing odour emissions by 70–95% (Massé *et al*., 2011). This process has other direct advantages of biogas production as renewable energy and the fortification of mineral fractions of N and P during digestion (Insam and Wett, 2008), resulting in a more balanced nutrient mix for plants as compared to undigested manure thus helps in more food production(Lied *et al*., 2006) (Fig.6)

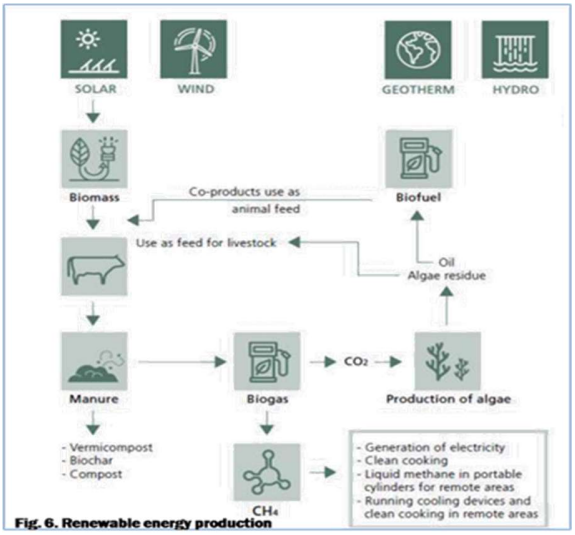


Fig. 6. Renewable energy production

iv. Slaughter house solid waste disposal - Solid waste generation is an alarming global issue associated with considerable rise in population, industrialization and urbanization. This waste has a tremendous pressure on the environment and public health and creates several environmental (water, air, and soil pollution) and health issues (waterborne diseases and respiratory illness), besides contribute 3% of global greenhouse gas emissions (Tahir *et al.*, 2015). Presently, the solid waste generation is estimated to be about 11.2 billion tons per year worldwide which is estimated to increase to 19 billion tons per year by 2025 (UNEP, 2021). Generally, slaughterhouse wastes are animal byproducts that remain unutilized after slaughtering and it has been estimated that about 30% to 50% of the total weight of slaughtered animal remains as slaughterhouse waste in livestock and poultry industry (Adhikari *et al.*, 2018; Meeker, 2009) that are well-recognized drivers of GHGs.The rising population and increased liking for animal foods ultimately increases the abattoir waste especially in urban areas. The management of wastes from slaughterhouses becomes a huge challenge that needs to be accounted for. In India, almost 3/4th of budget allocated to urban solid waste management is utilized for waste collection and transportation with little actually left for the effective treatment of solid wastes (Lahiry, 2019). Segregation of organic wastes collected from slaughterhouses or abattoir shops or centralized markets from other inorganic waste fractions is the main issue with solid waste disposal. As such mechanized slaughter houses with modern, hygienic processing units and efficient mechanism of slaughterhouse material collection, segregation, utilization and disposal is priority.The wastes from slaughterhouses and abattoir shops have huge potential for energy and product recoveries like protein hydrolysate synthesis, enzymes, and lipids. However, proper collection and treatment is of prime importance in order to harvest their maximum potency. Alonge (2005) has estimated that about 50–54% of each cow, 52% of each sheep or goat, 60–62% of each pig, 68–72% of each chicken, and 78% of each turkey is utilized for meat and the remaining is disposed off as waste. The slaughterhouse waste is majorly comprised of rumen (80%), dung/manure (12%), blood (5%), and others (3%) by weight (Fig. 7). The slaughterhouse waste disposal is through conventional methods of dumping, land filling, composting or incineration, however, anaerobic digestion is environment friendly and more valuable as it produces renewable energy. The slaughterhouse waste is produced in huge quantities and is increasing every other day, therefore some innovative technologies are always welcome in this sector to reduce environmental and health issues arising from the waste and at the same time increases the economy through its utilization. The slaughterhouse and poultry wastes are growing renewable energy resources and the production of renewable energy from such wastes will reduce the fossil fuel share in total energy supply and resultant reduction in carbon dioxide emissions. Therefore, research focussed on eco-friendly and sustainable energy from waste biomass is necessitated to

replace conventional fossil fuels (Demirbas *et al.*, 2009). Since both these wastes are rich in protein content, hence could be an ideal substrate for biofuel production. Several new innovative technologies have been researched and adopted and need to be strengthened. The few are described hereunder.

Biodiesel production Biodiesel is a green diesel, renewable energy, a replacement for petroleum diesel derived from animal fat, plant and vegetable oils through transesterification of long chain fatty acids producing methyl, ethyl or propyl esters.

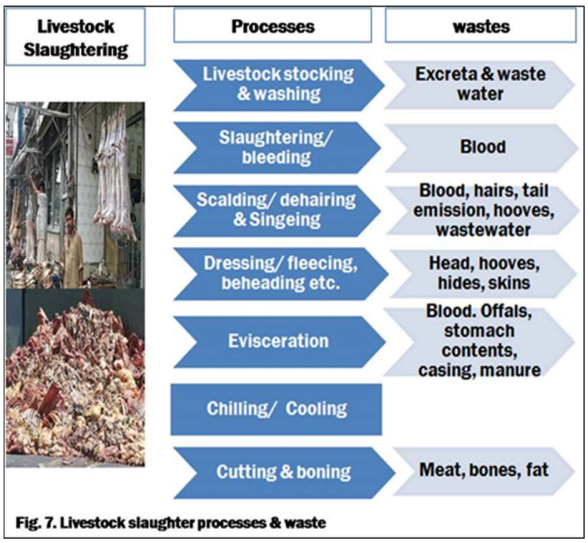


Fig. 7. Livestock slaughter processes & waste

These biodiesels can be blended with petroleum diesel or used in pure form as a renewable energy source. Further, the glycerol that is by-product of the trans-esterification process, can be used as a potential raw material for the synthesis of various chemicals, biodegradable polymers and also for energy production (Ashby *et al.*, 2009). Processes involved in biodiesel production from animal waste are shown in Fig. 8. Therefore, the technology of biodiesel production from slaughterhouse material can prove a vibrant technology in coming days reducing dependence on fossil fuel with resultant lower GHG emissions.

Pyrolysis: A process of thermal decomposition of organic wastes in absence of oxygen in a controlled environment also termed as devolatization. During the combustion at high temperature the organic waste is reduced to stable solid carbon residue called as bio-char that can sustain in soil for

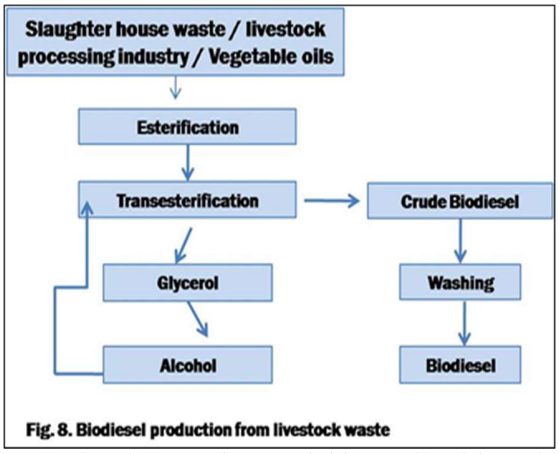
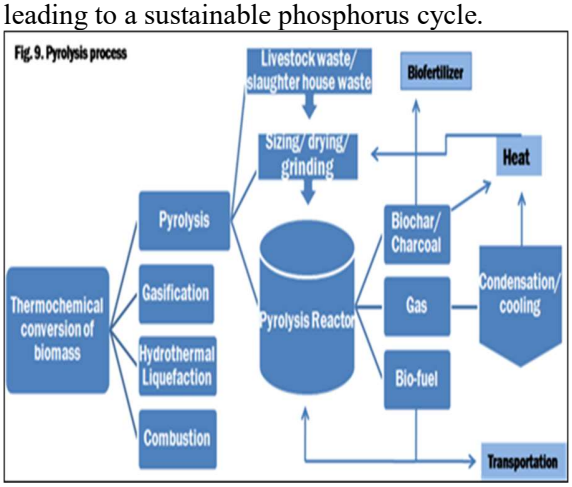


Fig. 8. Biodiesel production from livestock waste

thousands of years thus enriching soil with carbon and as a result increases production. Pyrolysis besides producing biochar also produces liquid (bio-oil) and gaseous products (Fig. 9). Pyrolysis of slaughterhouse wastes could also help in recovering phosphorus especially from bone char that can be used to produce phosphorus rich fertilizers hence leading to a sustainable phosphorus cycle.



Hydrothermal carbonization (HTC): A thermo-chemical treatment process where waste biomass is heated under low-temperature with pressurized water to produce a value added carbon-rich hydrochar material besides gaseous and water soluble products through hydrolysis, dehydration and decarboxylation processes. This method of converting waste biomass into a valuable solid hydrochar has an advantage over pyrolysis of using wet waste without pre-drying it. The carbon-rich hydro- char solid materials have high heating value, thermal stability, and the hydrophobic structure that makes their use in applications like solid fuels, adsorbents to remove pollutants (Kim *et al.*, 2018).

HTC further promotes nutrient recovery as both the hydrochar and the processed water contain essential nutrients like nitrogen, phosphorus and potassium that are very much important for plant growth.

Extraction of keratin/protein: Keratin is a natural structural fibrous protein also referred as scleroprotein found in vertebrates making up scales, hair, nails, feathers, wool, horns, claws and hooves and outer layer of skin in mammals, birds and reptiles. Keratin hydrolysate has been used in shampoos for strengthening the hair and keeping its good appearance. It has several applications in pharmaceutical, biomedical, food, and cosmetic industries. Since, the feathers are rich sources of keratin, poultry feather waste has great utilization potential in various applications (Li, 2019).

Production of fibre board: Fibreboard is an engineered wool product that is made of wood fibres. Research is being carried out for the fabrication of fibreboards from mixed waste poultry feathers and wood residues (Safari *et al.*, 2020). Fibreboard samples were prepared by mixing feathers with wood shavings (coarse structure) or mixed wood residues (finer and denser structure) in different proportions. Similarly, Bessa *et al.* (2017) studied the use of chicken feather fibres in the strengthening of polymeric matrices and reported the suitability in terms of good acoustic and thermal insulation. Therefore, the technologies or innovations in utilizing the solid organic animal wastes in production of fibreboards, mattresses or other insulating materials will be invited in coming years as the food of animal origin will be in great demand hence more waste production.

Fabrication of bioplastic sheets: Synthetic plastic use is now restricted due to its non-degradability and environmental issues, hence, are gradually being replaced by bioplastic materials. Bioplastic sheets are biodegradable in which degradation results from action of naturally occurring microorganisms. Slaughterhouse/poultry wastes are one of the renewable sources of protein that can be used for the fabrication of bioplastic films. Lukubira and Ogale (2013) evaluated the effect of chemical modification of plasticized meat and bone meal (with a composition of 4–7% moisture, 50% protein, 8–12% fat, and 35% ash) by the calcium hydroxide on bioplastic sheets for geo-structural uses. However, the bioplastic sheets thus produced were weak as compared to synthetic polymers in tensile strength. Extensive research is required to utilize the organic animal solid waste in biodegradable plastic with good tensile strength to replace synthetic plastic.

Innovative use of smart phone technology

Phenomics and its recording are not at its best in India. This is one of the major factors that limit the application of scientific breeding and management in Indian agriculture.

Several Android software applications and kiosks have been developed in India aiming at efficient knowledge dissemination to farmers. This technology post Covid has been used efficiently in telemedicine and information dissemination. Many Apps have been developed to benefit farmers providing them weather information, advisories for management practices, disease prevention, disease outbreaks, vaccination and market trends.The increasing human population demands more food production. Urbanization and improved incomes have shifted dietary preferences more towards food of animal origin. It will be essential to concentrate in livestock production systems to fulfil the demands of people and ensure their food & nutritional security. Livestock farming has been caught into a mess of greater contributor of climate change, hence livestock farming demands practices that are aimed to decrease carbon foot prints in its production system. Therefore, use of emerging technologies/ innovations like sensor based automation, robotics, AI-ML, IoT, ANN, smart phone in farm practices and similarly, technologies to minimize the waste and convert it to wealth through use of innovative techniques are inevitable.

Conclusion

It is clear beyond doubt that conventional livestock farming is not going to sustain safe, clean, sufficient, affordable and accessible food security. Ever increasing, health-conscious human population, shrinking agriculture land, soil degradation, environmental pollution and climate change are big challenges in providing sufficient and safe food and to alleviate the malnutrition. The introduction of mechanization and biotechnological tools in agriculture food production systems has increased food production tremendously and supported the everyday increasing human population. However, in this race the natural resources were not judicially used and thus resulted

into depletion of resources and degradation of soil. The use of modern tools and techniques in sustainable food production is inevitable to ensure food & nutritional security. New evolving technologies like internet of things, digitization, sensor technology, artificial intelligence, machine learning, robotics and data analysis have emerged as game-changer technologies that are affecting every sphere of life and have resulted into a significant shift in the ways the things were done. Livestock sector cannot afford to continue with all those traditional systems of farming, when the demands for food of animal origin are increasing continuously. Therefore, use of emerging technologies in the livestock farming and food production systems is obvious and inevitable to have sustainable food and nutritional security of increasing population and respecting the use of natural resources

Conflict of interest

The authors declare that they have no conflict of interest.

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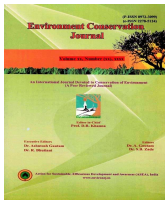

Conventional and molecular breeding strategies for improvement of drought tolerance cultivars in rice: Recent approaches and outlooks

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QTLs

ABSTRACT

Rice is a vital staple food, especially in Asia, but it is highly susceptible to drought, leading to significant yield losses. To ensure food sustainability, drought-tolerant rice varieties are essential. Conventional breeding methods improve drought tolerance by focusing on biometric traits like root depth, avoidance, escape, and tolerance. This involves screening and crossing drought-tolerant varieties with high-yielding ones, followed by selection and evaluation. Techniques such as pedigree selection, recurrent selection, and backcrossing introduce desirable genes to enhance drought tolerance. Induced mutation through radiation exposure is also used. The molecular basis of drought tolerance involves identifying and manipulating genes responsible for rice's response to water stress. Techniques like QTL analysis, transcriptomics, genomics, and proteomics identify genes and QTLs associated with drought tolerance. Important genes involved in drought response include DREB, LEA, and ROS scavenging genes. Identifying QTLs enables the development of molecular markers for efficient screening of drought-tolerant rice genotypes. In conclusion, conventional breeding and molecular approaches are employed to develop drought-tolerant rice varieties. Conventional breeding improves biometric traits, while molecular techniques identify and manipulate specific genes associated with drought tolerance. This combination holds promise for high-yielding and drought-tolerant rice cultivars, contributing to global food security. However, further research is needed to understand the complex genetic mechanisms underlying drought tolerance in rice and enhance breeding precision and efficiency.

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than one-third of the global population, with Asia being the leading producer and consumer of rice. Rice provides 80% of daily caloric needs for many

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people (Samal *et al*., 2018, Panda *et al*., 2021). However, rice is highly vulnerable to drought due to its tiny root system, thin cuticular wax, and quick stomata closure. Drought is one of the most destructive abiotic factors that can lead to complete yield losses, particularly during the reproductive development stage (Turral *et al*., 2011, Oladosu *et al*., 2019, Simkhada and Thapa, 2022). To achieve self-sufficiency in rice production and ensure food sustainability for the expanding population, there is a need to develop high-yielding rice varieties that are tolerant and resistant to both biotic and abiotic stressors, including drought (Asim *et al*., 2021, Gupta *et al*., 2020, Myers *et al*., 2017, Kaya *et al*., 2013). The increasing severity of droughts and the lack of high-yielding cultivars for drought-prone regions are the main challenges limiting rice output globally (Gupta *et al*., 2020). Progress in breeding drought-tolerant rice has been modest in the past due to the limited number of drought-tolerant variations discovered after screening a large number of germplasm samples (Myers *et al*., 2017, Pandey *et al*., 2015). Rice cultivation is seasonal and could be improved by developing drought-resistant rice cultivars and sustainable farming techniques (Singh *et al*., 2016).

The scarcity of high-yielding genotypes that can grow in drought-prone environments and the increasing severity of droughts are the two most significant limiting factors for low rice production globally (Oladosu *et al*., 2019, Pandey *et al*., 2015, Chaum and Kirdmanee, 2010). Breeding drought-tolerant rice cultivars has been challenging due to the quantitative and complex nature of the trait. Researchers have screened thousands of germplasms from different parts of the world and identified only a few drought-tolerant variants (Melandri *et al*., 2020, Cicek *et al*., 2012). The success of breeding drought-tolerant rice is hindered by the lack of appropriate screening techniques and a shortage of donors with a high level of drought tolerance. This review chapter aims to explain how drought stress affects rice plants and to highlight current developments in rice's molecular adaptation to drought tolerance. Additionally, the review describes the current process for creating a long-lasting rice variety that is drought-resistant through conventional breeding and the application of biotechnological tools. Lastly, the review conducts a thorough analysis of the information that is currently available on drought-resistant genes/QTLs, QTL analysis, gene introgression, and marker-assisted selection.

Conventional breeding approaches for developing drought-tolerant rice focus on improving biometrical traits

Conventional breeding approaches for improving biometrical traits such as root depth, drought avoidance, drought escape, and drought tolerance. These approaches include, screening of rice germplasm for drought tolerance is the first step in conventional breeding for drought tolerance. Rice breeders cross drought-tolerant varieties with high-yielding varieties to develop hybrid cultivars with both yield and drought tolerance. Rice breeders select the best plants from a population based on their drought tolerance and yield performance, and cross them to produce the next generation. The best drought-tolerant plants are crossed back to the original high-yielding variety to improve the yield of the drought-tolerant cultivar. Rice breeders evaluate the phenotype of the plants to select the best ones for further breeding. The final step in conventional breeding is to evaluate the performance of the drought-tolerant cultivars in field trials under drought conditions, these approaches are discussed below.

In conventional breeding methods, grain yield was traditionally used as a selection criterion for drought-resistant crops, but this has proven to be ineffective due to low heritability and strong genotype by environment interaction (Upadhyaya *et al*., 2019, Chourasia *et al*., 2017). Instead, selection has shifted towards physiological traits as they have a more direct link to crop performance and depend more on genetic variation (Chourasia *et al*., 2017, Atkinson *et al*., 2012, Dixit *et al*., 2014). The ultimate goal of crop breeding is to create high-yielding varieties in ideal water conditions, but well-yielding varieties can still maintain moderate to high yields during drought conditions (Khush *et al*., 1984).

The general selection process for developing drought-tolerant rice involves the use of pedigree selection, which is a traditional and popular breeding technique (Tatar *et al*., 2010, Khush *et al*., 1984). This approach is suitable for building resistance in rice when the trait is controlled by important genes. The main advantage of pedigree selection is the ability to combine numerous genes

affecting biotic and abiotic processes. However, it is a time-consuming process that requires evaluating numerous lines repeatedly over planting seasons while maintaining a record of the selection criteria. The approach is not appropriate for traits influenced by multiple genes, in which case the diallel mating design is more suitable for selection (Khush *et al*., 1984). Recurrent selection is a preferred method over pedigree selection in the development of drought-tolerant rice and other self-pollinated crops (Miah *et al*., 2013, Maqsood *et al*., 2013). This method is used to increase favourable allele frequencies while preserving genetic diversity. Recurrent selection offers more accurate genetic gains, faster and more defined breeding cycles, and the creation of highly diversified breeding lines. It has been extensively used in rice breeding and has been shown to be more effective than pedigree selection (Pang *et al*., 2017). The backcrossing technique is frequently employed in rice breeding to introduce desirable genes from the donor parent to the recipient parent. This method provides a precise and accurate way to create multiple superior breeding lines, and has been used to create rice cultivars that are drought-tolerant (Lafitte *et al*., 2006, Oladosu *et al*., 2014, Oladosu *et al*., 2015, Oladosu *et al*., 2016).Induced mutation is a technique used to supplement conventional breeding methods in the development of drought-tolerant rice. It has been shown to be effective in improving traits such as grain yield (Oladosu *et al*.,2014, Oladosu *et al*., 2015, Oladosu *et al*., 2016), resistance to pests and diseases, and physical grain quality. The main advantage of induced mutation is the ability to create new gene alleles that are not found in nature. This method has been used to create innovative rice varieties with improved characteristics. For example, exposure of Manawthukha rice to gamma radiation resulted in the creation of two mutant lines, *MK-D-2* and *MK-D-3*, which were determined to be drought-resistant after six generations of evaluation and selection. In Iran, 11 lines with drought-tolerant traits were chosen from the '*Tarom Mahalli*' rice landrace after exposure to gamma radiation. In Indonesia, induced mutation was used to create a super green rice mutant that is drought-resilient, high-yielding, and water-efficient (Hallajian *et al*., 2014). In Malaysia, two improved lines with high production potential and drought tolerance were developed from the common *MR219* rice variety (Gosal *et al*., 2009).These conventional breeding approaches have been successful in developing drought-tolerant rice cultivars, but they are time-consuming and have limited precision. New biotechnological tools are being developed to improve the efficiency and accuracy of breeding for drought tolerance.

The molecular basis for drought tolerance in rice

The molecular basis for the improvement of drought tolerance in rice involves the identification and manipulation of genes that regulate the plant's response to water stress. This is achieved through the use of various molecular techniques such as quantitative trait locus (*QTL*) analysis, transcriptomics, genomics, and proteomics (Fahliani *et al*., 2011, Zargar *et al*., 2011). In summary, the molecular basis for improving drought tolerance in rice involves the identification of genes and associated qualitative trait loci (*QTL*s) that are responsible for the trait and *QTL*s list presented in (Table 1). This can be achieved through DNA marker-based phenotyping studies and screening of large collections of germplasm (Kumar *et al*., 2017, Upadhyaya *et al*., 2019, Barik *et al*., 2019). The identified genes can then be introduced into the genetic background of suitable cultivars through genetic engineering techniques like Agrobacterium tumefaciens or gene gun, and hybridization with marker-assisted selection (Gosal *et al*., 2009). The goal is to create transgenic crops with improved drought tolerance and improved yields, ensuring high agronomic validity and safety.

***QTL*s associated with rice drought tolerance**

The plant genome has a number of genes known as *QTL*s that have extremely precise quantitative properties. (Table 2) displays many *QTL*s connected to various agronomic traits under drought. However, finding these *QTL*s for drought tolerance is not a simple task, as it involves complex interactions between different genes, and their effects on various physiological and biochemical processes in the plant (Dixit *et al*., 2014, Barik *et al*., 2019). Therefore, a combination of various molecular markers, phenotypic assays, and genomic data is used to identify and validate these *QTL*s for drought tolerance in rice (Singh *et al*., 2016).

Table 1: List of rice quantitative trait loci (QTLs) associated with various drought tolerance traits

Traits	QTLs	References
Grain yield (GY) and flag leaf (FL) photosynthesis under drought stress	*qDTY1.1, qDTY3.2, qDTY10.1, qDTY1.2, qDTY1.3, qDTY2.2, qDTY2.3, qDTY3.1, qDTY6.1, qDTY6.2*	Vikram *et al*., 2016
Leaf rolling (LR)	*qlr8.1, qLR9.1, qDLR8.1*	Lin *et al*., 2007
Leaf drying (LD)	*qLD9.1, qLD12.1*	Barik *et al*., 2019
Harvest index (HI)	*qHI9.1, qSf6, qPNF3.1*	Barik *et al*., 2019
Spikelet fertility (SF)	*qSF9.1*	Barik *et al*., 2019
Panicle number (PN)	*qgy3.1*	
Plant height (PH)	*qPH1.1*	Trijatmiko *et al*., 2014
Flowering day (FD)	*qHGW2.2*	Trijatmiko *et al*., 2014
PN & grain weight (GW)	*qGy7*	Trijatmiko *et al*., 2014
Panicle length (PL)	*qPL-9*	Sellamuthu *et al*., 2015
Grain number (GN)	*qDTY8.1*	Trijatmiko *et al*., 2014
Relative water content (RWC)	qRWC9.1	Vinod *et al*., 2019
Transpiration, root growth, number (RN) and length (RL) root volume	*qDTR8*	Xu *et al*., 2016
Shoot growth and biomass accumulation	*qSW5*	Vinod *et al*., 2019
PN & grain size (GS)	*qTL33*	Vikram *et al*., 2016
FL area and stomatal conductance	*qLPD6*	Vikram *et al*., 2016

This multi-disciplinary approach has enabled the identification of several drought-responsive genes, such as *DREB, LEA*, and *ROS* scavenging genes, that play key roles in drought tolerance (Dixit *et al*., 2014, Barik *et al*., 2019, Ramchander *et al*., 2016, Lin *et al*., 2007). By using these genes as targets, plant breeders can create more drought-tolerant rice varieties through genetic engineering or marker-assisted breeding (Pandey *et al*., 2015). The ultimate goal of this research is to provide improved rice varieties to farmers, particularly in areas that are frequently affected by drought, to enhance food security and reduce poverty.In conclusion, identifying *QTL*s associated with drought tolerance in rice is an important step towards creating drought-resistant rice varieties. The majority of the *QTL*s discovered so far come from non-elite genotypes, including *qDTY1.1* (Barik *et al*., 2019), *qDTY2.1, qDTY2.2* (Dixit *et al*., 2014), *qDTHI2.3* (Usman *et al*., 2017), *qDTY3.1, qDTY6.1* (Ramchander *et al*., 2016), *qDTR8* (Lin *et al*., 2007), *qLR9.1, qLD9.1, qHI9.1, qSF9.1*, and *qRWC9.1* (Barik *et al*., 2019). These *QTLs* control various morpho-physiological traits, including leaf rolling, leaf drying, harvest index, spikelet fertility, and relative water content. The use of DNA markers such as SSRs associated with these *QTL*s can aid in molecular screening of new rice genotypes for drought tolerance (Usman *et al*., 2017). This would result in a faster and more accurate profiling of rice lines, leading to the development of drought-resistant rice cultivars with high yield and agronomic validity.

Rice drought tolerance via transgenic/genetic engineering and genetic methods

For instance, overexpression of the rice *WRKY* transcription factor gene *OsWRKY22* improved drought tolerance by regulating stress-responsive genes, including those involved in water uptake, water transport, and energy metabolism.

Table 2: QTL/genes and their contributions to phenotypic variation in drought tolerance traits were studied in rice

Trait	Pedigree	Marker	Mapping population	No. of QTL	References
Drought resistance (DRs) in seeds	Indica × Azucena	RFLP & SSR	RIL	7	Zheng *et al.,* 2008
Stability of cellular membranes (CM)	IR62266 x CT9993	RFLP, AFLP, SSR	DH	9	Tripathy *et al*., 2000
Leaf rolling (LR) and leaf water relationships	Azucena × Bala	RFLP, AFLP, SSR	RIL	13	Khowaja & Price *et al*., 2008
Grain yield (GY), panicle and seed fertility	Teqing x Lemont	SNP	IL	5	Wang *et al*., 2014
Length, thickness, and root number	IR58821 ×IR52561	AFLP &RFLP	RIL	28	Ali *et al*., 2000
Root distribution & architecture, deep roots	IR64 x Azucena	RFLP	DH	39	Lou *et al*., 2015
Deep root (DR) architectural traits	3 populations	SSR, SNP	RIL	6	Lou *et al*., 2015
Root penetration, high root and tiller number	CO39 × Moroberekan	RFLP	RIL	39	Lou *et al*., 2015
High GY drought	Two population	SSR	BS	4	Wang *et al*., 2014
GY in aerobic environments (E)	Three populations	SSR	BS	1	Vikram *et al*., 2016
Yield traits at the reproductive stage (RS)	IR64 × Cabacu	SNP	RIL	1	Trijatmiko *et al*., 2014
GY under stress at RS	swarna x WAB	SSR	BIL	1	Wang *et al*., 2014
Grain yield under severe lowland drought	R77298 x Sabitri	SSR	BC1	1	Vikram *et al*., 2016
Yield at RS over factor E	Two populations	SSR	BSA	2	Vikram *et al*., 2016
Physio-morphological traits	IR64 × Azucena	RFLP	DH	15	Trijatmiko *et al*., 2014
Drought tolerance (DT) & Osmotic adjustment (OA)	CO39 × Moroberekan	RFLP	RIL	1	Vinod *et al*., 2019

Table 3: List of some of the genes that have been associated with different mechanisms of drought tolerance in rice

Gene	Function	References
DRO1	Induces root elongation and DR	Uga *et al*., 2013
OsDREB1C, OsDREB2B, OsDREB2C, OsDREB2D, OsDREB2E, OsDREB2F	Root growth and water uptake	Trijatmiko *et al*., 2014; Vinod *et al*., 2019
OsPP2C39, OsPP2C40	LR	Khowaja & Price *et al*., 2008
OsDREB1F	Maintains ABA-dependent signaling pathway	Fu *et al*., 2017
OsDREB2B	RL and RN of root increment	Xu *et al*., 2016
OsProT1, OsProT2	Proline biosynthesis	Chaum & Kirdmanee, 2000
CYP735A	Maintains cytokinin level	Kumar & Verslues, 2015
OsABI1, OsABI2, OsABI3	ABA signaling and regulation	Fu *et al*., 2017
OsNAC5	Enhances root diameter and GY	Wang *et al*., 2014
SNAC1	Enhances SF	Barik *et al*., 2019
OsGRX480, OsGRX581, OsGRXS21	Antioxidant defense	Uga *et al*., 2013
OsPIP2;3, OsPIP2;5, OsPIP2;6	Stomatal regulation (SR)	Xu *et al*., 2016
OsbZIP23, OsbZIP46	Increases GY	Vikram *et al*., 2016
AP37	Enhances seed filling and GW	Trijatmiko *et al*., 2014
OsbZIP71	Enhances seed setting (SS)	Barik *et al*., 2019
OsLIP9, OsLIP21	Water retention	Lou *et al*., 2015
EcNAC67	Increases RWC, delays LR, higher root and shoot mass	Xu *et al*., 2016
OsNHX1	CM stability	Tripathy *et al*., 2000
DsM1	Helps in Reactive oxygen species (ROS) scavenging, maintains drought tolerance (DT) at the seedling	Huang *et al*., 2009; Kim *et al*., 2020
OsPYL/RCAR5	Induces stomatal closure, regulates leaf fresh weight	Xu *et al*., 2016
OsWRKY47	Relatively low GY reduction	Wang *et al*., 2014
AtDREB1A	OA, chlorophyll maintenance, higher RWC and reduced ion leakage	Vinod *et al*., 2019
TlOsm	Maintains growth, retains higher RWC and membrane integrity and improves survival rate	Xu *et al*., 2016
OsMIOX	Higher ROS and proline content	Chaum & Kirdmanee, 2000
Coda	Better yield, higher photosystem II activity, increased detoxification of ROS	Kim *et al*., 2020
OsTPS1	Higher trehalose and proline accumulation	Chaum & Kirdmanee, 2000
OsCPK9	Increases DT through enhanced stomatal closure (SC) and better OA in transgenics	Vikram *et al*., 2016; Vinod *et al*., 2019
OsNAC10	Increases DT at vegetative stage, enlarges roots and improves GY	Vikram *et al*., 2016

Other functional proteins like heat shock proteins (HSPs) have also been found to play a role in the improvement of drought tolerance in rice (Wang *et al*., 2014). In addition to the genes mentioned, small non-coding RNAs have been found to regulate gene expression (Table 3) and contribute to drought tolerance in rice (Pang and Chen, 2017). Another important factor is the regulation of hormones such as abscisic acid (ABA) that play a crucial role in the regulation of water uptake, stomatal closure, and tolerance to drought stress (Usman *et al*., 2017, Efendi *et al*., 2013). Therefore, molecular studies on drought tolerance in rice have revealed numerous genes and pathways that contribute to the improvement of drought tolerance in rice. The use of molecular markers and genetic engineering techniques, in combination with traditional breeding techniques, could be utilized to create more drought-tolerant rice cultivars and contribute to global food security.

Zinc finger proteins are widely distributed in plants, including *WRKY* genes that regulate various abiotic stress responses (Sahebi *et al*., 2018, Huang *et al*., 2009). Rice zinc-finger protein (*dst* mutant) has demonstrated increased drought and salt tolerance by decreasing stomata density and improving stomata closure (Huang *et al*., 2009). The overexpression of *OsZFP252* has also been shown to improve drought tolerance by increasing proline and soluble sugar levels (Xu *et al*., 2008). After drought stress exposure, approximately 5000 genes are up-regulated and 6000 genes are down-regulated in rice (Oladosu *et al*., 2014, Joshi *et al*., 2016). These genes play a role in membrane transport, signalling, and transcriptional regulation (Upadhyaya *et al*., 2019, Kim *et al*., 2020) and are associated with drought tolerance, as listed in (Table 4).

In conclusion, the regulation of several genes, proteins and transcription factors play a vital role in the drought tolerance of rice plants (Gupta *et al*., 2020, Dash *et al*., 2018). Different genes and proteins have different functions, such as osmoregulation (Kumar *et al*., 2015, Upadhyaya *et al*., 2019), ABA signaling (Gupta *et al*., 2020, Dash *et al*., 2018), and stomata regulation, which improve drought tolerance. The overexpression or suppression of these genes have been studied to assess their impact on drought tolerance, and many have demonstrated increased chances of survival and better growth under drought stress (Fu *et al*., 2017). These findings highlight the importance of molecular screening for drought tolerance in rice and the potential for improving crop production in water-limited environments.

In summary, various genes and transcription factors play a role in regulating rice's response to drought stress. Transgenic methods have been used to introduce genes that improve root elongation (Kim *et al*., 2020, Joshi *et al*., 2016, Fu *et al*., 2017, Uga *et al*., 2013), osmoregulation, stomatal closure, water use efficiency, drought tolerance, and reduced levels of ROS. The overexpression of genes like *DREB2* (Huang *et al*., 2009), *OsDREB2B, CYP735A, OsDRAP1* (Kim *et al*., 2020), *OsNAC5, OsLEA3-1* (Liu *et al*., 2014), *OsCPK9* (Wei *et al*., 2014), *OsWRKY47* (Raineri *et al*., 2015) and *OsbZIP46* (Rahman *et al*., 2016) has been shown to improve drought tolerance in rice. Some genes like *OsITPK2* (Du *et al*., 2010) play a role in regulating ROS homeostasis under drought stress. Additionally, gene regulation is also influenced by ABA dependent and independent mechanisms *OsWRKY47* (Raineri *et al*., 2015), *OsbZIP46* (Rahman *et al*., 2016), as well as the interaction between regulatory proteins and signal transduction pathways (Xiang *et al*., 2008, Du *et al*., 2010).

Various genes have been tested for their ability to confer drought resistance in rice using transgenic techniques. Some of these genes include *OsJAZ1, DRO1, OsDREB2B, CYP735A, OsDREB1F, OsDRAP1, OsNAC5, OsLEA3-1, OsbZIP71, OsWRKY47, OsbZIP46, EDT1/HDG11, AtDREB1A, OsMIOX, OsTPS1, OsCPK9, OsDREB2A, CDPK7, CIPK03/CIPK12, OsITPK2* and *WRKY* genes (Panda *et al*., 2021). The buildup of trehalose has been shown to increase drought tolerance in rice by stabilizing proteins against denaturation and storing carbs. The fusion *TPP/TPS* gene from *E. coli* (*otsA* and *otsB*) has been introduced into rice and has been shown to increase trehalose, improve drought tolerance and decrease photo oxidation in the rice plant under cold and salt stress (Jang *et al*., 2003). Before being used in molecular breeding programs, these genes need to be tested in field conditions.

The summary of alternative breeding strategies for rice drought tolerance

Rice is one of the most important staple crops in the world, and drought stress can significantly impact rice production. To mitigate the effects of drought on rice cultivation, scientists and breeders have employed both conventional breeding techniques and molecular breeding methods to develop drought-tolerant rice cultivars (Table 5 and 6). Here are some examples of rice cultivars developed for drought tolerance through these approaches (Kumar *et al*., 2014).

Swarna-Sub1: Swarna-Sub1 is a popular drought-tolerant rice variety developed through conventional breeding at the International Rice Research Institute (IRRI). It was created by introgression the Sub1 gene from a wild rice species, Oryza rufipogon, into the popular rice variety Swarna. The Sub1 gene confers tolerance to prolonged submergence and also enhances drought tolerance in rice.

DRR Dhan 42: Developed by the Directorate of Rice Research (DRR) in India, this variety exhibits tolerance to both drought and submergence stresses. It was developed through a combination of conventional breeding and marker-assisted selection.

IR64-Sub1: IR64-Sub1 is another drought-tolerant rice cultivar developed through conventional breeding. It is a variant of the widely cultivated rice variety IR64, which was crossed with a wild rice relative carrying the Sub1 gene. IR64-Sub1 exhibits improved tolerance to both submergence and drought stress.

Vandana: Vandana is a drought-tolerant rice variety developed through conventional breeding at the International Rice Research Institute (IRRI). It is known for its tolerance to both drought and salinity stress. Vandana was developed by selecting and breeding from diverse rice germplasm for several generations to accumulate favorable traits for drought tolerance.

Sahbhagi Dhan: Sahbhagi Dhan is a popular drought-tolerant rice variety developed through molecular breeding techniques. It was developed by the scientists at the Bihar Agricultural University in India by introgression a major quantitative trait locus (QTL) for drought tolerance called "qDTY12.1" into a high-yielding rice variety. Sahbhagi Dhan exhibits improved yield and drought tolerance under water-limited conditions.

Sub1-2: Sub1-2 is a drought-tolerant rice cultivar developed through molecular breeding. It is a variant of the popular rice variety IR64, where the Sub1 gene was introduced using genetic engineering techniques. The Sub1-2 rice plants exhibit enhanced tolerance to submergence and also show improved drought tolerance.

Swarna-Sub1A: This variety is an improved version of Swarna-Sub1, developed using marker-assisted selection. It carries the SUB1A gene, providing tolerance to submergence and drought stress.

Vandana Sub1: Another example of a rice variety developed through marker-assisted selection, Vandana Sub1 carries the SUB1A gene and exhibits enhanced tolerance to submergence and drought.

Sahbhagi Dhan Sub1: This is a drought-tolerant variety developed through marker-assisted selection by the ICAR. It carries the SUB1A gene and has shown improved drought tolerance.

Nerica-4: This variety was developed through conventional breeding at the Africa Rice Center (WARDA). It is a drought-tolerant variety that combines the African parent Oryza glaberrima and the Asian parent Oryza sativa.

NERICA-L-19: Another drought-tolerant variety developed by the Africa Rice Center, NERICA-L-19 is known for its high cooking quality and suitability for table use.

WAB56-104: This variety, developed by the West Africa Rice Development Association (WARDA), is well adapted to drought-prone environments and has good cooking characteristics.

CT9993-5-10-1-1: This variety was developed using marker-assisted selection and contains the Sub1 gene for submergence tolerance and drought tolerance. It is known for its good cooking quality.

Azucena Sub1: Azucena Sub1 is a drought-tolerant variety developed through marker-assisted selection. It carries the Sub1 gene and exhibits good eating and cooking quality.

Samba Sub1: Developed through marker-assisted selection, Samba Sub1 is a table rice variety that carries the Sub1 gene, providing tolerance to submergence and drought stress. These examples highlight the successful efforts in developing drought-tolerant rice cultivars through both

Table 4: List of multiple genes that have been tested for their ability to confer drought tolerance in rice through genetic engineering or transgenic methods

Gene Action	Gene	Promoter	Gene transfer methods	Phenotype	References
Genes Encoding Enzymes that Synthesize Osmotic and Other Protectants					
Polyamine synthesis	*ADC, OsProT1 & OsProT2*	*Ubi-1*	*Biolistic*	Improved DT with high putrescine and spermine synthesis	Capell *et al*., 2004
abscisic acid Metabolism	*CaMV35SP*	*DSM2*	*Agrobacterium*	Oxidative and DRs and increase xanthophylls and non-photochemical quenching	Fu *et al*., 2017
Amino acid metabolism	*OsOAT*	*Ubi1*	*Agrobacterium*	Improve DT and increase SS	You *et al*., 2013
ROS	*OsSRO1c*	*Ubi1*	*Agrobacterium*	Oxidative ST and SC (R)	Kim *et al*., 2020
Protoporphyrinogen oxidase	*PPO*		*Agrobacterium*	Less oxidative damage, and DT	Phung *et al*., 2011
Late Embryogenesis Abundant (*LEA*) Related Genes					
LEA protein gene	*HVA1*	*Actin1*	*Agrobacterium*	CL stability, higher leaf RWC and increase in growth under DRs.	Babu *et al*., 2004
	HVA1	*Actin1*	*Agrobacterium*	DT and salinity tolerance (ST)	Rohila *et al*., 2002
	OsLEA3-2	*CaMV35S*	*Agrobacterium*	DRs and increase grain/panicle	Du *et al*., 2010
Various Regulatory Genes (VRG)					
Transcription factor	*HVA1 & OsbZIP72*	*CaMV35S*	*Agrobacterium*	DRs and ABA sensitivity	Xiang *et al*., 2008
Harpin protein	*Hrf1*	*CaMV 35S*	*Agrobacterium*	DRs through ABA signalling and antioxidants, and SC (R)	Zhang *et al*., 2011
Jasmonate and ethylene-responsive factor 1	*JERF1*	*CaMV35S*	*Agrobacterium*	DRs	Zhang *et al*., 2011
Ethylene-responsive factor 1	*TSRF1*	*CaMV35S*	*Agrobacterium*	Enhances the OA and DT	Zhang *et al*., 2011
Stress/zinc finger protein	*OsiSAP8*	*CaMV35S*	*Agrobacterium*	ST, DT and cold stress	Kanneganti, & Gupta, 2008

Table 5: IRRI identified drought-tolerant donors and developed high-yielding drought-tolerant rice varieties for conventional breeding, QTL mapping, and release in South and Southeast Asia and Africa (Kumar *et al*., 2014)

Variety	Suitability for use	Variety	Country, release year, situation
Basmati 370	Conventional breeding	Sahod Ulan 1	Philippines 2009, RL, UP
CT9993-5-10-1-M	QTL mapping and pre-breeding	Hardinath 1	Nepal 2009, RL
PSBRc 82	QTL mapping and pre-breeding	Sahbhagi dhan	India 2010, RL, UP
PSBRc 68	QTL mapping and pre-breeding	BRRI dhan56	Bangladesh 2011, RL
PSBRc 80	Conventional breeding	Sookha dhan 3	Nepal 2011, RL
Aus Bak Tulsi	QTL mapping and pre-breeding	Sookha dhan 1	Nepal 2011, RL
Kalia	QTL mapping and pre-breeding	Sookha dhan 2	Nepal 2011, RL
Lal Aus	QTL mapping and pre-breeding	Katihan 1	Philippines 2011, UP
IR83614-1007-B-B	QTL mapping and pre-breeding	Sahod Ulan 3	Philippines 2011, RL
Aus 257	Conventional breeding	Sahod Ulan 5	Philippines 2011, RL
Kali Aus	QTL mapping and pre-breeding	Sahod Ulan 6	Philippines 2011, RL
IR77298-14-1-2	Conventional breeding	Sahod Ulan 8	Philippines 2011, RL
Dular	Conventional breeding and QTL mapping	Inpago LIPI Go 1	Indonesia 2011, UP
IR83614-1002-B-B	Conventional breeding	Inpago LIPI Go 2	Indonesia 2011, UP
IR83614-1005-B-B	Conventional breeding	Sahod Ulan 12	Philippines 2013, RL, DS
IR57514-PMI-5-B-1-2	QTL mapping and pre-breeding	M'ZIVA	Mozambique 2013, RL
N22	Conventional breeding and QTL mapping	UPIA3	Nigeria 2013, RL
Apo	Conventional breeding and QTL mapping		

Table 6: *QTLs* for drought tolerance-high yield (DTY) and other stress tolerances were pyramided in popular rice varieties through marker-assisted breeding. (Kumar *et al*., 2014)

Variety	Target ecosystem	DTY *QTL*s used	Other *QTL*s	Current stage
IR64	Rainfed lowland	$qDTY_{2.2}$, $qDTY_{4.1}$ $qDTY_{1.1}$, $qDTY_{2.1}$, $qDTY_{3.1}$, $qDTY_{12.1}$ $qDTY_{6.2}$, $qDTY_{2.2}$, $qDTY_{4.1}$, $qDTY_{3.2}$, $qDTY_{12.1}$, $qDTY_{3.1}$, $qDTY_{6.1}$, $qDTY_{12.1}$, $qDTY_{2.3}$, $qDTY_{3.2}$	*Sub1*	Released in Nepal, identified for release in India, tested for release in Bangladesh
Swarna	Rainfed lowland			Testing and validation in progress
Vandana	Rainfed lowland			Testing and validation in progress
Sabitri Anjali	Rainfed lowland			Introgression ongoing
TDK1	Rainfed lowland			Testing and purification in progress
Sambha Mahsuri	Rainfed lowland			Testing and purification in progress
IR64	Rainfed lowland			Testing and purification in progress

conventional breeding techniques, such as introgression and selection, and molecular breeding methods, including marker-assisted selection and genetic engineering. These cultivars play a crucial role in ensuring stable rice production in drought-prone regions, ultimately contributing to food security.

In conclusion, the study of natural genotypic variation in rice and marker-assisted selection can help identify novel drought-tolerant genotypes and related genes/loci (Aldemir et al., 2017, Xu *et al*., 2016). The success of incorporating *QTL*s for drought tolerance into high-yielding rice cultivars through marker-assisted breeding has been limited (Singh *et al*., 2016, Swamy and Kumar *et al*., 2013, Dixit *et al*., 2014, Barik *et al*., 2019, Vikram *et al*., 2016), but the development of drought-tolerant rice varieties remains a focus due to the increasing significance of drought. Incorporating multiple *QTL*s into elite cultivars has shown promising results, such as the Malaysian rice cultivar *MR219* and the rice variety *TDK1* (Dixit *et al*., 2014, Singh *et al*., 2016, Shamsudin *et al*., 2016). However, there is still a need for more research and efforts to create practical and high-yielding drought-tolerant rice varieties that can adapt to a wide range of climatic conditions. In conclusion, the challenge of creating drought-tolerant rice varieties remains significant due to the difficulty in finding suitable donors with a high level of tolerance and the environment-specific nature of drought tolerance. Despite the success in incorporating QTLs for drought tolerance into high-yielding cultivars using marker-assisted breeding techniques, much more effort is needed to develop improved rice varieties that can withstand drought conditions and maintain high yields. The adoption of high-yielding cultivars like Swarna, Samba mahsuri, and IR36 in drought breeding efforts shows the potential for creating drought-tolerant rice varieties, but further research and development is necessary to achieve this goal.

Conclusion

In conclusion, the development of drought-tolerant rice varieties is a complex and challenging process that requires a combination of various techniques, including marker-assisted breeding, transgenic techniques, and field testing. Despite the progress that has been made, there is still much to be done to understand the mechanisms behind the whole-plant stress response and to create rice varieties that are highly tolerant to drought. To achieve this goal, it is essential to continue research and invest in new technologies in the field of molecular genetics and crop breeding. Despite the progress made in the development of drought-tolerant rice, there is still a long way to go in terms of developing rice varieties that are capable of resisting drought in various environments. The complex nature of drought stress and the multigenic regulation of drought tolerance make the breeding process challenging, but recent advances in functional genomics and marker-assisted selection can help overcome these challenges. Additionally, the integration of multiple abiotic stressors, such as high temperature and salt, and the assessment of both above- and below-ground characteristics, are crucial in creating successful drought-tolerant rice varieties. Field testing of the genes demonstrated to have drought tolerance should be carried out before incorporating them in breeding programs. Overall, further research and development in the field of rice breeding for drought tolerance is necessary to address the increasing need for food security in an ever-changing climate.

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Conflict of interest

The authors declare that they have no conflict of interest.

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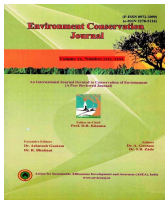

Effect of ayurvedic multimodal therapies on Plantar warts - a case report

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ABSTRACT

Deep plantar warts are the most common cutaneous lesions of the plantar aspect of the foot caused by Human papilloma Virus (HPV), mostly occur in children and adolescents. Most of the HPV infections are controlled by the humoral or cellular immune responses. But in few of the population groups these are manifested very frequently compared with other group of population. The virus sheds from the lesions and may infect the other sites of the plantar aspect or affect other parts of the body. Here we present a case of plantar warts which was successfully treated with multiple ayurvedic treatment modalities. This paper describes the case of a thirteen-year-old boy who presented to our hospital's outdoor department with plantar warts since seven days. The patient was treated holistically with ayurvedic treatment modalities including soaking feet in lukewarm *triphala* decoction, topical application of garlic paste with 777 oil and an ayurvedic oral medication, *pancha tikta ghrita guggulu,* and the patient was cured completely in 20 days with 100% clearance and even after four years of follow up there was no sign of recurrence. The holistic approach of these ayurvedic treatment modalities have proven as effective and safe in treating the plantar warts.

Introduction

Warts are the lesions caused by Human Papilloma Viruses (HPV). Over 100 strains of HPV have been identified (Lawley *et al.*, 2001). The virus infects the mucous membranes and skin epithelium and can be asymptomatic or be associated with both malignant and benign neoplasms or develop warts (Brown *et al.*, 2001). Most common cutaneous warts are common warts, plane or flat warts and deep plantar warts. Deep plantar warts also known as Verruca Plantaris (Brown *et al.*, 2001) (which means "ant hill" in Greek)/ Myrmecia (Mandell *et al.*,2000), mostly affect the plantar aspect of the foot in young adults and adolescents. HPV is released by plantar warts, which can spread to other places in the plantar region or other people (Witchey *et al.*, 2000) . The lesions are 2 mm to 1 cm in diameter and appear as raised bundles of soft keratotic fibres. Shaving of these lesions revealpunctate, bleeding blood vessels (Mandell *et al.*,2000). *Charmakeela*, a clinical ailment explained in Ayurveda that is similar to warts and is addressed in the context of *Arshoroga nidana* by *Susruta*, narrated it as a peg or nail-shaped, immovable lesion on the outside of the skin caused by exacerbated *vyana vayu* in association with *kapha.* The cardinal feature of charmakeela is extreme roughness. Conventionally, an armamentarium of wart treatments is available which include cryotherapy, application of keratinolytic agents such as salicylic acid plasters or solutions, podophyllin, topical imiquimod. Cryotherapy with liquid nitrogen (Brown *et al.*, 2001) is one of the most useful and convenient methods of treating warts in nearly any place. The location of the wart, the extent of the disease, the patient's age, and his or her immunological status are all factors that influence treatment options (Brown *et al.*, 2001). However, no single therapy appears to be uniformly or universally beneficial

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and wart recurrence appears to be a regular occurrence across all of these treatments.

In Ayurveda, treatment for *Charmakeela* was explained by author *Susruta* as *Chedana* (Excision) (Sharma.P.V.,1999), *Kshara Karma* (application of caustic agents) (Sharma.P.V.,1999) and *Agnikarma* (cauterization) (Sharma.P.V.,1999)). But in this case because of extensive involvement of the lesions in almost whole sole, none of the above recommended therapies were used for treatment. Only bheshaj chikitsa (conservative treatment) was adopted involving multimodal therapies such as *avagah swed* (soaking feet in medicated decoction), topical application and oral medication. Here we present a case of plantar warts which was successfully treated with above said comprehensive ayurvedic multiple treatment modalities.

Material and Methods

Case Presentation:

A healthy 13-year-old boy presented to National Institute of Ayurveda Hospital, Jaipur, Rajasthan, India on 27th January 2019, with multiple painless plantar lesions on sole of left foot for the past seven days. There were no other associated symptoms, such as itching or drainage. Patient's parents approached directly for Ayurvedic treatment without any prior interventions. On 20th January, 2019 while wearing shoes, he noticed few white lesions on the sole of his left foot. Over the next seven days, the lesions multiplied and expanded in size, encompassing nearly the whole medial aspect of sole of his left foot. Because of winter season he has been wearing linen socks throughout the day for the last four months. Socks that hadn't been washed were frequently used by the patient for school. There is no history of such lesions in the family.

On physical examination, multiple, medium to large, hyperkeratotic, confluent plaques filled with punctate black dots on the medial aspect of sole of the left foot extending from first, second and third toes down to the heel. The diagnosis was made as Plantar warts on the clinical ground. We treated the patient with ayurvedic multimodal therapies which comprised of soaking feet in lukewarm *triphala* decoction (*avagaha swedam*) twice daily for ten minutes each time; topical application of a paste of two crushed garlic cloves after mixing with sufficient quantity of 777 oil before bed and leaving it for overnight for twenty days; and an ayurvedic oral medication, *pancha tikta ghrita guggulu,* two tablets twice daily with lukewarm water after meals for one month (Suggested to consume for ten more days after complete clearance of lesions). The patient was educated on self-care practices such as to refrain from wearing socks or shoes, practice good personal hygiene, wash socks after each use, and properly dry shoes in between uses. Clinical improvement of the patient across different timelines is depicted in Figure 1.

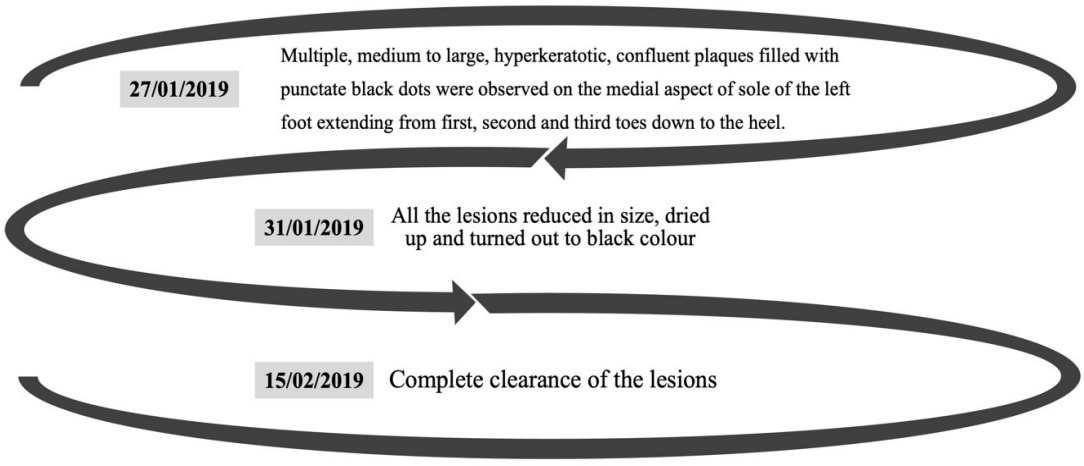

Figure- 1: Patient's clinical improvement across different timelines

Results and Discussion

A significant improvement was observed by the fifth day of treatment. All of the lesions dried up and turned out into black color, and by twentieth day, the lesions have completely healed and disappeared. Photographs were captured at presentation on the first day of visit (Figure 2), during treatment on the fifth day (Figure 3) and after completion of treatment on twentieth day (Figure 4). Although the patient was not strictly adherent to one of the interventions- soaking feet in *triphala khada* (followed only for ten days), but continued to take oral medication and topical application till the clearance of the lesions. The medication is well tolerated and has no local or systemic adverse events. After a four-year follow-up period, there was no sign of recurrence. Patient and patient's parents are ecstatic with the outcome of this ayurvedic treatment. Patient claims that he was very comfortable with every therapeutic modality and felt conducive in practicing the procedures like soaking feet in decoction, topical application and oral medication, which rendered complete yield clinically.

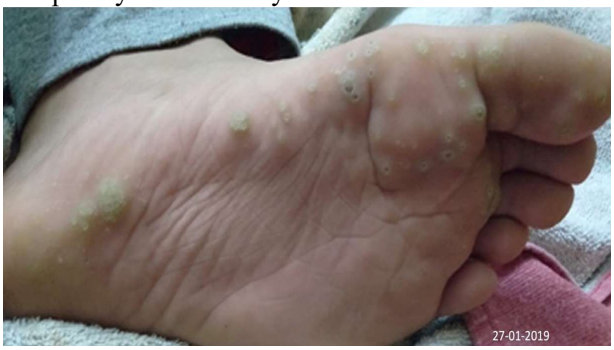

Figure 2: Showing the clinical presentation of plantar warts of patient on day one

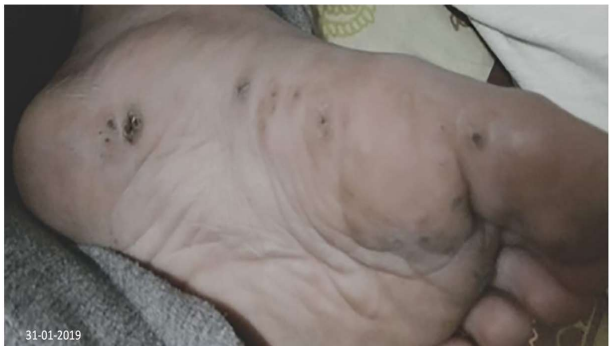

Figure 3: Showing the clinical improvement of the patient on day five

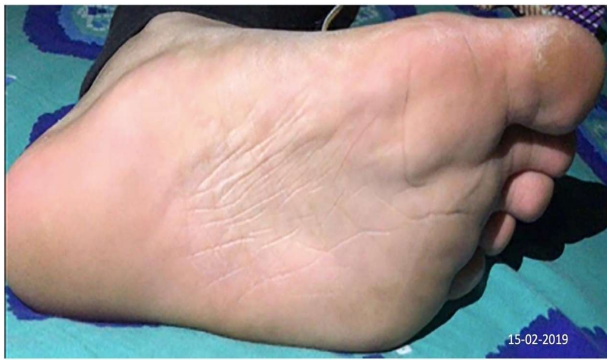

Figure 4: Showing the complete clearance of plantar warts on twentieth day of treatment

Despite widespread acceptability, the plantar warts clearance rate is just 50% following three months of treatment at three-week intervals. Furthermore, due to the ongoing irritation, high relapse rate, and cutaneous secondary bacterial infection, plantar warts patients are less likely to follow up (Witchey *et al.*, 2018). Majority of these ablative treatments are traumatic, causing local tissue loss, discomfort and inflammatory reactions on the surrounding skin (Jones *et al.*,1994). On the other hand, Ayurvedic multimodal treatments have not been associated with any local or systemic side effects. The drugs used for the treatment have potential antiviral properties. *Triphala,* the fruits of three medicinal plants; Terminalia chebula Retz. (*Hareetaki*), Terminalia bellerica Roxb. (*Vibheetaki*) and Emblica officinalis Gaertn. (*Amlaki*), is considered as most versatile of all herbal formulations had proven antibacterial, antiviral, antifungal, antihelmintic, antimutagenic and anticarcinogenic properties (Gupta, 2012). In a study T. chebula's hot water extract had shown antiviral activity against herpes simplex virus (HSV) in in-vivo and in both in-vivo and in-vitro antiviral activity against cytomegalovirus (CMV) (Yukawa *et al.*, 1996).

777 oil is a clinically proven medicine for psoriasis and other skin disorders, contains extracts of (*Shweta Kutaj*) Wrightia tinctoria 50% in coconut oil base (cocos nucifera) 50%. The methanolic extract of Wrightia tinctoria has shown flavanoids and alkaloids in it. "Wrightia tinctoria's instrumental analysis of Methanolic extract was carried out by using different analytical techniques such as HPLC, UV and TPLC which has shown the presence of few indole derivatives such as indurubine and isatin exhibiting potential anti-viral

activity" (Adake and Rao, 2011). In few studies wrightia tinctoria has shown anti-HIV and anti-HCV activity (Selvam *et al.*, 2009). Garlic: Garlic's (Allium Sativum) components have been proven to have antiviral activity (Rouf *et al.*, 2020) and impede the growth of virally infected cells. "In one placebo-controlled trial, topical application of chloroform extracts of garlic had shown complete eradication of cutaneous warts with no recurrence after 3- 4 months" (Dehghani *et al.*, 2005). In a clinical trial, complete clearance of male genital warts was observed with topical application of 10% of methanol garlic extract for 2 months (Mousavi *et al.*, 2018). Garlic's exact mode of action is unknown, although a few in-vitro studies have revealed that it can strengthen natural killer (NK) cells, which are regarded as a crucial component of the immune system in combating malignancies, viruses, and some bacteria (Agarwal, 1996).

Pancha tikta ghrita guggulu is an ayurvedic leading drug advisable in many of the skin diseases and works as a blood purifier. The cumulative effect of all these multimodal treatments had yielded marked results with total clearance of plantar warts in just twenty days and no relapse was observed after four years of follow up.

Conclusion

In conclusion, this ayurvedic multimodal treatment protocol can be a good alternative in the treatment of viral warts. Patient tolerance and satisfaction score are high without any local or systemic adverse reactions and the drugs are well accepted by the patients. As a result, these comprehensive therapy techniques are strongly recommended.

Conflict of interest

The authors declare that they have no conflict of interest.

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