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(A Peer Reviewed Journal)

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For book

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

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American Public Health Association (2012). Standard methods for examination of water and waste water.23th Ed. Washington DC, USA.

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Priyanka, Tiwari, Ramesh Chandra and Bhutiani, Rakesh (2021): Green tea and its effects with special reference to ayurvedic concept about tea and rasayana. In: Green Tea (pp: 99-109) Nova Publications.

Ph.D. Thesis, Dissertation and Reports

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.

For webpage

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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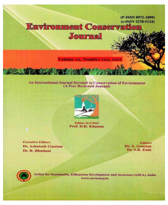

Effect of titanium dioxide (TiO_2) nanoparticles on the storability of onion (*Allium cepa* L.) seeds under ambient condition

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ABSTRACT

TiO_2 nanoparticles are highly stable, eco-friendly in nature, having low cost, act as a photo catalyst also having antimicrobial properties. Considering the effect of TiO_2 nanoparticles on seeds, a study was conducted during March 2022 to June 2022 at Department of Genetics and Plant Breeding, SHUATS, Prayagraj (U.P). In this study onion seeds of variety Nasik Red N-53 were collected to investigate the effect of TiO_2 nanoparticles on the seedling characters as well as on the biochemical characters under storage in ambient conditions. Onion seeds were treated with different concentration of TiO_2 nanoparticles (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130 and 140 ppm) along with control and stored in two containers; viz. tin container (C_1) and aluminum foil pouch (C_2). All the seedling parameters were evaluated every month during storage. The experiment was conducted in factorial CRD with 4 replications. The experimental result showed that the storage containers influenced the seedling characters of onion. Seeds stored in aluminum foil pouch (C_2) exhibited highest germination per cent (52.93 %), speed of germination (3.76), root length (2.27 cm), shoot length (4.11 cm), seedling length (6.38 cm), fresh weight (0.176 gm), dry weight (0.0205 gm), seed density (1.025 gm/cm^3), dehydrogenase activity (0.213 OD/g mL), catalase activity (0.0220 nmol/min/mg protein) and exhibited lowest moisture per cent (8.05 %) and lowest electrical conductivity (0.970 dS/m) as compared to tin container at the end of 3 months of storage. Seed treated with TiO_2 nanoparticles @40ppm (T_4) for 2 hours performed better in terms of seedling parameters; viz. germination per cent (58.5 %), speed of germination (4.20), root length (3.06 cm), shoot length (4.58 cm), seedling length (7.64 cm), fresh weight (0.222 gm), dry weight (0.0259 gm), seed density (1.056 gm/cm^3), dehydrogenase activity (0.337 OD/g mL), catalase activity (0.0375 nmol/min/mg protein) and recorded lowest moisture per cent (8.04 %) and lowest electrical conductivity (0.951 dS/m) as compared to control after 3 months of storage. The study concluded that seed treated with TiO_2 @40 ppm and stored in aluminum foil pouch can be used to expand the storability of onion seeds under ambient condition.

Introduction

Onion (*Allium cepa* L.) is one of the important vegetable crops that belongs to the family Amaryllidaceae. It is a cross pollinated crop having diploid chromosome number (2n = 16). It also known as the bulb onion or common onion, which is the most widely cultivated species of the genus *Allium* (Carl Linnaeus). Onion grows mainly in rabi season. It belongs to the biennial crop and requires two seasons for the production of seeds. Bulbs are produced in first year and stalks are grown, and seed are developed in second season. It is a long-day plant. The size of bulb and seedling

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length is affected by the day length but for seed production the day length has no influence. During early development of the bulb and during early growth of seed stalk the crop requires cool climate. To get high quality of bulbs, quality seeds are required at appropriate time. Loss of vigour in onion seeds due to moisture, relative humidity (RH) and temperature shifting, is the reason of poor storage life of the seeds. Seed deterioration mainly depends on the storage conditions of the seeds. If controlled storage facility is not possible then proper storage container must use to slow the deterioration. Rigid plastic container cannot absorb moisture, so they do not deteriorate and able to store seeds for long time (Flatman, 1977). Aluminum foil pouches and metal cans are most economical for packing small quantities like flower and vegetable hybrid seeds etc. (Khan *et al.,* 2018).

Nanotechnology is a connecting field between life sciences, material sciences and information technology. Among the advances in science, nanotechnology is visualized as a rapidly developing field that has the potential to improve agriculture and the food system (Mohanraj and Chen, 2006.). A nanoparticle is a small particle whose size is ranging between 1 and 100 nano-meters, undetectable to the human eyes. It poses high surface to volume ratio could be a reason for its reactivity, the ability to pierce the cell membranes and regulating biochemical activity of the seeds (Dubchak *et al.,* 2010). Seed priming method particularly nano-priming is more effective due to its small size and unique physiochemical property. Plant species differs physiologically so their uptake of nanoparticles and the effects of different concentration of nanoparticle on them may differs from plant to plant. Researchers have found that nanoparticle seed treatment could improve the seedling character (Singh *et al.,* 2012), induce growth and development of plants (Khater, 2014), increase the yield potential (Mathew *et al.,* 2021) and improve the storage life of seeds (Das and Dutta. 2022). Titanium dioxide (TiO_2) nanoparticles are the most common nanoparticles frequently used in diversified fields but there are less reviews on the effect of TiO_2 nanoparticles on plants compared to animals. TiO_2 act as a photo catalyst thus it helps in activating the photo reactions and helps in carbohydrate metabolism (Daood *et al.*, 1998). It was found to give resistant to some bacteria, fungi and other organisms (Navarro *et al.*, 2008) and it was reported in promoting the cell division at lower concentrations (Badiyeh *et al.*, 2017). Some previous research concluded that nanosized TiO_2 at lower concentration could promote the seed germination in wheatgrass (Azimi *et al.*, 2013) and in *Cannabis sativa* L. (Feizi and Javedanipour, 2021). Although the effect of nano priming with TiO_2 at different concentrations on onion seed germination is rarely reported but it is definite that use of nanoparticles can be one of the ways to retain the vigour and viability during storage by preventing the losses due to biotic and abiotic stress. Titanium dioxide (TiO_2) nanoparticles (NPs) are known for high stability and low costs, environmental-friendly in nature and found to be safe for human.

On the basis of above context, the study was conducted to find out the effect of different storage containers and TiO_2 nanoparticles treatments on the storage life of the onion seeds.

Material and Methods

Experimental site: The current study was conducted in a factorial CRD with four replications and the experiment was conducted at Seed Testing Laboratory (Notified by Govt. of Uttar Pradesh), Department of Genetics and Plant Breeding, Naini Agricultural Institute (NAI) and the preparation of nanoparticle solutions was done at Biochemistry laboratory, Department of Biochemistry & Biochemical Engineering, Sam Higginbottom University of Agriculture, Technology & Sciences Prayagraj (U.P.) during March 2022 to June 2022.

Source of seeds and TiO_2 nanoparticles: The seeds of onion variety Nasik Red N-53 were collected from Department of Genetics and Plant Breeding. The TiO_2 nanoparticle was purchased from Saveer Matrixnano Private Limited, Greater Noida, Uttar Pradesh with a purity of ≥98% and particle size of (50-80nm).

Preparation of TiO_2 nanoparticle solution: The required concentration of nanoparticles was prepared for the experiment as per the procedures given by Hao *et al.*, 2016.

Seed treatment and preparation of seeds for storage: After recording the initial seed quality parameters, seeds of onion variety Nasik red N-53 were soaked in different concentration of nanoparticle solution (T_1:10ppm, T_2:20 ppm, T_3:30 ppm, T_4:40 ppm, T_5:50 ppm, T_6:60 ppm, T_7:70 ppm, T_8:80 ppm, T_9:90 ppm, T_{10}:100 ppm, T_{11}:110 ppm, T_{12}:120 ppm, T_{13}:130 ppm and T_{14}:140 ppm) for 2 hours along with T_0:Control (untreated) and then seeds were dried over night to safe moisture limit and then was packed in two containers viz. tin container (C_1) and aluminum foil pouch (C_2) and then stored under ambient condition (25 ± 3°c) with 95% RH in Seed Testing Laboratory. Seed parameters were observed every month during storage up to 3 months.

The observations recorded during storage are

1. **Germination per cent**: It was measured using Top of Paper methods as per ISTA, 2015. It was calculated by the formula

$$\textbf{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Number of seed placed}} \times 100$$

2. **Speed of germination***:* It was measured by the formula given by Magurie, 1962. Speed of germination was calculated using formula:

$$SG = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots \frac{X_n - X_{n-1}}{Y_n}$$

X_1= Number of seeds germinated on first count; X_n= Number of seeds germinated on final count; Y_1= No of days from sowing to 1st count; Y_n= No of days from sowing to final count

3. **Moisture per cent**: Seed moisture content was determined by low constant oven method as per ISTA, 2015.

4. Root length (cm): 10 healthy seedlings from each replication were selected randomly on the 12th day and their root length was measured from the tip of the primary root to the base of hypocotyl (ISTA, 2015).

5. Shoot length (cm): 10 random seedlings selected for root length measurement. The shoot length was measured from the tip of the primary leaf to the base of the hypocotyls (ISTA, 2015).

6. Seedling length (cm): Seedling length was calculated by adding root and shoot length.

7. Fresh weight (gm): 10 healthy seedlings were picked randomly from each replication and the fresh weight of the seedlings was measured with the help of electronic balance (ISTA, 2015).

8. Dry weight (gm): It was measured using the methods given by ISTA, 2015.

9. Electrical conductivity: It was measured by the procedures of Brar *et al*., (2019) and it was expressed in dS/m.

10. Seed density (gm/cm^3): It was measured by the formula = (weight of the seed) / (volume of the seeds).

11. Dehydrogenase activity: DHA was measured as per the procedure given by Brar *et al*., (2019) using 0.5% tetrazolium solution. The absorbance was recorded at 480 nm in a spectrophotometer, and enzymatic activity was expressed as OD/g mL (Brar *et al*., 2019). The 200 mg flour prepared was soaked in 5 mL of 0.5% tetrazolium chloride solution at 38°C for 3-4 h and followed by centrifugation for 3 minutes at 10,000 rpm and the supernatant was discarded. The formazan from the sediment was extracted with 10 mL acetone and left for 16 h then followed by centrifugation and absorbance of the solution was recorded at 480 nm using spectrophotometer.

12. Catalase activity: The extraction process was used following protocol of Dhindsa *et al.,* (1981). The catalase activity was determined using following protocol of Aebi *et al.,* (1984). The reaction mixture was comprised of 0.1ml enzyme extract, 1.50ml phosphate buffer (50mM, pH 7.0) and 0.10ml H_2O_2 (10mM) with the final volume made to 3ml with distilled water. Soon after addition of H_2O_2 to the reaction mixture, decrease in the absorbance was noted at 240nm using spectrophotometer for one min at an interval of 15 seconds. The activity of catalase was expressed as nmol/min/mg protein (Brar *et al*., 2019)

Statistical analysis: All the data were analyzed by factorial CRD and the mean was subjected to the critical difference at 5 % level of significance, and the data was analyzed using OPSTAT software.

Results and Discussion

Effect of storage containers on seedling parameters: The storage containers significantly influenced the seed quality parameters after 3 months of storage. The study revealed that seeds stored in aluminum foil pouch (C_2) performed better than tin container (C_1) during the storage period. Reduction of germination per cent was observed in both containers but lowest reduction was found in aluminum foil pouch (66% to 52.93%) compared to tin container (66% to 50.53%) (Table 1).

Table 1. Influence of storage containers and seed treatments on germination per cent, speed of germination, moisture per cent and root length of onion seeds during storage

Containers (C)	Germination percent				Speed of germination				Moisture per cent				Root length (cm)			
	Initial	Months after storage			Initial	Months after storage			Initial	Months after storage			Initial	Months after storage		
		1	2	3		1	2	3		1	2	3		1	2	3
C_1	66.00	57.73	54.47	50.53	4.834	3.930	3.762	3.520	7.80	8.07	8.09	8.24	3.95	2.41	2.29	2.18
C_2		**58.07**	**56.57**	**52.93**		**4.057**	**3.909**	**3.765**		**8.00**	**8.03**	**8.05**		**2.54**	**2.44**	**2.27**
Mean		57.90	55.52	51.73		3.993	3.835	3.642		8.03	8.06	8.15		2.48	2.37	2.22
S.Em. (±)		0.55	0.35	0.20		0.040	0.040	0.010		0.01	0.01	0.01		0.06	0.04	0.01
S.Ed. (±)		0.78	0.50	0.29		0.050	0.060	0.010		0.01	0.01	0.01		0.08	0.05	0.01
CD @ 5%		NS	0.99	0.57		0.110	0.110	0.020		0.03	0.02	0.02		NS	0.10	0.02
Treatments (T)																
T_0	66.00	58.75	54.75	50.75	4.834	3.966	3.749	3.583	7.80	8.15	8.21	8.30	3.95	2.98	2.75	2.47
T_1		61.00	58.75	54.50		4.103	3.998	3.847		8.00	8.04	8.15		3.17	2.83	2.67
T_2		63.50	60.50	56.00		4.256	4.138	3.932		7.98	8.01	8.13		3.36	3.05	2.82
T_3		64.00	61.50	57.00		4.577	4.335	4.125		7.95	7.94	8.06		3.44	3.26	2.98
T_4		**64.25**	**62.25**	**58.50**		**4.620**	**4.540**	**4.209**		**7.91**	**7.90**	**8.04**		**3.53**	**3.31**	**3.07**
T_5		61.75	59.75	56.50		4.552	4.305	4.094		8.03	8.04	8.10		3.00	3.17	2.96
T_6		59.00	57.00	55.00		4.124	3.846	3.810		8.06	8.09	8.19		2.51	2.85	2.68
T_7		58.75	55.75	52.50		3.988	3.923	3.688		8.03	8.05	8.15		2.60	2.39	2.41
T_8		57.25	54.25	49.75		3.806	3.768	3.642		8.05	8.04	8.16		2.15	1.89	2.02
T_9		54.75	52.50	50.00		3.774	3.649	3.455		8.03	8.06	8.14		1.92	1.79	1.70
T_{10}		56.25	52.50	50.25		3.633	3.568	3.354		8.03	8.06	8.11		1.77	1.67	1.58
T_{11}		56.25	50.75	47.50		3.786	3.478	3.287		8.08	8.14	8.16		1.70	1.84	1.56
T_{12}		51.75	52.00	46.25		3.648	3.464	3.246		8.04	8.10	8.15		1.77	1.59	1.54
T_{13}		49.75	50.50	46.25		3.390	3.405	3.220		8.10	8.10	8.20		1.66	1.55	1.47
T_{14}		51.50	50.00	45.25		3.678	3.367	3.146		8.08	8.09	8.19		1.59	1.55	1.44
Mean		57.90	55.52	51.73		3.993	3.835	3.642		8.03	8.06	8.15		2.48	2.37	2.22
S.Em. (±)		1.50	0.96	0.56		0.100	0.110	0.020		0.03	0.02	0.02		0.15	0.10	0.02
S.Ed. (±)		2.12	1.36	0.79		0.150	0.150	0.030		0.04	0.03	0.03		0.21	0.14	0.03
CD @ 5%		4.22	2.71	1.56		0.290	0.300	0.070		0.07	0.06	0.06		0.43	0.28	0.05

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140ppm

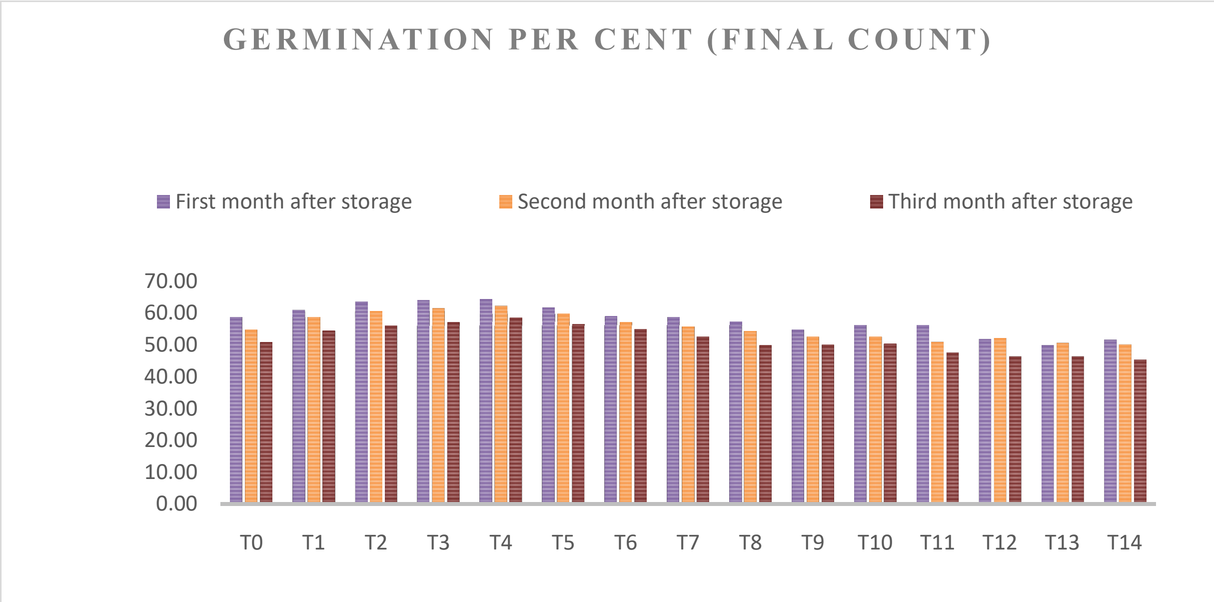

Figure 1. Effect of seed treatments on germination per cent (Final count) in onion variety Nasik Red N-53 during seed storage

Similar results were found by (Khan *et al.*, 2018), (Rolston *et al.*, 2012), (Kandil *et al.*, 2013) reported that aluminum foil pouch performed slightly better due to its moisture vapour proof nature, so seed deterioration was slow whereas tin container is moisture proof in nature (not vapour proof) (Monira *et al.*, 2012) so the deterioration was slightly on higher side. The initial speed of germination of the onion seed was 4.834 but with the advancement of the storage period it was decreased to 3.520 in tin container (C_1) and 3.765 in aluminum foil pouch (C_2). Initial moisture content before storage was 7.80% but it was increased with the increasing storage period. The inclination was lower in the seeds stored in aluminum foil pouch (7.80% to 8.05%) due its moisture vapour proof nature, so it does not absorb moisture from surroundings whereas slightly higher inclination was found in tin container (7.80% to 8.24%) (Kandil *et al.*, 2013) (Table 1). Initial root length (Table 1), shoot length (Table 3) and seedling length (Table 2) was declined with the advancement of storage period. Aluminum foil pouch recorded lowest declination in root length (3.95 cm to 2.27 cm), shoot length (5.47 cm to 4.12 cm) and seedling length (9.42 cm to 6.39 cm) as compare to tin container. Alike evident was reported by Hunje *et al.*, (2007). The initial fresh weight and dry weight was declined with increasing storage period. Lowest reduction in fresh weight (0.256 gm to 0.176 gm) and dry weight (0.0335 gm to 0.0205 gm) was found with the seeds stored in aluminum foil pouch (C_2) compared to tin container (C_1) (Table 2). An increasing trend was found in electrical conductivity during storage period. The enhancement was less in aluminum foil pouch (0.848 dS/mto 0.970 dS/m) compared to tin container (0.848 dS/mto 0.990 dS/m) (Table 3). Alike evident of electrical conductivity was reported by Hunje *et al.*, (2007), concluded that seeds stored in aluminum foil pouch continued to maintain the membrane integrity by reducing physiological and biochemical process thus improve the storage life of the seeds. The initial seed density was 1.077 gm/cm^3 which decreased to 1.025 gm/cm^3 in aluminum foil pouch and 1.016 gm/cm^3 in tin container at the end of the storage period (Table 3). The initial dehydrogenase and catalase activity was 0.446 OD/g mL and 0.0625 nmol/min/mg proteinbut it declined with the advancement of storage period. Although the declination was minimum in aluminum foil pouch (C_2) over tin container (C_1) (Table 3).

Table 2. Influence of storage containers and seed treatments on shoot length, seedling length, fresh weight and dry weight of onion seeds during storage

	Shoot length (cm)				Seedling length (cm)				Fresh weight (gm)				Dry weight (gm)			
Containers (C)	**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**		
		1	2	3		1	2	3		1	2	3		1	2	3
C_1	5.47	4.80	4.34	3.96	9.42	7.21	6.62	6.13	0.256	0.218	0.210	0.167	0.0335	0.0252	0.0243	0.0195
C_2		**4.96**	**4.45**	**4.12**		**7.50**	**6.89**	**6.39**		**0.227**	**0.218**	**0.176**		**0.0264**	**0.0251**	**0.0205**
Mean		4.88	4.39	4.04		7.36	6.76	6.26		0.223	0.214	0.171		0.0258	0.0247	0.0200
S.Em. (±)		0.01	0.01	0.01		0.06	0.04	0.01		0.001	0.002	0.000		0.0001	0.0003	0.0001
S.Ed. (±)		0.01	0.01	0.01		0.08	0.05	0.02		0.002	0.003	0.001		0.0002	0.0004	0.0001
CD @ 5%		0.02	0.03	0.02		0.15	0.11	0.03		0.004	0.007	0.001		0.0004	0.0008	0.0002
Treatments (T)																
T_0	5.47	4.84	4.48	4.06	9.42	7.82	7.23	6.53	0.256	0.219	0.196	0.165	0.0335	0.0254	0.0225	0.0194
T_1		5.14	4.75	4.34		8.32	7.58	7.01		0.224	0.214	0.183		0.0260	0.0248	0.0214
T_2		5.16	4.77	4.44		8.52	7.81	7.26		0.227	0.219	0.190		0.0264	0.0253	0.0223
T_3		5.22	4.86	4.52		8.66	8.12	7.50		0.246	0.242	0.209		0.0285	0.0281	0.0245
T_4		**5.27**	**4.97**	**4.58**		**8.79**	**8.29**	**7.65**		**0.260**	**0.245**	**0.222**		**0.0303**	**0.0289**	**0.0259**
T_5		5.00	4.72	4.48		8.00	7.88	7.45		0.246	0.235	0.208		0.0284	0.0274	0.0243
T_6		4.94	4.63	4.30		7.44	7.47	6.98		0.245	0.237	0.194		0.0281	0.0273	0.0228
T_7		4.91	4.52	4.20		7.51	6.91	6.61		0.240	0.234	0.184		0.0274	0.0270	0.0214
T_8		4.88	4.49	4.04		7.03	6.39	6.06		0.230	0.213	0.162		0.0268	0.0248	0.0189
T_9		4.77	4.34	3.86		6.69	6.13	5.57		0.217	0.227	0.154		0.0250	0.0264	0.0180
T_{10}		4.66	4.24	3.65		6.43	5.91	5.23		0.207	0.201	0.144		0.0239	0.0233	0.0170
T_{11}		4.62	3.94	3.62		6.32	5.78	5.18		0.201	0.192	0.146		0.0233	0.0223	0.0169
T_{12}		4.64	3.95	3.50		6.40	5.53	5.04		0.199	0.192	0.141		0.0231	0.0221	0.0163
T_{13}		4.60	3.65	3.52		6.26	5.20	4.99		0.196	0.186	0.135		0.0226	0.0209	0.0158
T_{14}		4.57	3.61	3.42		6.15	5.16	4.86		0.185	0.175	0.132		0.0215	0.0196	0.0151
Mean		4.88	4.39	4.04		7.36	6.76	6.26		0.223	0.214	0.171		0.0258	0.0247	0.0200
S.Em. (±)		0.02	0.02	0.02		0.15	0.11	0.03		0.004	0.007	0.001		0.0004	0.0008	0.0002
S.Ed. (±)		0.03	0.03	0.03		0.21	0.15	0.04		0.006	0.010	0.002		0.0010	0.0011	0.0003
CD @ 5%		0.05	0.07	0.06		0.42	0.30	0.08		0.012	0.021	0.004		0.0012	0.0022	0.0006

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140ppm

Table 3. Influence of storage containers and seed treatments on electrical conductivity, seed density, dehydrogenase activity and catalase activity of onion seeds during storage.

	Electrical conductivity (dS/m)				Seed density (gm/cm^3)				Dehydrogenase activity (OD/g mL)				Catalase activity (nmol/min/mg protein)			
Containers (C)	**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**		
		1	2	3		1	2	3		1	2	3		1	2	3
C_1	0.848	0.962	0.978	0.990	1.077	1.038	1.029	1.016	0.446	0.243	0.209	0.197	0.0625	0.0271	0.0226	0.0191
C_2		**0.938**	**0.947**	**0.970**		**1.042**	**1.033**	**1.025**		**0.256**	**0.229**	**0.213**		**0.0311**	**0.0245**	**0.0220**
Mean		0.950	0.962	0.980		1.040	1.031	1.021		0.250	0.219	0.205		0.0291	0.0235	0.0206
S.Em. (±)		0.002	0.003	0.001		0.001	0.001	0.001		0.006	0.001	0.001		0.001	0.0001	0.0001
S.Ed. (±)		0.003	0.005	0.001		0.002	0.001	0.002		0.008	0.001	0.001		0.001	0.0001	0.0001
CD @ 5%		0.006	0.010	0.002		0.003	0.003	0.003		NS	0.002	0.002		0.002	0.001	0.001
Treatments (T)																
T_0	0.848	0.984	1.014	1.077	1.077	1.018	1.010	0.990	0.446	0.292	0.223	0.209	0.0625	0.0164	0.0131	0.0114
T_1		0.962	0.959	0.982		1.031	1.021	1.011		0.320	0.272	0.255		0.0243	0.0206	0.0168
T_2		0.955	0.956	0.970		1.051	1.039	1.028		0.354	0.320	0.298		0.0285	0.0246	0.0224
T_3		0.939	0.948	0.961		1.060	1.048	1.043		0.383	0.337	0.318		0.0424	0.0377	0.0338
T_4		**0.913**	**0.934**	**0.951**		**1.075**	**1.066**	**1.056**		**0.399**	**0.365**	**0.337**		**0.0572**	**0.0436**	**0.0375**
T_5		0.944	0.952	0.963		1.052	1.045	1.039		0.378	0.335	0.316		0.0417	0.0373	0.0309
T_6		0.950	0.956	0.974		1.039	1.033	1.030		0.339	0.265	0.251		0.0295	0.0261	0.0233
T_7		0.960	0.967	0.978		1.038	1.027	1.023		0.270	0.234	0.218		0.0282	0.0215	0.0195
T_8		0.954	0.968	0.980		1.028	1.022	1.019		0.201	0.217	0.195		0.0240	0.0195	0.0176
T_9		0.950	0.955	0.980		1.032	1.028	1.019		0.175	0.136	0.134		0.0281	0.0213	0.0190
T_{10}		0.951	0.956	0.985		1.031	1.031	1.018		0.135	0.129	0.122		0.0260	0.0188	0.0173
T_{11}		0.949	0.960	0.969		1.032	1.039	1.019		0.139	0.118	0.116		0.0248	0.0207	0.0163
T_{12}		0.945	0.967	0.976		1.038	1.026	1.013		0.126	0.113	0.106		0.0218	0.0199	0.0155
T_{13}		0.948	0.976	0.973		1.037	1.021	1.004		0.115	0.110	0.103		0.0250	0.0142	0.0140
T_{14}		0.949	0.967	0.982		1.040	1.016	0.999		0.115	0.109	0.096		0.0184	0.0137	0.0133
Mean		0.950	0.962	0.980		1.040	1.031	1.021		0.250	0.219	0.205		0.0291	0.0235	0.0206
S.Em. (±)		0.006	0.01	0.002		0.003	0.003	0.003		0.016	0.002	0.002		0.002	0.001	0.001
S.Ed. (±)		0.009	0.013	0.003		0.004	0.004	0.004		0.023	0.003	0.002		0.003	0.001	0.001
CD @ 5%		0.018	0.027	0.006		0.009	0.008	0.008		0.046	0.005	0.005		0.006	0.003	0.002

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140ppm

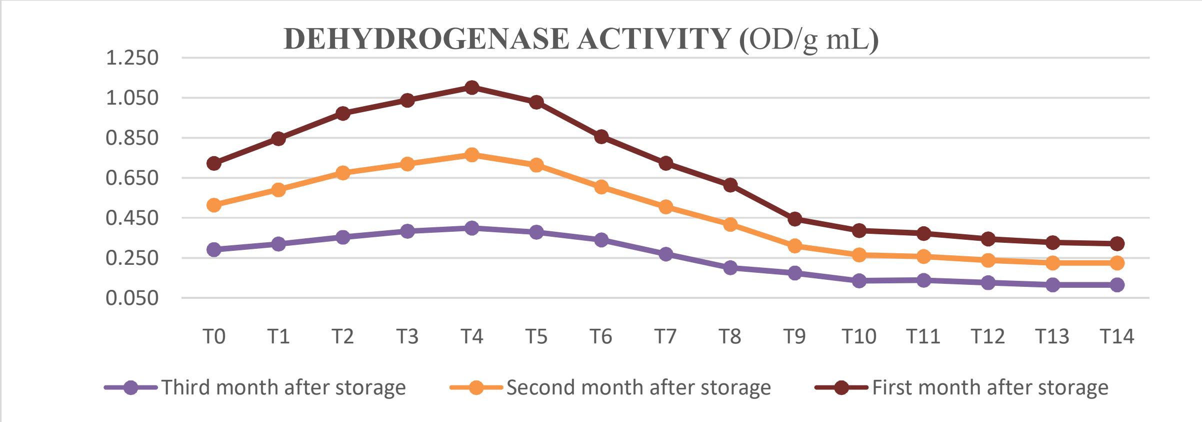

Figure 2. Effect of seed treatments on dehydrogenase activity (OD/g mL) **in onion variety Nasik Red N-53 during seed storage**

Effect of TiO_2 nanoparticle treatment on seedling parameters: A significant difference was observed for all the parameters of TiO_2 nanoparticle treated seeds. Germination per cent significantly reduced in all treatments from the initial to the end of the storage period. The lowest reduction trend was found in onion seeds treated with TiO_2 @40ppm (T_4) (66% to 58.50%) followed by TiO_2 @30ppm (T_3) and highest reduction was found in TiO_2 @140ppm (66% to 45.25%) (Table 1 and fig 1). The germination per cent was decreased in TiO_2 @80ppm and above concentration compared to the control. The reduction in germination per cent at higher concentrations might be due to the toxicity which affect the germination by reducing the water uptake (Eskandarinasab *et al.*, 2019). Alike evident was found by Azimi *et al.*, (2013) reported highest germination per cent (84%) in lower concentration of TiO_2 (40ppm) as compared to control in wheatgrass (*Agropyron desertorum*). The increase in the germination per cent at low concentration might be due to the photo sterilization and photo-generation of 'active oxygen like superoxide and hydroxide anions' which leads to increase of water and oxygen uptake by increasing the seed stress resistance and by promoting capsule penetration (Khot *et al.*, 2012) and by promoting activities of nitrate reductase enzyme and seed antioxidant system (Lu *et al.*, 2002). Similar reduction trend was found in speed of germination during storage period. The minimum decline in speed of germination was recorded withTiO_2 @ 40ppm (T_4) (4.834 to 4.209) followed by TiO_2 @30ppm (T_3) whereas maximum decline was found in TiO_2 @140ppm (4.834 to 3.146) (Table 1). It was found that speed of germination was significantly affected with TiO_2 nanoparticles at lower concentration (10ppm to 70ppm) which might be due to the increase in the cell division rate in the seed radicle meristem (Badiyeh *et al.*, 2017). Due to the hygroscopic nature of seeds, the initial moisture per cent was increased with the advancement of storage period. Seeds treated with TiO_2 @40ppm (T_4) recorded the lowest increase in moisture per cent (7.80% to8.04%) followed by TiO_2 @30ppm (T_3) whereas highest increase was recorded in control (T_0) (7.80% to 8.30%) (Table 1). Root, shoot and seedling length was significantly affected by TiO_2 nanoparticles, although the root, shoot and seedling length were decreased with the increasing storage period (Table 1 and Table 2). Application of TiO_2 @40ppm (T_4) recorded highest root length (3.07 cm), shoot length (4.58 cm) and seedling length (7.65 cm) at the end of the storage period. TiO_2 @80ppm and above concentration have showed reduction in root length, shoot length and seedling length which might be due to toxicity of TiO_2 nanoparticles (Azimi *et al.*, 2013). Feizi *et al.*, (2012) reported that seedling length is affected by lower concentrations of TiO_2 nanoparticles. Fresh weight and dry weight were significantly affected by TiO_2 nanoparticle treatment. The of storage period. The reduction was lower with TiO_2 @40ppm for fresh weight (0.256 gm to 0.222 gm) and for dry weight (0.0335 gm to 0.0259 gm) and followed by TiO_2 @30ppm. Use of TiO_2 @80ppm and above concentrations greatly reduced the fresh and dry weight, every month during storage (Table 2). Azimi *et al*., (2013) reported maximum fresh and dry weight at 40ppm of nano-TiO_2 and also reported alike decreasing trend in higher concentrations. Zhou *et al.,* (2022) stated that TiO_2 nanoparticles increase the fresh weight as compared to control. The downfall of fresh weight at higher concentration (100ppm) might be due to toxicity. Priya *et al.,* (2016) reported highest seedling dry weight at lower concentration of TiO_2 nanoparticles (20ppm, 40ppm) in cowpea might be due to the highest accumulation of dry matter content at lower concentration. Electrical conductivity was increased with the advancement of the storage period. At the end of the storage period lowest electrical conductivity (0.951 dS/m) was found at TiO_2 @40ppm (T_4) due to the less electrolyte leachate of seed coat at the particular concentration which shows less damage and more vigour but at the control (T_0) due to more damage of membrane, highest electrical conductivity (0.990 dS/m) was found (Table 3). A reduction trend was found in seed density with the advancement of the storage period. The lowest reduction was found with TiO_2 @40ppm (T_4) (1.077 gm/cm^3 to 1.075 gm/cm^3) followed by TiO_2 @30ppm (T_3) and the highest reduction was found in control (T_0) (1.077 gm/cm^{3}to 1.075 gm/cm^3) followed by TiO_2 @30ppm (T_3) and the highest reduction was found in control (T_0)(1.077 gm/cm^3 to 1.018 gm/cm^3). Shah *et al*., (2021) reported that TiO_2 nanoparticle priming significantly reduced the relative electrolyte leakages of the membrane in maize thus improve the storage. TiO_2 nanoparticle ensures the protections against the electrolyte leakages of membranes. The findings are similar with previous findings, stated that TiO_2 helps in maintaining membrane integrity (Mahmoodzadeh *et al*., 2013). Similar trend of reduction was found in dehydrogenase activity (OD value). The lowest decline was found in concentration TiO_2 @40ppm (0.446 to 0.337) followed by TiO_2 @30ppm as compared to control (Table 3 and figure 2). Dehydrogenase is a respiratory enzyme that is found in germinating seeds that catalases the food materials during respiration and yields energy to the growing seedling (Oikhena *et al*., 2013). With the increasing storage period the catalase activity was also declined. Highest catalase activity was found with TiO_2 @40ppm (T_4) (0.0375 nmol/min/mg protein) followed by TiO_2 @30ppm (T_3) over control (0.0114 nmol/min/mg protein) at the end of the storage period (Table 3 and figure 3). Catalase is an antioxidant enzyme that provides tolerance to the stress conditions (Kibinza *et al*., 2011) and its high presence in seeds indicates high seed quality (Bailly *et al*., 2001). It was suggested that priming with TiO_2 nanoparticles could improve the catalase activity in maize by scavenging reactive oxygen species (Mahmoodzadeh *et al*., 2013), (Shah *et al*., 2021). Sardar *et al*., (2021) reported an increase in catalase activity of coriander seeds treated with TiO_2 nanoparticles.

Interaction effect of storage containers and seed treatment on seedling parameters: Interaction effect due to the storage container and seed treatment was significant only after third month of storage except for the first count per cent, dry weight, seed density and catalase activity. All the seed quality parameters declined with the advancement of storage period except for electrical conductivity and moisture per cent. After 3 months of storage, the seeds treated with TiO_2 @40ppm and stored in aluminum foil pouch (C_2T_4) recorded highest germination per cent (60%) followed by seed treated with TiO_2 @30ppm and stored in aluminum foil pouch (C_2T_3) (Table 4). The same treatment combination (C_2T_4) also recorded highest speed of germination (4.358), root length (3.12 cm) (Table 5), shoot length (4.65 cm) and seedling length (7.77 cm) (Table 5) at the end of the storage period due to fast germination, cell division at radicle meristem region and root-shoot elongation. The seeds treated with TiO_2 @40ppm and stored in aluminum foil pouch (C_2T_4) recorded lowest moisture per cent (7.90%) whereas highest moisture per cent (8.40%) was recorded with untreated seeds stored in tin container (C_1T_0) after 3 months of storage (Table 4). The same treatment combination (C_2T_4) recorded highest fresh weight (0.227 gm) and dry weight (0.0265 gm) followed by the combination C_2T_3 and the lowest was recorded in the combination of seed treatment with TiO_2 @140ppm and tin container (C_1T_{14}) at the end of the storage period. Lowest electrical conductivity (0.994 dS/m) was found in the combination C_2T_4 and followed by C_2T_3 and highest electrical conductivity (1.123 dS/m) was recorded with the combination C_1T_0 at the end of the storage period (Table 6).

Table 4. Influence of interaction of storage containers and seed treatment on germination per cent, speed of germination, moisture per cent and root length of onion seeds during storage

	Germination percent				Speed of germination				Moisture per cent				Root length (cm)			
Interaction	**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**			**Initial**	**Months after storage**		
(CXT)		1	2	3		1	2	3		1	2	3		1	2	3
C_1T_0		58.50	53.50	49.50		3.937	3.746	3.527		8.15	8.23	8.40		2.84	2.41	2.36
C_1T_1		61.50	58.50	54.00		4.034	3.861	3.749		8.03	8.05	8.25		2.90	2.54	2.61
C_1T_2		63.50	59.00	54.50		4.106	3.916	3.790		8.00	8.03	8.23		3.23	2.97	2.80
C_1T_3		64.00	60.50	55.50		4.548	4.232	3.961		7.98	7.95	8.20		3.35	3.17	2.92
C_1T_4		64.00	61.50	57.00		4.599	4.473	4.060		7.95	7.93	8.18		3.42	3.22	3.01
C_1T_5		61.00	58.00	55.00		4.507	4.201	3.904		8.10	8.13	8.23		3.08	3.12	2.91
C_1T_6		57.50	55.00	52.50		3.978	3.824	3.658		8.10	8.15	8.33		2.83	3.02	2.75
C_1T_7		57.00	53.50	50.50		3.870	3.851	3.484		8.05	8.08	8.23		2.58	2.52	2.44
C_1T_8		56.50	53.50	48.00		3.736	3.689	3.510		8.08	8.08	8.30		1.87	1.71	2.00
C_1T_9		53.00	51.50	49.50		3.697	3.617	3.277		8.05	8.10	8.25		1.69	1.68	1.57
C_1T_{10}		56.50	52.00	49.50		3.525	3.572	3.226		8.13	8.15	8.23		1.65	1.58	1.47
C_1T_{11}		57.50	50.00	45.50		3.741	3.462	3.189		8.13	8.18	8.20		1.65	1.81	1.51
C_1T_{12}		53.50	52.00	46.50		3.649	3.438	3.157		8.08	8.10	8.20		1.85	1.49	1.49
C_1T_{13}		50.50	49.50	45.50		3.322	3.337	3.184		8.15	8.15	8.25		1.69	1.50	1.43
C_1T_{14}		51.50	49.00	45.00		3.703	3.208	3.126		8.08	8.08	8.20		1.58	1.59	1.40
C_2T_0	66.00	59.00	56.00	52.00	4.834	3.996	3.753	3.639	7.80	8.15	8.20	8.20	3.95	3.13	3.09	2.59
C_2T_1		60.50	59.00	55.00		4.173	4.136	3.946		7.98	8.03	8.05		3.45	3.12	2.73
C_2T_2		63.50	62.00	57.50		4.406	4.361	4.073		7.95	8.00	8.03		3.49	3.13	2.83
C_2T_3		64.00	62.50	58.50		4.606	4.438	4.289		7.93	7.93	7.93		3.54	3.35	3.04
C_2T_4		**64.50**	**63.00**	**60.00**		**4.641**	**4.607**	**4.358**		**7.88**	**7.88**	**7.90**		**3.64**	**3.41**	**3.12**
C_2T_5		62.50	61.50	58.00		4.597	4.410	4.285		7.95	7.95	7.98		2.92	3.22	3.02
C_2T_6		60.50	59.00	57.50		4.270	3.867	3.962		8.03	8.03	8.05		2.19	2.68	2.61
C_2T_7		60.50	58.00	54.50		4.107	3.994	3.892		8.00	8.03	8.08		2.62	2.27	2.38
C_2T_8		58.00	55.00	51.50		3.875	3.846	3.774		8.03	8.00	8.03		2.43	2.07	2.05
C_2T_9		56.50	53.50	50.50		3.851	3.681	3.633		8.00	8.03	8.03		2.15	1.90	1.84
C_2T_{10}		56.00	53.00	51.00		3.740	3.564	3.482		7.93	7.98	8.00		1.89	1.77	1.69
C_2T_{11}		55.00	51.50	49.50		3.831	3.494	3.386		8.03	8.10	8.13		1.75	1.87	1.61
C_2T_{12}		50.00	52.00	46.00		3.648	3.490	3.336		8.00	8.10	8.10		1.68	1.69	1.59
C_2T_{13}		49.00	51.50	47.00		3.458	3.473	3.256		8.05	8.05	8.15		1.63	1.60	1.51
C_2T_{14}		51.50	51.00	45.50		3.653	3.526	3.165		8.08	8.10	8.18		1.60	1.50	1.48
Mean		57.90	55.52	51.73		3.993	3.835	3.642		8.03	8.06	8.15		2.48	2.37	2.22
S.Em. (±)		2.12	1.36	0.79		0.15	0.15	0.03		0.04	0.03	0.03		0.21	0.14	0.03
S.Ed. (±)		3.00	1.93	1.11		0.21	0.21	0.05		0.05	0.04	0.04		0.3	0.2	0.04
CD @ 5%		NS	NS	2.21		NS	NS	0.09		NS	0.08	0.09		NS	NS	0.07

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140ppm

Table 5. Influence of interaction of storage containers and seed treatments on shoot length, seedling length, fresh weight and dry weight of onion seeds during storage

	Shoot length (cm)				Seedling length (cm)				Fresh weight (gm)				Dry weight (gm)			
Interaction	Initial	Months after storage			Initial	Months after storage			Initial	Months after storage			Initial	Months after storage		
CXT		1	2	3		1	2	3		1	2	3		1	2	3
C_1T_0	5.47	4.81	4.25	3.93	9.42	7.65	6.66	6.29	0.256	0.218	0.193	0.157	0.0335	0.0253	0.0223	0.0185
C_1T_1		5.04	4.55	4.25		7.93	7.09	6.86		0.221	0.210	0.179		0.0258	0.0243	0.0210
C_1T_2		5.06	4.58	4.37		8.29	7.55	7.17		0.223	0.216	0.187		0.0260	0.0250	0.0220
C_1T_3		5.11	4.64	4.44		8.47	7.81	7.36		0.240	0.238	0.205		0.0278	0.0278	0.0238
C_1T_4		5.15	4.77	4.52		8.56	7.99	7.53		0.258	0.243	0.217		0.0300	0.0283	0.0253
C_1T_5		5.02	4.58	4.42		8.10	7.69	7.32		0.240	0.235	0.204		0.0275	0.0275	0.0235
C_1T_6		4.84	4.56	4.20		7.67	7.58	6.95		0.240	0.240	0.192		0.0273	0.0275	0.0225
C_1T_7		4.83	4.56	4.07		7.40	7.08	6.51		0.232	0.235	0.178		0.0263	0.0273	0.0205
C_1T_8		4.81	4.55	3.92		6.68	6.27	5.91		0.233	0.202	0.154		0.0270	0.0233	0.0180
C_1T_9		4.72	4.54	3.83		6.40	6.22	5.39		0.207	0.228	0.146		0.0238	0.0263	0.0173
C_1T_{10}		4.57	4.49	3.61		6.22	6.07	5.08		0.199	0.197	0.138		0.0230	0.0228	0.0163
C_1T_{11}		4.54	3.92	3.58		6.18	5.73	5.10		0.194	0.182	0.144		0.0223	0.0210	0.0168
C_1T_{12}		4.55	3.99	3.40		6.40	5.48	4.89		0.198	0.182	0.140		0.0230	0.0210	0.0163
C_1T_{13}		4.51	3.57	3.47		6.20	5.07	4.90		0.194	0.177	0.133		0.0220	0.0205	0.0155
C_1T_{14}		4.48	3.53	3.34		6.06	5.12	4.74		0.179	0.168	0.130		0.0208	0.0193	0.0148
C_2T_0		4.86	4.72	4.18		7.99	7.81	6.77		0.221	0.199	0.173		0.0255	0.0228	0.0203
C_2T_1		5.25	4.96	4.44		8.70	8.07	7.17		0.226	0.219	0.188		0.0263	0.0253	0.0218
C_2T_2		5.27	4.96	4.52		8.76	8.08	7.35		0.231	0.222	0.193		0.0268	0.0255	0.0225
C_2T_3		5.33	5.09	4.60		8.86	8.44	7.64		0.253	0.246	0.214		0.0293	0.0285	0.0253
C_2T_4		**5.39**	**5.18**	**4.65**		**9.03**	**8.59**	**7.77**		**0.261**	**0.247**	**0.227**		**0.0305**	**0.0295**	**0.0265**
C_2T_5		4.98	4.86	4.55		7.90	8.07	7.57		0.252	0.235	0.213		0.0293	0.0273	0.0250
C_2T_6		5.03	4.69	4.40		7.22	7.37	7.01		0.251	0.234	0.196		0.0290	0.0270	0.0230
C_2T_7		4.99	4.48	4.34		7.61	6.75	6.72		0.248	0.233	0.191		0.0285	0.0268	0.0223
C_2T_8		4.96	4.43	4.17		7.39	6.51	6.22		0.227	0.224	0.170		0.0265	0.0263	0.0198
C_2T_9		4.83	4.14	3.90		6.98	6.04	5.74		0.226	0.226	0.162		0.0263	0.0265	0.0188
C_2T_{10}		4.76	3.98	3.69		6.64	5.75	5.37		0.215	0.205	0.151		0.0248	0.0238	0.0178
C_2T_{11}		4.71	3.96	3.67		6.46	5.83	5.27		0.209	0.203	0.148		0.0243	0.0235	0.0170
C_2T_{12}		4.73	3.91	3.60		6.41	5.59	5.19		0.200	0.201	0.142		0.0233	0.0233	0.0163
C_2T_{13}		4.69	3.73	3.57		6.32	5.34	5.08		0.199	0.195	0.136		0.0233	0.0213	0.0160
C_2T_{14}		4.66	3.70	3.49		6.25	5.20	4.97		0.192	0.183	0.134		0.0223	0.0200	0.0155
Mean		4.88	4.39	4.04		7.36	6.76	6.26		0.223	0.214	0.171		0.0258	0.0247	0.0200
S.Em. (±)		0.03	0.03	0.03		0.21	0.15	0.04		0.006	0.010	0.002		0.0010	0.0011	0.0003
S.Ed. (±)		0.04	0.05	0.05		0.30	0.21	0.06		0.008	0.015	0.003		0.0010	0.0016	0.0004
CD @ 5%		0.08	0.10	0.09		NS	0.42	0.12		NS	NS	0.005		NS	NS	NS

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140ppm

Table 6. Influence of interaction of storage containers and seed treatments on electrical conductivity, seed density, dehydrogenase activity and catalase activity of onion seeds during storage

	Electrical conductivity (dS/m)				Seed density (gm/cm^3)				Dehydrogenase activity (OD/g mL)				Catalase activity (nmol/min/mg protein)			
Interaction	Initial	Months after storage			Initial	Months after storage			Initial	Months after storage			Initial	Months after storage		
CXT		1	2	3		1	2	3		1	2	3		1	2	3
C_1T_0	0.848	0.983	1.030	1.123	1.077	1.017	1.008	0.983	0.446	0.286	0.215	0.201	0.0625	0.0145	0.0120	0.0100
C_1T_1		0.970	0.975	0.994		1.027	1.020	1.008		0.305	0.256	0.244		0.0198	0.0180	0.0143
C_1T_2		0.967	0.971	0.978		1.047	1.033	1.023		0.337	0.311	0.290		0.0263	0.0238	0.0215
C_1T_3		0.948	0.963	0.970		1.058	1.048	1.040		0.389	0.331	0.314		0.0393	0.0368	0.0318
C_1T_4		0.945	0.961	0.957		1.070	1.065	1.055		0.389	0.357	0.332		0.0545	0.0415	0.0360
C_1T_5		0.951	0.968	0.970		1.052	1.045	1.038		0.388	0.329	0.312		0.0383	0.0365	0.0288
C_1T_6		0.954	0.974	0.984		1.032	1.030	1.028		0.344	0.256	0.237		0.0253	0.0250	0.0210
C_1T_7		0.968	0.979	0.993		1.032	1.025	1.020		0.244	0.213	0.195		0.0250	0.0195	0.0178
C_1T_8		0.976	0.987	0.989		1.025	1.020	1.015		0.202	0.216	0.186		0.0270	0.0180	0.0165
C_1T_9		0.967	0.975	0.989		1.030	1.028	1.018		0.165	0.105	0.122		0.0303	0.0225	0.0170
C_1T_{10}		0.958	0.969	0.988		1.032	1.030	1.013		0.130	0.126	0.119		0.0253	0.0183	0.0165
C_1T_{11}		0.967	0.968	0.974		1.028	1.038	1.018		0.147	0.113	0.112		0.0190	0.0198	0.0148
C_1T_{12}		0.963	0.976	0.988		1.035	1.025	1.003		0.107	0.108	0.102		0.0200	0.0218	0.0145
C_1T_{13}		0.961	0.987	0.973		1.037	1.020	0.993		0.099	0.103	0.099		0.0253	0.0133	0.0130
C_1T_{14}		0.954	0.985	0.983		1.047	1.015	0.995		0.104	0.101	0.093		0.0165	0.0128	0.0128
C_2T_0		0.985	1.000	1.031		1.020	1.013	0.998		0.297	0.231	0.217		0.0183	0.0143	0.0128
C_2T_1		0.953	0.943	0.970		1.035	1.023	1.015		0.334	0.287	0.267		0.0290	0.0233	0.0193
C_2T_2		0.944	0.942	0.962		1.055	1.045	1.033		0.371	0.330	0.306		0.0308	0.0255	0.0233
C_2T_3		0.929	0.933	0.952		1.062	1.050	1.045		0.377	0.344	0.321		0.0455	0.0388	0.0358
C_2T_4		**0.882**	**0.908**	**0.944**		**1.080**	**1.068**	**1.058**		**0.409**	**0.374**	**0.343**		**0.0600**	**0.0458**	**0.0390**
C_2T_5		0.936	0.937	0.955		1.052	1.045	1.040		0.368	0.341	0.320		0.0453	0.0383	0.0330
C_2T_6		0.946	0.937	0.964		1.045	1.038	1.033		0.334	0.274	0.265		0.0338	0.0273	0.0255
C_2T_7		0.952	0.955	0.962		1.042	1.030	1.025		0.296	0.256	0.242		0.0315	0.0235	0.0213
C_2T_8		0.933	0.950	0.970		1.032	1.025	1.023		0.200	0.218	0.204		0.0210	0.0210	0.0188
C_2T_9		0.932	0.936	0.971		1.035	1.030	1.020		0.184	0.167	0.145		0.0260	0.0203	0.0210
C_2T_{10}		0.944	0.944	0.983		1.030	1.033	1.023		0.140	0.132	0.125		0.0268	0.0195	0.0180
C_2T_{11}		0.930	0.953	0.964		1.037	1.040	1.020		0.131	0.123	0.120		0.0305	0.0218	0.0178
C_2T_{12}		0.926	0.959	0.964		1.042	1.028	1.023		0.144	0.118	0.109		0.0238	0.0180	0.0165
C_2T_{13}		0.934	0.966	0.974		1.037	1.023	1.015		0.130	0.117	0.107		0.0248	0.0153	0.0150
C_2T_{14}		0.942	0.951	0.981		1.033	1.018	1.003		0.126	0.117	0.100		0.0203	0.0148	0.0138
Mean		0.950	0.963	0.980		1.040	1.032	1.021		0.249	0.219	0.205		0.0291	0.0235	0.0206
S.Em. (±)		0.009	0.013	0.003		0.004	0.004	0.004		0.023	0.003	0.002		0.003	0.001	0.001
S.Ed. (±)		0.013	0.019	0.004		0.006	0.006	0.006		0.032	0.004	0.003		0.004	0.002	0.001
CD @ 5%		NS	NS	0.008		NS	NS	NS		NS	0.008	0.007		NS	NS	NS

C_1= Tin container; C_2= Aluminum foil pouch; T_0= Control; T_1= TiO_2 @10ppm; T_2= TiO_2 @20ppm; T_3= TiO_2 @30ppm; T_4= TiO_2 @40ppm; T_5= TiO_2 @50ppm; T_6= TiO_2 @60ppm; T_7= TiO_2 @70ppm; T_8= TiO_2 @80ppm; T_9= TiO_2 @90ppm; T_{10}= TiO_2 @100ppm; T_{11}= TiO_2 @110ppm; T_{12}= TiO_2 @120ppm; T_{13}= TiO_2 @130ppm; T_{14}= TiO_2 @140

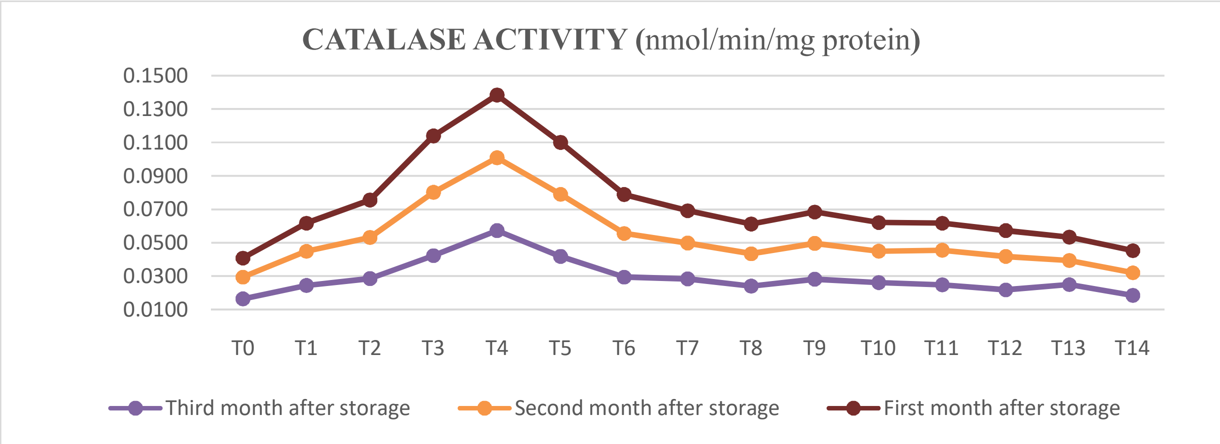

Figure 3. Effect of seed treatments on catalase activity (nmol/min/mg protein) **in onion variety Nasik Red N-53 during seed storage**

Similar treatment combination (C_2T_4) also exhibited highest seed density (1.058 gm/cm^3) followed by C_2T_3 after 3 months of storage and lowest seed density (0.983 gm/cm^3) was recorded with the treatment combination C_1T_0 at the end of the storage period. The combination C_2T_4 also recorded highest dehydrogenase activity (0.343 OD/g mL) and catalase activity (0.0390 nmol/min/mg protein) followed by C_2T_3 at the end of the storage period.

Conclusion

According to the current study, it is found that all the seedling parameters except electrical conductivity and moisture per cent expressed reduction trend with the advancement of storage period irrespective of storage containers and nano-TiO_2 seed treatment. It is concluded that seed treatment with TiO_2 nanoparticles have positive effect on seedling parameters as well as on the enzymatic activity (Dehydrogenase activity and catalase activity). Seed treatment with titanium dioxide (TiO_2) nanoparticles at lower concentration (10ppm to 70ppm) is beneficial for improving seedling parameters in onion and it is economically feasible. Among the containers, the seeds stored in aluminum foil pouch, exhibited highest seedling parameters and showed less damage of seeds (lowest moisture per cent and EC), so it performed well during the storage period. At the end of the storage period, it was found that the seeds treated with TiO_2 @40ppm and stored in aluminum foil pouch recorded highest seedling parameters and performed better in storage. So, this can be recommended for onion to improve seedling parameters and to extend the storage life of the seeds. These recommendations are based on three months experimentation and therefore further investigation is needed to arrive at valid recommendation.

Future prospects

In the field of agriculture, nanotechnology has been used to heighten the crop production with quality enrichment by improving farming systems. Low dose of nanoparticles can be assessed in order to encourage seed germination and seedling growth of different plant species. The potential of nanoparticles encourages a new green revolution with reduced farming risks. However, there are still huge gaps in our knowledge of the uptake capacity, permissible limit and the ecotoxicity of different nanoparticles on different crops. Therefore, further research is urgently needed to elucidate and develop potential.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Ecotoxicological studies of selected agrochemicals on the moulting stages of *Cyphoderus javanus* under laboratory conditions

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ABSTRACT

Indiscriminate use of toxic pesticides in agriculture has raised serious concerns worldwide, which led to a steady destruction of soil biota as well as the overall health of ecosystem. The present study was designed to evaluate the effects of selected agrochemicals such as cyfluthrin (insecticide), fytran (fungicide) and oxadiargyl (herbicide) on the moulting stages of soil springtail *Cyphoderus javnaus*. Laboratory analysis results indicated that the agrochemical treated organisms showed prolonged moulting intervals and need more days than normal to become a complete adult having sexual maturity. On treatment with the sub lethal concentration of oxadiargyl, first moult noticed after12th day, second moult after24 th day, third moult after 36 th day of egg hatching. The effect of herbicide oxadiargyl on Cyphoderus javanus was found to be comparatively higher than that of other two pesticides. The changes noticed in developmental stages, moulting period and egg laying pattern in *Cyphoderus javanus* when exposed to agrochemicals indicated that this soil collembolan is a potential biomarker of ecotoxicology investigations.

Introduction

Soil ecosystems are the crucial and basic assets of any kinds of anthropogenic needs and also serves as the essential constituents of agricultural sustainability (Bech *et al.*,2008). In addition to the fundamental aspects of soil biome, inhabiting microarthropods account for huge and ecologically relevant portion of soil biodiversity and they play an inevitable function in degradation by fragmenting and grazing on organic residues present in soil by fungal groups and aids to liberate nutritive elements for plant growth (Abbas and Parwez,2020). Among them, springtails are the most abundant groups and are considered as bioindicators of soil quality because of their rapid reaction to environmental shifts (Florian *et al.*,2019). They also performed a dominant role in paedogenesis, elevating air circulation, void fractions as well as the soil quality improvement by the disintegration of organic litters by complex activities of digestion (Banarjee *et al.*, 2009).The enormous agricultural yields currently obtained in many parts of the world are often achieved with the help of excessive fertilizer use (Ju *et al.,* 2009). According to Yanez *et al.* (2002), modern agricultural methods followed by unsustainable practices in many developing nations, have resulted in an enormous quantity of contaminated wastes being dumped into the natural habitats including air, water and land. Deep plowing and monocrop cultivation may result in resource exhaustion and large-scale soil weathering, excessive usage of inorganic pesticides as well as fertilizers have caused adulteration to our riverine ecosystems and soil environment (Baishya,2015). The results of intensive applications of agricultural products on soil inhabiting organisms can be estimated either as fluctuations in the population of organisms or as variations in the metabolic activities of individuals (Bunemann *et al.,*2006). *Cyphoderus javanus* is a tropical terrestrial collembolan present in decaying leaf litter and moist fertile soils. These

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collembolans play an essential link in detritus food chain and has a notable role in decomposition of organic matter. It's role as detritivores and biomarkers of soil ecosystem health makes them as an apt organism for ecotoxicological studies. Recently more researches were carried out focus the impacts of different pesticides on various aspects of life history in soil microarthropods by numerous investigators such as Bini (2019), Vinod (2019), Thomas and Kumar (2020 & 2022), D Lima e Silva *et al.* (2020 & 2022) and Aswin and Kumar (2022). However, toxicity studies on moulting stages of soil arthropods are comparatively very few. Therefore, the present study was mainly designed to study the adverse effects of commonly used some agrochemicals (Cyfluthrin, Fytran and Oxadiargyl) on pre adult moulting interval of common springtail *Cyphoderus javanus.*

Material and Methods

The live adult specimens of collembola, *Cyphoderus javanus* were collected from soil and leaf litters in various sites (Neyyar, Vithura and Agasthyavanam Biological Park) of Thiruvananthapuram district using soil augar. The obtained *Cyphoderus javanus* were extracted by using modified Tullgren funnel and reared in special plastic containers of 7×3cm size with a combination of Plaster of paris, activated charcoal and distilled water in the ratio 5:1:5 as base (Thomas and Kumar, 2020).

These culture vessels are kept under in a controlled environment with temperature of 28.9± 0.5 °C and light dark cycle 12:12 h in a BOD incubator. Moulting studies of *C. javanus* were carried out according to the standard procedures prescribed by Bini and Sanalkumar (2017). For estimating moulting intervals, juveniles arise from egg were cultured separately by transferring a group of ten organisms into new culture vessels with fine moistened camel hair brush. The laboratory cultured individuals of similar age groups were chosen for the investigation and fed with decaying jack leaves soaked with sublethal concentration of agro chemicals as food. Three replicates of *Cyphoderus javanus* and a control were maintained for each agrochemical testing. The number of days needed for each moult and mean number of days for each moult were carefully estimated. One-way ANOVA was conducted to determine the variations in the number of days in the replicates with respect to moulting period of *C. javanus* in normal and agrochemical treated groups (Thomas, 2022).

Results and Discussion

As shown in Table-1, the collembolan *Cyphoderus javanus* showed four successive moults under normal conditions. The first moult initiated after seven days of egg hatching, second moult after twelve days of hatching, third moult after fifteen days and fourth moult after twenty-three days of hatching and after thirty days of egg hatching, adult started egg deposition. Kaleka *et al.* (2019) cited that the insect larvae undergo moulting processes with number of times and environmental conditions such as humidity, temperature, photoperiod, food quality and quantity affect the number of instars. Butcher *et.al.* (1971) stated that the number of moults and duration needed to attain sexual maturity differs with individuals.

While comparing with control groups, experimental groups showed prolonged moulting period and takes more days to became adult (Table 2). After treatment with insecticide cyfluthrin four moults were obtained, first moult was noticed after ten days of hatching, second moult after sixteen days of hatching, third moult after nineteen days of hatching and four moult after twenty-nine days of hatching and adult laid eggs after thirty-six days of hatching. After exposure with fungicide fytran, first moult was recorded after twelve days of egg hatching and second moult after twenty-two days, third moult after twenty-six days, fourth moult after thirty-five days and after fourty three days adult stage started, having no ability to lay eggs. After treating with herbicide oxiadargyl, only three moults were observed, lacks fourth moult. The first moult was started on twenty third day of hatching, second moult on thirty fifth day and third moult on fifty third days of egg hatching. Similar to fytran treated sets, these experimental groups containing adults was notable to lay eggs. The results of one- way ANOVA indicated that there was no significant variation moulting periods between replicates in control sets and agrochemical treated sets ($P>0.05$) (Table 3).Bini and Sanalkumar (2017) and Aswin and Kumar (2022) have noticed the negative effects of herbicides on soil isopods, showed drastic reduction in fecundity and their

Table 1: Normal moulting interval of *Cyphoderus javanus* at 28.9±0.5^{0}C

Stage	Replicate I (Days)	Replicate II (Days)	Replicate III (Days)	Mean ± SE
Rest	5.0	6.4	7.2	6.2±0.44
1st moult	11.9	12.4	10.9	11.7±0.22
2nd moult	13.6	14.8	15.1	14.5±0.21
3rd moult	22.7	23.9	22.6	23.1±0.15
4th moult	30.1	28.9	28.3	29.1±0.17
Adult	Eggs laid			

Table 2: Moulting interval of *Cyphoderus javanus* exposed to different agrochemicals at 28.9±0.5^{0}C

Stage	Cyfluthrin				Fytran				Oxadiargyl			
	Replicate I (Days)	Replicate II (Days)	Replicate III (Days)	Mean ± SE	Replicate I (Days)	Replicate II (Days)	Replicate III (Days)	Mean ± SE	Replicate I (Days)	Replicate II (Days)	Replicate III (Days)	Mean ± SE
Rest	8.1	10.5	9.2	9.3 ±0.39	10.1	11.0	12.1	11.1 ±0.30	11.8	12.7	14.1	12.8 ±0.34
1st moult	15.2	16.4	16.5	16.0 ±0.18	19.6	20.8	22.2	20.9 ±0.75	25.4	25.2	23.8	24.8 ±0.17
2nd moult	17.9	19.5	19.3	18.9 ±0.21	24.6	25.8	26.1	25.5 ± 0.45	36.8	37.5	35.6	36.5 ±0.16
3rd moult	24.8	28.1	29.2	27.3 ±0.43	27.8	35.0	32.4	31.7 ±0.61	54.3	54.1	53.4	53.9 ±0.06
4th moult	36.1	33.2	34.4	34.5 ±0.25	43.9	41.5	43.1	42.8 ±0.19	-	-	-	-
Adult	Eggs laid				Eggs not laid				Eggs not laid			

Table 3: One-way ANOVA showing pre -adult moulting interval of *Cyphoderus javanus* in different treatments

Treatments	Between replicates		
	F value	P-value	F-crit value
Control	0.0038	0.9963*	3.68232
Cyfluthrin	0.0134	0.9867*	3.68232
Fytran	0.0201	0.9802*	3.68232
Oxadiargyl	0.0008	0.9992*	3.68232

*** No significant differences in moulting period between the different replicates (P >0.05)**

moulting intervals were prolonged. Haque *et al.* (2011) and Chakravorty *et al.* (2015) pointed that smaller longevity, elevated duration of moulting and early development on *Cyphoderus javanus* subjected to various concentration of pesticides. Liu *et al.* (2018) reported that the appearance or disappearance of highly sensitive and resistant organisms in an extremely polluted soil ecosystem may be act as a potential indicator of ecosystem fitness. Crouau and Moia (2006) studied the susceptibility of growth and fecundity of some chemicals on *Folsomia candida* and discovered adverse effects of these xenobiotics on numerous life history traits of *Folsomia* species. Ijumba *et al.* (2010) suggested that the insect growth regulator insecticides can disrupt the synthesis of chitin and the most influenced insects are not able to produce new cuticle and to undergoes complete moulting into the next phase. Saha and Joy (2014) observed the impact of insect growth regulator pesticides on soil springtail *Cyphoderus javanus* under microsom conditions affected growth rate of *C. javanus,* by decreasing the rate of moulting. Same patterns of results obtained in present study, agrochemicals treated *Cyphoderus javanus* were not able to moult and reproduce properly.

Conclusion

Soil microarthropods are the victim of collateral destruction resulting from extensive application of pesticides in crop fields which are used to eradicate insect pests. These pesticides cause an intense reduction in the abundance and diversity of soil biota particularly collembolans. The results of the laboratory experiments revealed that the selected pesticides cyfluthrin, oxadiargyl and fytran showed adverse toxic effects on the development of springtail *Cyphoderus javanus.* The moulting period of *Cyphoderus javanus* showed variations between normal and pesticidal treated experimental sets, which exhibited prolonged moulting interval and requires more days to become adult. This implies that restoration of ecosystem health after the overexposure of agrochemicals is not easy and may need several decades to regain its virgin condition. Therefore, it is highly advisable that these studied pesticides are to be utilized only in permissible amount (<0.05 ppm) suggested by the Department of agriculture, Government of India.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Screening of *Brassica* genotypes against mustard aphid under northern Indian Shivalik hill conditions

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ABSTRACT

Oilseed Brassica plays the important role in Indian agriculture in relation to oil production. The major insects in Brassicas are *Lipaphis erysimi*, *Bagrada hilaris*, *Athalia lugens proxima* (Klug), *Chromatomyia horticola* Goureau, *Pieris rapae*, *Bagrada cruciferarum* Kirkaldy are of practical importance. Out of these, the *Lipaphis erysimi* (Kalt.) [mustard aphid], a part of family Aphididae with order Hemiptera leads to great yield losses in rapeseed-mustard. To overcome this problem, the easiest solution is to grow resistant and tolerant varieties. The screening is done on the basis of aphid infestation index scale for categorization of the genotypes into resistance and tolerance. Experiments were carried out at the experimental farm of Division of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu during *rabi* of 2017–18 and 2018–19 to evaluate mustard germplasm against *Lipaphis erysimi* and selecting the resistant genotypes of Brassica for its cultivation and expansion purposes. A total of fifteen cultivars were evaluated based on population buildup of mustard aphid and infestation index. The genotypes RSPN-28, CNH-11-13, RL-1359, HNS-1101, GSC-101, CNH-11-2 and HNS-1102 were found moderately resistant during the *rabi* 2017–2018 and during *rabi* 2018–2019, due to weather conditions only two genotypes CNH-13-2 and RSPN-29 were found moderately resistant and susceptible against aphid infestation.

Introduction

Brassica belongs to tribe Brassicaceae (El-Esawi, 2016) is the important genus consisting of 238 genera and 3709 species (Poveda *et al.*, 2020) varied in respect of morphological as well as in genetical ways (Illardi *et al.*, 2020). They are known to be vegetable, fodder, oil, green manure as well as spice plants (Witzel *et al.*, 2021). This genus has 6 interrelated species. Out of six, three are diploid species namely, *Brassica nigra* (2n=16), *Brassica oleracea* (2n=18) and *Brassica rapa* (2n=20) and three are amphidiploid species namely, *Brassica carinata* (2n=34), *Brassica juncea* (2n=36) and *Brassica napus* (2n=38) (Sanlier *et al.*, 2018). The 'U' triangle defines the relationship among *Brassica*

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species (Zhang *et al*., 2022). The allopolyploid *Brassica napus* ($2n = 4x = 38$, AACC) is the third most (Javed *et al*., 2022) desirable oil crop in world comes after maize and oil palm obtained from interspecific hybridization between two species such as *Brassica rapa* having $2n= 2x= 20$, AA and *Brassica oleracea* having $2n = 2x = 18$, CC (Lu *et al*., 2019).

For oilseed brassica, India holds the second-place area wise and third place production wise in world and rapeseed-mustard produces 72.37 million tonnes from a stretch of 33.64 m ha (Anonymous, 2021). In India, Rapeseed-mustard is cultivated over an area of 6.23 m ha with production of 9.33 mt and productivity of 15 q/ha during 2018–19 (Anonymous, 2020). Each part of rapeseed such as seeds, flower, leaves, stem and root are useful for cosmetics, food, remedies and has other industrial applications. Especially the most useful part is the seed part because of its application for oil and protein source (Raboanatahiry *et al*., 2021).

The oil taken out from *Brassica napus* L. is high in quality wise (Hyder *et al*., 2021) and rich in terms of fatty acid profiling (Tiwari *et al.,* 2021). It is, therefore, subjected to various biotic as well as abiotic stresses, which brings down its productivity remarkably (Mahapatra *et al*., 2022). Keeping in view the growing demands of oils, every attempt is being put together for increasing the crop yield by adopting the modern agricultural practices, namely, utilization of high yielding varieties, heavy manuring and proper irrigation but these collaborated efforts are in vain if crop is not sheltered from the disastrous effect of insect-pests. The damage due to insect pest is one among the various major biotic factors leads to low productivity (Das *et al*., 2022). There are > 3 dozens of insect-pests are to be known in India in case of rapeseed mustard crop (Bakhetia and Sekhon, 1989) and among them the most disastrous is mustard aphid which however, solely prove as one of the limiting factors in the mustard production (Kumar *et al*., 2022).

Mustard aphid is a sucking pest and is the most destructive pest of brassica causing over 50 % yield loss (Fenning *et al*., 2020) by excessive phloem sap diversion (Duhlian *et al*., 2020). Especially, in case of rapeseed-mustard, it has occurred as the most atrocious insect-pest in the regions such as tropical and subtropical including India (Koramutla *et al*., 2016). This pest brings damage to plants by covering the entire flower bud, shoot and pod surface (Raj *et al*., 2017) at various stages such as vegetative, flowering, and also in pod formation stage (Taghizadeh, 2019). Commonly, sucking pest relatively become severe from the seedling stage. At time of heavy infestation, it causes the crop yield reduction to a large extent (Prasad and Ashwini, 2021). These aphids quantitatively and qualitatively affect plant production and processing through sap sucking, toxin injection and transmission of viruses from the Luteoviridae family, leading to leaf curling, shriveling and yellowing (Fidelis *et al*., 2018). The attack is extreme in those areas where the cloudy days are more in numbers throughout the pest activity period. In boosting the mustard aphid multiplication, environment makes the major contribution and out of these environmental parameters, rainfall, temperature and relative humidity have the major effect on the mustard aphid's survival and its multiplication (Kumar *et al*., 2000). Therefore, in present investigation, fifteen genotypes of *Brassica napus* were screened to detect the early tolerant varieties.

Material and Methods

Fifteen genotypes of *Brassica napus* mentioned (Table 1) were seeded at the experimental farm of Department of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu during the year 2017-18 and 2018-2019 with latitude 32°40 N and longitude 78°48 E. The experiment was remained for the causing the infestation of insects naturally and prevalence of aphid attack and for assuring their normal population growth, no insecticide was being used in contrary to the insect pests during the time. The first recording of mustard aphid was done after 50 days of sowing and subsequent data related to aphid population was taken on weekly basis, beginning from first incidence of pest and pursued up to the infestation come to an end (from February first week to March first week). Data of the aphid population was taken from randomly selected five plants in every replication on scale given by Bakhetia and Sandhu (Table 2). The observation of aphid population on each plant was done from 10 cm last shoot. By observing the aphids in every replication visually, population counts were being made on per plant basis by observing the number of aphids on different part of plant such as stem, leaves and inflorescence. Mean values was then calculated from the collected data to make the aphid population estimation based on per plant basis.

Aphid index was calculated by the following formula:

$$\textbf{Aphid Infestation Index}= \frac{0\times a+1\times b+2\times c+3\times d+4\times e+5\times f}{a+b+c+d+e+f}$$

Where a, b, c, d, e and f are the frequencies of the plants come under each grade from 0–5.

All the genotypes were categorized into different groups based on the aphid infestation index as mentioned in the table 3.

Table 1: List of Genotypes

SN	Genotypes	SN	Genotypes
1.	RSPN-29	9.	CNH-11-2
2.	RSPN-28	10.	GSC-6
3.	DGS-1	11.	EC552608
4.	RSPN-25	12.	CNH-13-2
5.	CNH-11-7	13.	HNS-1102
6.	CNH-11-13	14.	RL-1359
7.	GSL-1	15.	HNS-1101
8.	GSC-101		

Table 2: Screening of genotypes for aphid resistance by using the method of Bakhetia and Sandhu (1973)

Score	Injury symptoms
0	Free from aphid infestation.
1	Normal growth, no yellowing or curling of leaves, a few aphids without injury symptoms, flowering and pod setting almost normal.
2	Average growth, flowering and pod setting; curling and yellowing of few leaves.
3	Curling and yellowing of some branches, below average growth, poor flowering, with very little pod setting.
4	Very poor growth, heavy curling of leaves, stunting of plants, A little or no flowering and pod formation.
5	Heavy aphid colonies, severe stunting of plants, curling and yellowing of almost all leaves. No flowering and pod formation. Plants full of aphids.

Table 3: Categorization of genotypes on the basis of aphid infestation index:

Aphid Infestation Index	Designation
Up to 1.0	Highly resistant
1.1-2.0	Resistant
2.1-3.0	Moderately Resistant
Above 3	Susceptible

Results and Discussion

One of the most limiting factors in the rapeseed and mustard productivity is the losses brings by *Lipaphi serysimi* Kalt (mustard aphid) covering an extent of nearly 93% (Gupta *et al*., 2003). At times of the heavy infestation of *Lipaphis erysimi* causes the seed yield loss and reduction in oil content in mustard plants (Naga *et al*., 2022). The temperature is a crucial factor which affects directly the insect development, its reproduction and its survival. As the individual insect's behavior differs with climate change (increase in temperature), the prediction of the influence of global warming on insect plant interaction occur for enlarging the herbivore pressure intensity on plants (Dhaliwal, 2002).The aphid infestation index ranged from 2.4 to 3.3 during the year 2017–2018 (Table 4). None of the genotypes was found to be resistant against aphids. However, few genotypes viz., RSPN-29, RSPN-28, CNH-11-13, RL-1359, HNS-1101, GSC-101, CNH-11-2, HNS-1102 were found moderately resistant to aphids in the year 2017-18. The observations recorded during the year 2018-2019, only two genotypes namely, RSPN-29 and CNH-13-2 showed susceptible and moderately resistant reaction to aphid respectively and no aphid infestation was found on rest of the genotypes under study (Table 5).Favorable prevailing temperature as well as the relative humidity leads to the population buildup and aphid development and in turn, increase the aphid population. The prevalence of the *L. erysimi*, its growth and its multiplication are mainly affected by meteorological parameters such as temperature rainfall, relative humidity. During *rabi* 2017-2018, crop was sown on 20th and 30th October. The low temperature (10.2 to 19.6°C) and high relative humidity (56 to 88.2%) at vegetative as well as siliqua formation stages, favored aphid multiplication. During *rabi* 2018–2019, population

Table 4: Screening of Aphids based on Aphid Infestation Index.

Year	2017–2018	2018–2019
RSPN-29	2.6	4.25
RSPN-28	2.7	No infestation observed
DGS-1	3.2	No infestation observed
RSPN-25	3.3	No infestation observed
CNH 11-7	2.5	No infestation observed
CNH 11-13	2.4	No infestation observed
CNH 13-2	3.1	2.65
EC552608	3.2	No infestation observed
GSC-6	3.2	No infestation observed
RL1359	2.7	No infestation observed
HNS-1101	2.7	No infestation observed
GSC-101	3.0	No infestation observed
CNH 11-2	2.7	No infestation observed
GSL-1	3.2	No infestation observed
HNS-1102	2.6	No infestation observed

dynamics of *L. erysimi* was observed on mustard from 18th November 2018 to 19th April 2019. During this period the temperature fluctuated from 10.1 to 25.6 °C with relative humidity 63% on November 18th 2018. This increase of temperature and decrease in relative humidity continued till Feb 2019. At 19th March 2019 (siliqua formation stage), the temperature fluctuated between 5.2 to 17.4 with relative humidity 78% and on 19th April 2019, the

Table 5: Categorization of genotypes based on Aphid Infestation Index

Year	2017–2018	2018–2019
Highly Resistant	None of the genotypes	All these genotypes escaped aphid infestation
Resistant	None of the genotypes	None of the genotypes
Moderately Resistant	RSPN-29, RSPN-28, CNH-11-7, CNH-11-13, RL-1359, HNS-1101, GSC-101, CNH-11-2, HNS-1102	CNH-13-2
Susceptible	DGS-1, RSPN-25, CNH-13-2, EC552608, GSC-6,	RSPN-29

temperature ranged between 8.1 to 19.5with relative humidity 42.4% Therefore, this leads to declining of aphids during *rabi* 2018–2019. Hence, at vegetative stage the high temperature (25.6 to 26.6°C) and low relative humidity (52 to 63) and at siliqua formation, low temperature (17.4 to19.5°C) and high relative humidity (78 to 87%) might have helped these cultivars to break out from the severe attack of aphid and results in the development of healthy crops. Only two genotypes CNH-13-2 and RSPN-29 showed the aphid infestation (Table 5). These results are similar with Srivastava *et al*., 1995, Prasad, 2003 and Ali *et al*., 2011. By the results of the present study, identification of resistant as well as tolerant varieties to mustard aphid can be done by screening the rapeseed-mustard and can be used for the future endeavors.

Mustard aphid poses a serious threat to mustard cultivation and is a devastating pest of Brassica. There are over more than 200 different aphids type which are trouble-making for farmers and cause trouble by feeding on crops and also by infecting the plants with various diseases as well as viruses. Aphids can make colonies on the underside of leaves and also on growing tips. Aphid grow so fast that if get proper food supply, develops wings and fly and infects other plants. So the eco-friendly way for controlling the aphid infestation in Brassica is the application of resistance cultivars. Therefore, the screening of Brassica genotypes is the important aspect for finding the resistant and susceptible cultivars. The present study made by author are in conformity with the Jat *et al*., 2007 and Yadav *et al*., 2017 in which 240 mustard accessions were screened and 16 out of them were found resistant and 88 were found moderately resistant and remaining 102 were found susceptible and 39 were highly susceptible. Another study done by Chaudhary and Patel, 2016 revealed that three varieties namely, NRCM 120, NRCM 353 and Rayad 9602 found to be highly resistant with lowest aphid infestation index and one variety found resistant namely, Vardan and four varieties (GM-2, HYOLA-401, GM-3 and GM-1) found to be susceptible and highly susceptible. Other studies given by Pawar *et al*., 2009 and Sanwar *et al*., 2013 are also in support of the present study.

Conclusion

The mustard aphid leads to 97% yield losses in rapeseed- mustard. Pesticides application is not effective and deleterious as far the insecticide resistance is concerned. Germplasm screening is done to analyze the best germplasm for the development of tolerant or resistant varieties and for developing integrated pest management (IPM) program in oleiferous brassica crops, a good knowledge of insect pest and its interactions with plants is an important requirement. And for growers, the utilization of host plant resistance is the most useful, desirable, economical and practical method, with the assumption to be an efficient control element in IPM (insect pest management).

Conflict of interest

The authors declare that they have no conflict of interest.

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Evaluation of growth and seed yield of chickpea (*Cicer arietinum* L.)

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ABSTRACT

The field experiment was conducted during *rabi* 2020 at Zonal Agricultural Research Station, Mandya to study the impact of intercropping and nutrient dose on chickpea (*Cicer arietinum* L.) growth and seed yield. The results revealed the significant differences for growth and yield parameters of chickpea due to intercropping systems and fertilizer doses. A sole grown chickpea showed significantly higher plant height at harvest (35.73cm), number of pods per plant (42.02), hundred seed weight (23.20 g) and seed yield of chickpea (1733 kg/ha) compared to intercropping of chickpea with sorghum, safflower or linseed. Whereas, among fertilizer doses, application of 150 per cent recommended dose of fertilizer (RDF) produced noticeably taller plants at harvest (35.51 cm), number of pods (38.38), hundred seed weight (22.53 g) and seed yield of chickpea (1343 kg/ha) compared to other fertilizer doses. Therefore, sole cropping of chickpea with 150 per cent RDF could be recommended for certified seed production programme.

Introduction

The chickpea (*Cicer arietinum* L.), a crucial cool-season legume, is cultivated on 13.73 million hectares and produces an average yield of 982.1 kg/ha, ranking it as the third-most significant pulse in the world (Anon, 2019). India produces the most chickpeas worldwide, accounting for around 68 per cent of total production and 9.62 million hectares are used for cultivation, with an output of 974 kg/ha (Anon, 2019). Intercropping is the technique of growing two or more crops simultaneously in the same area. The most common benefit of intercropping is the ability to produce more yield on a given plot of land while using the natural resources more effectively by combining crops with different rooting abilities, canopy structures, heights, and nutrient requirements based on how well the component crops utilize their complementary growth resources. The fertilizers, which can be used to regulate the combined demand of both crops, may boost the efficiency of nutrients. Moreover, legumes can be intercropped to increase soil fertility through biological nitrogen fixation, soil conservation through increased ground cover over sole cropping, and insurance against crop failure or unstable market prices for a given commodity, particularly in regions vulnerable to extreme weather conditions like frost, drought, and flood. As a result, it provides more financial security than sole cropping, making the technique especially advantageous for labor-intensive small farms. Due to industrialization and urbanization the cultivable agriculture land is decreasing day by day and big size farmers are becoming small and marginal. Hence, in order to sustain the livelihood of each and every individual family they need to double their farm income within the available limited land holding with them. Hence, in order to address this problem, intercropping is one of the way where in the farmers can grow two crops within the same piece of land which differ in their root length, depth and with different durations which will not affect each other thereby they can get returns from both the crops and can double their farm

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income. In light of this, the current study was conducted to determine the impact of intercropping system and nutrient dosage on chickpea growth and seed yield.

Material and Methods

In *rabi* 2020, the field experiment was carried out at the Zonal Agricultural Research Station, Seed Unit, Mandya. The experiment was laid out in Two Factorial Randomized Complete Block Design with three replications. The trial included three different fertilizer doses of 100, 125 and 150 per cent recommended doses of fertilizers and four cropping systems, including solitary chickpea, chickpea + sorghum (4:2), chickpea + safflower (4:2), and chickpea + linseed (4:2). Before planting, chickpea seeds were treated with rhizobium and phosphate-solubilizing bacteria. The treated chickpea seeds and intercrop seeds were sown at a 4:2 row ratio in 3-5 cm deep soil, with a 30 cm row spacing and 10 cm between plants. Observations on its growth and yield attributes were recorded on five randomly selected plants from each plot (ISTA, 2015).

Results and Discussion

Plant height (cm)

Plant height of chickpea was significantly influenced by intercropping systems throughout its growth period (Table 1). Significantly higher plant height (23.34, 33.21 and 35.73 cm) was recorded in the sole crop of chickpea (C_1) at 30, 60 DAS and at harvest, respectively. However, it was found to be on par with chickpea + linseed (C_4) intercropping system but considerably reduced plant height of chickpea was observed in the intercropping system of chickpea and safflower (C_3) at 30, 60 DAS and at harvest (22.01, 31.54 and 33.95 cm, respectively).Similarly, a significant impact of fertilizer levels on plant height of chickpea was observed throughout the crop growing period. Application of 150 per cent recommended dose of fertilizers (F_3) showed highest plant height of chickpea (23.31, 32.88 and 35.51 cm) at 30, 60 DAS and at harvest, respectively which was statistically equivalent to the 125 per cent prescribed fertilizer dose (F_2). While, a considerable reduced plant height of chickpea was observed due to application of 100 per cent RDF (F_1) at 30, 60 DAS and at harvest (21.84, 31.72 and 34.02 cm, respectively). However, interaction between intercropping systems and levels of fertilizer application on plant height of chickpea was statistically non-significant.

Number of branches per plant

The number of branches per plant of chickpea differed significantly due to intercropping systems and levels of fertilizers application at advanced stage of crop growth (Table 2). A sole crop of chickpea (C_1) recorded significantly maximum number of branches per plant (10.24). However, it was found to be on par with chickpea + linseed (C_1) intercropping system (10.03). While, the significantly minimum number of branches per plant was noticed in chickpea + safflower (C_3) intercropping system (8.86).Among different fertilizer levels, the number of branches per plant significantly increased in 150 per cent RDF (10.00) followed by 125 per cent RDF (9.59). While, the significantly decreased number of branches per plant was noticed due to application of 100 per cent RDF (9.24). Interaction effect between intercropping systems and levels of fertilizer showed non-significant effect on number of branches per plant.

Number of pods per plant

Number of pods per plant observed in chickpea crop differed significantly due to intercropping systems (Table 2). In a single grown crop of chickpea (C_1), a significantly more number of pods per plant was observed (42.02) and significantly less numbers of pods per plant was recorded in chickpea + safflower (C_3) intercropping system (33.00).The amount of fertilizers had a substantial impact on the number of pods produced per plant in the chickpea intercropping systems. The number of pods per plant was found to be larger in 150 per cent RDF (38.38), and it was compared to 125 per cent RDF (37.47). The intercropping strategies and fertilizers levels exhibited a non-significant interaction effect on the number of pods produced per plant.

Seed yield per plant (g)

The variation in seed yield of chickpea per plant was significantly influenced by intercropping systems (Table 2). The sole crop of chickpea (C_1) recorded significantly higher seed yield per plant (10.23 g) over other intercropping systems. Lower seed yield per plant was recorded in chickpea + safflower (C_3) intercropping system (8.25 g).

Table 1: Effect of intercropping and fertilizer dose on plant height at different growth stages of chickpea

Treatments	Plant height (cm)		
	30DAS	60DAS	At harvest
Factor A: Intercrops (C)			
C_1- Sole chickpea	23.34	33.21	35.73
C_2- Chickpea+sorghum	22.49	32.02	34.57
C_3- Chickpea+safflower	22.01	31.54	33.95
C_4- Chickpea+linseed	22.65	32.24	34.73
S.Em±	0.27	0.37	0.39
CD at 5%	0.80	1.07	1.13
Factor B: Fertilizer dose (F)			
F_1- 100% RDF	21.94	31.72	34.02
F_2- 125% RDF	22.63	32.15	34.71
F_3- 150% RDF	23.31	32.88	35.51
S.Em±	0.24	0.32	0.34
CD at 5%	0.69	0.93	0.98
Interaction (C X F)			
C_1F_1	22.83	33.38	34.91
C_1F_2	23.10	32.63	35.18
C_1F_3	24.08	33.60	37.09
C_2F_1	21.53	31.06	33.61
C_2F_2	22.56	32.09	34.64
C_2F_3	23.28	32.90	35.46
C_3F_1	21.24	30.77	33.32
C_3F_2	22.07	31.59	34.15
C_3F_3	22.73	32.26	34.39
C_4F_1	22.14	31.66	34.22
C_4F_2	22.77	32.30	34.85
C_4F_3	23.04	32.77	35.12
S.Em±	0.47	0.63	0.67
CD at 5%	NS	NS	NS

Among different levels of fertilizer dose, the seed yield of chickpea per plant was significantly higher in 150 per cent RDF (9.10 g) followed by125 per cent RDF (8.98 g). The interaction between amounts of fertilizers and the intercropping systems had no appreciable impact on the number of seeds produced per plant.

Hundred seed weight (g)

The hundred seed weight of chickpea was assorted due to intercropping systems (Table 2). Significantly higher hundred seed weight of chickpea (23.20 g) was recorded in sole crop of chickpea (C_1). However, it was on par with chickpea + linseed (C_4) intercropping system (22.32 g). Significantly lowest seed weight of chickpea was recorded in chickpea + safflower (C_3) intercropping system (20.62 g).

Fertilizer levels showed significant effect on hundred seed weight of chickpea in intercropping systems. The hundred seed weight of chickpea was higher in 150 per cent RDF (22.53 g) and it was discovered to be equivalent to 125 percent RDF (21.74 g). Intercropping systems and fertilizers levels interaction had no discernible impact on the weight of chickpea seeds per hundred.

Seed yield per hectare (kg/ha)

The variation in the seed yield of chickpea per hectare was significantly influenced by intercropping systems (Table 2). The sole grown

Table 2: Effect of intercropping and fertilizer dose on number of branches per plant and seed yield attributes of chickpea

Treatments	No. branches per plant	No. of pods per plant	Seed yield (g/plant)	100 seed weight (g)	Seed yield (kg/ha)
Factor A: Intercrops (C)					
C_1- Sole chickpea	10.24	42.02	10.23	23.20	1733
C_2- Chickpea+sorghum	9.32	35.42	8.42	21.41	1090
C_3- Chickpea+safflower	8.86	33.00	8.25	20.62	952
C_4- Chickpea+linseed	10.03	39.69	9.04	22.32	1395
S.Em±	0.24	0.41	0.07	0.34	31
CD at 5%	0.69	1.20	0.20	1.00	92
Factor B: Fertilizer dose (F)					
F_1- 100% RDF	9.24	36.75	8.87	21.39	1241
F_2- 125% RDF	9.59	37.47	8.98	21.74	1294
F_3- 150% RDF	10.00	38.38	9.10	22.53	1343
S.Em±	0.20	0.35	0.06	0.29	27
CD at 5%	0.60	1.04	0.17	0.86	80
Interaction (C X F)					
C_1F_1	9.92	41.27	10.14	22.87	1684
C_1F_2	10.15	41.93	10.24	23.20	1733
C_1F_3	10.64	42.87	10.31	23.53	1781
C_2F_1	8.94	35.00	8.37	20.79	1007
C_2F_2	9.27	35.40	8.42	21.44	1098
C_2F_3	9.74	35.87	8.47	22.02	1164
C_3F_1	8.35	32.07	8.18	20.07	927
C_3F_2	8.93	33.07	8.26	20.30	946
C_3F_3	9.31	33.87	8.30	20.82	983
C_4F_1	9.75	38.67	8.79	21.49	1346
C_4F_2	10.01	39.47	9.00	22.02	1398
C_4F_3	10.33	40.93	9.32	23.09	1442
S.Em±	0.41	0.71	0.12	0.59	55
CD at 5%	NS	NS	NS	NS	NS

chickpea crop (C_1) gave significantly higher seed yield per hectare (1733 kg/ha) over other intercropping systems. Lower seed yield per hectare was recorded in chickpea + safflower (C_3) intercropping system (952 kg/ha). Among different levels of fertilizer dose, the seed yield of chickpea per hectare was significantly higher in 150 per cent RDF (1343 kg/ha) and also close result was noticed 125 per cent RDF (1294 kg/ha). The seed yield per hectare of chickpea crop was not significantly affected by the interaction impact of the intercropping systems and fertilizers levels. Crop growth performance is generally judged by plant height and number of branches produced which are governed by genotypic characters, nutritional and environmental factors. Intercropping systems had significantly affected the plant height and number of branches of chickpea. Sole crop of chickpea had taller plants and higher branches in comparison to intercropping systems tested that may be due to shading effect of intercrops on chickpea and also due to competitive nature which might have exploited light, water and nutrients for its own growth. These results were in accordance with reports made by Promod *et al.* (2018) in chickpea + mustard intercropping system, Dharmendra *et al.* (2018) in chickpea + linseed intercropping system and Nandhini *et al.* (2015) in pigeonpea + greengram intercropping system.The number of pods per plant, seed yield per plant, and test weight are important primary yield components, which were significantly influenced by intercropping systems and fertilizers levels. However, reduction of yield characteristics has been recorded in intercropping system compared to sole crop of chickpea. The results indicated the above were in accordance with reports made by Anitha *et al.* (2015) that reduction of yield attributes might be due to intensified interspecific competition for light and utilization of available resources offered by intercrops, resulting in etiolated growth and poor pod setting. Similar results were in accordance with reports made by Azar *et al.* (2013)

in chickpea + barley intercropping system, Manpreet *et al.* (2016) and Tanwar *et al.* (2011) in chickpea based cropping systems. Seed yield is the ultimate outcome of various physiological, biochemical and phenological functions occurring in the plant kingdom. Seed yield of chickpea significantly influenced by intercropping systems and fertilizer doses. It might be due to more number of plant population in the sole system than intercropping and also due to the increased rates of photosynthetic activity and the transfer of photosynthate to various plant sections caused by fertilizer application to intercrops. Kalaghatagi *et al.* (2017) in chickpea + linseed intercropping system, Dhadge *et al.* (2014) and Jani *et al.* (2015) in chickpea based cropping system reported similar results.

Conclusion
From the aforementioned findings, Chickpea crop grown as a sole (C_1) given higher seed yield over intercropping system. While for different doses of fertilizers, application of 150 per cent RDF found to be best.

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

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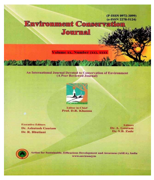

Spatiotemporal dynamics of land use/land cover changes and its drivers in *Bilate* watershed, central rift valley, Ethiopia

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ABSTRACT

Land use/land cover (LULC) changes influence the ecological function, consequence on ecosystem services, which are tightly linked to human wellbeing. However, quantification of the LULC changes and identifying the underlying factors remain patchy particularly in developing nations, despite this information is crucial to propose a feasible restoration action. Therefore, this study investigates the land use/land cover changes and its drivers in central Rift Valley, Ethiopia. GIS and Remote sensors i.e. Landsat 5 (TM), and Landsat 8 (OLI/TIRs) imagery sensors acquired from USGS, and field observation were used. Using the supervised classification method and the support of ArcGIS 10.5 and ERDAS IMAGINE 2014, all images were classified into various land cover types. Focus group discussions, key informant interviews, and structured questionnaire surveys were used to investigate the drivers of LULC change. NDVI was used to detect the vegetation cover change. Woodland, grassland, and barren lands were the major LULC types identified in this study. After 28 years, the woodland cover increased from 20.6% to 40.2% whereas the barren land decreased from 43.4% to 22.6%. Grassland showed very slight increment, i.e. from 35.9% to 36.9%. This implies that area enclosure plays a significant role in the restoration of degraded lands. The highest NDVI values (0.6) were determined in the year 2022 at the end of the classification. Focus group discussants and key informants confirmed that human-induced factors were the major drivers of LULC changes in the study area. Our findings indicated that human interventions are the key determinants of land use/land cover dynamics, and as a result, enforcement of the law and public education campaigns to change human behavior in support of the area enclosure approach are essential to restoring degraded land for the benefit and wellbeing of humans and nature while also advancing the achievement of the global goals.

Introduction

Changes in land use and land cover are very complicated processes that are greatly influenced by a variety of factors, including long-term natural climate changes, geomorphological and biological processes, and human-induced pressures on vegetation cover (Arsanjani, 2011; Gebrehiwot *et al.,* 2021). LULC changes have influenced the ecological conditions of the ecosystem (Yesuph & Dagnew, 2019). Consequently, it strongly affects ecosystem services and functioning (Wang *et al.,* 2015). In many semi-arid areas of sub-Saharan Africa, land degradation is a problem that needs to be addressed if it is to be restored (Emiru *et al.,* 2018; Yayneshet, 2011). Degradation of vegetation

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leading to eventually desertification is more pronounced in Africa than in any other continent (Mekuria *et al.,* 2019; Zeila & Jama, 2005). The same is true in Ethiopia (Gebreselassie *et al.,* 2016). Earlier studies (Gebrehiwot *et al.,* 2021; Kidane *et al.,* 2012) showed that population growth and settlement, agricultural expansion, deforestation, land clearing, and fire are the main causes of LULC change. Hence, (Feyisa *et al.,* 2017; Tesfaye *et al.,* 2016; Tsegay & Meng, 2021) argue that the use of area enclosure restoration practice is an effective strategy to reduce the degradation problem.The knowledge of temporal and spatial LULC change is crucial to understanding the current status of vegetation resources (Mohammed *et al.,* 2020) and predicting the consequences of vegetation degradation (Emiru *et al.,* 2018) which enables the development of other supportive applicable conservation strategies or enhances and improves conservation practice for further output. To plan locality-specific sustainable land use and resource management techniques in such area enclosures, precise and current spatiotemporal information on LULC dynamics, the underlying causes, and consequences of these changes are urgently required. The study area, *Bilate* watershed, is well experienced to be restored by area enclosure activities, but the area is still exposed to severe erosion and soil loss triggered by LULC changes. Therefore, it is essential to have a thorough understanding of the divers, consequences, and extent of LULC change to establish more appropriate environmental policies and land management strategies for the area and beyond. (Larjavaara & Muller-Landau, 2010). The degree of this change, its causes, and its effects are not fully understood because the LULC change in the Bilat watershed has not yet been fully examined. Therefore, the objective of this study was to detect, quantify, and map LULC dynamics and trends as well as to investigate the underlying causes of the LULC change.

Material and Methods

The study was carried out in the Central Ethiopian Rift Valley's Bilate Watershed. It covers 2032.11 ha. Three zones, i.e. Halaba, Kambata Temabaro, and Hadiya make up the Bilate watershed. One district was selected from each zone because of the presence of area enclosures and the adjacent open land. We selected *Weyera* district from Halaba zone. Six *Kebeles* (smallest administrative units: *Sheke Tena Woldia, Wanjana Woldia, Asore, Ashoka, Houlgeb kuke, Chambula*) were identified from this district, *Kedida Gamela* district was selected from *Kambata Tembaro* zone with *Hulegabezeto kebele. Misraq Badawacho* district was selected from *Hadyia* zone, and the kebele identified was 2nd *Keraniso*. The watershed is located between the latitudes of 7°12′33″ and 7°21′19″ and the longitudes of 38°06′00″ and 38°03′57″. The area features plain, sloppy, or undulating landscape types and an altitude range of 1654 to 1822 masl (Figure 1).

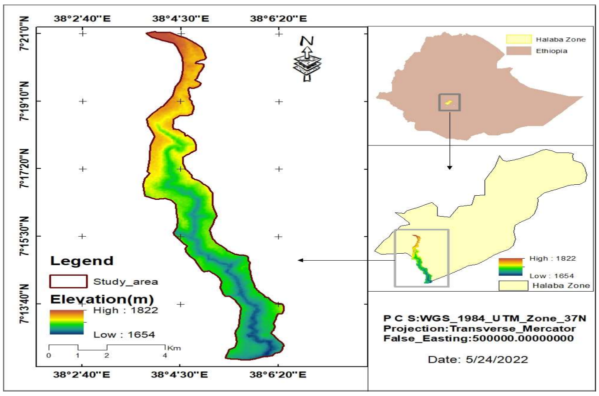

Figure 1: Map of the study area

The Ethiopian Rift, part of the Great Rift Valley's active rift system and characteristic of the area's warm climate with a width of roughly 1000 km, dominates the study area's geological formation (Wodaje, 2017). The soil types in the study area are *Chromic, orthic Luvsols,* and *Eutric Nitisols*, which have good potential for agricultural activities (Wodaje, 2017). The *Eutric Nitosols* is the most renounced for their fertility. *Ca.*, 80% of the soil is sandy loam, 15% is clay and 5% is sandy (Food *et al.,* 2003) in the study area. The long-term average rainfall in the watershed is approximately 1107 mm, and it has a bimodal pattern with *Belg* (a traditional division of the year with light rain) typically occurring from March to May and *Meher* (a traditional division of the year with heavy rain) typically occurring from June to September. In the research area, the annual average temperature is 26.2 °C.In general, there are two types of LULC in the study area right now: vegetation (trees, shrubs, grass, and herbs), and bare land. Natural and plantation forests are also present in the study area's vegetation cover, while areas of bare ground are covered by degraded grasslands and scattered rural settlements.

Data collection

To examine the LULC, path and raw satellite images of 1994, 2008, and 2022 years were used. Field observation and GPS point data (training sites and ground control points) were used within the study area. To understand the reality and the forces driving change and perceptions related to LULC changes, socioeconomic data were used to gather additional ancillary data from the elders, local community, and local administration through a set of questionnaires, interviews with key informants, and discussions in focus groups.

Sample size determination

The (Cochran, 1977) formula was used to determine the sample size for the household survey.

$$\boldsymbol{n = \frac{Z^2pq}{e}} \qquad (1)$$

Where: n= sample size; Z2= confidence interval (1.96, constant); p = population percentage (0.5, constant); q = 1 - p; e=margin of error which is fixed at 0.05. An error margin of 5% and a confidence interval of 95% were used.

The calculated sample size was 384 and the sample size for each *kebele* was allocated proportionally (Table 1). The respondents were selected using a simple random sampling technique.

Table 1: Target population and sample size of each study kebele in the *Bilate* watershed

SN	Kebele Name	Household			
		Male	Female	Total	Sample Size
1	Sheke Tena Woldia	197	87	284	37
2	Wanjana Woldia	258	198	456	59
3	Ashoka	312	98	410	53
4	Asore	243	103	346	45
5	Houlgeb kuke	189	110	299	39
6	Chambula	186	79	265	34
7	Hulegabezeto kebele	396	172	568	73
8	2nd Keraniso kebele	257	91	348	45
	Total	2038	938	2976	384

We used three primary sources to collect information on the factors driving LULC change and the historical background of the watershed : semi-structured household questionnaire focus groups, and key informant interviews. Eight focus group discussions (one group for each *kebele*) were held using checklists to generate more evidence on the selected topics.Four to ten discussants were included in each group. Village administrators, development experts of natural resources, male households, and female households participated in the FGD. Twenty-four key informants (three in each *kebele*) were selected purposively with the help of the local administration and agricultural development experts to gain in-depth information about the study area. The key informants, who were people who had lived in the area for more than three decades and were assumed to have essential knowledge about their area, were given a list of open-ended questions.

Data acquisition

To assess the LULC variations of the research area, images from Landsat 5 TM and Landsat 8 OLI/TIRS of three carefully chosen years of the previous 28 years (1994, 2008, and 2022) were obtained from the USGS Earth Explorer website (http://earthexplorer.usgs.gov) (Table 2). Data acquisition from January to March as cloud effect/clear sky during data acquisition. The year 1994 was selected as the reference year of a baseline because it represents the pre-restoration period, and 2008 represents the period of the green legacy movement of the Ethiopian millennium. It was a year when enclosures activities were widely carried out in the study area. After the images were downloaded, projected, and stacked (pre-processed) to be displayed in the ERDAS IMAGINE software interface, the land cover map was generated within the framework of the Geographic Information System (GIS) and Remote Sensing (RS) Environment. The acquired multi-temporal satellite image covers a large area with a sensor spatial resolution of 30 meters for all spectral bands except band six (thermal band), which has a resolution of 60 meters, and band 8, which has a resolution of 15 meters and was left out of the scene. The remaining bands were stacked to get false-color composite (FCC) images in ERDAS IMAGINE 2014 software (Qiu & Jensen, 2004; Szuster *et al.,* 2011) The study was divided into three time periods: 1994, 2008, and 2022 (28-year spans). For better

Table 2: Satellite data and sources used in this study

Satellite images	Date of acquisition	Path	Row	Resolution	Sources	Application
Landsat 5	03/02/ 1994	169	055	30m	USGS Website	LULC Map
Landsat 5	25/02/ 2008	169	055	30m		
Landsat 8	2/02/ 2022	169	055	30m		

interpretation, the image was enhanced by displaying it in RGB true color composite (band 3, 2, 1) and included two infrared channels (4, 5, 3). For vegetated and non-vegetated land areas, green vegetation, and vegetation discrimination mapping, the former band combination bands 3, 2, and 1 were used. The latter band combination bands 4, 5, and 3 were employed for vegetation moisture differentiation and NDVI classification. The LULC map of the region was validated using high-resolution images (Google Earth Image).

Processing and analysis of data

Image pre-processing and classification

Image pre-processing is essential to avoid bias caused by noise and instrument artifacts (Chander et al., 2009; Giri, 2012). To acquire a more accurate representation of the images, geometric and radiometric corrections were applied during pre-processing. Moreover, in ERDAS IMAGINE 2014, contrast stretching techniques were utilized to enhance the visual interpretation of multi-temporal satellite images, and aerial photographs were used for ground truth verification. Using equalizing histograms and masking cloud coverage, the image's quality can be enhanced. Image classification is important to remote sensing, image analysis, and pattern recognition (Figure 2). The technique of categorizing pixels in an image is known as image classification (Anand, 2017; Urgesa *et al.,* 2016). Supervised classification was used to group pixels of similar spectral domains into classes. This classification approach necessitates the selection of training regions as the classification basis. For supervised classifications to be successful, the computer needs to be trained on the scene area beforehand. To reflect a certain land cover class, the user defines the original pixels' corresponding spectral classifications (Akubia *et al.,* 2020; Lillesand *et al.,* 2015). The Supervised Maximum Likelihood classifier algorithm classification system was employed to complete the classification process. Each land cover type is determined by a combination of fieldwork and personal experience (Asokan & Anitha, 2019; Qiu & Jensen, 2004). The clusters' shape, size, and direction are all factors that this classifier considers in addition to the cluster centers. To do this, the statistical distance was calculated using the covariance matrix and mean values of the groups.

To classify and assess accuracy, 150 GPS points (50 GPS points in each LULC) were collected from the field using GPS. The analysis began by using signature editor tools to define and collect training samples with the same reflectance value, then save the signature to begin the classification activity. The training sample was used to collect signatures from the image for categorization. Each sampled pixel was collected with the automated optical inspection (AOI) instruments, then classified using the signature editor. The satellite images were then divided into three categories (Woodland, Grassland, and Barren land) (Table 3).

Table 3: Description of LULC classes

LULC classes	Description
Woodland	Areas are dominantly covered with trees and shrubs.
Grassland	Permanently grassed regions, such as those found along ridges, steep slopes, and plain areas utilized for grazing, are typically both private and community.
Barren land	Places with deteriorated grasses, some bare ground (rocks), and sporadic rural small villages and farms.

Accuracy assessment

When assessing the value of field data (reference point) and classified images, accuracy assessment is useful (Congalton & Green, 2019; Elias et al., 2019). In the field, these references were generated using GPS and Google Earth data. It is very important to determine how accurate the referenced point is agreed with classified images of the remotely sensed data. LULC maps derived from remote sensing always contain some sort of errors due to several factors from classification technique to method of satellite data capture. The most common accuracy assessment elements include overall accuracy, producer's accuracy, user's accuracy, and kappa coefficient (Lu *et al.,* 2004).

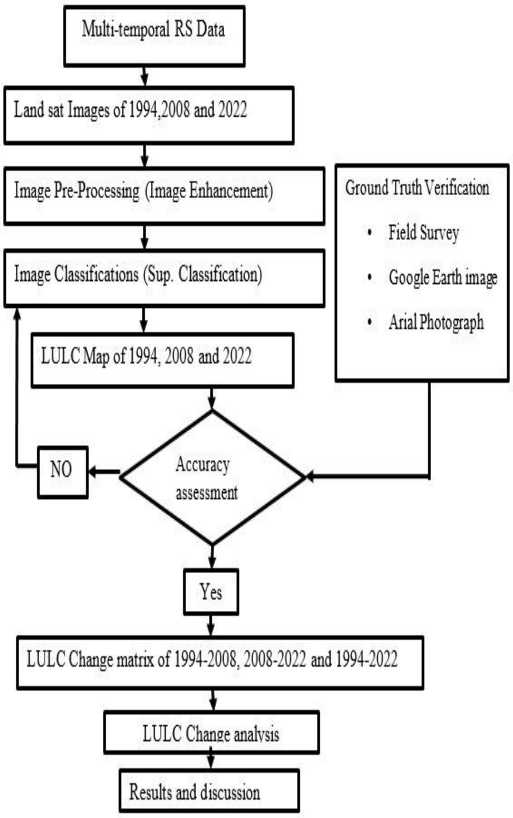

Figure 2: Flow chart of image classification

and the common tool to assess accuracy is the error matrix (Congalton & Green, 2019; Qiu & Jensen, 2004). These accuracy assessment elements were computed by using the following formula (Anand, 2017; Zewude *et al.,* 2022).

$$\textbf{Producer's Accuracy} = \frac{\textbf{total number of correct pixels in a category}}{\textbf{total number of pixels derived from reference data}}$$

$$\textbf{User's Accuracy} = \frac{\textbf{Total number of correct pixels in a category}}{\textbf{Total number of pixels derived from the reference data}}$$

$$\textbf{Overall accuracy} = \frac{\textbf{Sum of the diagonal elements}}{\textbf{Total number of accuracy sites (pixels)}}$$

Kappa coefficient (Khat) is a measure of the agreement between two maps taking into account all elements of the error matrix. It is defined in terms of an error matrix as given below:

$$(\mathbf{Khat}) = \frac{(N\sum_{i=1}^{r} xii) - (\sum_{i=1}^{r} (x_{i+} * x_{+i})}{N^2 - (\sum_{i=1}^{r} (x_{i+} * x_{+i}))} \quad (5)$$

Where;
r= number of rows in the error matrix
Xii = number of observations in row i and column i (on the major diagonal)
Xi+ = total observations in row (show as marginal total to right of the matrix)
X+I = total observations in column i (show as marginal total at bottom of the matrix)
N= total number of observations in included in the matrix

The kappa coefficient or statistics can be applied as a measure of how well the remotely sensed classification agrees with reference data. A value greater than 0.80 represents a strong or good classification; a value between 0.40 and 0.80 means moderate classification and a value less than 0.40 represents a poor classification or agreement (Anderson, 1976; Firdaus, 2014; Schowengerdt, 2012).

Change detection matrix

Pixel-based classified images were used to produce change information on the land classes and observed changes taking place. Thus, a change matrix was produced with the help of ERDAS IMAGINE 2014. The land cover map for the three-period series of images was analyzed based on LULC types of the study area using tables and graphs. To determine the magnitude, trend, and rate of LULC changes in the watershed, the area comparison analysis was made by subtracting the total area of each class of 1994 from 2008, 2008 from 2022, and 1994 from 2022 which the result could be positive (increasing) or negative (decreasing). The percent and rate of LULC change were computed by the following formula (Demissie *et al.,* 2017; Hegazy & Kaloop, 2015; Yesuph & Dagnew, 2019).

$$\textbf{Percent of change} = \frac{\mathbf{X - Y}}{\mathbf{Y}} \times \mathbf{100} \quad (6)$$

$$\textbf{Rate of change} \left(\frac{\mathbf{ha}}{\mathbf{year}}\right) = \frac{\mathbf{X - Y}}{\mathbf{Z}} \quad (7)$$

Where:
X = Recent area of the land use/land cover in ha,
Y = Previous area of the land use/land cover in ha and
Z = Time interval between X and Y in years.

Cross-tabulation matrix was used to differentiate the changes of each category at the expense of others

and its general structure follows the format displayed in Table 4. The rows display the categories of initial time, and the columns display the categories of subsequent or recent time. Entries on the diagonal (that is, P_{jj}) indicate the amount of LULC category which remained persistent in class j between the period and are used to calculate the gains and the losses of LULC classes while the diagonal entries show the size of the area that transitioned from category "i" to a different category "j" during the time interval (Aldwaik & Pontius Jr, 2012; Yesuph & Dagnew, 2019). For ease of reference, the equations and notation used to compute various components are presented as follows:
The proportion of the watershed Pi + that is occupied by class i, initial time is given by (Eq. 8):

$$\mathbf{Pi+} = \sum_{i=1}^{n} \mathbf{Pij} \quad (8)$$

Where n is the total number of LULC classes. Similarly, the proportion of the watershed P+j that is occupied by class j in recent times is given by (Eq. 9)

$$\mathbf{P+j} = \sum_{i=1}^{n} \mathbf{Pij} \quad (9)$$

Similarly, the following equations were used to determine the gain, loss, persistence, swap, and total change for all four-classified imagery (Braimoh, 2006; Kindu *et al.,* 2013).
The Gain (G_{ij}) was calculated through the difference between the total value for recent time (P_{+j}) and the persistence (P_{ij}), using Eq. 10:

$$\mathbf{G_{ij} = G_{+j} - G_{jj}} \quad (10)$$

On the other hand, the Loss (L_{ij}) was the difference between the total values for the initial time file (P_{j+}) and the persistence, using Eq. 11:

$$\mathbf{G_{ij} = G_{+j} - G_{jj}} \quad (11)$$

The swapping (S_j) is the exchange between the categories i.e. the proportion of a given class that changes location, while the total surface area remains the same. It denotes concurrent gain (i.e., the difference between class i and persistence) and loss (i.e., the difference between class j and persistence) of a given LULC class. Swap shows the fact that a lack of net change does not necessarily imply a lack of change in LULC in the watershed. Through the use of Eq. 11, it was determined to be two times the minimum value of the gains and losses.

$$\mathbf{S_j = 2 \times MIN(P_{j+} - P_{jj}, P_{+j} - P_{jj}} \quad (11)$$

The net change shows the definite change between the two time periods. The total was determined by subtracting the Total row from the Total column. The total change for each category (Cj) was the sum of net change (Dj) and the swapping (Sj), or the sum of gain and loss (Eq. 12).

$$\mathbf{C_j = (D_j + S_j)} \quad (12)$$

If the net change is zero (implying gain is equal to loss), then the swap is twice the loss or gain.

Table 4: A 3 × 3 LULC Conversion matrix for comparing two maps from different points in time

	Recent time					
Initial time	**LULC1**	**LULC2**	**LULC3**	**Total initial**	**Loss**	**LULC1**
LULC1	P11	P12	P13	P1+	P1+-P11	P11
LULC2	P21	P22	P23	P2+	P2+-P22	P21
LULC3	P31	P32	P33	P3+	P3+-P33	P31
Total Recent	P+1	P+2	P+3	1		P+1
Gain	P+1-P11	P+2-P22	P+3-P33			P+1-P11

Note: "P" refers to any conversion from one LULC to another and the number refers to columns and rows of LULC categories Source: Modified from (Adugna et al., 2017; Yesuph & Dagnew, 2019).

The exposure of each LULC class for a change was evaluated using the loss to persistence ratio (Lp = loss/persistence); gain to persistence ratio (Gp = gain/persistence) and net change to persistence ratio (Np = net change/persistence) (Randolph, 2004; Yesuph & Dagnew, 2019). A given land use or cover class has a higher probability of changing to another LULC than persisting in its current condition when G_P and L_P values are greater than one (Dibaba et al., 2020; Talukdar *et al.*, 2020). The land use/cover class is more likely to lose area to other LULC classes than to gain from them if Np had a negative value.Finally, two types of data were produced;

namely, three LULC maps, which illustrate the changes in a spatial context and various tables which exhibit the amount of areas for each LULC category, and a cross-tabulation matrix which demonstrates the LULC transition from category to a category at different study periods. ArcGIS 10.5 was used for data analysis, management, spatial referencing, geo-referencing, and layout for final mapping, and ERDAS IMAGINE 2014 for image processing, classification, and change detection of the final LULC maps as well as the socioeconomic data, also analyzed by SPSS v 21.

NDVI (Normalized difference vegetation index) data

One of the most widely used indices for computing green vegetation is the Normalized Difference Vegetation Index (NDVI) (Gandhi *et al.,* 2015; Warkineh & Hailemicael, 2021). It is useful for identifying vegetation from non-vegetation land cover. Areas of barren rock, sand, or snow usually show very low NDVI values (for example, 0.1 or less). Sparse vegetation such as shrubs and grasslands or senescing crops may result in moderate NDVI values (approximately 0.2 to 0.5). High NDVI values (approximately 0.6 to 0.9) correspond to dense vegetation such as that found in temperate and tropical forests or crops at their peak growth stage (Ghorbani *et al.,* 2012; Helbich, 2019). NDVI values were calculated on composite image and used band 3 (Red) and 4 (Near Infrared) for Landsat 5, and band 4 (Red) comes with band 5 (Near Infrared) for Landsat 8. NDVI approaching calculation of greenness degree of image correlates with vegetation crown density. NDVI correlates with chlorophyll content and its value is between -1 to 1. NDVI is calculated as follows (Costa *et al.,* 2020; Eastman *et al.,* 2013; Gandhi *et al.,* 2015; Zaitunah *et al.,* 2018).

$$\mathbf{NDVI} = \frac{\mathbf{near\ infrared - red}}{\mathbf{near\ infrared + red}} \qquad (12)$$

Where
NIR is the near-infrared reflectance and RED is the red reflectance.

In this NDVI analysis, the higher positive values are classified as vegetation and values close to zero are classified as mixed vegetation. While the negative value including zero is classified as barren land (Gandhi *et al.,* 2015).

Results and Discussion

Land use/land cover distribution (1994 to 2022)

The distribution of land use/land cover categories in the study area was examined from January to March using satellite images. These LULC categories were divided into three land classes (i.e. Woodland, Grassland, and Barren land). The final LULC categories (1994 to 2022) showed that woodland was the most predominant LULC category in the study area followed by grassland and barren land. Focus group discussion and key informants' interviews showed that the increment of woodland cover could be linked with the practice of area enclosure restoration activities in the *Bilate* watershed. Earlier studies (e. g. Emiru *et al.,* 2018; Feyisa *et al.,* 2017; Warkineh & Hailemicael, 2021) showed that the use of area enclosure for vegetation restoration was the key contributor to the increase in vegetation coverage along the time gradient.The temporal trend of LULC change in the last three decades showed a magnificent change (Figure 3). In addition to that, the rate of woodland change was fast compared to the other LULC categories (i.e. Grassland and Barren land in the last three decades due to area enclosure restoration practiced). In 1994, barren land accounted for the largest area coverage (883 ha = 43.4% of the total area) in the study area. Conversely, the woodland cover was the least (419 ha = 20.6%) (Figure 3 and Table 5). Our findings may imply that the study area was severely degraded prior to the application of the area enclosure. Grassland counted for 730 ha or 35.9% in 1994. Moreover, in 2008, the woodland and grassland attained the highest coverage, being 770.1 ha (37.9%) and 747 ha (36.8%) of the total area, respectively. In the same year, barren land covered the smallest share (515 ha = 25.3%) of the total area. Our findings indicated that after 28 years, the LULC dynamics of the study area were significantly changed. In 2022, Woodland (816.03 ha = 40.2%) nearly covered twice the area of barren land (466.38 ha = 22.9%). Grassland (749.7 ha, or 36.9%) occupied the remaining space in the research area. Previous studies (e.g. Abera *et al.,* 2016; Araya, 2014; Feyisa *et al.,* 2017; Solomon *et al.,* 2022; Yayneshet, 2011) reported the increment of woodland and grassland due to the area enclosure confirming that it is a promising approach to the reverse lost vegetation in a specific area.

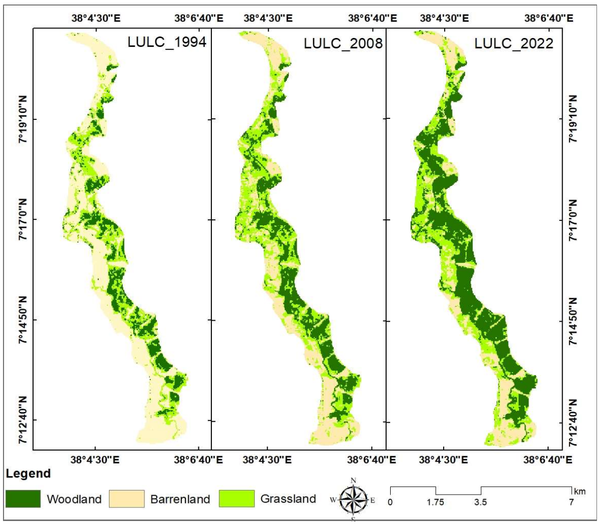

Figure 3: Land use/land cover map of *Bilate* watershed under different periods

Table 5: Area coverage by LULC classes in the *Bilate* watershed during various periods

LULC Class	1994		2008		2022	
	Area (ha)	% of total	Area (ha)	% of total	Area (ha)	% of total
Woodland	419	20.6	770	37.9	816	40.2
Grassland	730	35.9	747	36.8	750	36.9
Barren Land	883	43.4	515	25.3	466	23.0
Total	2032	100	2032	100	2032	100

Table 6: Error matrix classification accuracy assessments of images from 1994, 2008, and 2022

LULC Types	1994		2008		2022	
	P (%)	U (%)	P (%)	U (%)	P (%)	U (%)
Woodland	92.2	94	95.9	94	96.1	98
Grassland	90.6	96	93.9	92	91.8	90
Barren land	97.8	90	92.3	96	94	94
Overall accuracy (%)	93		94		94	
Kappa coefficients	0.90		0.91		0.91	

U=user's accuracies, P=producer's accuracies

Furthermore, as stated by the elders and confirmed in the ground, continuous community based integrated watershed management interventions and restoration programs appear to make significant contributions to the enhancements of the watershed's vegetation cover. A restoration trend was also visible in the watershed's enclosed areas, which was caused by livestock and human interference is avoided. However, studies (e.g. Bufebo & Elias, 2021; Dingamo *et al.,* 2021; Tewabe & Fentahun, 2020; Warkineh & Hailemicael, 2021) showed that the major depletion trend was observed degradation of woodland and expansion of farmland/agriculture.

Accuracy assessment

To assess the classification's accuracy, the LULC map was put up against the reference data. As a result, overall classification accuracies scored were 93%, 94%, and 94%, for the classified Landsat imageries of 1994, 2008, and 2022, respectively (Table 6). The overall accuracy standard of 85% was first proposed by (Anderson, 1976), and it is currently acknowledged and used as a benchmark in map accuracy assessment. Our analysis's total accuracy rating is higher than the accepted threshold. The outcome indicated that there is a reasonable correspondence between the classified image and the reality it represents. For the years 1994, 2008, and 2022, respectively, a kappa coefficient result was found to be 0.90, 0.91, and 0.91. The Kappa coefficients revealed that the three classified images each indicated higher or good classification performance or strong agreement, with a Kappa value ranging from 0.40 to 0.85 (Anderson, 1976; Congalton, 1991; Congalton & Green, 2019; Elias *et al.,* 2019; Tewabe & Fentahun, 2020).

Rate of land use/land cover change

Our findings show that throughout the entire study period (1994–2022), the area of grassland and woodland rose while the area of barren land significantly decreased (Figure 4 and Table 7). In the initial period (1994–2008), the amount of woodland vegetation increased by 351.3 ha, or approximately 25.1 ha/year, whilst the amount of barren land decreased by -369.8 ha (-26.3 ha/year). The rate of growth of woodland was 45.9 ha (3.3 ha/year) between 2008 and 2022. In contrast, both the first and second periods saw a significant decrease in the barren land. Under this year (2008), area enclosures activities were widely practiced in the study area, (i.e. there is no degradation, woodland increment also not too much visible). Given that the area has been protected from human and animal interference, woodland was found to show the highest rate of change, increasing by 28.4 ha/year, followed by grassland at 1.4 ha/year.

Table 7: Rate of changes in land use/land cover classes (1994 to 2022)

LULC class	1994		2008		2022	
	Area (ha)	Change rate (ha year^{-1})	Area (ha)	Change rate (ha year^{-1})	Area (ha)	Change rate (ha year^{-1})
Woodland	351	+25.1	45.9	+3.3	397.2	+28.4
Grassland	16.6	+1.2	2.8	+0.2	19.4	+1.4
Barren Land	-369.8	-26.3	-48.5	-3.5	-416.5	-29.8

Note. "+" = increased, "-" = decreased the magnitude of particular land use/land cover type

During the entire period (1994-2022), the barren land class had the highest negative value (-416.5 ha/year). In general, the results verified a series of LULC rate changes in the study area over the past 28 years (1994-2022). The study indicates that woodland has grown significantly over the past 28 years as a result of the area enclosure restoration practices used. Consequently, areas that had before been barren had transformed to a larger extent into woodland and grassland. These findings are in agreement with the studies (e.g. Araya, 2014; Mekuria *et al.,* 2020; Tsegay & Meng, 2021) that depicted the area enclosure reverses land degradation into a productive landscape and increases vegetation cover.

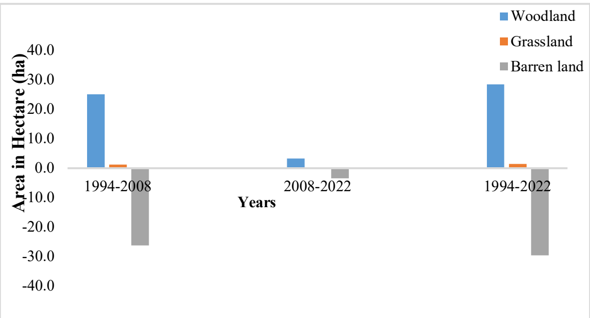

Figure 4: The extent and rate of change in the study area land use/land cover

From the result, grassland had been relatively high persistence value (426.9 ha) in the *Bilate* watershed from 1994 to 2022time period. Similar to how barren land displayed the highest values for losses of LULC classes and woods the highest values for gains, In addition, from 1994 to 2022, most of the additional woodland came from grassland and barren land (Table 8 and 5). This implies that the area within the watershed had the highest overall change. Besides, the barren land class was mainly replaced by grassland and woodland. Studies (e.g. Dessie & Christiansson, 2008; Shiferaw & Singh, 2011; Zewude *et al.,* 2022) said that many studies have been carried out in the highlands of the country's central and northern regions, where land degradation and deforestation were already a big concern many years ago.In contrast, these studies noted a rise in vegetation cover over the preceding three decades as a result of area enclosure restoration initiatives taking place in the studied area. Similarly, studies (e.g. Kasim *et al.,* 2015; Mohammed *et al.,* 2020; Tsegay & Meng, 2021) show that the vegetation cover was increased due to area enclosure restoration activities and community-managed forests. Therefore, based on this finding, it can be noted that human interventions are what ultimately decide how the LULC changes. Studies (e. g, Abera *et al.,* 2016; Urgesa *et al.,* 2016) stated that the conservation of biological diversity in protected areas was successful through the intervention of local communities through management activities. Our findings are in support of these studies.

Table 8: LULC gain, loss, and absolute net changes in ha for *Bilate* watershed (1994–2022)

LULC Class	Total 1994	Total 2022	Persistence	Gain	Loss	Total change	Absolute net change
Woodland	419	816	377.3	438.8	41.6	480.4	397.2
Grassland	730	750	426.9	322.8	303.5	626.3	19.3
Barren land	883	466	406.4	59.9	476.5	536.4	416
Total	4026	4054	1210.6	821.5	821.6	1643.1	832.5

Persistence and vulnerability of land use/land cover dynamics

The ratio's magnitude always displays the gains to persistence ratio, loss to persistence ratio, and net change to persistence ratio (which tells how many times the LULC types gain/loss than its persistence) (Kasim *et al.,* 2015; Zewdie & Csaplovies, 2015). In this study, woodland classes have gain to persistence ratios (G/P) that are greater than one

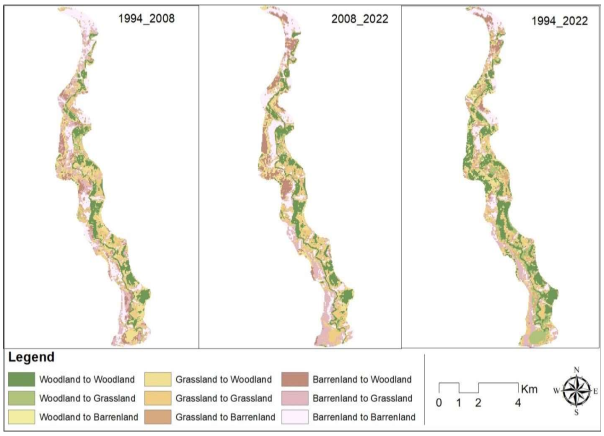

Figure 5: Map of changes in land use/land cover between 1994 and 2022

showing a tendency toward gain rather than loss. The loss to persistence (L/P) ratio of barren lands, on the other hand, is more than 1, showing that the LULC is vulnerable to changes in other land cover groups. This implies a higher restoration tendency of the watershed and increment of vegetation cover rather than degradation. Gain to persistence ratio and loss to the persistence of barren land is closer to zero value, indicating that the barren land class is insignificant compared to its persistence (Table 9).

Table 9: Gain to persistent (G/P), loss to persistent (L/P), and net change to persistent (N/P) ratio of land use/land cover classes in *Bilate* watershed (1994–2022)

LULC Class	Persistence (P)	Gain (G)	Loss (L)	G/P	L/P	N/P
Woodland	377.3 ha	438.8 ha	41.6 ha	1.2	0.1	1.05
Grassland	426.9 ha	322.8 ha	303.5 ha	0.8	0.7	0.05
Barren land	406.4 ha	59.9 ha	476.5 ha	0.1	1.2	-1.03
Total	1210.6 ha	821.5 ha	821.6 ha	2.1	2.0	0.07

When the gain to persistence ratio (G/P) is larger than one, the LULC has a higher chance of gaining than of persisting. Moreover, the LULC is vulnerable to changes in other land cover classes since the loss to persistence ratio (L/P) value is higher than one (Akubia *et al.,* 2020; Viana & Rocha, 2020). Also, grassland LULC classes have gain to persistence and loss to persistence values that are both lower than one, indicating that they are less vulnerable to both of these outcomes. The net change to persistence (N/P) is negative barren land, showing net loss compared to persistence. The loss of barren land may be related to restoration activities due to the increase of woodland and grassland in the study area. The net change to persistence (N/P) woodland area is significantly increased. Grassland also experienced a net increase in size, but it also showed a comparable loss in the same period. Similarly, studies (e.g. Birhane *et al.,* 2017; Mengistu *et al.,* 2005; Tesfay, 2018) stated that the area of the enclosure is currently a meaningful solution for the restoration of degraded lands.

Normalized difference vegetation index (NDVI)

In this study, it has been observed that the vegetation cover was more in 2008 and 2022 with maximum NDVI values of 0.42 and 0.63, respectively. This indicated that during the period of the study restoration activities were increased. The highest value shows high vegetation cover. Studies (e.g. Abebe *et al.,* 2014; Asmare & Gure, 2019; Yimer *et al.,* 2015) reported that area enclosure restoration activities promoted vegetation coverage and enhanced restoration processes and degraded lands were replaced by vegetation. However, the result of the NDVI value had 0.11 in 1994 which indicates that the area had lower vegetation covers during the first decade (Figure 6).

The area, which is placed in the northern, northwest, and northeast parts of the study area resulted in a higher NDVI value and the area is woodland area enclosure and grassland. This report is also in line with (Araya, 2014; Fikadu & Argaw, 2021) who reported that area enclosure was one of the reason factors for the increase of vegetation coverage and the NDVI value increment. However, barren land was placed in the southern part of the study area indicating relatively low NDVI values. (Eastman *et al.,* 2013; Ya'acob *et al.,* 2014) stated that the value of NDVI closed to zero indicates that the area is devoid of vegetation or barren land. According to the NDVI results from our study, there was a significant change in the amount of vegetation cover; the amount of high-moderate density vegetation cover increased by 40.2 %, while the amount of barren land decreased by 22.9 % from the entire area of the *Bilate* watershed.

Land use/land cover change drivers profile of the respondent

In the *Bilate* watershed, families ranged in size from 2 to 14 persons per household, or 5 people on average.

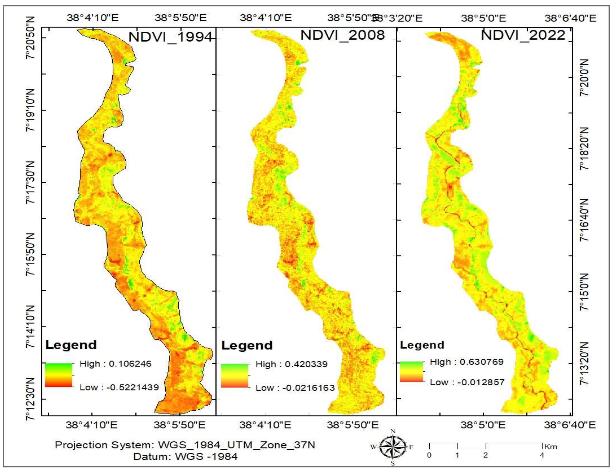

Figure 6: Normalized difference vegetation index maps of the study area (1994-2022)

The age range of the respondents was 18 to 69, with the majority falling between the ages of 41 and 69 (70%). Almost participants were married. According to their gender distribution, 69% of respondents were men and 31% were women. The respondents represent 14 % of the sampled households who were formally educated and 76% of the sampled households' non-educated members. This represented a serious limitation to the transfer of technology and emphasized the value of perhaps inadequate education. The majority of the studied household members, three fourth were involved in livestock and mixed crop production. Only a small percentage of the respondents, however, said they were only engaged in farming and related occupations besides raising their income. This confirmed earlier findings from various regions of Ethiopia and showed that crop and livestock output accounted for more than half of total household income(Asresie *et al.,* 2015; Taffesse *et al.,* 2012).

Perception of local community towards land use/land cover change drivers

The majority of respondents (n=354: 92.19%) indicated that the study area's current vegetation cover is denser than it was at the start of the previous three decades. This implies that respondents generally had a positive perception of the study area's historical land cover pattern. The community is resistant to adapting technologies since 76% of the respondents did not have a formal education, as a result, the local community's influence on the vegetation cover finally leads to a change in LULC. This result is also in line with the LULC change observed in the remote sensing data interpretation. Similarly, studies (e.g. (Asmare & Gure, 2019; Gebregziabher & Soltani, 2019; Kassaye Mekonen *et al.,* 2022) indicated that people's perceptions about area enclosure play an important role in landscape features and land use/land cover dynamics.Our findings also revealed that the insight of respondents on drivers of land use/land cover change showed a significant association with sources of income (x^2=19.21, df = 2, P < 0.001), educational level (x^2= 12.01, df = 2, P < 0.01), and gender (x^2=15.11, df = 1, P < 0.001). While the distance from the area enclosure or watershed and family sizes were independent or there is not associated with the existing drivers of land use/land cover change in the community. Studies (e.g. Ali, 2009; Gebrehiwot *et al.,* 2021; Warkineh & Hailemicael, 2021) also revealed a relationship between the number of families, distance from the protected area, and the land holding size of respondents was not significantly influenced by the perception of respondents on the drivers of LULC change.

Drivers of LULC change in *Bilate* watershed

The FGD participants and key informants in the study area engaged in a series of discussions and interviews, and the results showed that the management system of the area enclosures in the watershed is the primary cause of LULCC. The management strategy and system worked well; they conserved or protected the increase of agricultural investment, illegal fuel wood cutting and extraction, illegal stone extraction, overgrazing, and expansion of illegal and unplanned settlements. Hence the LULC change is driven by the expansion of agriculture investment, illegal logging and fuel wood extraction, illegal stone extraction, overgrazing, and expansion of illegal and unplanned settlements (Figures 7) (i.e. However, these driving forces are not significantly processed in Remote sensing imagery, but from local community perspective these factors were affected the area enclosure, and on the future, it will be expanded, e.g. recently some part of area enclosure was transferred to the local agricultural investor yet, theses induce factor are not processed by remote sensing but the problem exists now a day). Studies (e.g. Kindu *et al.,* 2015; Sewnet, 2015; Zewude *et al.,* 2022) show that the management approach and strategy were significantly important for the improved and sustained protected areas. Moreover, (Melese, 2016; Minale, 2013) stated that the discussion and interviews with focus groups discussion and key informants indicated that the expansion of illegal firewood extraction and the expansion of illegal and unplanned settlements were the major drivers of LULC change in natural vegetation. On the other hand, other studies (e.g. Elias *et al.,* 2019; Gebreselassie *et al.,* 2016) reported that population increase, poverty, and food insecurity were the main forces for LULC change throughout time. Such illegal cuttings have also happened as a result of t rapid human population demand for large amounts of wood for construction. Anthropogenic pressure is cited as the primary source of vegetation changes (Alemu *et al.,* 2015; Elias *et al.,* 2019; Tesfaye *et al.,* 2014). (e. g. illegal cutting and fuelwood extraction). Overgrazing by cattle inside the enclosure may result in the trampling and browsing of seedlings and saplings of some plant species, as well as damaging the vegetation cover. Similarly, studies (e.g. Feyisa *et al.,* 2017; Melese, 2016) stated that the main reason for the vegetation change in this central Ethiopian Rift valley is due to agricultural activities. According to the field observation, most of the farmlands were located near *Bilate* watershed

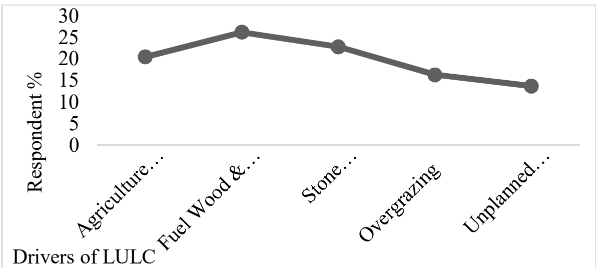

Figure 7: Drivers of LULC changed from 1994 to 2010 in *Bilate* watersheds

which allowed the owner of the farm to gain access to the nearby area enclosure. Previous studies (e.g. Othow *et al.,* 2017; Rahmato, 2011; Zewude *et al.,* 2022) also indicated that the investors are leasing is situated close to national parks, woods, and other protected areas. According to the findings of survey interviews and focus groups, the fragmentation of forests and the establishment of unauthorized settlements inside of area enclosures by the local population are the other two primary proximal causes of vegetation degradation in the Bilate watershed.

Conclusion
LULC affiliated with human demand increases land degradation thereby affecting the ecological functions of the ecosystems. The ecological and socioeconomic conditions were impacted by the LULC changes that were seen in the research area. Here, the LULC analysis showed that before the area enclosure was put into place, there had been significant land degradation. However, the final LULC change maps showed a progressive change in vegetation cover between the study periods. As Enclosures in the study area were not fully protected, some factors were still driving LULC change. The results of FGDs, KIIs, and field observations showed that LULC changes and socioeconomic dynamics have a strong relationship; as the population increases, there is an increased need for agricultural land, grazing land, fuel wood, and settlement areas to meet the growing demand for food and energy, as well as an increased population of livestock. Based on our findings the following recommendations are forwarded:

- Establishing and implementing Community-based area enclosure restoration activities in the watershed.
- Supporting local initiatives, such as alternative income and off-farm economic activity that aim to boost household income in the local community.
- Encourage the adoption of modern stoves for effective energy utilization.
- Implementing effective enforcement of forest laws, policies, and awareness raising campaigns.

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

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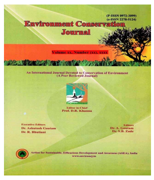

Bioinvasion of *Mytella strigata* (Hanley, 1843) in Ashtamudi Lake, Kerala, India – is pollution aggravating environmental degradation in Ramsar wetland?

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ABSTRACT

Polluted or degraded aquatic ecosystems accumulate more invasive species than less polluted areas. The alien invasive mussel *Mytella strigata* (=*charruana*) was reported to be affecting wild spats of mussels as a dominant competitor for space on floating substrates which is native to Central and South America later outspread to east coast of central Florida and subsequently reported from Philippines. The tremendous increase of *M. strigata* population in Ashtamudi Lake is raising an alarming situation over the native bivalve species as it compete for space, breeding ground, substrates and food. The broad salinity ranges allows them to invade via ballast exchange of water and the fouled ship hulls. When salinity levels goes beyond the thresholds of the native species, *M. strigata* can sometimes outcompete them. Moreover, the molecular phylogenetic analysis via COI sequences shows a close genetic relationship shared between the native mussel *Perna viridis* and the alien invasive mussel *M. strigata*. The increasing pollution load along with the solid waste disposal in the Lake, is accelerating the spat fall of *M. strigata* over the native species and therefore should be a priority in the bioinvasion control, otherwise can lead to displacement or local extinction of the native species.

Introduction

Ashtamudi Lake (8°59’N 76°36’E) is the second largest and deepest wetland ecosystem in Kerala, well known for its unique biodiversity which has been considered as one among the Ramsar sites in India. The Neendakara Fisheries Harbour of Ashtamudi Lake is the second biggest fish trading centre in the State and it provides landing and berthing facilities to the existing flet of mechanized crafts operating from Kollam region. The establishment of the new industrial projects, have increased the pollution load on the Ashtamudi Lake which is adversely effecting native bivalve populations. Municipal solid waste and sewage from Kollam Municipal Corporation oil spillage, hydrocarbon/ fuel pollution, sand mining, pollution by effluents from various industries situated in the banks of estuary are the major environmental issues facing by the Lake (Sitaram, 2014) and thereby increases the risk of invasive species. *M. strigata* which is native to Central and South America (Boehs *et al.,* 2004), was first observed in Jacksonville, FL, United States in 1986 and was considered eliminated after 1987. However in 2004, few individuals were reported along the east coast of central Florida which was 212 km south of the previous site (Boudreaux & Walters 2006). In 2014, *M. strigata* was reported to be affecting wild spats of mussel and other bivalves in the Philippines which was facing serious environmental threats by land and sea based pollution (Vallejo *et al.,* 2017).

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The invasive mussel *M. strigata* was known as *M. charruana* (d'Orbigny, 1846) in the previous literature. However, currently *M. charruana* is known to be a synonym of *M. strigata*. The first report of *M. strigata* in Indian waters came from Cochin backwaters, Kerala (Jayachandran *et al.,* 2019) and followed by the reports from Ashtamudi Lake and other Kerala coasts by Biju et al. (2019). However, the genetic basis for the invasion of *M. strigata* from Indian waters is very scare and impeded by the lack of molecular taxonomic studies and phylogenetic utility. Since, bivalves show phenotypic plasticity, it becomes a prerequisite to provide an integrated taxonomic information including a solid molecular evidence for the proper identification of the invasive mussel and for the management of the native bivalves especially the native *Perna viridis* population. Since, Ashtamudi Lake is one among the major contributors of bivalve fishery of India, it is imperative to analyse the genetic relatedness of *M. strigata* with that of the native mussel *P. viridis.* Therefore, this paper documents the COI gene sequencing data of *M. strigata* for the first time from Ashtamudi Lake, Kerala, along with the sequence data of native Asian green mussel *P. viridis* and discusses its genetic relationship through molecular phylogenetic analysis. Furthermore, the environmental indices have been discussed along with the possible threats that may arise in the near future.

Material and Methods

Individuals of *M. strigata* were collected from Ashtamudi Lake (Lat- 8.961508, Long- 76.607392) in March, 2019, which was primarily attached to the hardy shells of Oysters. The spats of *M. strigata* was found to be increasing tremendously and the fouling effects of *M. strigata* were clearly visible as it were attached to the hard substrates like the native bivalve shells, damaged boats and other concrete remains (Figure 1). The collected specimens were brought to the laboratory and the species identification were done according to Medioda *et al.,* 2017 and Vallejo *et al.,* 2017. The water quality parameters such as Surface water temperature, pH, Salinity, Dissolved oxygen, Hardness and TDS were estimated according to (APHA, 1989). Total genomic DNA from the muscle tissue was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). A partial fragment of mitochondrial COI was amplified via PCR using the primers LCO1490 (GGTCAACAAATCATA AAGATATTGG) and HCO2198 (TAACTTCAGG GTGACCAAAAAATCA) (Folmer *et al.,* 1994). Polymerase chain reactions was carried out in 10µl volume including 1 µl DNA template, 0.2 µl dNTP mix, 2.0 µl 5x phire buffer, 0.25 µl of primer and 0.2 µl (1 U) Taq phire polymerase, 0.15 µl of 5 % DMSO, 1 µl BSA and the PCR cycles were performed in a Mastercycler PCR System (Eppendorf) with a pre-denaturation for 1 min at 94°C, followed by 35 cycles for 20 sec at 94°C, annealing temperature for 20 sec at 45-55°C, extension at 72°C for 1 min followed by a final extension step for 5 min at 72°C. Using ExoSAP-IT (GE Healthcare) the PCR products were purified and the sequencing reaction was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) by the Big Dye Terminator V.3.1 Cycle sequencing kit (Applied Biosystems). For checking the sequence quality, Sequence Scanner Software v1 (Applied Bio systems) was employed and the sequence alignment were carried out using Bioedit (Hall 1999). Analysis for homology of the CO1 gene sequence against nucleotide databases was performed with the help of NCBI- BLAST server. The taxonomy of the sequences were thus confirmed and the sequences were deposited in GenBank under the accession numbers MN603972 (*M. strigata =charruana*) and MW722974 (*P. viridis*). Using MEGA 7 the Maximum Likelihood phylogenetic tree was constructed using Tamura-Nei model (Kumar *et al.,* 2016) with a bootstrap values for 1000 replicates.

Results and Discussion

M. strigata in Ashtamudi Lake was observed with a temperature range of 32 to 36°C, Salinity from 8.337 ppt to 32.066 ppt and dissolved oxygen from 5.236 to 7.653 mg/L (Table 1).

The diagnostic characters such as morphology and other images are given below.

Morphology

Moderately large sized, thick and equivalve shells, with mytiliform and narrow-wedge shaped shell outline, having smooth and shiny shell surface characterised by the concentric or semi-circular

Table 1: Water Quality parameters of the Study Site.

Environmental parameter	Range
Surface water temperature	32 - 36°C
pH	7.6 - 8
Salinity	8.337 ppt - 32.066 ppt
Dissolved Oxygen	5.236 - 7.653 mg/L
Hardness	225 - 310 mg/L
TDS	36.25 - 40.83 mg/L

rings (Fig. 2). Posterior dorsal margin crescent shaped with thick and dark black coloured periostracum of wavy dark pattern. Downturned beaks with curved umbones having narrow hinge area, without teeth in the anterior region bearing pitted resilial edge. Internal colouration white with a broad band of iridescent purple and deep purplish black at the smooth posterior margin. Foot is characterised by deep orange colour along with brown pigmentation at mid-hinged area, bearing byssal threads protruding outside. Pallial line curved towards the adductor scar and the byssal retractor scar seen beneath the adductor muscle which forms a thick straight line going towards the middle portion of the shell, while anterior retractor is greatly reduced in the umbonal area (Fig. 3).

Figure 1: Cluster of *M. strigata* collected from Ashtamudi Lake

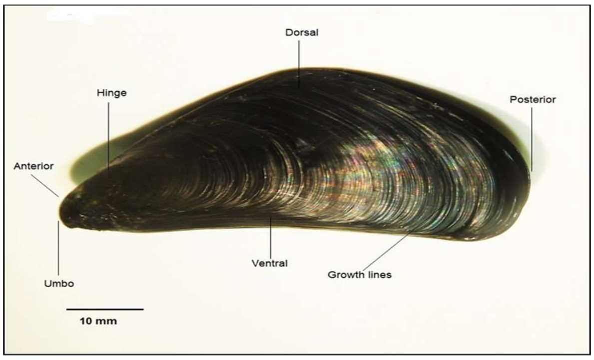

Figure 2: External shell morphology of *M. strigata*

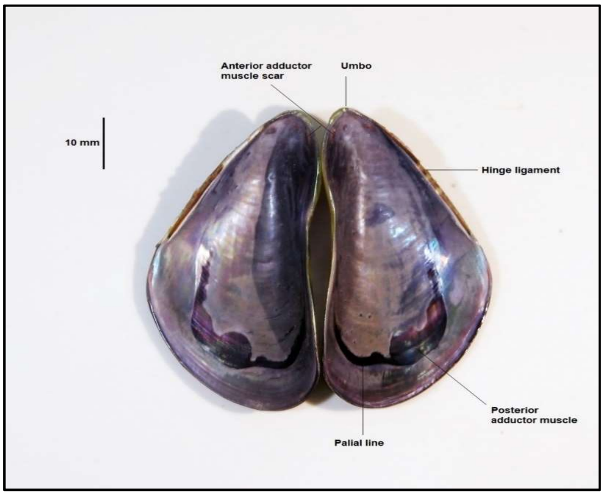

Figure 3: Internal shell morphology of *M. strigata*

Molecular Phylogenetic Analysis

From the chromatogram, FASTA sequences with 533 (*M. strigata*) and 558 (*P. viridis*) base pairs were interpreted and the taxonomy were confirmed through BLAST analysis and deposited in GenBank under accession numbers MN603972 (*Mytella strigata= charruana*) and MW722974 (*Perna viridis*). The COI gene sequence analysis showed the nucleotide frequencies as 24.4 (A), 42.4 (T), 18.6 (G), 14.6 (C) for *M. strigata* and 24.2 (A), 42.5 (T), 20.8 (G), 12.5 (C) for *P. viridis*. Maximum likelihood tree was constructed to infer the evolutionary history of *M. strigata*. Analysis of CO1 gene dataset involved, 2 original sequences and 11 related sequences retrieved from NCBI-GenBank. The molecular sequencing data of *M. strigata* was reported for the first time from Ashtamudi Lake, Kerala. The relationships of *M. strigata* with other Mytilids are illustrated in the Fig. 4. The members of the tree fall under two major well-resolved clades as *Perna*-I and *Mytilus*-II respectively with well supported bootstrap values. It is noteworthy to observe *M. strigata* under the *Perna* clade and not under the *Mytilus* clade. The K2P genetic distance between the *Perna* clade was found to be 0.202 and only a distance of 0.193 was observed between *M. strigata* and *P. viridis*. The observed sequence of *M. strigata* (MN603972) was compared between the Philippine sequence (EU917168) as well the sequence from Kochin (MN165292) backwaters, Kerala, India and the closest distance was observed

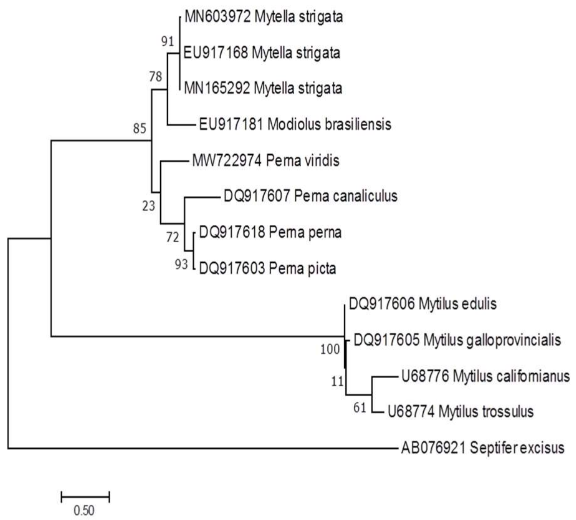

Figure 4: Maximum-likelihood tree (Tamura-Nei Model) using COI sequences of Mytilids

between the Philippine sequence (*M. strigata =charruana*) rather than the Kochin sequence with a bootstrap value of 100. The mean K2P distance between *Mytilus* and *Perna* clade was observed as 0.399.Among the other closely related species, *M. strigata* showed the closest relationship with *Modiolus brasiliensis* (Gray) (=*Mytella guyanensis*). The curve towards the adductor scar created by the pallial line is the unique morphological means of identification of *M. strigata (=charruana)* (Medioda *et al.,* 2017). Also the curved pallial line is unique to *M. strigata* compared to other species like *Perna canaliculus*, *P. viridis*, *P. perna* and *Mytilus edulis* (Quayle and Newkirk 1989). Moreover, distinct brown to black colouration of the shell observed in the samples confirmed the species identification of Rice et al. (2016) and Vallejo et al. (2017) as *M. strigata* (=*charruana*). External features described were the same as those of Gosling (2003) and Spinuzzi et al. (2013) for *M. strigata*. The phylogenetic relationships of *M. strigata* is congruent with the findings of Rice *et al.,* 2016 and Vallejo *et al.,* 2017 and fall under the lineage of *Perna* and the alien invasive mussel *M. strigata* was found to share a close relationship with the native mussel *P. viridis*. The closest relationship between the *M. strigata* with the sequence from Philippines rather than from Kochi indicate the possibility of an independent invasion of *M. strigata* through the Neendakara harbor.

Impact on the ecosystem

The observed salinity ranges of *M. strigata* agrees with the previous records from salinities ranging from 2–40 ppt and even to 55 ppt (Rice *et al.,* 2016). Therefore it can invade and survive in broad saline environments (Yuan *et al.,* 2010; Sanpanich and Wells, 2019). As far as coastal ecosystems are concerned, increase in salinization likely possess a significant effect on the pathways of invasive species. Increased physiological cost has to incur by the salinity-intolerant species to keep-up the osmotic balance when salinity increases, and therefore will gradually grow slower than the tolerant ones. Therefore, increase in salinity may favour invasive species when they are tolerant than the native species. Therefore, the foremost means to bring down the *M. strigata* spread is by means of open-ocean ballast-water exchange (Rahel and Olden, 2008). An emergency action on this invasive species is highly recommended as it has been considered as the potent species to compete with the native species for living space and food resources and can lead to the displacement or the local extinction of the indigenous species (Gurevitch & Padilla, 2004; Spinuzzi *et al.,* 2013). Moreover, the ability of *M. strigata* to accumulate in high densities as 11000 m^2 could also lead to the native species displacement (Pereira *et al.,* 2003). This will worsen when the native species is larger in size like *P. viridis* that may rise up the competitive edge of the species for space and food and has reported to be economically costly (Spinnuzi *et al.,* 2013). This makes *M. strigata* as a dominant competitor and henceforth must be a preference in the bioinvasion control management (Rocha *et al.,* 2010). However, no successful control strategies has been so far reported.The potential of *M. strigata* to outcompete the *P. viridis* is a serious concern that urgently needs to be assessed (Lim *et al.,* 2018; Sanpanich and Wells, 2019) as they occupy similar ecological niche (Vallejo *et al.,* 2017). It is a growing matter of concern that the concentration of the invasive species during the low-flow conditions especially during the hot summer seasons may also rise up the hybridization rate between the non-native and native aquatic species. However, more studies are needed in this concern, to understand its long-term effects. Apart from the consequences on species richness of the invasive species and the corresponding native species extinction, few invasions may govern multiple influences that lead to gross ecosystem functioning, together with factors like primary production, material flow in between the trophic groups, expansion of organic material decomposition and of benthic-pelagic coupling (Occhipinti-Ambrogi, 2007) and even lead to cascading effects of ecosystem (Ivanov *et al.,* 2000; Shiganova *et al.,* 2001). However, the advantages offered to the invasive species by human-activities in any ecosystem cannot be overlooked. The habitat disturbance by human activities and increasing emission of greenhouse gases, deposition of nitrogen and pollutants thereby global climatic change can effect resource dynamics and species distribution in both aquatic and terrestrial ecosystems and subsequently leads to bioinvasion (Dukes and Mooney, 1999, Occhipinti-Ambrogi, 2007). The rapidly evolving ones in these are the total alkalinity, changing pH and temperature. Furthermore, the reverberation on ecosystem and climate will be carrying a long-term effect, even if human activities slowdown in the near future.

Conclusion

Since the studied sites are the breeding grounds of the native Bivalves such as *Villorita cyprinoides*, *Marcia recens*, *Magallana bilineata*, *P. viridis* etc., *M. strigata* puts a high risk over their vital habitats by rapid increasing and also by competing for space, substrates and food. The broad salinity tolerance in turn allows this species to spread through the ballast water exchange process and fouled ship hulls and sometimes can outcompete the native species when their salinity levels goes beyond the thresholds. Therefore, an incessant monitoring on the ecosystem dynamics of Ashtamudi Lake should be guaranteed allied with environmental factors that promote the steady pace in the establishment of *M. strigata* in the Lake. The change in the alkalinity, salinity, pH and temperature associated with the increasing pollution load has been a growing concern and now the advantages offered by the organic pollution and plastic and other solid waste disposal may favour the spat-fall and growth of the invasive species to flourish and outcompete the native species. Therefore, the invasion of *M. strigata* in Ashtamudi Lake can be assessed as an indicator of environmental degradation of this Ramsar Wetland. Henceforth, an effective solution should be derived through the co-operation of the natives and fisher folk of Ashtamudi Lake to mitigate further spat-fall of *M. strigata* through removal of substrates and to ensure a viable population of *P. viridis* and other indigenous bivalves in the wild.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Study of correlation and path coefficient analysis for yield attributing traits in selected rils of diverse wheat (*Triticum aestivum* L.) genotypes for heat tolerance

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ABSTRACT

Rising temperature has adversely affected wheat production globally. In this realm we studied various morpho-physiological traits governing heat tolerance using thirty bread wheat RILs developed via crossing LOK-1 x HUW- 468, LOK 1 x HUW 234 and Raj-4014 x PBN-51. Correlation studies reflected that traits like number of tillers per plant, plant height, and chlorophyll content were significantly positive correlated with grain yield per plant, on the other hand, path coefficient analysis revealed that the chlorophyll content (0.362) and tillers per plant (0.222) showed a significant positive correlation with grain yield per plant, whereas significant negative correlation for grain yield was exhibited with relative water loss (-0.392) and canopy temperature (-0.402) at genotypic level.

Introduction

Bread wheat (*Tritium aestivum* L.) being a hexaploid (AABBDD) is an annual *Rabi* crop of vital importance among staple food crops globally. Wheat, a multipurpose crop is a primary source of gluten (protein) for diverse range of world population. Global warming, a cataract in the world eye via which a nebulae of abiotic as well as biotic stresses have cruised their way out. Abiotic stresses such as high temperature stress (20%), salinity stress (10%), drought stress (9%), and chilling stress (7%) retard wheat grain yield in the manner of 50% cumulatively (Thilert *et al.,* 2006). Among the wholesome of the abiotic stresses, heat stress is of prime importance as it limits crop production via hampering plant development and simultaneously hitting crop yield. With the rise of a unit degree global temperature, as much as 4 million tonnes of global wheat production is retarded (Khairnar *et al.,* 2018). Heat tolerance refers to a plant's capacity to produce desirable yield under elevated temperature environment by combating higher temperatures (Thapa *et al.,* 2020). The consequences of extreme temperatures on crop growth depends on various factors like temperature intensity, duration of high temperature, development stage and the interaction of these factors with the growth stage (Wahid *et*

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al., 2007). High temperature stress reduces number of kernels per ear and the weight of kernel hence affecting grain yield per plant (Gupta *et al.,* 2001). Direct exposure to high temperature leads to chlorophyll degradation that in turn hampers grain yield (Tripathy *et al.,* 2012), also, loss of chlorophyll content damages cell membrane and ultimately leads to leaf senescence (Ristic *et al.,* 2007).Grain yield is not only pretentious to environmental factors but also lies on numerous yield attributing characters which fluctuate yield in even Steven manner. To study the influence of each of these independent traits affecting the grain yield and hence provide a criterion for the selection of the desirable genotypes emphasis is laid on the correlations as well as path correlation (Chowdhury *et al.,* 2019). The correlation coefficient assesses the degree of similarity between any two characters and. hence is vital for calculating the dependence of grain yield on these characters as well as between other characters. However, the study of correlation coefficients alone does not meet the requirements and thus is insufficient to explain the relationships between different traits because it is not able to evaluate the direct and indirect effects of independent variables on dependent variables. To overcome these limitations, path coefficient analysis is used to evaluate the effect of each independent variable (cause) on the dependent variable (effect), i.e. yield (Abdulhamed *et al.,* 2021). The current study was undertaken to ascertain the correlation and the direct and indirect effects of twelve morph-physiological variables on yield.

Material and Methods

Plant material and field trails

The present study was conducted at the Research Farm of Division of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. A population of advanced Recombinant Inbred Lines was used as the germplasm for the current investigation. 30 RILs derived from three crosses between LOK-1 × HUW- 468, LOK 1 × HUW 234 and Raj-4014 × PBN-51 along with two checks namely PBN 51 (heat tolerant) and HUW 234 (heat susceptible) line were used. Among these, LOK -1 and PBN-51 were used as heat-tolerant lines while as HUW-234, HUW-468, and Raj-4014 were used as heat susceptible lines. Ten selected genotypes from these crosses were planted during *Rabi* 2018-2019 using RBD design.The maximum temperature of 43.1ºC was recorded in the fourth week of the May, while as the lowest temperature of 1.7ºC was recorded in the last week of December. Among the total tally of days of the crop stand, as many as 77 days were recorded with temperatures above 25ºC. Fortunately, or unfortunately, these days of high temperature coincided with the most sensitive stage of crop growth that are flowering and grain filling stages, which directly determine yield. Correlation analysis was performed with windostat using Pearson’s correlation coefficient method at both 5% and 1% levels of significance. (* $P<0.05$, ** $P<0.01$).

Results and Discussion

Character association

The correlation coefficient analysis of different trait is presented in table 1 for genotypic and phenotypic correlations respectively.Grain yield per plant showed positive correlation with plant height (0.036), number of tillers per plant (0.222) and flag leaf area (0.064) at genotypic level. Similarly, Majoul *et al.* **(**2004) observed positive correlation of grain yield with similar characters. Grain yield showed positive correlation with yield at phenotypic level (0.026). (Akram. 2011) concluded significant positive correlation of yield with relative water content. According to (Molnar *et al.,* 2002), a decline in relative water content causes a decrease in plant yield. Wheat genotypes' leaf water capacities were examined by Ashraf *et al.* (1994), who came to the conclusion that cultivars with higher water retention capacities were more heat-tolerant. A cooler canopy reduces the detrimental effects of heat stress on grain yield, as seen by the canopy temperature's strong negative association with grain yield at genotypic level (-0.402) in current experiment. Grain yield was found to be significantly negatively correlated with both canopy temperature (-0.402) and relative water loss (-0.392) at genotypic level. Reduced canopy temperature during crucial stages, such as the grain filling stage, is a crucial physiological trait to take into account when breeding for high-temperature tolerant cultivars (Munjal and Rena's 2003). According to Zhang and Oweis (1998), optimal leaf water during or after anthesis enhances photosynthetic rate and aids in the transfer of carbohydrates to grains, improving grain quality and increasing yield. (Bahar *et al.,* 2008) for canopy temperature and Khazaei *et al*. (2010) for relative water loss, also produced similar findings. Relative water loss shows negative correlation relation with grain yield (Kashik *et al.,* 2004). Relative water content showed a positive correlation with test weight (0.221) and tillers per plant (0.194) at genotypic level. Similar to our findings, Jaiswal *et al*. (2010) similarly observed positive correlation between tillers per plant and relative water content. Chlorophyll content showed a genotypically significant positive correlation with flag leaf area (0.221), tillers per plant (0.537) and relative water content (0.065). A substantial positive correlation between flag leaf area and the number of tillers per plant with chlorophyll content, as well as a positive association with relative water content at the genotypic level, was observed by Ali *et al*. in 2008. Significant amount of significant positive correlation was observed between number of days to maturity with tillers per plant and flag leaf area, as well as a positive correlation with canopy temperature at the genotypic level. In accordance with our findings, Anwar *et al*. (2009) reported similar results for tillers per plant. Compared to significant negative correlation with days to 50% blooming (-0.338), thousand grain weight showed positive correlation with chlorophyll concentration (0.019) and positive significant correlation with relative water content (0.221) at genotypic level. Asif *et al.* (2003) also concluded same results for relative water content. Flag leaf area displayed significant positive association with height of plant (0.244), relative water content (0.309), tillers per plant (0.529), and chlorophyll content (0.275), whereas negative correlation with canopy temperature (0.024) at genotypic level. Leaf area increases the contribution to canopy temperature. More is the leaf area index, more is the area of leaf, a greater photo-synthetically active surface area is available, which would result in high production rate**.** This showed that increase in leaf area index declines canopy temperature because canopy of plants increases with increase in flag leaf area which improves transpiration rate and assists in lowering down the canopy temperature and maintaining cooler temperature than ambient temperature.

Therefore, it can be inferred from the current study that there is a positive association between flag leaf area and grain output, and significant positive correlation between plant height, chlorophyll content and number of tillers per plant with grain yield. For an increase in grain yield, choosing any one of these physio-morphological characteristics would be crucial.

Path coefficient analysis

In the current experiment path coefficient studies were performed for grain yield. Genotypic path diagram of yield attributing characters on grain yield in wheat for thirty-two wheat genotypes is presented in fig 1. The direct and indirect effect of different component characters and physiological characters with grain yield per plant at genotypic level are tabulated in table 2. Since, the correlation studies alone are not sufficient to study association analysis very clear, hence the study of real effect of an independent character towards grain yield per plant becomes essential. Correlation studies cannot interpret the precise results predicted by path coefficient. Path correlation considers both the direct and indirect effects of one independent variable on the dependent variable, such as the effect of yield through other indirect factors. In the present experiment study of path coefficient analysis in which diagonal values are direct effects and non-diagonal indirect effects, showed that the flag leaf area (1.470) had highest positive direct effect on grain yield followed by plant height (0.742), number of days to fifty percent flowering (0.185) and number of days to maturity (0.104). Similar results were obtained by Kashif *et al.* (2004) for flag leaf area and plant height. The negative direct effect was recorded for canopy temperature (-0.046), relative water content (-0.463), chlorophyll content (-0.599), grains per ear (-0.381), tillers per plant (-1.680), relative water loss (-0.491) and canopy temperature depression (-1.100). Alike results were reported by Ojha *et al.,* (2018) for chlorophyll content and thousand grain weight, Kashif *et al.* (2004) for number of tillers per plant and Saleem *et al.* (2015) for relative water loss.

Characters *viz* canopy temperature, chlorophyll content, flag leaf area and days to maturity showed indirect positive effect via relative water loss. Relative water content and days to maturity showed positive indirect effect via canopy

Table 1: **Genotypic (upper diagonal) and Phenotypic correlations (lower diagonal) coefficient among thirteen characters with grain yield**

	Number of days to fifty percent flowering	Number of days to maturity	Plant height (cm)	Number of tillers per plant	Number of grains per ear	Thousand grain weight (g)	Flag leaf area (cm^2)	Chlorophyll content (SPAD Unit)	Relative water content (%)	Relative water loss (%)	Canopy temperature (^{0}C)	Canopy temperature depression (^{0}C)	Grain yield $^{-1}$ (g)
Number of days to fifty percent flowering		0.474**	0.059	0.186	0.123	-0.338**	-0.047**	0.153	0.026	-0.287**	0.134	-0.011	-0.009
Number of days to maturity	--0.269**		0.062	0.324**	-0.089	-0.324**	0.350**	-0.085	0.241*	0.191	0.060	0.226*	-0.152
Plant height (cm)	0.078	-0.073		0.709**	-0.264*	0.148	0.244*	0.320**	0.474**	-0.489**	-0.173	-0.534**	0.367**
Number of tillers per plant	0.243*	0.145	0.318**		-0.029	0.384**	0529**	0.537**	0.194	-0.370**	0,024	-0.478**	0.222*
Number of grains per ear	0.087	0.179	-0.149	0.002		-0.227*	0.360**	-0.664**	-0.157	0.605**	0.249*	0.007**	-0.799**
Thousand grain weight (g)	-0.217*	-0.142	0.157	-0.124	0.119		-0.087	0.019	0.221*	0.321*	0.134	-0.040	-0.197
Flag leaf area (cm^2)	-0.053	0.19	0.182	0.303**	0.142	-0.069		0.275**	0.309**	0.513**	-0.089	0.076	0.064
Chlorophyll content (SPAD Unit)	0.052	0.055	0.075	0.188	-0.08	0.043	0.026 *		0.082	-0.617**	0.310**	-0.646	0.362**
Relative water content (%)	0.104	0.15	0.248*	0.265**	-0.082	0.105	0.216 *	0.051		0.065	-0.042	-0.084	-0.008
Relative water loss (%)	-0.099	-0.005	-0.055	-0.215*	0.178	0.17	0.187	-0.222*	0.067		-0.350**	0.744**	-0.392**
Canopy temperature (^{0} C)	0.111	0.107	-0.105	0.088	0.226*	0.078	0.084	0.066	0.052	-0.044		-0.073	-0.402**
Canopy temperature depression (^{0} C)	0.027	0.211*	-0.154	-0.028	0.286**	-0.094	-0.082	-0.203*	-0.016	0.234*	0.113		-0.857*
Grain yield $^{-1}$ (g)	0.037	-0.041	0.194	0.116	-0.326**	-0.774	0.003	0.166	0.026	-0.235*	-0.174	-0.409**	

***,** significantly at 5% and 1% levels, respectively.**

Table 2: Genotypic path showing direct (diagonal) and indirect (off diagonal) effect of twelve characters on grain yield for 32 wheat genotype at phenotypic level

	Canopy temperature (^{0}C)	Plant height (cm)	Relative water content (%)	Chlorophyll content (SPAD Unit)	Number of Grains per ear	Number of tillers per plant	Flag leaf area (cm^2)	Relative water Loss (%)	Canopy temperature depression (^{0}C)	Number of days to fifty percent flowering	Number of days to maturity	Thousand grain weight (g)	Genotypic correlation with Grain yield^{-1}
Canopy temperature (^{0}C)	**-0.046**	-0.128	0.020	-0.186	-0.095	-0.041	-0.130	0.172	0.081	0.025	0.006	-0.078	-0.402**
Plant height (cm)	0.008	**0.742**	-0.219	-0.191	0.101	-1.191	0.359	0.240	0.588	0.011	0.007	-0.086	0.367**
Relative water content (%)	0.002	0.351	**-0.463**	-0.049	0.060	-0.325	0.455	-0.032	0.092	0.005	0.025	-0.128	-0.008
Chlorophyll content (SPAD Unit)	-0.014	0.237	-0.038	**-0.599**	0.253	-0.903	0.404	0.303	0.711	0.028	-0.009	-0.011	0.362**
Number of grains per ear	-0.012	-0.196	0.073	0.398	**-0.381**	0.049	0.530	-0.297	-1.108	0.023	-0.009	0.132	-0.799**
Number of tillers per plant	-0.001	0.526	-0.090	-0.322	0.011	**-1.680**	0.778	0.182	0.526	0.034	0.034	0.224	0.222*
Flag leaf area (cm^2)	0.004	0.181	-0.143	-0.165	-0.137	-0.890	**1.470**	-0.252	-0.083	-0.009	0.036	0.051	0.064
Relative water loss (%)	0.016	-0.363	-0.030	0.369	-0.231	0.622	0.753	**-0.491**	-0.818	-0.053	0.020	-0.187	-0.392**
Canopy temperature depression (^{0}C)	0.003	-0.396	0.039	0.387	-0.384	0.804	0.111	-0.365	**-1.100**	-0.002	0.023	0.023	-0.857**
Number ofdays to fifty per cent flowering	-0.006	0.044	-0.012	-0.092	-0.047	-0.312	-0.069	0.141	0.012	**0.185**	-0.049	0.197	-0.009
Number of days to maturity	-0.003	0.046	-0.111	0.051	0.034	-0.545	0.514	-0.094	-0.248	-0.088	**0.104**	0.189	-0.152
Thousand grain weight (g)	-0.006	0.110	-0.102	-0.011	0.087	0.646	-0.128	-0.158	0.044	-0.063	-0.034	**-0.582**	-0.197

***,**significantly at 5% and 1% levels, respectively, Residual effect = 0.856**

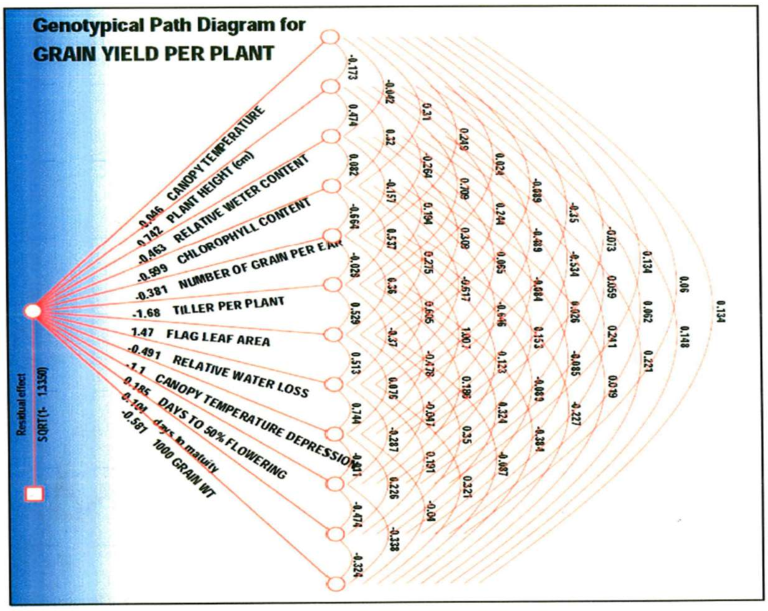

Fig.1: Genotypic Path diagram of yield component effect on grain yield in wheat for thirty-two wheat genotypes

temperature at genotypic level. It indicates that factors such as ability to maintain relative high tissue water status during period of heat stress and an ability to maintain developmental plasticity are partially responsible for heat resistance by reducing canopy temperature. These results were in accordance with Anwar *et al. (*2009) for relative water loss. Characters *viz* chlorophyll content, flag leaf area and days to maturity showed indirect positive effect via relative water loss. Ahmadizadeh *et al.* (2011) also concluded similar results for chlorophyll content. Grain yield correlated positively with tillers per plant, chlorophyll content, and flag leaf, but significantly negatively with canopy temperature, relative water loss, and canopy temperature depression at the genotypic level.

Conclusion

The results of the current study demonstrate that various traits such as plant height, chlorophyll content, flag leaf area and canopy temperature depression, can be used to create high yielding wheat varieties under extremely high temperatures. A critical perusal of path coefficient showed that days to fifty percent flowering and number days to maturity had positive direct effect on grain yield at genotypic level, hence selection for such traits would prove beneficial for getting heat tolerant recombinants during selection programme for grain yield under heat stress situation. Future efforts should be focused to fully explore the relationship between physio-morphological traits with yield, this will assist breeders to identify the physio-morphological pathways to sustain sustainable production of wheat against heat stress. Further studies about interrelationships studied by incorporating correlation and path correlation in genetic studies can positively affect the breeding programmes through the incorporation of desirable selection indices that help in successful breeding programme.

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Conflict of interest

The authors declare that they have no conflict of interest.

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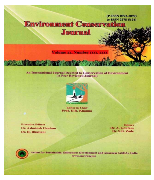

Performance evaluation of tractor mounted boom sprayer on chilly crop

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ABSTRACT

Chilli (*Capsicum annuum L.*) is an important vegetable and spice crop belongs to Solanaceae family which grown all over the world. Dried pods contain 36 gms of carbohydrates, 18 gms of proteins, and excellent source of vitamin A and vitamin C which together provide roughly 160 calories of energy per 100 gms (Narayanan *et al.*, 1999). Area, production and productivity of Chilli in India was 7.43 Lakh ha, 19.14 Lakh Tonnes and 2576 kg/ha, respectively (Anonymous, 2018). Keeping in mind, above parameters the performance of tractor operated boom sprayer was tested on Chilly crop. Under laboratory conditions, the designed boom sprayer performed excellently at 0.90 l/min nozzle discharge and 689.5 kPa operating pressure. It was observed that droplet size, spray uniformity, and droplet density was influenced by the nozzle discharge rate and pressure of 0.45, 0.70, 0.90, and 1.35 l/min and 275.8, 413.7, 551.6, and 689.5 kPa, respectively. The Volume Median Daimater (VMD), Uniformity Coefficient (UC) and Droplet Density (DD) of the existing boom sprayer have 130.9-206.36 µm, 0.98-1.39 and 11-27 number of droplets/cm^2, respectively, for nozzle 0.9 lpm and pressure 689.5 kPa. The modified sprayer has droplet sizes between 125.04 and 181.42 m, droplet densities between 16 and 27 number of droplets/cm^2, and uniformity coefficients between 0.99 and 1.25 at nozzle discharges of 0.90 l/min. With the 689.5 kPa working pressure and 0.90 l/min nozzle discharge, the designed boom sprayer offers notably higher discharge and nozzle pressure in each individual nozzle than the existing sprayer.

Introduction

Chilli (*Capsicum annuum L.*) is an essential vegetable and spice crop grown all over the world and prized for its aroma, taste, flavour, and pungency. Chilli belongs to solanaceae family with chromosome number 2n=24. It is a basic ingredient in many Indian curries and chutneys, as well as in vegetables, spices, medical products, condiments, sauces, and pickles. In terms of nutrients, Chilles are high in vitamins, particularly vitamin A and C (Raju and Luckose, 1991). For every 100 gms of dried pods, there are 36 gms of carbohydrates, 18 gms of proteins, 16 gms of lipids, 480 mgms of calcium, 3.1 mgms of phosphorous, 31 mgms of iron, 2.5 mgms of niacin, 640 I.U. of vitamin "A," and 40 mgms of vitamin "C", it gives about 160 calories of energy (Narayanan *et al.,* 1999). There are more than 400 different types of Chilli grown around the world, with the "Carolina reaper" variety from the USA

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being the spiciest (Bindu and Nayak, 2021). Nationwide, 1.776 million hectares are used to cultivate Chillies, yielding 7.182 million tonnes yearly. With a 25% and a 24% share of all global exports, respectively, India and China are the two biggest chilli exporters in the world (Anonymous, 2018). Area, production and productivity of chilli in India was 7.43 Lakh ha, 19.14 Lakh Tonnes and 2576 kg/ha, respectively (Anonymous, 2018). In India, Andhra Pradesh stands first in both production and area of Chilli which accopies 49%, followed by Orissa, Maharashtra and Madhya Pradesh 7, 6 4% of Chilli production in the India (Gade *et al.,* 2020).

Pest, disease, and weed infestations are the main causes of decreased crop productivity. The most common technique used to manage most insects, weeds, and diseases is chemical control. Either spraying or dusting the chemicals on is used to apply them. One of the best and most efficient ways to apply a tiny amount of spray liquid to protect crops is through spraying. When using the traditional spraying technique, it is challenging to evenly and successfully apply the insecticide throughout the tree. Although this method effectively controls pests, it requires a lot of time and labour and consumes a lot of liquid per plant. Nowadays, agriculture is facing significant challenges, due to growing public concern over how agricultural production practices affect the environment and our ability to live in a safe and secure environment. To safeguard crops against insects and pests, a number of sprayers are available on the market. Numerous technological, technical, and environmental aspects affect how well a sprayer performs. These consist of the nozzle type, suitable spray parameters, temperature, humidity, and plant protection product directions.

Ground surface deposition and off-target drift are the main obstacles to plant protection with spraying equipment. This drift commonly causes a hazard to both human and animal health as well as a source of environmental contamination (Maski and Durairaj 2010). A tractor-based spray application system can be promoted to reduce the multiple - input and achieve uniform deposition, distribution, and vertical fluid distribution (Sedlar *et al.,* 2013). As a result, it's important to encourage the usage of time-saving machinery operated by tractors (Raut *et al.,* 2013). Inadequate nozzle pressure, discharge, height, and other factors all contribute to pesticide loss. Therefore, in order to minimize pesticide losses from sprayers, the optimum discharge rate and pressure must be determined (Gholap *et al.,* 2013).Keeping in mind, above parameters the performance of tractor operated boom sprayer was tested in chilly crop. In order to study the effects of nozzle discharge rate (i.e., 0.45, 0.70, 0.90, and 1.35 l/min) and nozzle pressure (i.e., 275.8, 413.7, 551.6, and 689.5 kPa) on spray uniformity, the hydraulic boom sprayer was tested for Chilli crop. Tires from tractors cause very little crop damage since they can move easily between rows (Nalavade *et al.,* 2008). The efficiency of insecticides can be improved with a good sprayer (Singh *et al.,* 2019). In developed nations, spraying enables people to enjoy high-quality products that are free of pest contamination and blemishes (Prokop and Kejklicek, 2002). In order to increase the sprayer's efficacy on the guar crop in the context of the local environment, it is necessary to evaluate and improve the performance of various spray parameters, including pressure, nozzle height, swath width, and discharge (Nuyttens *et al.,* 2007; Narang *et al.,* 2015).

Material and Methods

Study area: The present study was carried out in ASPEE Research Farm, Wada, Thane.

Sprayer setup: Using three point linkage, a 35 HP tractor was equipped with a 12 m boom sprayer. A universal joint was used to link the sprayer's v-belt pulley to the tractor P. T. O. unit. An experiment was conducted in a lab using a 12 m tractor mounted boom sprayer, descriptions of the sprayer is shown in Table 1. The tractor's power take-off (PTO) shaft provided the required energy for the boom sprayer to operate it.

Table 1: Specifications of the tractor mounted boom sprayer

Description	Boom sprayer
Tank capacity	400 litre
Operating pressure	689.5 kPa
Maximum pressure	2758 kPa
Power required	35 HP
Weight of sprayer (without liquid)	270 kg
Size of sprayer (l × w × h)	1364x1000x1212 mm
Application Rate	580-700 lit/ha
Type and number of nozzles used	Hollow cone nozzle, 25

Instrument used: A pump tester was used to determine the pump flow rate, and a master pressure gauge tester was used to calibrate the pressure gauge. The nozzle discharge rate of 0.45, 0.7, 0.9, and 1.35 l/min, respectively, were independent variables for spray deposition on Chilli crop, while operating pressures were 689.5 kPa. Three times the experiment was conducted on chilly crops in a lab.

Field preparation: To assess the spray deposition of the boom sprayer on the Chilli crop, a plot size of 100x100 m^2 was selected. At a distance of 25 m length and 12 m width, two poles were built.

Nozzle discharge: The discharge of the sprayer nozzles at the desired pressure was measured in the lab using a test bench. The discharge of the nozzles was measured and noted using a timer and graduated beaker. Spray discharge was collected in still air for a period of 60 seconds.

Spray angle: Another crucial nozzle performance factor, spray angle which determines the proper nozzle spacing, application height, and overlapping. The type of nozzle, orifice size, and operating pressure all affect the spray angle. Spray angle and swath width also increase with pressure. Using a patternator, the spray angle of the nozzles was computed in the lab.

Control valve assembly

Control panel set-up 1:

The pressure regulator (CVA), suck-back valve, and four on/off valves make up the control panel setup. When the hydraulic agitator is operated, it makes it easier to spray from any part of the boom, and it also helps in sucking back. For every six nozzles that follow one another, four on/off valves are provided for delivery, and one is for the auto-filling assembly. Four on/off valves are provided for delivery for every six consecutive nozzles and one is provided for auto filling assembly. The conceptual sketch of control panel set-up 1 has shown in Figure 1 (a) and the original set-up 1 has shown in Figure 1 (b).

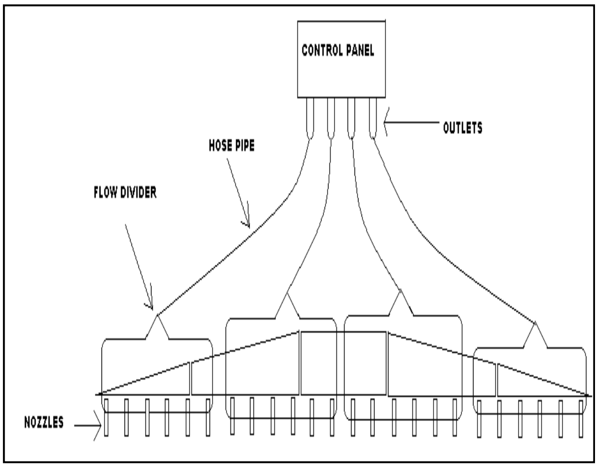

Figure 1(a): Conceptual sketch of control panel set-up 1

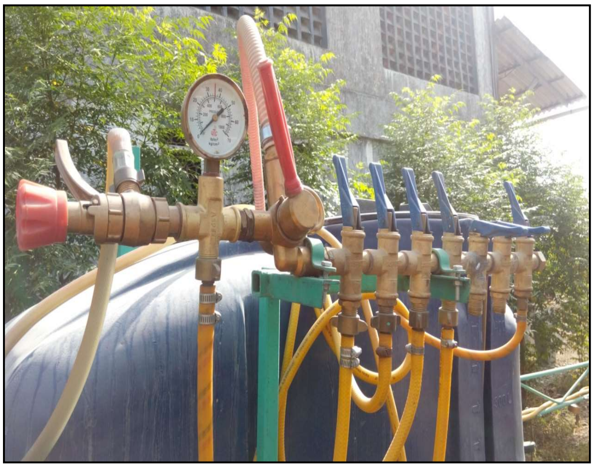

Figure 1(b): Set-up 1 Four control valve assembly

Control panel set-up 2:

The pressure regulator (CVA), suck back valve, and six on/off valves make up the control panel setup. For every four successive nozzles, there are six on/off valves provided for distribution, and one is also available for the auto filling assembly. There is a pressure regulator available for controlling the pressure. Reduced spray fluctuation is the primary use of pressure vessels. The primary frame of thesprayer's spraying was where the control assembly was attached. The conceptual sketch of control panel set-up 1 has seen in Figure 2 (a) and the original set-up 1 has shown in Figure 2 (b).

Nozzle:

Generally hollow cone nozzle was used in the boom sprayer, this nozzle available in standard size of 0.4 to 1.35 l/min discharge rate. So, Hollow cone nozzles of 0.4, 0.7, 0.9 and 1.35 l/min were selected for the testing. The nozzle discharge rate,

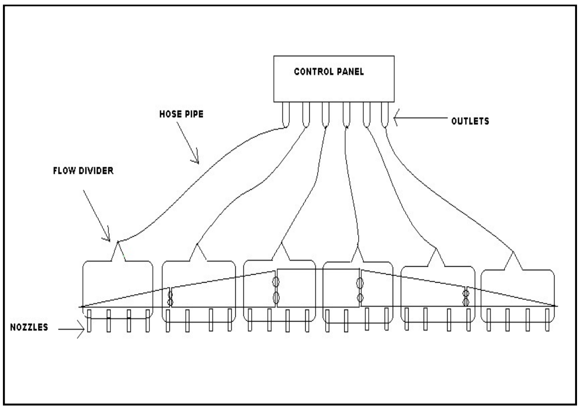

Figure 2(a): Conceptual sketch of control panel set-up 1

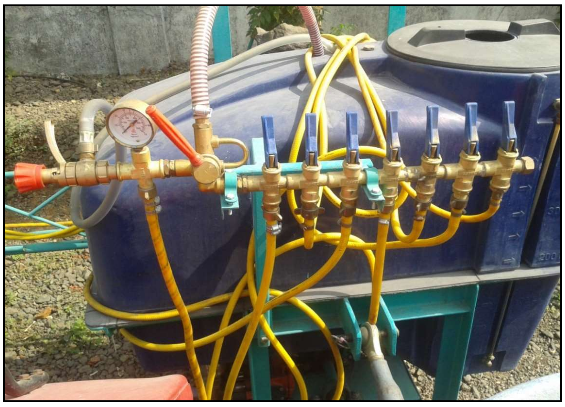

Figure 2(b): Set-up 2 Six control valve assembly

spray angle, droplet size distribution, and spray intensity distribution under specific standard pressure are such distinguishing characteristics. The Hollow nozzle was selected, based on type of chemical being sprayed. The recommended angle of hollow cone nozzle is 65^o to 110^o (Sanchavat *et al.,* 2020).

Spacing:

Consideration of appropriate nozzle spacing is important, as it affects the boom height and overlap. Generally, for 110^o spray angle nozzle spacing is considered as 500 mm (Matthews, 1994).

Pump flow rate Measurement:

The flow rate of pump was measured using instrument AAMS pump tester. The pump was dismounted from the sprayer and connected to pump tester. The flow rate was measured for four pump rpm speeds as 800, 850, 900 and 950.

Pressure gauge testing:

The manometer of the sprayer is dismounted and attached to a quick coupling that can be fitted on the manometer tester. The gauge was calibrated for the 1, 2, 3, 4, 5, 6, and 7 kPa.

Analysis of water sensitive paper strips:

For determination of droplets size of each sprayer, a blue-coloured dye was mixed with water and the impression of droplets was taken on glossy paper. For the purpose of observing the droplets fall, three glossy papers were attached to each place. Before starting the experiments, the equipment was calibrated as necessary and operated for 30 minutes. The set up was started 3 meter before the canopy and the sample was gathered on sample cards made of glossy paper with measurements of 62 mm x 44 mm. This was done to ensure that the crop was exposed to the spraying uniformly. To create a coloured spray solution, royal blue indigo dye was combined with water. Onto the sample glossy photographic paper, the coloured spray was allowed to fall. After experiment, sample cards were carefully taken out and taken to the lab for analysis. The Digital image analyzer was used to determine the size of droplets i.e. NMD, VMD, spray uniformity etc.

Measurement of Droplet Deposition:

The most powerful electronic imaging software, "Image Pro Plus," was used to evaluate glossy paper. The program's advanced image processing abilities are made available by Microsoft Windows, which includes a microscope which was connected to the computer software via a Graphical Interface Card, allowing us to view the image on computer screen directly, as shown in Figure 3. Following computer processing of these photos, droplet size and density were directly determined.

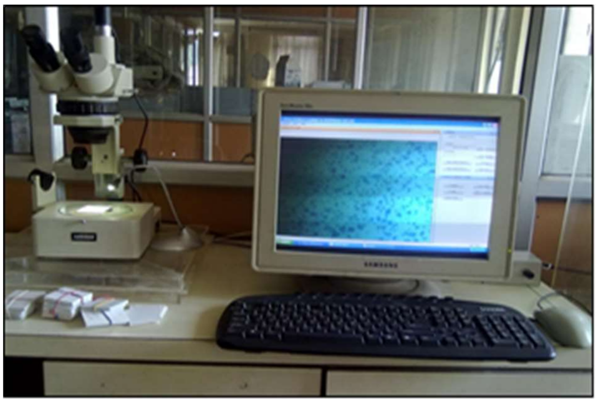

Figure 3: Electronic imaging instrument

Data Analysis

M.S. Excel was used to store and analyze the data. The droplet spectrum recorded on the sample card at a particular place had its VMD and Mean diameter calculated using the image-pro application. Utilizing factorial CRD statistical software tools, the data were analyzed on a computer. At the 1% level, it was discovered that the data for VMD, NMD, DD, and UC was significant.

Figure 4: Modified hydraulic boom sprayer

Plant Position:

The boom sprayer has mostly used for spraying pesticide on vegetable, pulses, catch crops etc. so for the measurement of the droplet deposition the chilly plant was selected for the study. The arrangement of the glossy paper on chilly plant has seen in Figure 5. To ease the assessment of spray penetration into the canopy of the Chilly plant, the plant was separated into six distinct portions depending upon the area where the influence of independent variable on spray deposition was to be examined.

Figure 5: Chilly plant with glossy paper

Actual field capacity: Real work time and time lost to other operations like turning and tank filling were both taken into account when determining the actual field capacity. The field capacity was given by (Sanchavat *et al.,* 2017)

$$\textit{Actual field capacity}\ (ha/h) = \frac{\textbf{Width (m)} \times \textbf{Speed}\ (km/h)}{\textbf{10}} \times \textit{Efficiency}\ (\%)$$

Theoretical field capacity: Theoretical field capacity was calculated by (Sanchavat *et al.,* 2017)

$$\textit{Therotical field capacity}\ (ha/h) = \frac{\textbf{Width (m)} \times \textbf{Speed}\ (km/h)}{\textbf{10}}$$

Field efficiency: Field efficiency is calculated by (Sanchavat *et al.,* 2017)

$$\textit{Field Efficiency}\ (\%) = \frac{\textbf{Therotical field capacity}}{\textbf{Actual field capacity}}$$

Results and Discussion

To evaluate the performance of the developed tractor mounted boom sprayer, various tests were carried out in laboratory.

Pump flow rate measurement

In a laboratory, the discharge of the sprayer pump at each of its four rpm settings was measured. The pump's rpm could be adjusted by using pulleys of various diameters. For each pressure of 275.8 kPa, 423.7 kPa, 551.6 kPa, and 689.5 kPa, the pump's rotational speed was selected as 800, 850, 900, and 950. Pump discharge was found to be 36 l/min at 689.5 kPa pressure. The results showed that for increase in pressure flow rate increases (Hofman, 2004). It was found that the minimum values of flow rate were 32.40 l/min for 850 pump rpm and maximum value 35.94 l/min for 950 pump rpm. The pump was able to maintain appropriate flow rate but failed to maintain sufficient pressure in the nozzles. The test results are shown in Figure 6.

Pressure gauge testing

The AAMS master gauge was used to test the sprayer's pressure gauge. The gauge was calibrated for the 0, 10, 20, 30, 40, 50, 60 and 70 kPa pressures. The commercial pressure gauge of the existing boom sprayer showed linear relationship when calibrated with AAMS master gauge.

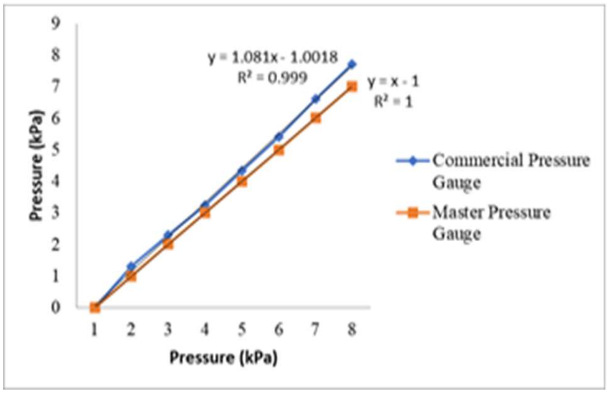

Figure 6: Pressure curve for commercial pressure gauge and master gauge

Effect of nozzle discharge rates and pressures on droplet density (DD), uniformity co-efficient (UC), and droplet size (VMD) of existing boom sprayer

Effect on droplet size (VMD)

With nozzle discharge rates of 0.45, 0.7, 0.9, and 1.35 l/min and a nozzle pressure of 689.5 kPa, the boom sprayer's VMD ranged from 130.9 µm to 288.33 µm. The droplet sizes (VMD) with 0.9 l/m nozzle discharge and 689.5 kPa pressure were extremely near to the practical range of 150 µm to 250 µm. The droplet sizes at a 0.9 l/min nozzle discharge were found to be 206.36 µm and 199.5 µm for the top upper and bottom lower plant positions, 178.67 µm and 160.5 µm for the middle higher and lower plant positions, and 145.3 µm and 130.9 µm for the bottom upper and lower plant positions. The droplet size reduced as a result of rising operating pressure, which also caused an increase in the number of droplets (Kumar *et al.,* 2021).

Effect on uniformity coefficient (UC):

At nozzle discharge rate of 0.45, 0.7, 0.9, and 1.35 l/min, the sprayer's uniformity coefficient was found to be in the range of 0.82 to 1.84. 0.9 l/min nozzle discharge and 689.5 kPa pressure resulted in a uniformity coefficient (UC) that was extremely near to one. The middle upper and middle lower plant positions had uniformity coefficients of 1.35 and 1.25, the top upper and top lower plant positions had uniformity coefficients of 1.39 and 1.29, and the bottom upper and bottom lower plant positions had uniformity coefficients of 1.18 and 0.98 at 0.9 l/min discharge.

Effect on droplet density (DD):

At nozzle discharge rates of 0.45, 0.7, 0.9, and 1.35 l/min, the boom sprayer's droplet density ranged from 10-29 number of droplets/cm^2. The effective range of 16 to 30 number of droplets/cm^2 was very nearly achieved by the droplet density (DD) at 0.9 l/min nozzle discharge and 689.5 kPa pressure. At 0.9 l/min nozzle discharge, the droplet density of the top upper and top lower leaves varied between 27 and 19 number of droplets/cm^2, while that of the middle upper and middle lower leaves was 17 and 14 number of droplets/cm^2, and the bottom upper and bottom lower leaves had a droplet density between 12 and 11 number of droplets/cm^2. The droplet quantity may have increased due to a decrease in the mean droplet size (Kumar *et al.,* 2021).

Measurements of Pressure and discharge of nozzles:

The boom sprayer was run at 689.5 kPa working pressure for laboratory testing. For each individual nozzle, the tractor mounted boom sprayer pressure and discharge were measured from left to right in front of the driver seat (Nalavade, 2008). The modified set-up 2 boom sprayer's nozzle discharge with an operating pressure of 689.5 kPa is depicted in Figure 7(a). The discharge rate of the nozzle varied from 0.87 to 0.92 l/min when the boom is shifted from left to right. The variation was found to be 4.5% as compared to 29.8% in the boom sprayer that was already in use. The nozzle pressure of a modified set-up 2 boom sprayer with an operating pressure of 689.5 kPa is shown in Figure 7(b). From 490 to 525 kPa, the nozzle pressure varies. The modified set-up 2 boom sprayer's nozzle discharge with an operating pressure of 689.5 kPa is depicted in Figure 7(a). The discharge rate of the nozzle varied from 0.87 to 0.92 l/min when the boom is changed from left to right. The variation was found to be 4.5 % as compared to 29.8 % in the boom sprayer that was already in use. The nozzle pressure of a modified set-up 2 boom sprayer with an operating pressure of 689.5 kPa is shown in Figure 7(b). The nozzle pressure ranges from 490 to 525 kPa. Figure 8(a) illustrates the mean nozzle discharge performance of the original and modified (set-up 1 and set-up 2) boom sprayers.

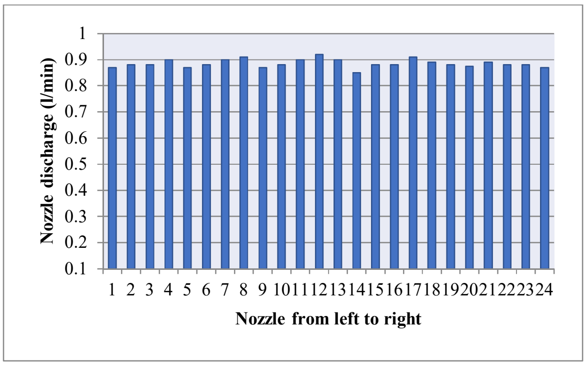

Figure 7 (a): Nozzle discharge of modified set-up 1 boom sprayer for 689.5 kPa working pressure

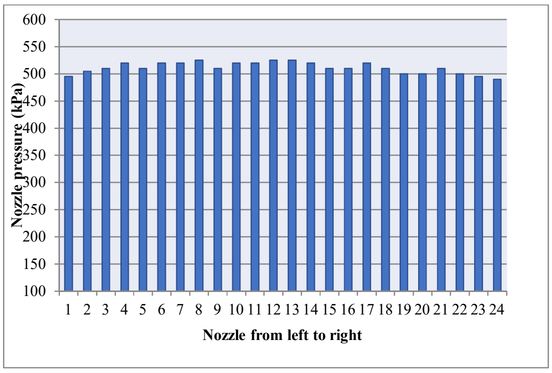

Figure 7 (b): Nozzle pressure of modified set-up 1 boom sprayer for 689.5 kPa working pressure

Comparative Performance of Modified set-up 1 and set-up 2 Boom Sprayer with Existing Boom Sprayer

The figure demonstrates that, compared to the existing boom sprayer, the mean nozzle discharge of the modified set-up 1 and set-up 2 boom sprayers improved by 29.5 and 44.2%, respectively.Figure 8 (b) compares the performance of existing and modified (set-up 1 and set-up 2) boom sprayers. The Figure illustrates that the mean nozzle pressure of the modified setups 1 and 2 boom sprayers increased by 70 and 160% of the baseline boom sprayer, respectively. It was observed that the top position of the plant, which was followed by the middle location and the bottom location, achieved the largest droplet size (VMD). The results also showed that the droplet size at the plant's upper and lower leaf surfaces was within the acceptable range. The top location of the plant yielded the maximum droplet density (DD), which was followed by the middle and bottom locations. The results also showed that the droplet density at the plant's upper and lower leaf surfaces was within acceptable limits at the top, middle, and bottom locations.

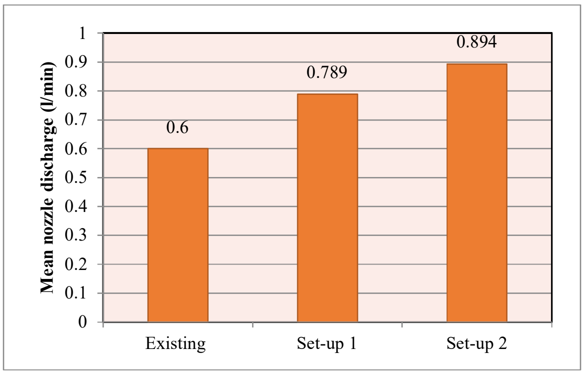

Figure 8 (a): Comparative performance of nozzle discharge for existing and modified (set-up 1 and set-up 2) boom sprayer.

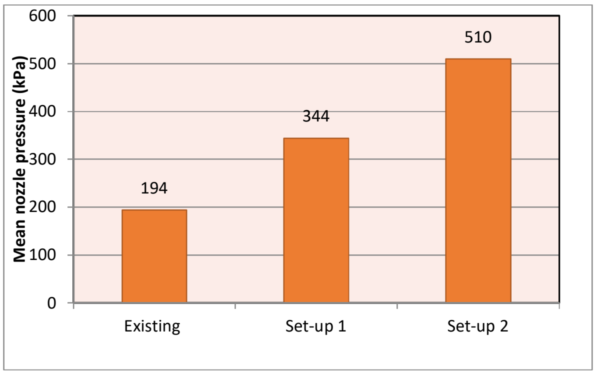

Figure 8 (b): Comparative performance of nozzle pressure for existing and modified (set-up 1 and set-up 2) boom sprayer.

Economical parameter:

The developed tractor mounted boom sprayer was tested in chilly crop**.** The theoretical field capacity and field efficiency of tractor mounted boom sprayer was 3.5 ha/h and 72.4%, respectively. An average of three trials showed that the theoretical field capacity was 3.3 ha/h and field efficiency of 63.03% (Sanchavat *et al.,* 2020). The cost of operation of tractor operated booms sprayer was Rs.208.12/ha for

Chilli crop. For the cotton crop, the cost of running a tractor-operated boom sprayer was Rs. 220.79/ha (Sanchavat *et al.,* 2020).

Conclusion

The performance evaluation of tractor mounted boom sprayer was found satisfactorily for Chilly crop. The nozzle discharge of 0.9 l/m and pressure of 689.5 kPa the values of VMD, UC and DD of the existing boom sprayer were 130.9-206.36 µm, 0.98-1.39 and 11-27 number of droplets/cm^2, respectively. It has been found that using a tractor-mounted sprayer operating at 600 kPa results in more evenly dispersing insecticides across the cotton crop's field. Modified sprayer has the droplet size in the range 125.04 to 181.42 µm, droplet density 16 to 27 Number of droplets/cm^2 and uniformity coefficient 0.99 to 1.25 at 0.90 l/min nozzle discharge. Therefore, it can be concluded that the modified boom sprayer gives the desired spray deposition and highly efficient as compared to existing boom sprayer and its performance was found up to mark. The pressure and discharge of each individual nozzle was significantly increased and was able to maintain uniform pressure and discharge in each section of boom.

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Conflict of interest

The authors declare that they have no conflict of interest.

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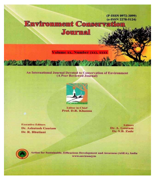

Association and path analysis studies for yield contributing and fibre quality traits in the F_1 population of *Gossypium hirsutum* L.

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ABSTRACT

The present investigation on correlation and path analysis studies helps to study the relationship among the yield and yield contributing traits along with the quality parameters. The observations were taken in the F_1 population of 35 hybrids along with lines (7 lines) and testers (5 testers) for the traits *viz.* plant height (cm), number of sympodial branches per plant, the number of bolls per plant, boll weight (g), lint index (g), single cotton yield per plant (g), ginning outturn (%), upper half mean length (cm), elongation percent (%) and micronaire value (µg per inch). The results revealed that seed cotton yield per plant had strong and positive association with the traits namely number of sympodial branches per plant (0.646), number of bolls (0.633) and boll weight (0.652). Path analysis study revealed that number of sympodial branches per plant (1.5396), boll weight (0.6285), lint index (1.3526) and upper half mean length (0.3392) had high direct positive effects on single plant yield and indirect very high positive effects through the traits *viz.*, number of sympodial branches per plant *via.*, number of bolls per plant (1.5125) and boll weight (1.0121). Hence selection based on these traits that are positively associated and direct positive effect with the yield would produce the best outcome in the genotypes in further breeding programmes.

Introduction

Cotton (*Gossypium hirsutum* L.), often known as "White Gold" belong to Malvaceae family which is mostly farmed in the tropics and subtropics as a fibre crop. Cotton enhancement programmes all around the world have always adapted to the demands of the market as suggested by Faylet *et al.* (2014). Farmers and industries worked together to achieve a high output while maintaining decent fibre quality. India is a country where a major goal is to create high-yielding cotton types with improved fibre quality. Many cotton improvement programmes have this as their goal. Among the species of cotton, *G. hirsutum* was estimated to account 90% of world commerce from its highly improved modern cultivars. The current study also aims to investigate the relationship between various yield and fibre quality factors in order to strengthen the cotton breeding research.

Correlation studies in plant breeding open the path for a deeper understanding of the relationship. Association studies between highly heritable traits and the most economic ones such as fibre quality provide superior results comprehending the role of

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each attribute in the development of the crop's genetic make-up. When selecting plants for breeding, there is an indirect association between the different characters under study. Path coefficient analysis is a useful tool for selection since it divides the correlation into segments of both direct and indirect components.

Material and Methods

The present study was carried out at Department of Cotton, Tamilnadu Agricultural University, Coimbatore - 03 during 2021. The F_1 hybrids were obtained by the crosses made between lines (BSH 18, MCU 5, CO 14, CO 17, TCH 1828, TCH 1199 and Suraj) and testers (KC2, KC 3, GTHV 15-32, GISV 323 and RHC 1409). Spacing of 120x 60 cm was followed and one plant per hill was maintained. All agronomic and cultural operations were completed on schedule. The data was collected in the F_1 hybrids for the following characters namely, days to first flowering (days), plant height (cm), number of monopodial branches per plant, number of sympodial branches per plant, the number of bolls per plant , boll weight (g), number of locules per boll, seed index (g), lint index (g), 100 seed weight (g), seed cotton yield per plant (g), ginning outturn (%), upper half mean length (mm), uniformity index (%), bundle strength (g/tex), elongation percent (%) and micronaire value (µg per inch). Correlation studies were done using the SPSS software 16.0 and the path analysis was carried out in the TNAU STAT developed by Manivannan (2014).

Results and Discussion

Correlation

Correlation helps to identify the linear association between different traits statistically hence the selection could be made effective through direct selection of the trait. Correlation identifies the relationship between yield and its attributing traits and paves way for indirect selection of best genotype. In this study, days to first flowering showed negative significant correlation with boll weight (-0.468) and seed cotton yield per plant (-0.436). The character, number of sympodial branches per plant (0.547) was found to be positively and highly significantly associated with the plant height as reported by Gauswami Jyoti *et al*. (2021), Attiq Ur Rehman Gohar, M. (2020) and Rai *et al*. (2020) . The trait plant height exhibited positive highly significant value with the traits *viz*, number of bolls per plant (0.541) and boll weight (0.628) as reported by Mudhalvan *et al.,*(2021) and number of locules per boll (0.550). The trait number of sympodial branches increases with the increase in height of the plant, thereby indirectly contributing to the yield of cotton. Hence farmers would fetch more income with the increase in number of bolls per plant and boll weight with the increase in number of sympodial branches. Number of monopodial branches per plant had negative and highly significant correlation with various traits *viz.,* boll weight (-0.325), ginning outturn (-0.398), upper half mean length (-0.427) and uniformity index (-0.462). The same trait contributed negative correlation with bundle strength (-0.297) and positive correlation with seed index (0.380). Number of sympodial branches per plant showed positive highly significant correlation with number of bolls per plant (0.982) as reported by Monisha *et al*., (2018) and Baloch *et al.* (2020). Number of bolls would channelize to the increased fibre yield of cotton hence favouring the textile industries with higher outcomes of yarn. Same trait also had positive significant correlation with the traits *viz.,* boll weight (0.657) and number of locules per bolls (0.526). The trait number of bolls per plant had positive and highly significant value with boll weight (0.670) and number of locules per boll (0.534). The boll weight per plant showed positive correlation value with the traits namely, number of locules per boll (0.683) and uniformity index (0.314). The trait number of locules per boll revealed positive correlation value for the traits *viz.,* upper half mean length (0.288), uniformity index (0.391) and seed yield of cotton (0.559). The trait seed index reported negative highly significant correlation with two traits, ginning outturn (-0.672) and elongation percent (-0.372). The same trait was found to have positive correlation value with lint index (0.448) and micronaire value (0.301). The traits ginning outturn (0.358) and upper half mean length (0.326) have positive significant correlation with lint index. The trait upper half mean length showed positive and significant correlation with bundle strength (0.456) and negative significant correlation with micronaire value (-0.370).The bundle strength exhibited highly significant positive

Table 1: Phenotypic correlation of 35 F_1 hybrids for yield , yield attributing and fibre quality characters in cotton (*Gossypium hirsutum* L.)

	DFF	PH	M	S	NB	BW	NLB	SI	LI	GOT	UHML	UI	STR	EL	MIC	Y
DFF	1															
PH	-0.068	1														
M	0.234	0.063	1													
S	-0.268	$.547^{**}$	-0.248	1												
NB	-0.26	$.541^{**}$	-0.202	$.982^{**}$	1											
BW	$-.468^{**}$	$.628^{**}$	$-.325^{*}$	$.657^{**}$	$.670^{**}$	1										
NLB	-0.274	$.550^{**}$	-0.151	$.526^{**}$	$.534^{**}$	$.683^{**}$	1									
SI	-0.11	$.318^{*}$	$.380^{**}$	0.032	0.025	0.039	0.136	1								
LI	-0.042	0.268	0.003	0.037	0.012	-0.013	0.012	$.448^{**}$	1							
GOT	0.071	-0.104	$-.398^{**}$	-0.004	-0.016	-0.046	-0.123	$-.672^{**}$	$.358^{*}$	1						
UHML	-0.229	-0.039	$-.427^{**}$	0.123	0.108	0.212	$.288^{*}$	-0.009	$.326^{*}$	0.28	1					
UI	-0.149	0.15	$-.462^{**}$	0.147	0.14	$.314^{*}$	$.391^{**}$	-0.104	-0.033	0.092	0.095	1				
STR	-0.131	-0.101	$-.297^{*}$	0.087	0.068	0.218	0.236	-0.134	-0.132	0.033	$.456^{**}$	0.242	1			
EL	-0.16	-0.133	-0.274	0.239	0.267	0.171	0.135	$-.372^{**}$	-0.139	0.283	0.234	0.237	$.444^{**}$	1		
MIC	-0.12	0.09	$.377^{**}$	-0.135	-0.1	-0.123	-0.179	$.301^{*}$	0.065	-0.261	$-.370^{**}$	-0.095	$-.434^{**}$	-0.242	1	
Y	$-.436^{**}$	0.214	-0.24	$.646^{**}$	$.633^{**}$	$.652^{**}$	$.559^{**}$	-0.014	-0.018	-0.007	$.414^{**}$	0.217	$.321^{*}$	$.402^{**}$	-0.048	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

DFF: Days to first flowering **M: Number of monopodial branches (nos.)** **PH: Plant height (cm)**

S: Number of sympodial branches (nos.) **GOT: Ginning outturn (%)** **SI: Seed index (g)**

LI: Lint Index (g) **EL: Elongation percent (%)** **NBPP: Number of bolls per plant (Nos.)**

NLB: Number of locules per boll (nos.) **Y: Seed Cotton yield per plant (g)** **BW: Boll Weight (g)**

UHML: Upper Half Mean Length (mm **MIC: Micronaire value (µg/inch)** **UI: Uniformity index (%)**

STR: Bundle strength (g/tex)

correlation with elongation percent (0.444) and negative correlation with micronaire value (-0.434). The seed cotton yield per plant showed positive significant values with the quality parameters *viz.,* upper half mean length (0.414), bundle strength (0.321) and elongation percent (0.402). The single plant yield showed highly positive significance for the trait number of sympodial branches per plant (0.646) as reported by Kakar *et al.* (2021) and Rehman *et al.* (2020). Number of bolls per plant (0.633), boll weight (0.652) as reported by Nikhil *et al*. (2018) and Baloch (2015) showed highly positive significance correlation for single plant yield. Similarly the same traits *viz.,* number of bolls per plant, boll weight and number of sympodial branches per plant exhibited a significant positive correlation with seed cotton yield per plant as reported by Mudasir *et al*. (2021), Hampannavar et al. (2020), Saritha *et al*. (2019) and Handi (2017). Hence it was evident that breeding of a particular trait would increase the related traits that contributes to the increased seed cotton yield per plant.

Path analysis

Path analysis was used to assess the partitioning of the correlation coefficient of different components with different yield and quality traits into direct and indirect effects. Trait analysis on a path which has a direct influence on single plant yield output may be deemed the best selection criteria for increasing the population's yield output.Direct effect refers to the influence of an independent character on the dependent character, whereas indirect effect refers to the effect of an independent character on the dependent character via other independent traits.

Direct effects

From the table 2, among the characters, number of sympodial branches per plant (1.5396) had very high direct positive effect on yield per plant followed by lint index (1.3526), this was in accordance with the findings of Monicashree *et al*. (2018). Similar result of high direct effect of number of sympodial branches per plant was reported by Arunkumar *et al.* (2020) and Manonmani *et al*. (2019). Direct effect relates to the trait that directly contributes to the dependent trait *i.e.,* yield of the crop. Hence by selection of higher number of sympodial branches per plant, seed cotton yield per plant could be maximized. High direct effect for seed cotton yield was also recorded through the traits namely boll weight (0.6285) and upper half mean length (0.3392). Increasing the boll weight and upper half mean length the increased seed cotton yield could be obtained.The traits *viz.,* number of monopodial branches per plant (0.2828), elongation percent (0.2096) and micronaire value (0.2835) exhibited moderate positive direct effect on yield. High negative direct effect on single plant yield was contributed by plant height (-0.5029) as reported by Arunkumar *et al.* (2020) and Jangid, K. (2019). Increased plant height do not favour to the yield of the crop, hence it had high negative direct effect. Hence selection should not be performed for higher plant height. The traits seed index (-1.7377), number of bolls per plant (-1.1177) and ginning outturn (-1.6504) revealed negative high direct effect with seed cotton yield per plant The remaining characters exhibited only low and negligible direct effects on the single plant yield. The path analysis of 35 F_1 hybrids has the residual effect of 0.4225.

Indirect effects

Positive very high indirect effect for number of sympodial branches per plant with single plant yield *via* the trait boll weight (1.0121) was high through the traits number of bolls per plant (1.5125) which was similar as reported by Satish *et al.* (2020). The same trait reported positive high indirect effect with single plant yield through the traits *viz.,* plant height (0.8424) as reported by Farooq *et al.* 2018, Gulhane and Wadikar *et al.* 2017 and further also with the traits, number of locules per boll (0.8099) and elongation percent (0.3686). High indirect positive effect suggested that selection made for the aforementioned traits would contribute indirectly to seed cotton yield through other traits. The number of bolls per plant exhibited negative very high indirect effect through the trait sympodial branches per plant (-1.0979) and high indirect negative effect through the trait boll weight (-0.7492) and number of locules per boll (-0.5963). Boll weight contributed high indirect positive effect linking through the traits *viz*., plant height (0.3946), number of sympodial branches per plant (0.4132), number of bolls per plant (0.4213) and number of locules per boll (0.4296). Very high positive indirect effect of seed index with single plant yield was high through the trait ginning out turn (1.1672) and high positive

Table 2: Phenotypic path analysis of 35 F_1 hybrids for yield, yield attributing and fibre quality characters in cotton (*Gossypium hirsutum* L.)

	DFF	PH	M	S	NB	BW	NLB	SI	LI	GOT	UHML	UI	STR	EL	MIC	Y
DFF	**0.0614**	0.0342	0.0662	-0.412	0.2909	-0.2941	-0.047	0.1917	-0.0568	-0.1178	-0.0775	-0.0091	0.0016	-0.0336	-0.034	-0.436
PH	-0.0042	**-0.5029**	0.0178	0.8424	-0.605	0.3946	0.0944	-0.5528	0.3629	0.1721	-0.0134	0.0092	0.0012	-0.0279	0.0256	0.2138
M	0.0144	-0.0316	**0.2828**	-0.382	0.2254	-0.2041	-0.026	-0.6604	0.0045	0.6573	-0.1448	-0.0284	0.0036	-0.0574	0.107	-0.2398
S	-0.0164	-0.2751	-0.0702	**1.5396**	-1.0979	0.4132	0.0903	-0.055	0.0497	0.0064	0.0416	0.009	-0.0011	0.0502	-0.0384	0.6459
NB	-0.016	-0.2722	-0.057	1.5125	**-1.1177**	0.4213	0.0915	-0.0438	0.0162	0.0261	0.0365	0.0086	-0.0008	0.056	-0.0283	0.633
BW	-0.0287	-0.3157	-0.0918	1.0121	-0.7492	**0.6285**	0.1173	-0.0685	-0.0173	0.0755	0.0721	0.0193	-0.0026	0.0358	-0.0348	0.6517
NLB	-0.0168	-0.2766	-0.0428	0.8099	-0.5963	0.4296	**0.1716**	-0.2356	0.0162	0.2035	0.0976	0.0241	-0.0029	0.0282	-0.0508	0.5588
SI	-0.0068	-0.16	0.1075	0.0488	-0.0282	0.0248	0.0233	**-1.7377**	0.6064	1.1086	-0.0029	-0.0064	0.0016	-0.0781	0.0854	-0.0137
LI	-0.0026	-0.1349	0.0009	0.0566	-0.0134	-0.008	0.0021	-0.7791	**1.3526**	-0.5916	0.1105	-0.002	0.0016	-0.0291	0.0185	-0.018
GOT	0.0044	0.0524	-0.1126	-0.006	0.0177	-0.0287	-0.0212	1.1672	0.4849	**-1.6504**	0.095	0.0056	-0.0004	0.0593	-0.0741	-0.0069
UHML	-0.014	0.0199	-0.1207	0.1888	-0.1204	0.1335	0.0494	0.0149	0.4408	-0.4622	**0.3392**	0.0059	-0.0055	0.0489	-0.1049	0.4136
UI	-0.0091	-0.0752	-0.1306	0.2264	-0.1566	0.1971	0.0672	0.1807	-0.0451	-0.1514	0.0323	**0.0614**	-0.0029	0.0497	-0.0268	0.2171
STR	-0.008	0.0509	-0.084	0.1341	-0.0765	0.1373	0.0405	0.2331	-0.1788	-0.0546	0.1547	0.0149	**-0.0121**	0.0931	-0.1231	0.3212
EL	-0.0099	0.067	-0.0775	0.3686	-0.2988	0.1073	0.0231	0.6472	-0.1879	-0.4669	0.0792	0.0146	-0.0054	**0.2096**	-0.0685	0.4019
MIC	-0.0074	-0.0454	0.1067	-0.2084	0.1115	-0.0772	-0.0307	-0.5233	0.0881	0.4314	-0.1255	-0.0058	0.0053	-0.0506	**0.2835**	-0.0479

Residual effect = 0.4225

DFF: Days to first flowering
M: Number of monopodial branches (nos.)
PH: Plant height (cm)
S: Number of sympodial branches (nos.)
GOT: Ginning outturn (%)
SI: Seed index (g)
LI: Lint Index (g)
EL: Elongation percent (%)
NBPP: Number of bolls per plant (Nos.)
NLB: Number of locules per boll (nos.)
Y: Seed Cotton yield per plant (g)
BW: Boll Weight (g)
UHML: Upper Half Mean Length (mm
MIC: Micronaire value (µg/inch)
UI: Uniformity index (%)
STR: Bundle strength (g/tex)

Indirect effect through the trait elongation percentage (0.6472). The same trait had negative high indirect effect via the traits *viz*., plant height (-0.5528), number of monopodial branches per plant (-0.6604), lint index (-0.7791) and micronaire value (-0.5233). Positive high indirect effect of lint index was contributed via the traits *viz*., plant height (0.0629), ginning outturn (0.4849) and upper half mean length (0.4408). Very high positive indirect effect of ginning outturn was high channelizing through the trait seed index (1.1086) and high positive indirect effect *via.,* number of monopodial branches per plant (0.6573) and micronaire value (0.4314) for the seed cotton yield of the plant. The same trait contributed negative high indirect effect to the traits namely, lint index (-0.5916), upper half mean length (-0.4622) and elongation percent (-0.4669). High indirect negative effect for seed cotton yield per plant was contributed by the trait boll weight through plant height (-0.3157). Moderate indirect negative effect for single plant yield was contributed through the trait plant height *via.,* the trait sympodial branches (-0.2751) and also for the traits *viz*., number of bolls per plant (-0.2722) and number of locules per boll (-0.2766).

Hence from the studies of correlation and path analysis, the most promising traits that enhances the yield could be identified and the selection criteria based on those traits would confer desirable genotypes. The traits namely, number of sympodial branches per plant, number of bolls per plant and boll weight had greatest impact on the yield of the cotton which had positive correlation with direct and indirect effects on increasing the yield of cotton. Hence, correlations and direct and indirect effects estimation would provide useful information for planning a successful breeding programme for simultaneously enhancing both yield and fibre quality traits through selection of these traits.

Conclusion

As a concluding remark, seed cotton yield per plant showed strong and positive association with the traits namely number of sympodial branches per plant, number of bolls per plant and boll weight. Path analysis study revealed that number of sympodia, lint index, boll weight and upperhalf mean length had direct high positive effects on single plant yield and indirect high positive effects through the traits *viz.,* plant height *via.,* number of sympodial branches per plant and boll weight) and number of sympodial branches per plant *via.,* the traits namely, number of bolls per plant, boll weight, number of locules per boll and elongation percent. High indirect positive effect on seed cotton yield per plant for number of bolls per plant was directed *via* the traits *viz.,* boll weight and number of locules per boll and also ginning outturn *via* seed index. Hence these characters can be accounted as the best criterion for selection of yield enhancing traits in further breeding programmes. As a result, the current study, which was based on correlation and path co-efficient analysis, confined that simultaneous selection based on important yield traits in terms of yield and quality attributes may be promising.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Effect of herbicides on plant growth and seed yield and quality of soybean (*Glycine max* L. Merr.)

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ABSTRACT

Weeds create a great problem for soybean production and to overcome this problem a large number of weedicides are being applied prevalently worldwide. Gradually increasing the use of chemical herbicides, not only creating resistance against these chemicals among the weeds and polluting the environment, but also influencing the main crop and deteriorating the produce quality. To focus this problem a two-year experiment was laid down during '*kharif*', at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, to evaluate the detrimental effect of herbicides on soybean plant growth and seed quality. The study come up with the results that the growth parameters during 60-90 DAS (days after sowing) *viz.,* crop growth rate (CGR 0.235 g/day), relative growth rate (RGR 0.014 g/ g/ day) and net assimilation rate (NAR 2.250 g/cm^2/day) decreased due to higher weed competition in weedy plot. Weed competition also significantly reduced yield parameters like number of branches (3.8) and pod per plant (25.4), seed per pod (2.4), seed yield (386 kg/ ha), seed index (11.8 %), and straw yield (1611 kg/ha). The highest seed yield (2665 kg/ha) was recorded with application of diclosulam (as pre-emergence) followed by one hand weeding, which was statistically equal with the treatment in which diclosulam followed by haloxyfop (as post-emergence) was applied. These herbicides also significantly alter the fatty acid composition of soybean seed oil.

Introduction

Soybean (*Glycine max* (L.) Merrill), an important source of protein (approximately 40-42 %) and oil (approximately 18-20 %), plays a great role in oilseed production and stands an important place in consuming as cooking oil, in the country. Meanwhile, weeds are the major limiting factor, creating hindrance in soybean production, causes around 35-40 % yield reduction (Oerke and Dehne, 2004). Sometimes these weeds causes losses up to the extent higher than other insect, pests and diseases, as competition among the plants and weeds for natural resources like soil nutrients, water, space, sunlight etc. Studies shows around 50-60 % reduction in yield due to weeds (Peer *et al.,* 2013). *Cyperus rotundus* (nut grass) and *Echinochloa colona* (jungle rice) among the monocots, while *Celosia argentea* (silver cock's comb or safed murg) among the dicot weeds are majorly reported for this crop (Singh *et al*., 2004). During the early stage of the crop this crop weed competition is not so much strong, so if weed growth is controlled or checked during this stage, the main crop suppress them afterwards and get benefitted (Halford *et al*., 2001). However selective chemicals (pre and post emergence herbicide) like fluchloralin, pendimethalin, alachlor, imazethapyr, quizalofop ethyl etc., are some recommended selective herbicides for controlling the weeds in soybean. The main advantage of using these herbicides for crop production is to reduce the crop weed competition during early stage of the plant growth and minimizing the seed yield loss (Knezevic *et al.,*

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2019) however sometime plant injury at early growth stage due to over dose of chemicals, is a great concern, which later reflected as poor plant growth resulting loss in seed yield and poor seed quality (Mahoney *et al*., 2014).

Therefore, at this stage it become necessary to investigate and explore the repercussion of herbicides on the soybean plant, applied to reduce the weed problems and enhance the plant growth and good quality of seed yield of the soybean. Therefore, an experiment was conducted to study the detrimental effect of herbicides and crop weed competition on plant growth, seed yield and quality of the soybean crop.

Material and Methods

The experiment was laid out at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The soil test of the experiment plots was done before the sowing of the crop and found the soil was neutral (pH=7.2) with silty clay loam texture and with following chemical properties:

Table 1: Initial soil chemical properties before seed sowing

Organic Carbon	0.54 %
Total Nitrogen	0.066 %
Available Phosphorus	24.18 kg/ha
Available Potassium	190.8 kg/ha

The experiment consisted ten treatments of different weedicides doses and their combinations with four replications, was laid out in randomized block design in field. The details of the treatments were as Control plot (weedy); weed free; fluchloralin (1000 g/ha); pendimethalin (1000 g/ha); diclosulam (26 g/ha); diclosulam (18 g/ha) followed by (fb) one hand weeding (20 DAS); fluchloralin (1000 g/ha) fb diclosulam (18 g/ha); haloxyfop (100 g/ha); fluchloralin (1000 g/ha) fb haloxyfop (100 g/ha); diclosulam (18 g/ha) fb haloxyfop (100 g/ha). The seed (variety PS-1347) sowing at the rate of 75 kg/ha was done at 45 cm of row spacing. The spraying of pre-emergence herbicides was done, 24 hr. after seed sowing however, fluchloralin was sprayed one day before of sowing as pre plant incorporated (PPI) herbicide. Haloxyfop as post emergence herbicide was sprayed in standing crop at 25 DAS. The chemical fertilizers were applied as per recommendation (25 kg N + 75 kg P +30 kg S + 10 kg Zn /ha) as basal dose. Plant population was maintained by thinning of the plants at 15-20 DAS. Trizophos 40 EC (insecticide) at the rate of 500 ml /ha in 700 litre of water was sprayed before flowering and pod formation, to protect the crop from insect.

Weed index (WI) was calculated as

$$\boldsymbol{WI = \frac{(A-B)}{A} X100}$$

Where, A and B indicates the seed yield in weed-free and other treated plot, respectively.

Plant growth performance were studied with following growth parameters:

$$\textbf{Crop growth rate } (\boldsymbol{CGR}) = \frac{w_2-w_1}{t_2-t_1}\ \textbf{(g/day)}$$

Where, W_1 & W_2 are plant dry weight at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.

$$\textbf{Relative growth rate } (\boldsymbol{RGR}) = \frac{\log_e w_2 - log_{3e}\, w_1}{t_2-t_1}\ \textbf{(g/ g /day)}$$

Where, W_1 & W_2 are plant dry weight at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.

$$\textbf{Net assimilation rate } (\boldsymbol{NAR}) = \frac{w_2-w_1}{t_2-t_1} \times \frac{log_e A_2 - log_e A_1}{A_2-A_1}\ \textbf{(g /cm}^2\textbf{/day)}$$

Where, W_1 & W_2 are plant dry weight at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.

A_1 & A_2 are Leaf area (cm^2/ plant) of plant at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.

$$\textbf{Leaf area ratio (LAR)} = \frac{(A_2-A_1)}{(w_2-w_1)} \times \frac{(log_e W_2 - log_e W_1)}{(log_e A_2 - log_e A_1)}\ \textbf{(cm}^2\textbf{ /g)}$$

Where, W_1 & W_2 are plant dry weight at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.
A_1 & A_2 are Leaf area (cm^2/ plant) of plant at interval of t_1 & t_2 days after sowing (DAS), respectively. T_1 =30 DAS; t_2 =60 DAS.

The data generated from the study were analyzed with average values of respective treatments and compared by Tukey test ($p < 0.05$), as per procedures of Pimentel-Gomes & Garcia (2002).

Results and Discussion

Weed population and weed index (WI)

The major weed species found in the experiment plot were *Echinochloa colona* (27%) and *Celosia argentia* (34%). The other weeds were *Brachiaria ramose, Digitaria sanguinalis, Eclipta alba, Eleusine indica* and *Trianthema monogyna*. However, the occurrence and intensity of these weed species was varied in different treatments, might be due to herbicide application and manual weeding plots. The highest (87%) for the weed index were obtained from control plot indicates the significantly higher weed population. However, the lowest value, indicating the lower weed population, recorded application of Diclosulam 18g/ha followed by one hand weeding (Table 2). It shows the effectiveness of diclosulam as pre emergence herbicide, to control the wide spectrum of weed flora including monocots and dicots as well (Golubev, 2021).

Growth parameters

During the experiment, higher values for growth parameters showing the better growth performance of the soybean crop as comparatively control plot. Less interference of weeds in treatments resulted increase in more leaf surface area and higher number of trifoliate per plant, which leads to comparatively more plant dry matter production. These findings were corroborated with Prajapati and Patel (2001). The higher plant growth in terms of CGR, RGR and NAR were also obtained due to increase in leaf size and dry matter per plant. Higher values for growth parameters *viz.*, CGR, RGR, NAR and LAR during 30-60 DAS were also observed as compared to 60-90 (table 2), might be due to more assimilation per unit area of optimum input resources at early stage of plant growth, which was decreased due to shading of leaves in later stage. Osipe *et al.* (2014) also evaluated diclosulam as pre-emergence herbicide to weed control in soybean and reported some phytotoxic effect of higher dose (>38g a.i./ha) of the chemical like leaf injuries and reduction and mortality in the plant stand. In general, studies indicate minimal effects on soybean agronomic performance with application of diclosulam (18 g a.i./ ha) (Braz *et al.,* 2017). Less competition of crop-weed during growth in weed control treatments leads to higher values of dry matter production and higher seed yield per plant (Clewis *et al.,* 2017).

Yield and yield attributes

Significantly higher values of the different yield parameters like branches and pods per plant and number of seed per pod were obtained weed free plot followed by the application diclosulam followed by with HW at 20 DAS (table 3) might be reduction in weed crop competition (Gazola *et al.,* 2016). It shows the effectiveness of the diclosulam to suppress the weed growth and proper utilization of natural resources by crop plants. Reduction in crop weed competition may provide the opportunity to more utilization of natural resources like air, water, sunlight etc., and resulting the increased synthesis and translocation of metabolites for the more pod development and grain formation in the same treatment (Grey & Prostko, 2015). This high amount of photosynthates mobilization from source to sink leads to significantly higher values for seed index (Singh *et al.* 2001). Significantly higher seed and straw yield was obtained from weed free plot which was 81 % more than weedy (control) plot. The seed yield obtained from weed free plot was at par with application of diclosulam followed by one hand weeding followed by application of diclosulam as pre emergence and haloxyfop as post emergence (Singh *et al.,* 2009). The lowest seed yield (454 kg/ha) and straw yield (1611 kg/ha) obtained from weedy plot showed the lowest harvest index (21%, might be due to the higher impact of weed infestation on the crop. According to Ehteshami (1998) results, seed yield dipped while dry weight of weeds went up. Tiwari and Kurchania (1990) also reported that weed infestation in soybean field may reduce yield up to 77 per cent depending upon the intensity, nature and the duration of weed competition.

Effect on seed quality

Significant higher values for seed protein and oil content obtained from herbicidal treated plots (table 4). Competition from ambient weeds reduced oil and protein concentration as weed free condition gave about 12 per cent higher oil content and 15 percent protein content, than control. Similar findings were reported with Abd El-Rhman (2004), Saudy and El-Metwally (2009) and Movahedpour *et al.,* (2011). Among the herbicide treated plots, significantly higher seed protein and oil content were reported from diclosulam treated plots.

Table 2: Effect of herbicides on CGR (g/day), RGR (g/ g/ day), NAR (g/ cm^2/day) and LAR (cm^2/g) at different growth stages of soybean (pooled mean of 2 years)

Treatments	CGR		RGR		NAR		LAR		Weed index
	30-60	60-90	30-60	60-90	30-60	60-90	30-60	60-90	
Control	0.295	0.235	0.026	0.014	2.900	2.250	100.8	61.8	87
Weed free	0.445	0.460	0.028	0.015	4.050	3.700	78.3	41.5	0
Fluchloralin	0.310	0.300	0.026	0.015	3.350	3.200	79.0	44.8	69
Pendimethalin	0.370	0.350	0.027	0.014	3.500	3.150	82.1	46.6	55
Diclosulam	0.400	0.385	0.027	0.014	3.600	3.500	79.7	45.3	23
Diclosulam + HW	0.415	0.415	0.027	0.015	3.800	3.650	80.2	43.9	14
Fluchloralin + Diclosulam	0.370	0.375	0.027	0.015	3.650	3.400	72.4	42.7	31
Haloxyfop	0.340	0.295	0.026	0.014	3.400	3.050	79.4	47.3	64
Fluchloralin + Haloxyfop	0.325	0.285	0.027	0.014	3.350	2.650	88.4	53.8	58
Diclosulam + Haloxyfop	0.380	0.370	0.026	0.014	3.800	3.550	78.1	44.9	20
C.D. at 5%	0.050	0.060	NS	0.001	NS	0.600	8.5	9.1	-

Table 3: **Effect of different weed control treatments on oil and protein content and fatty acid composition (%) of soybean (pooled mean of 2 years)**

Treatments	Oil content	Protein content	Fatty acid composition			
			Saturated fatty acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
Control	17.8	35.2	10.13	15.07	63.69	10.09
Weed free	19.9	40.5	12.11	16.87	64.10	8.02
Fluchloralin	18.7	37.7	10.97	17.16	62.68	10.47
Pendimethalin	18.3	38.6	11.02	17.33	62.08	10.31
Diclosulam	18.4	38.4	10.79	18.33	62.31	10.00
Diclosulam + HW	19.2	38.5	10.23	16.56	61.89	11.59
Fluchloralin + Diclosulam	19.1	39.2	11.28	19.71	60.28	9.34
Haloxyfop	19.0	37.8	11.16	13.80	66.41	8.82
Fluchloralin + Haloxyfop	19.3	38.4	11.67	18.61	61.17	9.53
Diclosulam + Haloxyfop	19.8	38.0	11.17	16.74	63.03	9.09
C.D. at 5%	0.9	1.3	0.31	1.75	2.04	1.24

Table 4: Effect of herbicides and crop weed competition on yield and yield attributes of soybean (pooled mean of 2 years)

Treatments	Branches/ plant	Pods/ plant	Grain/ pod	Seed index (%)	Seed yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)
Control	3.8	25.4	2.4	11.8	386	1611	21
Weed free	5.9	60.6	2.8	12.4	2853	4499	39
Fluchloralin	5.1	40.4	2.5	11.9	886	1641	37
Pendimethalin	4.5	41.4	2.5	11.0	1235	2638	32
Diclosulam	5.5	48.6	2.6	12.0	2172	3637	38
Diclosulam + HW	5.4	52.6	2.7	12.4	2665	4226	38
Fluchloralin + Diclosulam	4.9	39.7	2.5	12.3	1963	3257	38
Haloxyfop	3.5	33.5	2.4	11.6	1042	1661	39
Fluchloralin + Haloxyfop	4.0	36.6	2.3	12.0	1209	1786	42
Diclosulam + Haloxyfop	5.1	53.4	2.7	12.4	2303	4129	36
C.D. at 5%	1.5	14.8	0.4	0.8	453	1158	11

Better weed control measures reduce the weed interference for soybean crop growth and improved the seed quality in terms of seed protein might be due to higher number of nodules and their dry weight leading to more nitrogen fixation and assimilation and its translocation to seed. Porwar *et al*. (1999) also reported higher oil and protein content in seed by using weed control treatments as compare to weedy. Herbicidal treatments also affect the saturated fatty acid composition of soybean seed oil, ranged from 10 to 12 %. The higher values of total fatty acid were reported by weed free plot. The lower values of total saturated fatty acid in some herbicidal treatments, might be due to altering or reducing the rate of lipid metabolism by these chemicals. Farag *et al*. (2006) also reported that fatty acid composition of soybean oil was altered by herbicides application. However, diclosulam treated treatments showed not much phytotoxic symptoms to soybean plant and alteration in fatty acid composition (Golubev, 2021).

Conclusion

Significantly higher quality of seed yield obtained by the spraying of diclosulam followed by hand weeding showed the effectiveness of the weedicide to suppress all type of weeds when applied as pre emergence. Thus application of diclosulam at the rate 18g/ha followed by one manual weeding at 20-25 DAS may be recommended in soybean to minimize the weed load and provide higher crop yield in sustainable manner as well as up to some extent eco-friendly in nature.

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Conflict of interest

The authors declare that they have no conflict of interest.

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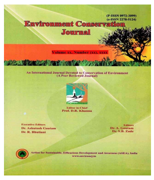

Revitalization of Rawa Pening lake, Indonesia

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ABSTRACT

Rawa Pening Lake has been in critical condition, caused by high sedimentation levels, declining water quality, damage to the watershed, and excessive riparian occupation. Therefore, this study aims to identify the main problems of the revitalization of Rawa Pening lake. Data and information collection were carried out through direct observation and interviews. The results showed that the main problems of the lake were the removal of the water weeds/water hyacinths, tidal rice fields, and buildings on the lake and riparian areas. The solution to the three main problems is lake revitalization. The revitalization concept that can be proposed regarding the control of water hyacinth is by mechanical means (using water hyacinth lifting equipment, boats, and cutting water hyacinth) and by biological methods, namely the cultivation of koan fish that will eat water hyacinth. At the tidal rice fields, it is necessary to determine the water level limit wisely. The areas with elevation of 462.30 m3-463.25 m3 (covering an area of 812 Ha) can be planted twice a year. The areas with a height of 462.05 m3-462.30 m3 (covering an area of 200 Ha) can be planted once in the rainy season. The areas with elevation of 462.05 m3 cannot be planted because it is in a state of flooding. Incommensurate areas and lakes, there is a lot of land and buildings consisting of settlements, tourism destinations, places of worship, and restaurants. Then the proposed alternative solution is the land acquisition compensation of land and buildings in commensurate areas.

Introduction

Indonesia has many lakes which are naturally and artificially formed. The exact number of lakes and their areas has not been known yet, but it is estimated that there are more than 1,575 lakes, consisting of 840 large and 735 small lakes (Kementerian Negara Lingkungan Hidup, 2007). In general, most lakes are facing environmental problems such as eutrophication, sedimentation (Soeprobowati, 2015; Sulastri *et al.,* 2016), the decline in surface area, degradation problems caused by land conversion in the watershed, high soil erosion, and water pollution from the agriculture and households (Trisakti *et al.,* 2014). Revitalization efforts on lake management were agreed during the Indonesian Lake National Conference held in Bali in 2009 which stipulated that 15 lakes e to be saved from damage, one of which was Rawa Pening Lake in Semarang Regency, Central Java. The current problems of Rawa Pening Lake are eutrophication and sedimentation (Soeprobowati, 2012). Eutrophication is a process in which human activities contribute to the increasing amount of nutrition in the water ecosystem and result in undesirable changes in the quality of the surface water which is influenced by biogeochemical carbon, nitrogen, and phosphorus caused by agricultural fertilizers, fisheries, and household waste (Ekdahl *et al.,* 2004; Scholten *et al.,* 2005; Ayele and Atlabachew, 2021). Meanwhile, sedimentation is mainly caused by land erosion and the decomposition of water hyacinth (Sanjoto *et al.*, 2020). In 1991 sedimentation caused by land erosion reached 4,084,484.59 tons, in 2011 the sedimentation rate of the lake decreased to 3,688,480.45 tons due to the decrease in rainfall, and the sedimentation rate of water hyacinth 2011 to continuously increased from 153,745.30 tons in

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171,349.90 tons in 2011 (Apriliyana, 2015).
Rawa Pening Lake has many strategic functions including economic functions as a power plant, freshwater fishery, irrigation, source of drinking water, and tourist attraction air (Budihardjo & Huboyo, 2007; Dwisapta & Sri, 2013; Samudra *et al.,* 2013; Schmieder, 2004). As for socio-cultural function, the lake usually became a source of inspiration for the birth of cultural and religious traditions of the people around the lake. Meanwhile, to support ecosystem functions, the lake supports the availability of biodiversity, the sources and places of protein formation, toxicity control in water bodies, dampening flood fluctuations in rivers, and microclimate control (Dewan Sumber Daya Air Nasional, 2020). In addition, the agricultural sector increases its production because of this lake. In Semarang Regency, the agricultural sector played the most significant role in supporting the economy, used as rice fields, farms, embankments, ponds/dams, plantations, and forests. Meanwhile, the rest are used as yards and buildings, grassland, swamps, and vacant land for business.
Saving the lake from damage has become a national issue. Various approaches to the study related to Rawa Pening lake have been carried out. Sulastri et al. (2016) mention that Rawa Pening lake is a eutrophic lake, where phosphorus plays a role in causing eutrophication and the growth of more water hyacinth. Hidayati et al. (2018) show that the presence of water hyacinth has been a concern for many years. Water hyacinth control failed and even tended to increase during the period 2012 to 2016. Then Sittadewi (2008) examined tidal land and stated that the land formed due to processes of the rising and falling water levels of Rawa Pening lake occur the fluctuations between the dry season and the rainy season. The use of tidal land is for rice fields. Some of the studies above present interesting issues to look at the problem of Lake Rawa Pening. However, this research is different from previous research. In general, it discusses the problem of water hyacinth and tidal land, but the substance and data presented are different. No research specifically examines the revitalization of Rawa Pening lake. This study aims to analyze the main problems of Rawa Pening which is currently experiencing damage. Efforts are needed to save the lake from damage and maintain the function of the lake based on the principle of ecosystem balance and supporting the carrying capacity of the environment.

Material and Methods

This study was conducted in the area around Lake Rawa Pening, located in four sub-districts, namely the sub-districts of Bawen, Ambarawa, Tuntang, and Banyubiru, Semarang Regency, Central Java province. Geographically, it is located at coordinates 7° 4' 00" South Latitude – 7° 30' 00" and 110° 24' 46" East Longitude – 110° 49' 06" East Longitude and is at an altitude between 455-465 meters above sea level (dpl). The location of this lake is strategic because it is on the edge of the Semarang-Solo and Semarang-Yogyakarta National highways, and is on the road between Ambarawa-Salatiga City (Kementerian Lingkungan Hidup, 2011). Data were obtained during pre-research and research implementation stages. The pre-research was conducted in April 2022, in the pre-research or preliminary study aimed at mapping concepts and mapping problems. Then the research will be carried out starting from May 23 to May 31, 2022, although the research was conducted for nine days, this research does not reduce the quality of research data and information because in the field it only takes field data, because previously there was a preliminary/pre-research study. The research method used a combination of interviews and direct observation of the subjects and objects of research. Interviews were conducted with in-depth interviews with a face-to-face interview process. Field observations and interviews with lake managers, the National Land Agency, village heads, and local communities to obtain data related to the revitalization of Rawa Pening lake are presented descriptively.

Results and Discussion

Water weeds removal

Water hyacinth is a dangerous weed due to its rapid spread and dense growth (Malik, 2007; Safauldeen *et al.,* 2019), consuming nutrients and oxygen from the water bodies that affect other plants and animals' growth (Sindhu *et al.,* 2017), which is influenced by temperature, wind water nutrients, water currents, waves, and seasons (Higgins & Richardson, 1996; Wilson *et al.,* 2005). Many efforts have been made to eliminate these weeds either by chemical or biological methods, but the maximum result has not been achieved. On the other hand, water hyacinth has the potential to be used as phytoremediation, paper, organic fertilizer, biogas production, human food, fiber, livestock feed, as an ornamental plant and even its biomass has the potential to be a source of renewable energy (Jafari, 2010; Rezania *et al.,* 2015).

The same thing happened to Rawa Pening Lake with the hyacinth growth rate ranging from 6.40-7.26%/day (Prasetyo *et al.,* 2022), which can trigger the lake siltation because the root system of hyacinth allows high sediment trapping. The measurements of sedimentation and water quality of Rawa Pening Lake conducted in 2015 revealed that the lake underwent shrinking and siltation which resulted in the effective area of the lake becoming 1,850 ha with a reservoir volume of 48.15 million m3 (elevation +463). The surface of the lake was covered by water hyacinth with an elevation of +463m ± 755 ha or about 47.7% of the total surface area, while the sedimentation rate was 1.77mm/ha/year (Balai Besar Wilayah Sungai Pemali Juana, 2015). Water hyacinth removal was necessarily needed because its rapid growth produced a lot of unutilized water hyacinth. In 2015, the water hyacinth area was ± 755 ha or about 47.7%, in 2017 the water hyacinth area reduced to 400 ha or about 25.27% and in 2018 it reduced again to 300 ha or about 20% (Balai Besar Wilayah Sungai Pemali Juana, 2018). In 2020, 550 ha area of water hyacinth was removed (Balai Besar Wilayah Sungai Pemali Juana, 2020). Receding water was the obstacle to the water hyacinth removal process since the equipment could not be operated at the disposal site, while the nearest disposal site is in the Bukit Cinta area. However, another disposal is still required due to the rapid growth of water hyacinth.

Various lake cleaning efforts have been initiated from dredging lakes and construction of check dams, controlling water weed by periodically removing the water hyacinth, herbicides spraying, drifting water weed through Tuntang River, relocating water weed to the temporary disposal which later thrown to landfills, and many other methods. Nevertheless, all these efforts did not show significant results. There is a more effective and efficient method that saves energy, costs, and time does not cause traffic disruption, road damage, environmental pollution, and other impacts that can trigger social conflicts, that is, the creation of embankments delimiting the lake area with citizens' land and the creation of Rawa Pening wildlife conservation island (Interview with the Head of Kesonggo Village, 2022).

Water hyacinth is considered a nuisance weed, so various ways (mechanical and biological) are carried out to overcome it. They are: a) Herbicides. b) Lifting water hyacinth directly from the aquatic environment. c) Predators (animals that eat water hyacinths). The predator is the koan fish. Koan fish disturb the balance of weeds on the water surface. So, their leaves touch the surface of the water. The decomposition occurs and is then eaten by fish. d) Utilizes water hyacinth as a material for making paper, compost, biogas, furniture, and handicrafts, as a growth medium for edible mushrooms.

Tidal rice field ownership

The tidal area was formed due to the tide of the water surface (Sittadewi, 2008), and becomes a part of the wetland ecosystem (Noor, 2015). The tidal area in Rawa Pening Lake is used by the surrounding community for rice farming activities with an area of about 1,030.51 ha. The use of tidal land as rice farms improved the economy of the community, but it might result in environmental degradation that will threaten the sustainability of the lake ecosystem as a whole such as flooding, narrowing the area of the lake, the leakage of nutrients into water caused by agricultural activities, hastening the eutrophication of the lake, and increasing the acidity level of the land due to the decomposing organic materials and various chemicals used in agricultural activities (Cahyaningrum, 2020; Nugroho *et al.,* 2014). Many rice fields were found in Banyubiru Village with the area of 186.90 ha, Kebumen Village 185.13 ha, Pojoksari Village 146.98 ha, Tambakboyo Village 119.49 ha, Asinan Village 93.00 ha, and Candirejo Village 149.771 ha (Balitbang Provinsi Jawa Tengah, 2003). This tidal area is closely related to the determination of elevation. Based on the Decree of the Minister of Public Works and Public Housing Number 365/2020, the elevation limit of 462.7 m is higher than the previously set limit. As a consequence of the regulation, the community could not plant rice for two years because the tidal rice fields managed by the community were waterlogged, such as in Bejalen Village where the puddle covers 700 meters of the land, whereas in Asinan Village it covered 1 kilometer of the land. Farmers have lost their livelihoods and things are getting more difficult with the COVID-19 pandemic (Interview with the residents of Bejalan Village, 2022).Regarding the elevation limit, the community has demanded it to be lowered since 2020, and it was approved that the elevation limit is lowered to 461.30 m. The lowered elevation enables the community to replant whimsical land at least once a year. Siswanto (2022), sluicegate keeper of Jelok Dam, said that for tidal rice field farmers, the lower the water level, the more the farmers will be benefited because they can harvest twice a year. However, if the elevation is raised, it will have the potential for water to flood the tidal rice fields so that the community will experience loss due to harvest failure or because the tidal rice fields cannot be replanted. On the other hand, lower elevation of the water discharge in Rawa Pening Lake might result in the obstruction of the Hydroelectric Power Plants (PLTA) operation. The decrease in elevation is carried out gradually by the targets that the community wants to achieve. In fact, the community participated in monitoring the entrance of the Jelok Dam so that they could directly see and supervise the activity through a schedule of farmer pickets made by themselves**.** However, the elevation might be changed at any time during the rainy season. During the decrease in elevation, the condition of the residents' lands that were flooded gradually receded. There were even those that were not flooded anymore. The following are the data from the community related to the current condition of citizen lands at elevation 461.32 as of June 3, 2022 (Table 1).

The demand of the community to meet the needs of tidal rice fields affected: a) the non-fulfillment of water needs in rice fields covering an area of 20,067 ha, which is downstream; b) the harvest from rice fields covering an area of 20,067 ha was not optimal to support rice self-sufficiency in Central Java Province; c) users who utilize water from Rawa Pening Lake, such as Hydroelectric Power Plants, Local Water Supply Utility, do not meet their needs; d) increasing water deficit; e) BERKY harvester equipment cannot work optimally because it requires elevation above +461.90 m in order to work properly (Balai Besar Wilayah Sungai Pemali Juana, 2021). The changing of water elevation level must be carried out wisely because it affects the area around the lake as well as the output of water used as an irrigation source for 2,000 acres of rice fields in the Grobogan and Demak Regencies. Especially during the rainy season, water discharges clearly increase so that the lands around the lake that are originally dry will potentially be flooded. In this condition, the community wanted the water gate to be opened immediately so that the water immediately recedes to prevent flood in the downstream area, considering the amount of water needed by the Hydropower Plant to optimally supply the electricity. The decrease in water surface elevation has been tested by BBWS (2021) at a

Table 1: **Plot at elevation 461.32**

SN.	Village/Ward	Description
1.	Bejalen Village	There are still waterlogged
2.	Asinan Village	There are still waterlogged
3.	Tuntang Village	No waterlog found
4.	Tambakoyo Ward	No waterlog found
5.	Banyubiru Village	There are still waterlogged
6.	Rowoboni Village	No waterlog found
7.	Rowosari Village	No waterlog found
8.	Sraten Village	There are still waterlogged
9.	Pojoksari Ward	There are still waterlogged
10.	Candirejo Village	No waterlog found
11.	Kebumen Village	No waterlog found
12.	Lopait Village	No waterlog found
13.	Kesongo Village	No waterlog found
14.	Kebondowo Village	There are still waterlogged

minimum elevation of +461.90 m and minimum elevation of +461.65 m, as follows (Table 2).

Land and building ownership of the Lake and Riparian areas

The effective area of Rawa Pening Lake has shrunk by 820 ha (about 30%) from the initial area of 2,670 ha to 1,850.10 ha (Balai Besar Wilayah Sungai Pemali Juana, 2018). Based on the Decree of the Minister of Public Works and Public Housing Number 365 of 2020 concerning the Determination of Rawa Pening Lake Riparian Borders, the area of this lake is 2507 ha with a water body area of 2387 ha and the riparian area of 120 ha. The riparian area of the lake is 50 meters from the edge of the highest water table ever with a height of 463.3 m. In the lake and riparian areas, there are 251 buildings, consisting of settlements, tourism spots, places of worship and restaurants. The total area of the entire building located at 50 m from elevation 463.3 is 6.19 ha. The number of rice fields that enter the riparian area of the lake is 65.6 ha in all villages around Rawa Pening Lake. The total length of the road entering the riparian area is 2,519 meters. The number of rice fields in the water body is 386.82 ha. The number of Floating Net Cages is 3223 buah (Balai Besar Wilayah Sungai Pemali Juana, 2020).

Table 2: Alternative lowering the elevation of water level of Jelok Dam

Alternative	**Alternative I Min. Elevation +461.90 m**	**Alternative II Min. Elevation +461.65 m**
Volume	The elevation of the Jelok Dam based on the Reservoir Annual Operation Plan September II is +462.11m so the water to be released is ± 3.78 million m3	The elevation of the Jelok Dam based on the Reservoir Annual Operation Plan September II is +462.11 m so the water to be released is ± 8.28 million m3
Fieldwork on the revitalization project of Rawa Pening Lake	The tools were working but it was not effective	Currently, most of the water hyacinth has been brought to the lakeside which caused the tool cannot be operated.
Hydroelectric Power Plant	Electricity production decreased to 7.5 MW (50% of 15 MW)	Electricity production decreased to 4.7 MW (31% of 15 MW)
Raw Water	It can still be served entirely	It can still be served entirely

Table 3: Inventory of certified land parcels within the lake and riparian areas.

SN	**Village/Ward**	**District**	**Number of Plots**	**Total Area Per Plot (M2)**
1	Asinan Village	Ambarawa	175	310.600
2	Bejalen Village		121	221,418
3	Kupang Ward		46	94.084
4	Candirejo Village		191	411.872
5	Kebondowo Village	Banyubiru	28	105.225
6	Banyubiru Village		71	205.877
7	Rowoboni Village		0	0
8	Lopait Village	Tuntang	34	61.928
9	Rowoboni Village		130	229.572
10	Rowosari Village		152	283.499
11	Tambakboyo Ward		10	113.409
12	Tuntang Village		424	397.778
Total Number			1382	2435262

The riparian area can only be used for: a) water resources infrastructure buildings; b) road access, bridges, and docks; c) gas and drinking water pipelines; d) electricity and telecommunication cable stretches; e) tourism, sports, religious, research, and development science infrastructure; f) sanitation infrastructure and facilities, and g) electricity development. Buildings entering the riparian area are declared as a status quo, meaning that it is not allowed to change, add, and improve the area because they will gradually be ordered to restore the function of the riparian area. Practically, since 1967 there have been buildings and ownership rights to land in the form of property rights that are on the lake boundary. The forms of land ownership around Rawa Pening Lake are as follows: first, the Letter C document, is a land register book that exists in the village for generations and is stored in the respective village office. Meanwhile, residents who own the lands are only given the excerpts of Letter C. Before the land registration activity, Letter C was a basis for the withdrawal of land tax and strong evidence that explained that the land was a customary property right and the name listed in Letter C was the taxpayer so that the registration was through conversion. Letter C is the basis for the issuance of land title certificates (Figure 1).

Yellow peg in front of the community houses | Yellow peg on community rice fields | Yellow peg on the community land

Figure 1: Yellow pegs on the riparian area

Second. Village Treasury Land, is land controlled or owned by the village government as one of the original sources of village income for social benefit. Provision of Village Treasury Land: a) it is used for the sake of village development and community service; b) position land which is the provision to village officials as additional income for their service while serving as village officials; c) as a tribute to officials who have completed their duties; b) lands that have general functions and social functions such as fields, cemeteries, village halls, and so on. Kesongo Village located in Tuntang District is one that has village treasury land that is used for public interest to increase the village's original income and carry out social functions. Village treasury land is used for rice fields that are managed by the community, parking lots by fishermen and fish cage owners, and culinary tourism (Figure 2).

Daringan Culinary Park, Kesongo Village | Blue peg on the community house | Blue peg on resident's rice field

Figure 2: Blue pegs on the boundary of the lake area.

Yellow and blue pegs mark the corresponding lake and riparian areas which aim to protect the lake area and restore the lake's function as it supposed to be. However, in its implementation, it causes anxiety in the community because the pegs that have been installed are in buildings and land owned by the community that has been certified and is still in the form of letter C, which has the potential to cause conflicts between citizens and state officials. On the other hand, it has an economic impact in the form of decreasing income and shifting livelihoods because people lose land rights. The following is an inventory of certified land parcels downloaded from the Semarang Regency Land Office in 2022 (Table 3). Land ownership status within the lake or riparian areas is as follows: 1) If the lake boundary land is used for city facilities, buildings, roads, or public facilities, it will remain unaltered, maintained as long as no reason is found that is more important than its current benefits; 2) If the lake boundary land is owned by the community, its original purpose must be returned as a riparian, as long as the ownership rights to the land are validly recognized, but the landowner must comply with the designation of the land as a riparian area; 3) Buildings that have already been established on the riparian area are declared in the status quo, i.e., cannot be changed, added, and repaired and new construction permits will no longer be issued; 4) Community land acquisition with a comprehensive land procurement mechanism both in lake bodies and riparian areas

Conclusion

The revitalization of the Rawa Pening Lake was carried out by *first,* removal of the water weeds/water hyacinth that caused the silting. Once the water hyacinth is removed, it sinks and dispositions. This deposition will cause sedimentation and a decrease in the capacity of the water reservoir. In 2020, 550 ha area of water hyacinth has been removed. The cleaning of water hyacinth is difficult because of its rapid growth. Various efforts have been made such as dredging the lake, building dam checks, cleaning by spraying with herbicides, drifting weeds through the Tuntang River, and pulling and holding weeds to final disposal, but the results are not significant. *Second,* tidal rice fields are closely related to elevation. The determination of the elevation limit by the government is 462.7 m, causing tidal rice fields cannot be planted by the community, then on the demand of the community, the elevation is lowered to 461.30 m, but the decrease in elevation hinders the operation of the Hydroelectric Power Plant. *Third,* ownership of land and buildings in the lake and riparian areas. Ownership of land and buildings can only be done for land acquisition (compensation) to the land owner. The revitalization of the riparian areas was carried out at the highest elevation of the lake water body of 463.3 m which was then pulled out along 50 m. This riparian area will later be made an embankment that can function as a jogging track and also a public space.

Conflict of interest

The authors declare that they have no conflict of interest.

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Impact of long-term fertilization on soil organic carbon dynamics and biological health of soils under Pearl millet -mustard-cowpea cropping sequence in an inceptisol of Gujarat

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ABSTRACT

The study was undertaken by utilizing an ongoing long-term experiment on continuous cropping at Anand Agricultural University that began in 1980. From 1994 onwards, a modification was made by including Farmyard Manure (FYM) treatments for studying the following objectives: long-term effect of fertility levels with and without FYM on changes in soil organic carbon pools for assessing the role of organics and chemical fertilizers on soil organic carbon buildup and their interrelationship with soil aggregate stability under the pearl millet-mustard-cowpea (F) cropping sequence. Under F1 (FYM @ 10 t/ha) and FL3 (NP application @150 percent of RDF), there was a considerable improvement in the status of Walkley and Black C (WBC), Soil Microbial Biomass Carbon (SMBC), and Total Organic Carbon (TOC) compared to the control in both depths (0-15 and 15-30 cm). Long-term manuring and fertilization practices affect aggregate development and stabilization. In all depths, the highest soil macroaggregates and microaggregates were found when FYM @ 10 t/ha and FL3 (150 percent NP) were applied. Under FYM treated plots and with the greater dose of NP (NP application @150 percent of RDF) in both the surface and sub-surface layers, the maximum water-stable aggregate expressed as mean weight diameter (MWD) was recorded. Furthermore, a significantly positive correlation was observed between SMBC and enzymatic activities (phosphatase, urease, and dehydrogenase) in both the soil depths; indicating the effect of labile C on the biological activities of soil which might be achieved by means of changes in microbial diversity of the soil.

Introduction

In the post-green revolution era, the overexploitation of natural resources to feed an exponentially rising population has posed a serious challenge to Indian agriculture systems. Some soils have lost up to 80 tonnes of carbon per hectare around the planet, with most of it being exhaled into the atmosphere (Lal, 2004). The influence of C transfer from soil to the atmosphere is important not just for the global C cycle, but also for soil's ability to produce food, fiber, fuel, and construction materials, and it is a cause of concern for future productivity. Soils contain approximately twice as much carbon (C) as the atmosphere (800 Pg C) and three times as much C as above-ground and terrestrial vegetation (500 Pg C) (Anonymous, 2015). The dynamics of the soil carbon pool affect carbon dioxide concentrations in the atmosphere, causing global climate changes (Lal, 2011). Declining soil organic C is a major issue of concern due to its vital role in maintaining beneficial physical properties of soil, such as soil porosity, aggregation, and bulk density, as well as soil water storage (Benbi *et al.,* 1998; Chenu *et al.,* 2000).

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Organic C also affects soil chemical properties such as availability and mobilization of nutrients, cation exchange, and buffering capacity along with maintaining biological activities of soil. In order to comprehend the mechanisms driving C stabilization, SOC is divided into labile and recalcitrant C pools. The labile pool is the one that fluctuates most quickly. Very labile carbon, hot water-soluble carbon, particulate organic matter-carbon (POM-C), and microbial biomass carbon are just a few examples of the several types of labile carbon that can be found in soils. Labile C is a reliable measure of variations in SOC concentration as this pool is subject to rapid alteration in response to changes in soil management (Bhattacharyya *et al.,* 2011, Dutta *et al.,* 2018). As a result, labile C may be used as an easy yet useful indicator to understand the connection between various soil management options and SOC development under a particular management strategy. The most resilient carbon pools are passive ones, which are made up of extremely stable material that has been present in the soil for hundreds or even thousands of years. Most of the humus that is physically protected in clay-humus complexes falls under this category which accounts for 60-90% of the organic matter. The colloidal characteristics of soil humus, which account for the majority of the cations and water-holding capacities added to the soil by organic matter, are most closely associated with the passive pool. For assessment of soil physical quality, the distribution, as well as stability of soil aggregates, are considered essential, emphasizing the importance of soil management on particle aggregation and disaggregation (Silva *et al.,* 2014). The aggregate stability is generally strongly correlated with SOC content by playing a vital role as the organic polymer binding agents (Haynes & Swift, 1990; Majumder *et al*., 2010) and the physical trapping of particles by fine roots and fungal hyphae (Helfrich *et al.,*2015) which promote aggregate cohesion. Additionally, fertilization and irrigation practices that boost crop productivity, biomass, and root production may indirectly enhance the system's carbon inputs and contributes to the aggregate formation (Yadav *et al*., 2017).

In addition to fertilizer application, one of the most significant factors affecting SOC content in soil is the cropping system (Bhutiani and Ahamad 2019; Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022). Continuous cereal farming could affect productivity, carbon pools, and soil health. Adding organics and legumes to cropping systems enhances soil aggregation and boosts C and N sequestration, according to research (Bandyopadhyay, *et al.,* 2010). Bajra, or pearl millet, and mustard are important food grain crops in India, and they are primarily grown in semi-arid tropic regions with sandy loam soils. Pearl millet is grown on 7.12 million hectares and produces 8.06 million tonnes of grain with the country's productivity of 1132 kg/ha. In Gujarat, the total planted area is 4.31 lakh acres with a total productivity of 9.31 lakh hectares tonnes, with a yield of 2158 kg/ha (Anonymous, 2018). In India's tropical and subtropical climates, cowpea is a common green fodder legume. However, due to increased soil temperature and increased soil erosion issues, respectively, the SOC pools are lower in the tropical and sub-temperate regions of India compared to temperate soils worldwide (Kumar *et al*., 2013; Nianpeng *et al*., 2013; Dixit *et al.,* 2019). Several research highlighted that nutrient management strategies have the ability to alter the C storage capacity of agricultural soils depending on climate, soil conditions, and cropping systems (Bhattacharyya *et al*., 2011; Dutta *et al.,* 2018; Ghosh *et al.,* 2019; Dixit *et al*., 2019). Therefore, it is necessary to determine and implement the optimal management strategies to maintain or raise SOC levels, especially in those areas where production systems are naturally poor in soil fertility (Manna *et al*., 2013; Dixit *et al*. 2019).

Long-term fertilizer experiments (LTFEs) are essential for evaluating how chemical fertilizers and organic additives affect the soil carbon buildup by the transformation of SOC into different pools, which is important for quantifying soil quality and system sustainability. Keeping all of the aforementioned, the current study was designed to investigate the effect of continuous organic and inorganic fertilizer applications on changes in soil organic carbon fractions, aggregate stability, and enzymatic activities during the pearl millet-mustard-cowpea (F) cropping sequence.

Material and Methods

The Micronutrient Research Project (ICAR) started the long-term experiment in 1980 at Anand

Agricultural University in Anand, Gujarat, which was located at $22^0 35'$ North latitude and $72^0 55'$East longitude with an elevation of 45.1 meters above mean sea level on loamy sand soil (81.10 percent sand, 12.20 percent silt and 6.70 percent clay). Later in 1994, the experiment was changed to a Randomized Block Design with two Factors to investigate the effect of Farmyard manure in the maintaining of soil fertility and productivity at various levels of fertility during intense cropping. Under the pearl millet (*kharif*) -mustard (rabi) - cowpea (F) (summer) cropping sequence, FYM treatments were applied in half of the replications. The experiment contained four fertility levels and two levels of FYM, namely F0 (no-FYM) and F1 (FYM @ 10 t/ha) to be delivered to the *kharif* (pearl millet) crop once a year. The eight fertilizer treatments included in the experiment were: F_0FL_0: Control, $F_0 FL_1$:50 % NPK (50% RDF), $F_0 FL_2$: 100 % NPK (100% RDF), F_0 FL_3 :150 % NPK (150% RDF), F_1 FL_0: FYM 10 t/ ha, F_1 FL_1: FYM 10 t/ha+ 50 % NPK (50% RDF), F_1 FL_2: FYM 10 t/ha+ 100 % NPK (100% RDF), F_1 FL_3: FYM 10 t/ha+ 150 % NP (150% RDF). The recommended doses of N-P fertilizer for pearl millet: 80 kg N/ha, 40 kg P_2O_5/ha, Mustard:50 kg N/ha, 50 kg P_2O_5/ha and Cowpea: 25 kg N/ha, 50 kg P_2O_5/ha were applied. The FYM was given before *kharif* season @ 10 t/ha to F_1 treatment. The basal dose of Nitrogen fertilizer was applied in the form of urea as per recommendation. The plow furrow was treated with the required amount of P_2O_5 via diammonium phosphate. The remaining Nitrogen was added in two splits to pearl millet and one split to mustard. The experimental site's soil reaction was moderately alkaline (pH 8.35), and the EC was normal (0.16 dS/m). The highest monthly average rainfall (312 mm) has been received during the growing season of bajra, with a well-distributed pattern.

Following the harvest of cowpea (2018), soil samples were collected from two depths, 0-15 and 15-30 cm, and were prepared by mixing samples collected randomly from four spots in each plot. For storing the soil samples were air-dried and passed through a 2 mm sieve and then kept in polythene bags for further analysis of the physicochemical properties. For the biological parameter analysis, collected fresh soil samples from both depths were immediately transferred to the refrigerator, where they were kept at 4°C.

Analysis of Physico-chemical parameters:

Soil pH and EC were measured by using a soil water suspension ratio of 1:2.5. (Jackson, 1979).

The procedure used for aggregate analysis was Modified Yoder's wet sieving method (Yoder, 1936). To calculate the mean weight diameter (MWD), oven dry weight of the fractions of the aggregates retained on each sieve was determined using the formula:

$$\text{MWD} = \sum_{i=1}^{i=n}(\text{Xi Wi}) \,/\, \sum_{i=1}^{i=n}(\text{Wi})$$

Where, X_i = Mean opening of the sieve (i.e. 0.05, 0.175, 0.375, 0.75, 1.5, 3.5 mm for 0.1, 0.1-0.25, 0.25-0.5, 0.5-1.0, 1-2, 2-5 mm size classes, respectively); W_i = Weight of retained aggregates (g) and n = Number of size classes.

Large macroaggregates were classified as soil held between 4 and 2 mm, whereas small macroaggregates were classified as soil retained between 2 mm and 250 µm. Small micro aggregates were defined as soils held between 250 µm and 53 µm, whereas silt and clay fractions were defined as soils passing through 53 µm sieve and retained on the pan. However, macro-aggregates include both large and small macroaggregates.

Organic Carbon Fractions and total organic carbon:

The Organic carbon in soil was determined by Walkley and Black (1934) rapid titration method.

The fumigation extraction method was used to determine the SMBC (Vance *et al.,* 1987). In 100-mL capacity beakers, two portions of moist soil, each containing 50 g oven-dry soil, were weighed. One portion was used as a control and was immediately extracted with 0.5 M K_2SO_4. Another portion was fumigated. For 30 minutes, the fumigated soil samples were extracted with 200 mL 0.5 M K_2SO_4. Organic carbon in soil extracts was evaluated using dichromate oxidation, and SMBC was calculated.

Hot Water-soluble C (HWSC) was determined using the method described by Mc Gill *et al.,* (1986). Five grams of soil were centrifuged for 30 minutes at 10,000 rpm with 10 ml of distilled water. A portion of the supernatant was filtered. The filtrate was then treated with 5 ml of 0.07 N $K_2Cr_2O_7$, 10 ml of 98 percent H_2SO_4, and 5 mL of 88 percent H_3PO_3, and digested for 30 minutes at 150^0C. After cooling, the samples were titrated with 0.01 N ferrous ammonium sulphate in 0.4 N H_2SO_4 using diphenylamine as an indicator.

TOC content of the soils was determined by the method of Yeomas and Bremmer (1989) after treating the soil with dilute acid. In this method, one gram of soil was oxidized with 5 mL of 1*N* $K_2Cr_2O_7$ and 7.5 mL concentrated H_2SO_4 utilizing external heat (150 ^0C for 30 minutes). The tubes were removed from the block digester and cooled down for 15 minutes before being filled with water to a capacity of 50 mL. The contents were determined by titrating with ferrous ammonium sulphate (0.2 N) to a bright green endpoint using diphenylamine as an indicator. To compensate for the loss of $K_2Cr_2O_7$ due to boiling, two controls were included: one boiled and one unboiled. TOC was calculated as

$$\text{TOC (\%)} = \text{A (N of F. A. S.)} \times (0.003) \times \frac{100}{\text{Sample weight (g)}}$$

Where, A= (BC- S) × (UC - BC) / UC) + (BC –S)
BC = Boiled control
UC = Unboiled control
S = F. A. S. used in sample titration

Organic carbon stock

The following formula was utilized to calculate the Soil organic carbon stock:

C stock (Mg/ha) = [Total organic carbon (%)/100] × bulk density (Mg/m^3) × depth (m) ×10,000 (m^2/ha).

Enzymatic activity of soil:

Acid and alkaline phosphates activity in soil were assayed by the method described by Tabatabai and Bremnar (1969).

Urease activity in soil samples was estimated according to the “determination of urea remaining” methodology given by Tabatabai and Bremnar (1972) and expressed as µg urea hydrolyzed/g/h.

Soil dehydrogenase activity was determined by the method described by Cassida *et al.,* (1964) and results were expressed as µg TPF formed/ha/g of soil.

Results and Discussion

The application of FYM @ 10 t/ha over no FYM (F0) enhanced soil WBC by 26.3 percent in the 0-15 cm depth and 6.0 percent in the 15-30 cm depth, according to data in Table.1. Long-term application of FYM led to enhanced root development, which resulted in the concentration of organic residue in the soil and decomposition may have added to the organic carbon content of the soil. Ghosh *et al.,* (2018) also concluded that the Walkley-Black C (oxidizable organic C) concentration of surface soils decreased by 51%relative to the initial value when a wheat-based cropping system was used for 44 years without fertilization. However, the balanced fertilization (NPK) sustained or even increased the Walkley-Black C concentration. The amount of oxidizable organic C in the surface soil layer was higher in the NPK, 150 percent NPK, and NPK + FYM treatments compared to the unfertilized control plots by up to 103, 116, and 141 percent, respectively. In the subsurface soil layer, a similar pattern was observed.

In both the surface and subsurface depths, the HWSC status improved significantly with the treatment FYM 10 t/ha compared to the control. The improvement was 35.4 percent in the Surface depth, while subsurface depth was improved by 38.0 percent. In the manure-treated plots, the freshly humified organic carbon from FYM addition may have sustained a higher amount of HWSC than in the control plots (Liang *et al.,* 2012). In this investigation, soil microbial biomass carbon was considerably higher in the FYM treated plots (290.18 and 255.69 µg/g) in both surface and subsurface soil. This result is in agreement with numerous earlier observations (Chakraborty *et al.*, 2011; Marschner *et al.,* 2003). The presence of fast metabolizable C and N in FYM had the greatest impact on microbial biomass carbon levels, and increased root biomass and root exudates led to improved crop production in manure-treated plots (Benbi *et al.,* 2015). TOC increased by 31.6 percent on the surface and 18.2 percent on the subsurface under FYM (10 t/ha). The amount of TOC dropped as depth was increased, and the difference in TOC between fertilization treatments was more noticeable at 0-15 cm depths than at 15-30 cm depths. The SOC stock under FYM @ 10 t/ha(F1) was greater by 28.1 and 15.84 percent over control for profile depths of 0-15 and 15-30 cm, respectively. The rise in carbon content could be related to the addition of organic manure, which increases SOC stock.

Table 1: Long-term effect of FYM and fertilizer application on different carbon fractions in two soil depths after harvest of cowpea in a cropping sequence

Treatments	Soil Microbial Biomass C (µg/g)		Walkley & Black C (g/kg)		Water Soluble C (mg/kg)		Total Organic Carbon (g/kg)		Soil Organic Carbon stock (Mg/ha)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels										
F_0 (Without FYM)	**270**	**242**	**3.65**	**3.13**	**26.02**	**23.04**	**5.62**	**4.83**	**11.63**	**10.1**
F_1 (FYM 10 t ha^{-1})	**290**	**256**	**4.61**	**3.32**	**35.24**	**31.84**	**7.40**	**5.71**	**14.90**	**11.7**
S.Em ±	5.4	3.4	0.073	0.029	0.568	0.435	0.073	0.109	0.19	0.31
C.D.(P=0.05)	16	10	0.22	0.08	1.72	1.31	0.22	0.32	0.57	0.94
Fertility levels										
FL_0 (Control)	**243**	**221**	**3.66**	**3.15**	**16.51**	**19.84**	**5.48**	**4.86**	**10.51**	**10.13**
FL_1 (50 % NP)	**271**	**246**	**3.95**	**3.20**	**22.26**	**24.92**	**6.37**	**5.06**	**11.80**	**10.85**
FL_2 (100 % NP)	**286**	**256**	**4.20**	**3.24**	**25.66**	**28.86**	**6.96**	**5.30**	**12.57**	**11.12**
FL_3 (150 % NP)	**320**	**273**	**4.71**	**3.29**	**31.46**	**36.78**	**7.24**	**5.86**	**12.99**	**11.49**
S.Em ±	7.6	4.8	0.103	0.041	0.803	0.615	0.103	0.153	0.17	0.44
C.D.(P=0.05)	23	14	0.31	NS	2.43	1.86	0.31	0.46	0.31	1.33
Mean	**280**	**249**	**4.13**	**3.22**	**30.63**	**27.44**	**6.51**	**5.27**	**13.26**	**10.90**
F x FL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV %	**6.67**	**4.70**	**6.12**	**3.09**	**6.42**	**5.49**	**3.88**	**7.13**	**4.90**	**9.88**

Table 2: Long-term effect of FYM and fertilizer application on the size distribution of soil aggregate and mean weight diameter (MWD) of in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	Macroaggregates (g 100 g^{-1})		Microaggregates (g 100 g^{-1})		Silt + clay-sized fraction (g 100 g^{-1})		MWD (mm)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels								
F_0 (Without FYM)	**19.56**	**18.44**	**41.48**	**43.79**	**37.10**	**36.46**	**0.63**	**0.46**
F_1 (FYM 10 t ha^{-1})	**22.92**	**21.30**	**45.64**	**49.20**	**29.10**	**28.63**	**0.77**	**0.59**
S.Em ±	0.643	0.641	0.803	1.552	1.048	1.217	0.022	0.019
C.D.(P=0.05)	1.95	1.94	2.43	4.70	3.18	3.69	0.07	0.05
Fertility levels								
FL_0 (Control)	**15.99**	**14.86**	**32.59**	**33.97**	**45.48**	**41.39**	**0.45**	**0.41**
FL_1 (50 % NP)	**19.51**	**19.50**	**41.23**	**43.09**	**35.60**	**37.00**	**0.61**	**0.59**
FL_2 (100 % NP)	**22.63**	**21.25**	**47.93**	**52.42**	**28.00**	**29.42**	**0.79**	**0.63**
FL_3 (150 % NP)	**25.83**	**23.89**	**51.00**	**56.74**	**22.83**	**22.36**	**0.94**	**0.74**
S.Em ±	0.910	0.906	1.135	2.194	1.482	1.722	0.031	0.027
C.D.(P=0.05)	2.76	2.75	3.44	6.65	4.49	5.22	0.10	0.08
Mean	**21.24**	**19.87**	**43.56**	**46.49**	**33.10**	**32.54**	**0.70**	**0.52**
F x FL	NS	NS	NS	NS	NS	NS	NS	NS
CV %	**10.49**	**11.17**	**6.44**	**11.56**	**11.01**	**12.96**	**10.98**	**12.23**

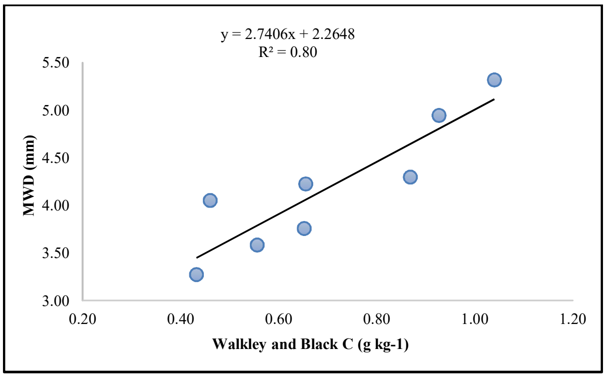

Figure 1: Soil organic carbon (Walkley and Black carbon) relationship with MWD at 0-15 cm soil depth

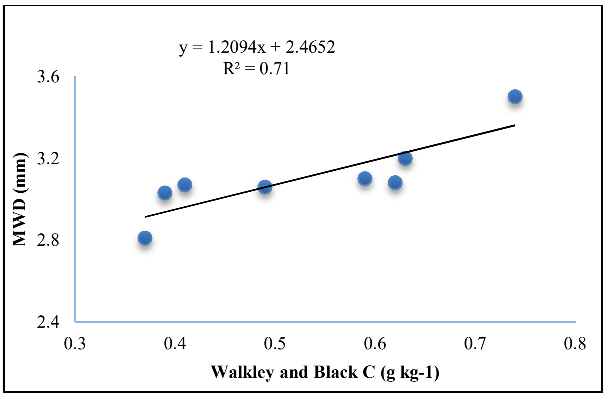

Figure 2: Soil organic carbon (Walkley and Black carbon) relationship with MWD at 15-30 cm soil depth

Among the different fertility levels, FL3 (NP application @ 150 percent RDF) had the highest WBC (4.71 g/kg) for surface soil, but the influence of fertility levels on changes in WBC at subsurface depths of the soil profile was non-significant. The changes in OC concentration caused by graded fertilizer application could be related to differences in organic matter quantity and rate of oxidation by microorganisms (Peacock *et al.,* 2001; Zhang *et al.,* 2010). The significantly highest values for HWSC were recorder with NP application @ 150 % RDF i.e. FL_3 in 0-15 cm (31.46 mg/ kg) and 15-30 cm (36.78 mg/ kg), respectively. The highest TOC accumulation was seen with NP @ 150 percent of RDF, while the control plot had the lowest TOC. In both soil depths, 150 percent RDF (NP) significantly enhanced TOC content in soil compared to the control and 50 percent NP. Increased fertilizer dose raises TOC content considerably over control regardless of depth. Furthermore, FL3 (NP application @ 150 percent RDF) had the highest SOC stock of fertility doses. Over the control, the SOC stock increased by 23.6 at 0-15 cm depth layer and 13.42 percent at15-30 cm depth, respectively in the highest fertility level i.e. FL3 (NP application @ 150 percent RDF). Fertilizer additions contributed to carbon accumulation, mostly by increasing root biomass; crop leftovers eventually resulted in carbon accumulation over time (Kundu *et al.,* 2007, Ghosh *et al.,* 2012).

Soil macroaggregates and microaggregates were significantly higher under FYM application @ 10 t/ha, with an increase of 17.1% and 10% over unfertilized control plots in the surface soil, and 15.5 and 12.2% increases in the sub-surface soil, respectively. FL3 (150 percent NP) was observed to contribute significantly to the development of macro and microaggregates in both depths over control and rest of fertility levels. For subsurface layers, however, this treatment impact was comparable to FL2 (100 percent NP). The silt + clay-sized fractions of aggregates decreased significantly in FYM and fertilizer-treated plots compared to the untreated control. There are some primary mechanisms for the formation and stabilization of soil macroaggregate under integrated nutrient management. The by-products of FYM decomposition, glue compounds generated by roots, fungal hyphae, and polysaccharides' action as a binding agent to bind microaggregates together to form soil macroaggregates are among them (Liao *et al.,* 2006 and Tripathi *et al.,* 2014). In FYM-treated plots, the formation of micro and macroaggregates by soil clay particles could result in very low silt + clay fractions. Organic fertilizer application over time increases SOC content and macroaggregate proportion (Saha *et al*., 2014).

In both soil layers, manure and a super-optimal fertilizer dose significantly increased water-stable aggregate (WSA). Significantly, FYM treated plots had 22.2 and 28.2 percent higher MWD in the surface and subsurface layers, respectively than control plots. Under NP application @ 150 percent RDF (FL3), the increase in MWD for surface and sub-surface layers was 108.8 and 80.4 percent over control, respectively. The continuous application of chemical fertilizers also showed improvement in the MWD in the long run indicating the role played by phosphate ions in bindings soil particles or due

Table 3: Effect of FYM and fertilizer application on microbial count in in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	Bacteria (CFU g^{-1} x 10^8)		Actinomycetes (CFU g^{-1} x 10^1)		Fungi (CFU g^{-1} x 10^3)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels						
F_0 (Without FYM)	**146**	**100**	**83**	**57**	**5**	**4**
F_1 (FYM 10 t ha^{-1})	**182**	**111**	**103**	**62**	**7**	**5**
S.Em ±	5.2	3.2	2.7	1.6	0.2	0.2
C.D.(P=0.05)	15.9	9.8	8.3	5.0	0.6	0.5
Fertility levels						
FL_0 (Control)	**106**	**93**	**63**	**44**	**4**	**3**
FL_1 (50 % NP)	**139**	**101**	**88**	**57**	**5**	**4**
FL_2 (100 % NP)	**184**	**108**	**104**	**67**	**6**	**5**
FL_3 (150 % NP)	**227**	**118**	**118**	**70**	**9**	**7**
S.Em ±	7.4	4.5	3.8	2.3	0.3	0.3
C.D.(P=0.05)	22.4	13.8	11.7	7.1	0.8	0.8
Mean	**164**	**105**	**93**	**60**	**6**	**4**
F x FL (Interaction)	NS	NS	NS	NS	NS	NS
CV %	**11.07**	**10.66**	**10.19**	**9.60**	**11.86**	**13.90**

***Significant at P 0.05; NS- Non-Significant at P > 0.05**

Table 4: Long-term effect of FYM and fertilizer application on soil enzymatic activities in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	Alkaline Phosphatase (µg pNP g-1 hr-1)		Acid Phosphatase (µg pNP g-1 hr-1)		Dehydrogenase (µg TPF g-1 hr-1)		Urease (µg urea g-1 hr-1)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels								
F0 (Without FYM)	**37.82**	**25.34**	**7.47**	**7.34**	**24.97**	**23.32**	**170.29**	**131.01**
F1 (FYM 10 t ha-1)	**41.67**	**27.70**	**11.64**	**8.03**	**26.73**	**26.01**	**190.32**	**147.72**
S.Em ±	1.146	0.748	0.278	0.184	0.548	0.820	4.789	2.643
C.D.(P=0.05)	3.47	2.27	0.84	0.56	1.67	2.48	14.52	8.01
Fertility levels								
FL0 (Control)	**34.23**	**22.30**	**8.42**	**7.06**	**21.37**	**20.21**	**151.61**	**114.40**
FL 1 (50 % NPK)	**39.97**	**25.79**	**9.18**	**7.45**	**24.11**	**24.02**	**173.60**	**136.84**
FL 2 (100 % NPK)	**40.60**	**28.16**	**9.63**	**7.93**	**26.08**	**26.14**	**182.22**	**149.28**
FL 3 (150 % NPK)	**44.16**	**29.82**	**10.99**	**8.31**	**31.84**	**28.28**	**213.78**	**156.72**
S.Em ±	1.620	1.058	0.393	0.261	0.774	1.160	6.773	3.738
C.D.(P=0.05)	4.91	3.21	1.193	0.79	2.34	3.52	20.54	11.34
Mean	**39.74**	**26.52**	**9.55**	**7.68**	**25.85**	**24.66**	**180.30**	**139.36**
F x FL(Interaction)	NS	NS	NS	NS	NS	NS	NS	NS
CV %	**9.99**	**9.77**	**10.08**	**8.31**	**7.34**	**11.52**	**9.20**	**6.57**

may be due to a greater amount of organic residues produced due to balanced fertilization (Bandyopadhyay *et al.*, 2010). Our findings also revealed a strong and positive relationship between SOC and MWD (Fig. 1 & 2).The soil microbial activities have been significantly affected by long-term manuring in the surface as well as subsurface depths (Table 3). With the long-term application of FYM, the increase in the bacterial, fungal and actinomycetes population were to the tune of 24.8, 52.6 and 23.0 percent for the 0-15 while 11.0, 27.0 and 10.2 percent for 15-30 cm depths, respectively. Among the fertility doses, FL_3 (150 % NP) showed a significantly highest population of bacteria, fungi and actinomycetes and the lowest were recorded under control. Again, for 15-30 cm depth the maximum population of microbes was recorded under the same fertility level i.e. NP application @ 150 % RDF. However, it was at par with NP application @ 100 % RDF for bacteria and actinomycetes.

For the enzymatic activity (Table 4) it has been observed that irrespective of depths the alkaline phosphatase activity was higher than acid phosphatase. Again, FYM application @ 10 t/ha improved the alkaline phosphatase and acid phosphatase activities to the tune of 10.1, 9.3 for surface and 55.8, 9.4 percent for subsurface layers, respectively over the control (F_0). The application of 150% NP resulted in higher phosphatase enzyme activity as compared to control and rest of fertility levels which was increased to the tune of 29.0 percent over the control for 0-15 cm depths. In the case of subsurface depth (15-30 cm), maximum activity for Alkaline and acid phosphatase was found in treatment FL_3 (150 % NP) which was at par with FL_2 (100 % NP). Regardless of treatment, higher enzyme activity was observed in the surface soil layer than in the subsurface soil. The significantly higher alkaline and acid phosphatase activities in the organically treated plots could be attributed to increased microbial activity and diversity of phosphate solubilizing bacteria as a result of organic matter input over the period. These results are similar to the findings of Kanchikerimath and Singh (2001); Mandal *et al.,* (2007), and Manna *et al.,* (2007). Similarly, long-term application of FYM @ 10 t/ha significantly increased dehydrogenase and urease activity by 7.0, 11.5, and 11.8,12.7 percent over control in the surface and subsurface layers, respectively. The beneficial effects of FYM on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms and the increase in microbial growth with the addition of carbon source, which is in conformity with the findings of Manna *et al.,* (2005) and Mandal *et al.,*(2007). The graded levels of fertility enhanced dehydrogenase and urease enzyme activity by 40.9, 39.9, and 41.0, 36.9 percent under higher fertility levels (FL_3) over FL_0 in the surface and subsurface layers, respectively. However, in the subsurface, FL_3 was at par with FL_2. The results are like the findings of Bhatt *et al.,* (2016) as they reported that, fertilizer treatment with 100% NPK and 150 % NPK were comparable and significantly superior to 50 % NPK for dehydrogenase activity of soil. Different soil organic carbon fractions were found to be positively and significantly correlated with soil microbial populations and enzymatic activities (Table 5).

Table 5: Correlation between total organic C (TOC, g kg^{-1}), Walkley & Black C (g kg^{-1}), microbial biomass C (MBC, g kg^{-1}), microbial count and enzymatic activity at 0-15 cm depth

.

0-15 cm	TOC	WBC	SMBC	Bacteria	Fungi	Actinomycetes	Alkaline P	Acid P	Dehydrogenase	Urease
TOC	1									
WBC	0.961**	1								
SMBC	0.785*	0.814*	1							
Bacteria	0.828*	0.832*	0.970**	1						
Fungi	0.788*	0.852**	0.983**	0.933**	1					
Actinomycetes	0.882**	0.871**	0.942**	0.953**	0.936**	1				
Alkaline P	0.859**	0.879**	0.923**	0.873**	0.944**	0.943**	1			
Acid P	0.944**	0.961**	0.943**	0.693**	0.735*	0.718*	0.778*	1		
Dehydrogenase	0.694**	0.776*	0.954**	0.871**	0.943**	0.914**	0.871**	0.592	1	
Urease	0.831*	0.885**	0.946**	0.929**	0.970**	0.966**	0.939**	0.739*	0.972**	1

Table 6: Correlation between total organic C (TOC, g kg^{-1}), Walkley & Black C (g kg^{-1}), microbial biomass C (MBC, g kg^{-1}), microbial count and enzymatic activity at 15-30 cm depth

15-30 cm	TOC	WBC	SMBC	bacteria	fungi	Actinomycetes	alkaline p	acid p	dehydrogenase	urease
TOC	1									
WBC	0.854**	1								
SMBC	0.790*	0.720*	1							
Bacteria	0.880**	0.839*	0.960 **	1						
Fungi	0.733*	0.780*	0.956**	0.945**	1					
Actinomycetes	0.692*	0.700**	0.933**	0.906**	0.936**	1				
Alkaline p	0.683*	0.789**	0.914**	0.896**	0.944**	0.943**	1			
Acid p	0.909**	0.857**	0.929**	0.990**	0.735*	0.895*	0.868*	1		
Dehydrogenase	0.741*	0.805*	0.910**	0.940**	0.943**	0.948**	0.988**	0.916**	1	
Urease	0.777*	0.821**	0.944**	0.924**	0.970**	0.9072**	0.978**	0.919**	0.990**	1

**. significant at the 0.01 level *. significant at the 0.05 level

Significantly the highest correlation (Table 6) was observed between SMBC and microbial populations (bacteria, fungi, actinomycete; R^2 = 0.97, 0.98 and 0.94 for surface and R^2 = 0.96, 0.95 and 0.93 for sub-surface, respectively). Similarly, SMBC revealed a positive and significantly the highest correlation with soil enzymatic activity.For all of the above-mentioned parameters, the interaction effect of FYM and fertility levels has been found to be non-significant for both the surface and subsurface layers.

Conclusion

After 15 years of long-term fertilization on the pearl millet-mustard-cowpea cropping system, the soil carbon buildup in different labile fractions from the surface and sub-surface depths was assessed. The results reveal that long-term cultivation with NP + FYM treatments increased SOC and its selected labile fractions (WBC, HWSC, and SMBC) over no fertilization (control) and treatments with a solitary application of inorganic fertilizers. Such augmentation of SOC and almost all its labile fractions were higher with the application of FYM @10 t/ha + 150 % NP in the system. The significantly higher accumulation of TOC as well as SOC stock with higher doses of inorganic fertilizer conjugated with FYM also signifies the contribution of inorganic fertilizer in oil C buildup. Again, soil aggregate stability and biological activity were also significantly higher under the integrated application of FYM @10 t/ha + 150 % NP in the cropping system. The labile C fractions and activities of soil enzymes and microbes were predominated in the near-surface soil layers but declined significantly as depth increased, which could be due more to biomass accumulation in the surface soil layer. Moreover, the significant positive correlations of labile C fractions to microbial and biological activity also indicate the crucial role played by these fractions for regulating biological health of soil. Although these overall advantages may be viewed as an opportunity to improve soil quality and productivity, there is still a need to evaluate and control any negative consequences associated with the long-term use of organic amendments, such as excessive nutrient accumulation or leaching and phytotoxicity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Field bio-efficacy, phytotoxicity of different insecticides against invasive leafminer, *Liriomyza huidobrensis* in potato and its safety to natural enemies

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ABSTRACT

In order to assess the efficiency of different pesticides, their phytotoxicity, and the safety of natural enemies in potato fields of the Nilgiris district against the recently existing invasive pest, *Liriomyza huidobrensis*, two field experiments were conducted at two different locations, namely Kukkal in Kotagiri and Kappachi in Ooty, both located in the district Nilgiris, Tamil Nadu. The outcomes showed that cyantraniliprole 10.26 OD @ 75 g a.i. /ha and chlorantraniliprole 18.5 S @ 30 g a.i. /ha were helpful for managing *L. huidobrensis*. Following spraying, coccinellid and spider populations first declined, but gradually rose. Following it, Profenofos 50 EC @ 500 g a.i. /ha was also demonstrated to yield favourable results, but it was rejected because it significantly reduced the population of natural enemies. Additionally, it was discovered that none of the pesticides had any phototoxic effects on potato during the trial. In light of the fact that profenofos 50 EC has a similar impact on the natural enemy population as cyantraniliprole 10.26 OD and chlorantraniliprole 18.5 S @ 30 g a.i. ha^{-1}, they can be used successfully as a management strategy for potato leaf miner.

Introduction

The fourth-most significant food crop in the world is the potato (*Solanum tuberosum*), which belongs to the Solanaceae family. China is the world's finest producer of potato, followed by India, Ukraine, Russia, and the United States. In fiscal year 2020, India has over two million hectares of land available for potato cultivation, producing roughly 508.57 lakh tonnes (Anonymous, 2020). Each year, pests destroy potato tubers worth around 60 billion rupees (US$1.2 billion), or 10 to 20 percent of the crop's total production. Pests that affect this crop include aphid, leafhopper, potato cutworms, potato tuber moth, and leafminers (Simpson, 1977). It is thought that *Liriomyza* leafminers (Agromyzidae: Diptera) are the most serious pest in the majority of horticultural crops worldwide (Bader *et al.*, 2006). A thorough investigation was carried out in potato fields at Nilgiris, Tamil Nadu, during 2020–2021, when it was discovered that a new leafminer species, *L. huidobrensis*, causes significant yield losses in potato. Up to 20% of the entire cost of production for potato might be attributed to pesticide use. Chemical control is one of the widely utilized strategies for pest management in the production of potato.

Chemical control remained the most frequently implemented methods for the control of arthropod pests, despite the certainty that the registration of any novel insecticides will be decided based on the environmental and human safety concerns and the identification of successful non-chemical strategies against the majority of pests (Alyokhin, 2011). In order to prevent the pest from spreading and taking on the role of a significant pest, it is crucial that investigations be performed in potato fields given the severity of the damage caused by *Liriomyza huidobrensis*. This study's objective is to assess several synthetic pesticides for the efficient control of *L. huidobrensis*, the potato leafminer.

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Material and Methods

Two field trials were carried out at farmer's holdings in the Nilgiris region of Tamil Nadu in Kukkal village, Kotagiri (11.46°N 76.88°E and 1,847 MSL), and Kappachi, Ooty (11.43°N 76.76°E and 2,209 MSL). With a plot size of 25 m^2, the experiment was carried out using a Randomized Block Design (RBD) on the potato variety Kufri Jyothi. Insecticides were applied initially when the pest passed the ETL (10% leaf damage), and a second application was made 15 days later (TNAU Agritech. portal). The treatment details were:

T_1	Chlorantraniliprole 18.5 SC @ 30 g ai. / ha
T_2	Cyantraniliprole 10.26 OD @ 75 g ai. / ha
T_3	Flubendiamide 20 WG @ 48 g ai. / ha
T_4	Spinosad 45 SC @ 75 g ai. / ha
T_5	Profenofos 50 EC @ 500 g ai. / ha
T_6	Spinetoram 11.7 SC @ 50 g ai. / ha
T_7	Emamectin benzoate 5 SG @ 10 g ai. / ha
T8	Control

Pretreatment assessments of the % leaf damage were made on five randomly selected plants from each plot, as well as 3, 5, 7, 10, and 14 days after each spray. In order to evaluate the safety of insecticides at five randomly chosen plants, the populations of natural enemies, such as spiders and coccinellids, were also counted. Phytotoxic symptoms were also examined. Prior to analysis, the experiment's data were converted into an arc sine (angular transformation) for the percentage of leaf damage and a square root (analytical transformation) for the population of natural enemies. The data was then subjected to a variance analysis (ANOVA). Using Duccan's Multiple Range Test, the means of the substantially different treatments (P = 0.05) were separated (DMRT). The significance threshold was set at = 0.05. These processes were performed using the SPSS Statistics 28.0.1 (IBM Corp, 2021).

Results and Discussion

Table 1 summarises the findings of field trials with eight distinct treatments carried out in the Nilgiris district of Tamil Nadu in Kotagiri and Ooty. Based on the percentage reduction in leaf damage compared to the untreated control at Kotagiri, the following treatments were found to be most effective: cyantraniliprole 10.26 OD @ 75 g a.i. /ha (85.50%) > chlorantraniliprole 18.5 SC @ 30 g a.i. /ha (80.23%) > profenofos 50 EC @ 500 g a.i. /ha (72.92%) > emamectin benzoate 5 SG @ 10 g a.i. /ha (70.05%) > flubendiamide 20 WG @ 48 g a.i. /ha (66.49%) > spinetoram 11.7 SC @ 50 g a.i. ha^{-1}(45.74%) > spinosad 45 SC @ 75 g a.i. /ha (42.38%) (Fig.1). At Kappachi, Ooty, plots treated with cyantraniliprole 10.26 OD @ 75 g a.i. /ha showed the greatest percentage reduction compared to controls (92.24 %). According to the percentage reduction in leaf damage compared to the untreated control, the following treatments were found to be the most effective: cyantraniliprole 10.26 OD @ 75 g a.i. /ha (92.24%) > chlorantraniliprole 18.5 SC @ 30 g a.i. /ha (91.96%) > profenofos 50 EC @ 500 g a.i. /ha (88.30%) > emamectin benzoate 5 SG @ 10 g a.i. /ha (79.98%) > flubendiamide 20 WG @ 48 g a.i. /ha (79.00%) > spinetoram 11.7 SC @ 50 g a.i. /ha (73.47%) > spinosad 45 SC @ 75 g a.i. /ha (69.42%). Based on the findings, it was reported that cyantraniliprole 10.26 OD @ 75 g a.i./ ha and chlorantraniliprole 18.5 S @ 30 g a.i./ ha were found to be effective insecticides among various treatments tested for their efficacy in management of leaf miner, *L. huidobrensis* in the potato ecosystem. Similar findings were given by Mohan and Anitha (2017) whom reported that chlorantraniliprole 18.5 SC 0.03 % at 10 days interval reduces the leaf minor damage, number of mines and larvae per plant in tomato. Selvaraj *et al.* (2017) identified that in tomato, chlorantraniliprole 4.3 % as a combination insecticide with Abamectin 1.7% SC was significantly effective while spraying twice fortnightly. The insecticides like profenofos, buprofezin, spinosad, chlorantraniliprole, thiame thoxam, acephate, malathion along with NSKE @ 5% and azadirachtin 1500 ppm were shown to reduce the leaf miner population in ridge gourd to considerable level (Hirekurubar and Tatagar, 2018), which was found to be in accordance with our results. Ramesh *et al.* (2020) also stated that the insecticide cyantraniliprole 10.26 OD registered lower leaf miner infestation in watermelon. Generalist predators in the potato ecosystem, such spiders and coccinellids, were evident during the cropping season. Spiders or coccinellids were significantly impacted by various pesticide treatments. Tables 2 and 3 explain the findings of the impact of various insecticidal treatments on thepopulation of spiders and coccinellids in two distinct locales.

Table 1: Efficacy of different insecticides against *Liriomyza* leafminer in Potato

Treatment	Location I - Kotagiri								Location II - Ooty							
	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)
T1	35.31	22.29	18.04	14.91	11.36	8.45	15.01	80.23	21.12	12.18	10.26	7.18	5.30	3.84	7.75	91.96
	(36.46)	(28.17	(25.14)	(22.72)	(19.69)	(16.89)	(22.79)^{de}		(27.36)	(20.42)	(18.68)	(15.54)	(13.31)	(11.31)	(16.17)^{g}	
T2	36.84	21.73	15.14	11.92	8.87	6.19	12.77	85.50	27.81	13.64	10.22	8.11	5.59	3.71	8.25	92.24
	(37.37)	(27.78)	(22.90)	(20.20)	(17.32)	(14.41)	(20.94)^{e}		(31.82)	(21.67)	(18.64)	(16.55)	(13.67)	(11.11)	(16.69)^{f}	
T3	38.05	28.08	22.66	19.54	17.69	16.02	20.80	62.49	24.06	15.62	13.59	12.13	11.09	10.05	12.4	79.00
	(38.08)	(32.00)	(28.42)	(26.23)	(24.87)	(23.59)	(27.13)^{c}		(29.38)	(23.28)	(21.63)	(20.38)	(19.46)	(18.48)	(20.70)^{d}	
T4	40.15	30.68	29.82	28.30	26.08	24.61	27.90	42.38	27.22	21.14	19.05	17.04	15.53	14.63	17.48	69.42
	(39.32)	(33.63)	(33.10)	(32.14)	(30.71)	(29.74)	(31.88)^{b}		(31.45)	(27.37)	(25.88)	(24.38)	(23.21)	(22.49)	(24.71)^{b}	
T5	39.03	26.16	20.57	16.73	14.30	11.57	17.86	72.92	23.13	12.02	10.14	8.69	7.14	5.60	8.72	88.30
	(38.66)	(30.76)	(26.97)	(24.14)	(22.22)	(19.88)	(25.00)^{cd}		(28.74)	(20.29)	(18.57)	(17.14)	(15.49)	(13.68)	(17.17)^{e}	
T6	34.91	30.65	28.57	26.63	24.62	23.18	26.73	45.74	26.53	19.03	16.65	15.21	13.64	12.69	15.44	73.47
	(36.22)	(33.62)	(32.31)	(31.07)	(29.75)	(28.78)	(31.13)^{b}		(31.00)	(25.86)	(24.08)	(22.95)	(21.67)	(20.87)	(23.14)^{c}	
T7	38.11	24.44	20.75	17.08	14.59	12.79	17.93	70.05	23.20	15.56	13.19	11.98	10.60	9.58	12.18	79.98
	(38.12)	(29.63)	(27.10)	(24.41)	(22.45)	(20.95)	(25.05)^{cd}		(28.79)	(23.23)	(21.29)	(20.25)	(19.00)	(18.03)	(20.43)^{d}	
T8	37.66	41.90	42.50	43.01	43.50	42.71	42.72	-	22.05	33.63	37.48	40.48	43.67	47.83	40.62	-
	(37.85)	(40.34)	(40.69)	(40.98)	(41.27)	(40.81)	(40.82)^{a}		(28.01)	(35.44)	(37.75)	(39.51)	(41.36)	(43.76)	(39.59)^{a}	
S Em	0.386	1.347	1.682	1.699	1.744	1.784	1.65		0.257	0.159	0.188	0.24	0.21	0.232	0.155	
CD	1.183	4.126	5.15	5.203	5.434	5.463	5.054		0.787	0.488	0.577	0.734	0.634	0.71	0.476	

PRC is for percent reduction over untreated control; PTC stands for pre-treatment count; DAS stands for days after spraying; and the numbers are pooled from two sprays. Arc sine converted values represent the figures in parenthesis. By DMRT at the 5% level of significance, treatment means having a letter in common are not considered significant. a, b, c, d, e, f and g are letters used to denote the significance by DMRT.

Table 2: Efficacy of different insecticides against predatory spiders in potato

Treatment	Location I - Kotagiri								Location II - Ooty							
	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)
T1	1.72	1.52	1.64	1.76	1.83	1.93	1.73	29.69	1.28	1.24	1.34	1.45	1.54	1.63	1.44	31.34
	(1.49)	(1.42)	(1.46)	(1.50)	(1.53)	(1.56)	(1.49)^{f}		(1.33)	(1.32)	(1.36)	(1.40)	(1.43)	(1.46)	(1.39)^{d}	
T2	1.62	1.47	1.55	1.59	1.69	1.78	1.62	34.27	1.27	1.25	1.33	1.45	1.55	1.65	1.44	30.64
	(1.46)	(1.40)	(1.43)	(1.45)	(1.48)	(1.51)	(1.45)^{g}		(1.33)	(1.32)	(1.35)	(1.39)	(1.43)	(1.46)	(1.39)^{d}	
T3	1.82	1.63	1.71	1.83	1.95	2.04	1.83	25.62	1.26	1.31	1.36	1.50	1.59	1.68	1.49	29.23
	(1.52)	(1.46)	(1.49)	(1.52)	(1.56)	(1.59)	(1.53)^{d}		(1.33)	(1.34)	(1.36)	(1.41)	(1.45)	(1.48)	(1.41)^{c}	
T4	1.81	1.87	1.99	2.13	2.21	2.31	2.10	14.57	1.32	1.41	1.52	1.62	1.73	1.80	1.61	24.10
	(1.52)	(1.54)	(1.58)	(1.62)	(1.65)	(1.68	(1.61)^{b}		(1.35)	(1.38)	(1.42)	(1.46)	(1.49)	(1.52)	(1.45)^{b}	

T5	1.55	1.27	1.36	1.40	1.47	1.52	1.40	42.99	1.14	0.99	1.12	1.17	1.27	1.34	1.18	43.71
	(1.43)	(1.33	(1.36)	(1.38)	(1.40)	(1.42)	(1.38)^{h}		(1.28)	(1.22)	(1.27)	(1.29)	(1.33)	(1.35)	(1.29)^{e}	
T6	1.63	1.69	1.79	1.88)	2.01	2.09	1.89	23.14	(1.27	1.32	1.52	1.62	1.76	1.89	1.62	20.24
	(1.46)	(1.48)	(1.51)	(1.54	(1.58)	(1.61)	(1.55)^{c}		(1.33)	(1.35)	(1.42)	(1.45)	(1.50)	(1.55)	(1.46)^{b}	
T7	1.70	1.57	1.66	1.74)	1.85	2.00	1.76	28.28	1.38	1.26	1.39	1.51	1.57	1.69	1.48	28.60
	(1.48)	(1.44)	(1.47)	(1.50	(1.53)	(1.58)	(1.50)^{e}		(1.37)	(1.33)	(1.38)	(1.42)	(1.44)	(1.48)	(1.41)^{c}	
T8	1.75	2.21	2.26	2.49)	2.59	2.75	2.46	-	1.32	1.79	1.93	2.10	2.24	2.37	2.08	-
	(1.50)	(1.65)	(1.66)	(1.73)	(1.76)	(1.80)	(1.72)^{a}		(1.35)	(1.51)	(1.56)	(1.61)	(1.66)	(1.69)	(1.61)^{a}	
S Em	0.009	0.003	0.003	0.002	0.004	0.002	0.003		0.002	0.002	0.003	0.003	0.003	0.003	0.002	
CD	0.027	0.01	0.008	0.007	0.011	0.007	0.008		0.006	0.006	0.008	0.009	0.008	0.008	0.007	

PRC is for percent reduction over untreated control; PTC stands for pre-treatment count; DAS stands for days after spraying; and the numbers are pooled from two sprays. Arc sine converted values represent the figures in parenthesis. By DMRT at the 5% level of significance, treatment means having a letter in common are not considered significant. a, b, c, d, e, f, g and h are letters used to denote the significance by DMRT.

Table 3: Efficacy of different insecticides against predatory coccinellids in potato

Treatment	Location I - Kotagiri								Location II - Ooty							
	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)	PTC	3 DAS	5 DAS	7 DAS	10 DAS	14 DAS	Mean	PRC (%)
T1	1.92	1.77	1.81	1.88	1.94	2.06	1.89	34.17	1.95	1.88	1.99	2.13	2.18	2.31	2.10	33.12
	(1.55)	(1.50)	(1.52)	(1.54)	(1.56)	(1.60)	(1.55)^{e}		(1.56)	(1.54)	(1.58)	(1.62)	(1.64)	(1.68)	(1.61)^{d}	
T2	1.66	1.53	1.63	1.71	1.80	1.92	1.72	38.65	1.95	1.89	2.00	2.12	2.21	2.32	2.11	32.79
	(1.47)	(1.42)	(1.46)	(1.49)	(1.52)	(1.55)	(1.49)^{f}		(1.56)	(1.55)	(1.58)	(1.62)	(1.65)	(1.68)	(1.62)^{d}	
T3	2.02	1.81	1.90	1.95	2.09	2.20	1.99	29.47	2.06	2.02	2.10	2.17	2.28	2.38	2.19	31.10
	(1.59)	(1.52)	(1.55)	(1.57)	(1.61)	(1.64)	(1.58)^{c}		(1.60)	(1.590	(1.61)	(1.63)	(1.67)	(1.70)	(1.64)^{cd}	
T4	1.98	1.83	1.93	2.01	2.15	2.27	2.04	27.28	2.34	2.32	2.43	2.51	2.58	2.71	2.51	21.54
	(1.57)	(1.53)	(1.56)	(1.58)	(1.63)	(1.66)	(1.59)^{b}		(1.69)	(1.68)	(1.71)	(1.73)	(1.76)	(1.79)	(1.73)^{b}	
T5	1.51	1.27	1.37	1.43	1.47	1.56	1.42	50.13	1.82	1.49	1.61	1.67	1.72	1.95	1.69	43.60
	(1.42)	(1.33)	(1.37)	(1.39)	(1.40)	(1.43)	(1.38)^{g}		(1.52)	(1.41)	(1.45)	(1.47)	(1.49)	(1.56)	(1.48)^{e}	
T6	1.87	1.81	1.92	1.99	2.09	2.21	2.00	29.20	2.25	2.20	2.27	2.37	2.50	2.59	2.39	24.92
	(1.54)	(1.52)	(1.56)	(1.58)	(1.61)	(1.65)	(1.58)^{c}		(1.66)	(1.64)	(1.66)	(1.69)	(1.73)	(1.76)	(1.70)^{bc}	
T7	1.82	1.66	1.91	1.98	2.03	2.13	1.94	31.93	2.19	2.12	2.21	2.30	2.90	2.50	2.41	27.67
	(1.52)	(1.47)	(1.55)	(1.57)	(1.59)	(1.62)	(1.56)^{d}		(1.64)	(1.62)	(1.65	(1.67)	(1.84)	(1.73)	(1.70)^{bc}	
T8	1.92	2.48	2.56	2.68	2.97	3.12	2.76	-	2.51	3.03	2.60	3.20	3.33	3.45	3.12	-
	(1.56)	(1.72)	(1.75)	(1.78)	(1.86)	(1.90)	(1.81)^{a}		(1.74)	(1.88)	(1.76)	(1.92)	(1.96)	(1.99)	(1.90)^{a}	
S Em	0.003	0.002	0.002	0.005	0.005	0.003	0.002		0.004	0.003	0.003	0.002	0.003	0.003	0.025	
CD	0.008	0.007	0.007	0.014	0.014	0.008	0.005		0.013	0.008	0.008	0.008	0.008	0.01	0.076	

PRC is for percent reduction over untreated control; PTC stands for pre-treatment count; DAS stands for days after spraying; and the numbers are pooled from two sprays. Arc sine converted values represent the figures in parenthesis. By DMRT at the 5% level of significance, treatment means having a letter in common are not considered significant. a, b, c, d, e, f and g are letters used to denote the significance by DMRT.

The proportion of spiders that were reduced by over 1% for profenofos 50 EC @ 500 g a.i /ha was the highest (27.28 percent). The spider population decreased in treatment plots over control plots in the following order: profenofos 50 EC @ 500 g a.i. /ha > cyantraniliprole 10.26 OD @ 75 g a.i. ha^{-1}> chlorantraniliprole 18.5 SC @ 30 g a.i. /ha > emamectin benzoate 5 SG @ 10 g a.i. /ha > flubendiamide 20 WG @ 48 g a.i. /ha > spinetoram 11.7 SC @ 50 g a.i. /ha > spinosad 45 SC @ 75 g a.i. /ha at both the locations. Similarly, the order of reduction in population of coccinellids in treated plots over control were profenofos 50 EC @ 500 g a.i. /ha > chlorantraniliprole 18.5 SC @ 30 g a.i. /ha > cyantraniliprole 10.26 OD @ 75 g a.i. /ha > flubendiamide 20 WG @ 48 g a.i. ha^{-1}> emamectin benzoate 5 SG @ 10 g a.i. /ha > spinetoram 11.7 SC @ 50 g a.i. ha^{1} > spinosad 45 SC @ 75 g a.i. /ha in both the locations. Profenofos 50 EC was found to cause higher reduction in natural enemy population at both the locations. Whereas, cyantraniliprole 10.26 OD and chlorantraniliprole 18.5 S @ 30 g a.i./ ha doesn't cause much harm to the natural enemy population comparable to the effect of profenofos 50 EC. Cyazypyr 10% OD @ 45–105 g a.i./ha has not significantly reduced the population of natural enemies even @ 360 g a.i./ha and was found to be safer to the natural enemies (Mandal, 2012). During the post-application period, the predatory coccinellid population did not significantly differ from the control population, demonstrating the safety of cyantraniliprole 10% OD @ 90 and 105 g a.i./ha and spinosad at the tested levels to the predators, with the exception of other treatment (Misra, 2013).The results of the field trial in each of the tested locations showed that potato plants sprayed with concentrations of chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}, cyantraniliprole 10.26 OD @ 75 g a.i. ha^{-1}, flubendiamide 20 WG @ 48 g a.i. ha^{-1}, spinosad 45 SC @ 75 g a.i. ha^{-1}, profenofos 50 EC @ 500 g a.i. ha^{-1}, spinetoram 11.7 SC @ 50 g a.i. /ha and emamectin benzoate 5 SG @ 10 g a.i. /ha concentrations had not caused any phytotoxic effects. None of the insecticides were found to cause any phytotoxic symptoms. In his assessment of the bio-efficacy of cyantraniliprole 10 % OD against major sucking pests and potato armyworms in the potato environment, Bojan (2021) concluded that the compound exhibited no phytotoxic effects at any dose. According to Mandal (2012), cyazypyr 10 percent% OD did not cause phytotoxicity in the treated tomato crop, even at a dosage of 360 g a.i./ ha.

Conclusion

The results indicated that the insecticides cyantraniliprole 10.26 OD @ 75 g a.i. /ha and chlorantraniliprole 18.5 S @ 30 g a.i. /ha were successful in controlling leafminers in the potato environment. Additionally, it was discovered that none of the pesticides had any phototoxic effects on the ecosystem of potato during the trial. The method validation addressed the specificity, linearity, recovery, repeatability, and ruggedness. Therefore, both pesticides may be used at the required dosage to manage the Liriomyza huidobrensis leafminer effectively in the potato habitat.

Conflict of interest

The authors declare that they have no conflict of interest.

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Relative bioefficacy of different insecticides against sucking pest complex of tomato (*Lycopersicum esculentum* L.) and their effect on natural enemies present under field condition

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ABSTRACT

The field experiment was carried out on tomato in the *Rabi* season of 2019-20 at the Vegetable Research Farm, Institute of Agricultural Science, Banaras Hindu University, Varanasi, India. The crop variety, Arka Vikas (Selection 22) was selected to evaluate the bioefficacy of nine different insecticidal treatments against the sucking pest complex and the natural enemies in tomato under field conditions. Two sprays at 15 days intervals of ten treatments with three replications were applied. The treatments were Diafenthiuron 50% WP, Abamectin 1.8% EC, Buprofezin 25% SC, Indoxacarb 14.5% SC, Spinosad 45% SC, Chlorantraniliprole 18.5% SC, Pymetrozine 50% WG, Flonicamid 50% WG, and Lancer Gold (50 + 1.8) % SP and control (water spray). Observations were recorded one day before and 1st, 3rd, 5th, 7th, 10th, and 15th days after each spray. For controlling Whitefly (*B. tabaci*), Lancer gold was observed to be the best (76.98%), followed by Pymetrozine (69.03%) and Flonicamid (59.39%). At the same time, Flonicamid was excellent (70.62%) in controlling Aphids (*A. gossypii*), followed by Lancer gold (67.15%) and Pymetrozine (65.48%). In case of a reduction of damage by Thrips (*T. tabaci*), Lancer gold showed the best result (75.60%), followed by Buprofezin (68.45%) and Flonicamid (65.69%). However, all the treatments showed minute toxicity for the natural enemies; yet Flonicamid and Lancer gold were significantly safer among all of them.

Introduction

Tomato (*Lycopersicon esculentum* L.) is a vital crop among the different vegetables grown throughout the year in our country due to its high commercial and nutritional importance and its wide range of environmental versatility. Tomato has become an important food crop in the world in less than a century. Though tomato is considered as a vegetable, it is a fruit. After the potato, it is the second most widely produced vegetable globally. West Bengal, Andhra Pradesh, Bihar, Karnataka, Uttar Pradesh, Orissa, Maharashtra, Madhya Pradesh, and Assam are the leading states in our country in tomato production. With the growing demand for this vegetable crop in India and internationally, there has been a significant increase in the region where it was previously uninhabited. As a result, there has been a significant increase in previously documented pests and the emergence of novel invasive pests such as the South American tomato leaf miner, *Tuta absoluta* (Sridhar *et al.,* 2014). Among the many

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pests recorded in India, as many as sixteen have been observed feeding from germination through harvesting, reducing productivity while also degrading the quality. Fruit borer (*Helicoverpa armigera* Hub.), Aphid (*Aphis gossypii* Glover, *Myzus persicae* Sulzer), Jassid (*Amarasca biguttula* Ishida), Serpentine leaf miner (*Liriomyza trifolii* Burgess), Tobacco caterpillar (*Spodoptera litura* Fabricius), Whitefly (*Bemisia tabaci* Gennadius), Thrips (*Thrips tabaci* Lindeman), and Hadda beetle (*Epilachana dodecastigma*) are the most common insect pests of tomatoes. The sucking pests damage plant's cells by sucking the phloem sap directly (Abdel-Baky and Al-Deghairi, 2008), secreting honeydews, and transmitting a variety of viral infections (Khan and Ahmad, 2005). Among the various management methods of these pests, the use of plant products and chemical insecticides are the most popular. Crop protection agents from the organochlorine, organophosphate, and carbamate groups have been utilized to manage insect pests. However, the application of these insecticides produced a coating of persistent poison over the foliage and fruits (Dikshit *et al.,* 2000), and insects developed resistance to them (Cahill *et al.,* 1996; Kramer *et al*., 2012). Their widespread abuse and misuse have resulted in the three, viz: pesticide resistance, pest resurgence, and residues, as well as toxicity dangers to non-target species. As a result, newer compounds with a lower dose of a few grams per hectare must be used to replace these traditional pesticides. This study aimed to assess the efficacy of several pesticides to control the sucking pest complex of tomato, including Diafenthiuron, Abamectin, Buprofezin, Indoxacarb, Spinosad, Chlorantraniliprole, Pymetrozine, Flonicamid 50%, and Lancer Gold.

Material and Methods

The experimental investigation on the bio-efficacy of insecticides against sucking pests, jassid and thrips infesting tomato was carried out at the Vegetable Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India during *Rabi*, 2020, under field conditions adopting Randomized Block Design. Tomato variety Arka Vikas (Selection 22) of 8-10 cm length were transplanted from the nursery to the main field and standard agronomic practices were followed to raise the crop. The crop was grown without applying any insecticide either in the soil or as a seed treatment. The detail of the insecticides used for the study is described in table 1.

Table 1: List of Insecticides and their doses

SN	Insecticides	Formulations	Trade name	Group of Chemicals	Dosage (g a.i./ha)
1.	Diafenthiuron	50% WP	Pegasus	Thio-urea derivatives	300
2.	Abamectin	1.8% EC	Vertimec	Avermectins	10
3.	Buprofezin	25% SC	Appalaud	Thiadiaznes	250
4.	Indoxacarb	14.5% SC	Avaunt	Oxadiazines	300
5.	Spinosad	45% SC	Tracer	Spinosyns	73
6.	Chlorantraniliprole	18.5% SC	Coragen	Anthanilic Diamides	30
7.	Pymetrozine	50% WG	Chess	Pyridine Azomethine derivatives	200
8.	Flonicamid	50% WG	Ulala	Pyridine carboxamides	60
9.	Acephate 50% + Imidacloprid 1.8% SP	(50 + 1.8) % SP	Lancer gold	Mixture of OP and Neonicotinoid group	518

Application of treatments:

All the insecticides were applied as a foliar spray using a knapsack sprayer. The amount of spray fluid required per plot was estimated by spraying the control plot with water and calculating the required spray fluids. Spray fluid was made by combining a specific amount of water with a pesticide.

$$\text{Amount of formulation} = \frac{\text{Percentage of required concentration x volume required (lit)}}{\text{Concentration of toxicant in insecticide}}$$

The first spray was applied when the pest first reached its economic threshold level (ETL), and the second spray was applied 15 days following the first.

Spraying and observations taken:

Pest population observations were taken on five randomly selected plants before 24 hours and after 1, 3, 5, 7, 10, and 15 days of spraying. The populations were counted on five tagged plants from each plot with three leaves each from the top, three from the middle, and three from the bottom canopy of the plant, with the help of a hand lens. The percent reduction in pest population over control was calculated by using the following formula:

$$\textbf{Percent reduction in population} = \frac{x_1 - x_2}{x_1} \times 100$$

Where,
X_1 = population in control plots
X_2 = population in treated plots

The data were assembled to determine the mean pest infestation in the respective treatment and statistical analysis was used to determine the overall effect of each treatment, standard error, and CD at a 5% level of significance.

Results and Discussion

Effect of newer insecticides against the population of whitefly, *Bemisia tabaci* (Gennadius) in tomato:

First spray:

Table 2 shows that all insecticidal treatments were more effective at reducing whitefly populations than the untreated control. Pre-treatment whitefly populations were uniformly distributed throughout all plots, ranging from 4.20 - 4.80 (average number of whitefly) per plant. The whitefly population after one day of insecticidal spray varied between 3.60 and 4.67 per plant in different insecticidal treatments, while it was 5.07 per plant in the untreated control. After three and five days of spraying, the decreasing rate of the whitefly population remained the same with a range of 3.00 - 4.47 per plant and 1.80 - 4.13 per plant, respectively, and all the treatments were superior to control (5.73 and 6.67 per plant, respectively). After seven days of spraying, the mean population of whitefly gradually increased, and the same trend was also observed in case of ten and fifteen days after spraying. At seven, ten, and fifteen days, the mean population of whitefly varied from 2.40 - 4.73, 2.80 - 5.53, and 3.47 - 6.67 per plant, respectively, whereas in the untreated plot, this population was varied from 7.27, 8.00 and 8.27 per plant respectively. After fifteen days of first spraying, the overall mean population of whiteflies in all the treated plots varied between 3.04 - 5.00 per plant, which is significantly superior to the untreated control (6.58 per plant).After the first, third, fifth, seventh, tenth, and fifteenth day of spray, Lancer gold (T_9) was found to be significantly superior over the rest of the treatments, whereas Pymetrozine (T_7), Flonicamid (T_8), Buprofezin (T_3), Diafenthiuron (T_1), Abamectin (T_2) were found statistically at par with each other in most of the days. Indoxacarb 14.5% SC @ 300 g a.i ha^{-1} was inferior among all insecticidal treatments. Further, the mean percent reduction in whitefly population over control after the first, third, fifth, seventh, tenth and fifteenth days of spray was in descending order: Lancer gold (53.79%)>Pymetrozine(48.78%)>Flonicamid(43.31%)Buprofezin(40.42%)>Diafenthiuron(37.23%)>Abamectin(36.95%)>Spinosad(32.37%)>Chlorantraniliprole (31.30%)> Indoxacarb (24.01%).

Second spray:

The data presented in table 2 showed that all the insecticidal treatments were significantly superior to the untreated control in reducing the whitefly population after the second spray. The average number of whiteflies one day before the second insecticidal spray in different insecticidal treatments ranged from 3.47-8.87 per plant. The whitefly population after one day of insecticidal spray varied between 2.73 and 5.47 per plant in different insecticidal treatments, while it was 6.60 per plant in the untreated control. After three and five days after spraying, the decreasing rate of the whitefly population remained the same, and it ranged from 1.93-4.87 and 0.73-4.47 per plant, respectively, and all the treatments were superior to control (6.13 and 5.60 per plant respectively). Unlike the first spray, after seven days of the second spraying, the mean population of whitefly gradually decreased, and the same trend was also observed in ten and fifteen days after spraying. At seven, ten, and fifteen days, the mean population of whitefly varied from 0.33-4.00, 0.20-3.80, and 0.13-3.33 per plant, respectively, whereas in the untreated plot, this population was varied from 5.00, 4.73 and 4.47 per plant respectively. After fifteen days of first spraying, the overall mean population of whiteflies in all the treated plots varied between 1.36-4.66 per plant, which is significantly superior to the untreated control (5.91 per plant). After the first, third, fifth, seventh, tenth, and fifteenth day of spray, Lancer gold (T_9) was found to be significantly superior over the rest of the treatments,

Table 2: Effect of newer molecules of insecticides against the population of whitefly, *Bemisia tabaci* (Gennadius) in tomato after first and second application during 2019-20

Treat. No.	Treatments	Dose (g a.i. ha^{-1})	First spray									Second spray								
			Mean population of whitefly per plant*							Post-treatment mean	MRC (%)	Mean population of whitefly per plant*							Post-treatment mean	MRC (%)
			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS		
T_1	Diafenthiuron	300	4.73 (2.39)	4.40 (2.32)	3.87 (2.20)	3.13 (2.03)	3.67 (2.15)	4.20 (2.27)	4.93 (2.43)	4.13	37.23	4.93 (2.43)	4.53 (2.35)	4.07 (2.25)	3.13 (2.03)	2.40 (1.84)	1.80 (1.67)	1.20 (1.48)	3.15	46.70
T_2	Abamectin	10	4.60 (2.36)	4.40 (2.32)	4.13 (2.26)	3.40 (2.09)	3.93 (2.22)	4.33 (2.30)	5.13 (2.47)	4.28	36.95	5.13 (2.47)	4.67 (2.37)	4.27 (2.29)	3.60 (2.14)	3.07 (2.01)	2.47 (1.86)	1.80 (1.66)	3.57	39.59
T_3	Buprofezin	250	4.47 (2.33)	4.20 (2.28)	3.73 (2.17)	2.87 (1.96)	3.47 (2.11)	4.00 (2.23)	4.73 (2.39)	3.92	40.42	4.73 (2.39)	4.13 (2.26)	3.67 (2.15)	2.87 (1.96)	2.07 (1.74)	1.47 (1.56)	0.93 (1.38)	2.84	51.94
T_4	Indoxacarb	300	4.80 (2.40)	4.67 (2.38)	4.47 (2.33)	4.13 (2.26)	4.73 (2.39)	5.53 (2.55)	6.67 (2.76)	5.00	24.01	6.67 (2.76)	5.47 (2.54)	4.87 (2.42)	4.47 (2.33)	4.00 (2.23)	3.80 (2.19)	3.33 (2.08)	4.66	21.15
T_5	Spinosad	73	4.80 (2.40)	4.60 (2.36)	4.27 (2.29)	3.60 (2.14)	4.07 (2.25)	4.53 (2.35)	5.27 (2.50)	4.45	32.37	5.27 (2.50)	4.93 (2.43)	4.40 (2.32)	4.00 (2.23)	3.40 (2.09)	3.00 (2.00)	2.40 (1.84)	3.91	33.84
T_6	Clorantraniliprole	30	4.67 (2.38)	4.60 (2.36)	4.40 (2.32)	3.60 (2.14)	4.27 (2.29)	4.73 (2.39)	5.40 (2.53)	4.52	31.30	5.40 (2.53)	5.13 (2.47)	4.60 (2.36)	4.20 (2.28)	3.93 (2.22)	3.80 (2.19)	3.00 (2.00)	4.30	27.24
T_7	Pymetrozine	200	4.27 (2.29)	3.87 (2.20)	3.40 (2.09)	2.20 (1.78)	2.80 (1.94)	3.20 (2.04)	3.87 (2.20)	3.37	48.78	3.87 (2.20)	3.27 (2.06)	2.53 (1.87)	1.60 (1.60)	0.73 (1.31)	0.47 (1.20)	0.33 (1.15)	1.83	69.03
T_8	Flonicamid	60	4.27 (2.29)	4.00 (2.23)	3.60 (2.14)	2.67 (1.91)	3.33 (2.08)	3.87 (2.200	4.40 (2.32)	3.73	43.31	4.40 (2.32)	3.93 (2.22)	3.13 (2.03)	2.13 (1.76)	1.60 (1.61)	1.00 (1.41)	0.60 (1.26)	2.40	59.39
T_9	Lancer gold	518	4.20 (2.28)	3.60 (2.14)	3.00 (2.00)	1.80 (1.67)	2.40 (1.84)	2.80 (1.94)	3.47 (2.11)	3.04	53.79	3.47 (2.11)	2.73 (1.93)	1.93 (1.71)	0.73 (1.31)	0.33 (1.15)	0.20 (1.09)	0.13 (1.06)	1.36	76.98
T_{10}	Control	-	4.47 (2.33)	5.07 (2.46)	5.73 (2.59)	6.67 (2.76)	7.27 (2.87)	8.00 (3.00)	8.87 (3.14)	6.58	-	8.87 (3.14)	6.60 (2.75)	6.13 (2.67)	5.60 (2.56)	5.00 (2.44)	4.73 (2.39)	4.47 (2.33)	5.91	-
SEm±			0.048	0.045	0.043	0.060	0.047	0.041	0.039			0.039	0.043	0.043	0.051	0.047	0.05	0.055		
CD @ 5%			NS	0.134	0.127	0.180	0.141	0.122	0.116			0.116	0.130	0.129	0.153	0.141	0.149	0.165		

PTC- Pre-treatment count;
DAS- Days after spraying;
MRC – Mean reduction over control;
*** Mean of three replications**
Values in parenthesis are square root transformed values;
NS- non-significant

whereas Pymetrozine (T_7), Flonicamid (T_8), Buprofezin (T_3), Diafenthiuron (T_1), Abamectin (T_2) were found statistically at par with each other in most of the days. Indoxacarb 14.5% SC @ 300 g a.i ha^{-1} was inferior among all insecticidal treatments. Further, the mean percent reduction in whitefly population over control after the first, third, fifth, seventh, tenth and fifteenth days of spray was in descending order: Lancer gold (76.98%)> Pymetrozine(69.03%)>Flonicamid(59.39%)>Buprofezin(51.94%)>Diafenthiuron(46.70%)> Abamectin (39.59%)>Spinosad(33.84%)>Chlorantraniliprole(27.24%)>Indoxacarb (21.15%).These findings were strongly supported by Kar (2017) remarking that the whitefly population became zero after three continuous sprayings of Imidacloprid 17.8 % SL@ 175 ml ha $^{-1}$. Dhar and Bhattacharya (2015) also recorded that spraying Imidacloprid 17.8 % SL for once followed by spraying Spinosad (45 % SC) twice resulted in the highest reduction of whitefly infestation in both in okra and tomato.

Effect of newer insecticides against the population of aphid, *Aphis gossypii* Glover, in tomato: First spray:

The data shown in table 3 revealed that the insect population in all experimental plots were similar (varied from 20.13-21.00 per plant) prior to the imposition of treatments. After spraying, all the test insecticides were determined to be significantly better than the untreated control. The order of effectiveness of various treatments was similar or consistent at various intervals after spraying, with the lowest whitefly population (overall mean value) (irrespective of days after spraying (DAS)) being observed in Flonicamid 50% WG (13.18 aphids/plant) followed by Lancer Gold (50 + 1.8) % SP (13.87 aphids/plant), Pymetrozine 50% WG (14.00 aphids/plant), Diafenthiuron 50% WP (15.19 aphids/plant), which in turn were at par with each other. The next group in terms of effectiveness consisted of Buprofezin 25% SC (15.37 aphids/plant) followed by Abamectin 1.8% EC (15.72 aphids/plant), Spinosad 45% SC (16.29 aphids/plant) and Indoxacarb 14.5% SC (18.50 aphids/plant). On the other hand, the untreated control plot recorded the highest aphid population at 35.74 per plant. The overall mean per cent reduction in aphid population over control was in descending order: Flonicamid 50% WG (63.12%)> Lancer Gold (50 + 1.8) % SP (61.19%)> Pymetrozine 50% WG (60.82%)> Diafenthiuron 50% WP (57.49%)> Buprofezin 25% SC (56.99%)> Abamectin 1.8% EC (56.01%)> Spinosad 45% SC (54.42%)> Chlorantraniliprole 18.5% SC (52.23%)> Indoxacarb 14.5% SC (48.23%).

Second spray:

The data shown in table 3 revealed that the insect population in all experimental plots varied from 17.33-45.33 per plant before the imposition of treatments. After spraying, all the test insecticides were determined to be significantly better than the untreated control. The order of effectiveness of various treatments were similar or consistent at various intervals after spraying, with the lowest whitefly population (overall mean value) (irrespective of DAS) being observed in Flonicamid 50% WG @ 60 g a.i ha^{-1} (8.64 aphids/plant) followed by Lancer Gold (50 + 1.8) % SP (9.66 aphids/plant), Pymetrozine 50% WG (10.15 aphids/plant), Diafenthiuron 50% WP (10.65 aphids/plant). The next group in terms of effectiveness consisted of Buprofezin 25% SC (10.73 aphids/plant), followed by Abamectin 1.8% EC (11.03 aphids/plant), Spinosad 45% SC (11.50 aphids/plant) and Indoxacarb 14.5% SC (13.68 aphids/plant), which in turn were at par with each other. On the other hand, the untreated control plot recorded the highest aphid population at 29.41 per plant. The overall mean per cent reduction in aphid population over control was in descending order: Flonicamid 50% WG (70.62%)> Lancer Gold (50 + 1.8) % SP (67.15%)> Pymetrozine 50% WG (65.48%)> Diafenthiuron 50% WP (63.78%)> Buprofezin 25% SC (63.51%)> Abamectin 1.8% EC (62.49%)> Spinosad 45% SC (60.89%)> Chlorantraniliprole 18.5% SC (56.88%)> Indoxacarb 14.5% SC (53.48%). Similarly, Joost *et al.* (2006) observed that aphids and plant bugs cease their feeding within 15 to 30 minutes upon exposure to Flonicamid and subsequently recorded the death of these insects after some time depending upon the existing environmental conditions. Morita *et al.* (2007) said that the nymphs born from adults when exposed to Flonicamid for 3 hours displayed significantly higher mortality. Koo *et al.* (2014) noted the effects of sublethal exposure to Flonicamid and Imidacloprid and the mechanisms by which these insecticides affect the feeding behaviour of *A.*

gossypii. The lowest net reproductive rate was recorded in *A. gossypii* treated with the LC_{30} of Flonicamid in their result.

Effect of newer insecticides against the population of thrips, *Thrips tabaci* Lindeman, in tomato:First spray:

The thrips population was nearly uniform one day before treatment was imposed. table 4 shows that all insecticidal treatments were much more effective at reducing thrips populations than the untreated control. Pre-treatment thrips populations were uniformly distributed throughout all plots, ranging from 5.60 to 5.80 (average number of thrips) per plant. The thrips population after one day after insecticidal spray varied between 4.47 and 5.53 per plant in different insecticidal treatments, while it was 5.87 per plant in the untreated control. After three and five days after spraying, the decreasing rate of the thrips population remained the same. It ranged from 3.53-4.80 and 1.73-4.00 per plant, respectively, and all the treatments were superior to the control (6.20 and 6.47 per plant, respectively). After seven days of spraying, the mean population of thrips gradually increases, and the same trend is also observed in ten and fifteen days after spraying. At seven, ten, and fifteen days, the mean population of thrips varied from 2.27-4.47, 2.67-4.60, and 3.13-5.40 per plant, respectively, whereas in the untreated plot, this population was varied from 6.73, 7.00 and 7.33 per plant respectively. After fifteen days of first spraying, the overall mean population of thrips in all the treated plots varied between 3.36-4.90 per plant, which is significantly much more superior to the untreated control (6.46 per plant).After the first, third, fifth, seventh, tenth, and fifteenth day of spray, Lancer gold (T_9) was found to be significantly superior over the rest of the treatments, followed by Buprofezin (T_3), Flonicamid (T_8), Pymetrozine (T_7), Diafenthiuron (T_1), Spinosad (T_5), Abamectin (T_2), Chlorantraniliprole (T_6) were found statistically at par with each other in most of the days. Indoxacarb 14.5% SC @ 300 g a.i ha^{-1} was inferior among all insecticidal treatments. Further, the mean per cent reduction in whitefly population over control after first, third, fifth, seventh, tenth and fifteenth days of spray was in descending order: Lancer gold (47.98%) > Buprofezin (42.41%) > Flonicamid(38.85)>Pymetrozine(34.98%)>Diafenthiuron(32.19%)>Spinosad(30.49%)>Abamectin(29.72%) > Chlorantraniliprole(26.78%)> Indoxacarb (24.14%).

Second spray:

The thrips population was nearly uniform one day before treatment was imposed. table 4 shows that all insecticidal treatments were much more effective at reducing thrips populations than the untreated control. Pre-treatment thrips populations ranged from 3.13 to 7.33 (average number of thrips) per plant. After one day after insecticidal spray, the population of thrips varied between 2.60 and 4.87 per plant in different insecticidal treatments, while it was 7.40 thrips per plant in the untreated control. After three and five days after spraying, the decreasing rate of the thrips population remained the same. It ranged from 2.07-4.20 and 1.27-3.80 per plant, respectively, and all the treatments were superior to the control (7.00 and 6.53 per plant, respectively). The decreasing trend of the thrips population was also observed on seven, ten, and fifteen days after spraying. At seven, ten, and fifteen days, the mean population of thrips varied from 0.73-3.40, 0.40-3.00, and 0.27-2.87 per plant, respectively, whereas in the untreated plot, this population was varied from 5.53, 4.93 and 4.33 per plant respectively. After fifteen days of first spraying, the overall mean population of thrips in all the treated plots varied between 1.50-3.93 per plant, which was significantly much more superior to the untreated control (6.15 per plant).After the first, third, fifth, seventh, tenth, and fifteenth day of spray, Lancer gold (T_9) was found to be significantly superior over the rest of the treatments. Indoxacarb 14.5% SC @ 300 g a.i ha^{-1} was inferior among all the insecticidal treatments. Further, the mean per cent reduction in whitefly population over control after first, third, fifth, seventh, tenth and fifteenth days of spray was in descending order: Lancer gold (75.60%)>Buprofezin(68.45%)>Flonicamid(65.69) > Pymetrozine (61.30%)> Diafenthiuron (53.98%)> Spinosad(51.54%)>Abamectin(45.36%)> hlorantraniliprole (43.25%)>Indoxacarb(36.09%). These results show similarity with previous works like, Vikas *et al.* (2005) reported, the green chilli yield was highest from the plots applied with imidacloprid and gave significantly higher green chilli yield than the other insecticides. Dey *et al.* (2005) also stated that Imidacloprid 70 WS successfully controlled the initial sucking pest complex of okra when applied as a seed treatment.

Table 3: Effect of newer molecules of insecticides against the population of aphid, *Aphis gossypii* Glover in tomato after first and second application during 2019-20

Treat. No.	Treatments	Dose (g a.i. ha^{-1})	First spray: Mean population of aphid per plant* PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS	Post-treatment mean	MRC (%)	Second spray: Mean population of aphid per plant* PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS	Post-treatment mean	MRC (%)
T_1	**Diafenthiuron**	300	20.47 (4.63)	16.27 (5.15)	12.07 (3.61)	6.87 (2.80)	13.40 (3.79)	16.87 (4.22)	20.40 (4.62)	15.19	57.49	20.40 (4.62)	18.47 (4.41)	14.67 (3.95)	8.87 (3.14)	6.27 (2.69)	4.27 (2.29)	1.60 (1.60)	10.65	63.78
T_2	**Abamectin**	10	20.13 (4.59)	17.20 (4.26)	13.60 (3.82)	7.33 (2.88)	13.93 (3.86)	18.13 (4.37)	19.73 (4.55)	15.72	56.01	19.73 (4.55)	18.80 (4.45)	15.00 (4.00)	9.40 (3.22)	7.07 (2.83)	4.80 (2.40)	2.40 (1.83)	11.03	62.49
T_3	**Buprofezin**	250	20.33 (4.61)	16.67 (4.20)	13.27 (3.77)	6.93 (2.81)	13.60 (3.82)	17.87 (4.34)	18.93 (4.46)	15.37	56.99	18.93 (4.46)	18.60 (4.42)	14.80 (3.97)	9.27 (3.20)	6.80 (2.78)	4.53 (2.35)	2.20 (1.78)	10.73	63.51
T_4	**Indoxacarb**	300	20.33 (4.61)	18.87 (4.45)	17.33 (4.28)	15.13 (4.01)	16.33 (4.16)	19.47 (4.52)	22.07 (4.80)	18.50	48.23	22.07 (4.80)	20.73 (4.66)	17.20 (4.26)	13.40 (3.79)	10.13 (3.33)	7.73 (2.95)	4.47 (2.33)	13.68	53.48
T_5	**Spinosad**	73	20.53 (4.64)	17.67 (4.32)	13.93 (3.86)	8.67 (3.12)	14.27 (3.90)	18.80 (4.45)	20.13 (4.59)	16.29	54.42	20.13 (4.59)	18.87 (4.45)	16.00 (4.12)	10.07 (3.32)	7.47 (2.90)	5.13 (2.47)	2.80 (1.94)	11.50	60.89
T_6	**Clorantraniliprole**	30	21.93 (4.78)	18.20 (4.38)	14.27 (3.90)	8.73 (3.11)	15.73 (4.09)	19.00 (4.47)	21.60 (4.75)	17.07	52.23	21.60 (4.75)	20.73 (4.66)	16.73 (4.21)	11.93 (3.59)	8.47 (3.07)	5.93 (2.63)	3.33 (2.07)	12.68	56.88
T_7	**Pymetrozine**	200	19.80 (4.56)	16.33 (4.16)	10.13 (3.33)	6.40 (2.71)	10.53 (3.39)	16.00 (4.12)	18.80 (4.45)	14.00	60.82	18.80 (4.45)	18.20 (4.38)	14.40 (3.92)	8.13 (3.01)	5.93 (2.62)	4.07 (2.25)	1.53 (1.59)	10.15	65.48
T_8	**Flonicamid**	60	20.27 (4.60)	15.53 (4.06)	9.53 (3.24)	4.93 (2.43)	9.93 (3.30)	14.73 (3.96)	17.33 (4.28)	13.18	63.12	17.33 (4.28)	16.53 (4.18)	11.00 (3.46)	7.47 (2.90)	4.87 (2.42)	2.47 (1.86)	0.80 (1.33)	8.64	70.62
T_9	**Lancer gold**	518	21.00 (4.69)	16.27 (4.15)	9.87 (3.29)	5.93 (2.63)	10.27 (3.35)	15.67 (4.08)	18.07 (4.36)	13.87	61.19	18.07 (4.36)	17.73 (4.32)	13.53 (3.81)	8.07 (3.01)	5.47 (2.54)	3.60 (2.14)	1.13 (1.45)	9.66	67.15
T_{10}	**Control**	-	20.07 (4.58)	28.27 (5.40)	33.13 (5.83)	37.73 (6.220	41.80 (6.53)	43.87 (6.69)	45.33 (6.80)	35.74	-	45.33 (6.80)	41.13 (6.48)	37.20 (6.17)	29.07 (5.47)	22.60 (4.84)	18.07 (4.36)	12.47 (3.66)	29.41	-
SEm±			0.054	0.044	0.052	0.068	0.087	0.076	0.071			0.071	0.076	0.085	0.078	0.104	0.074	0.101		
CD @ 5%			NS	0.132	0.154	0.204	0.261	0.227	0.212			0.212	0.226	0.254	0.235	0.311	0.221	0.303		

Table 4: Effect of newer molecules of insecticides against the population of thrips, *Thrips tabaci* Lindeman in tomato after first and second application during 2019-20

Treat. No.	Treatments	Dose (g a.i. ha^{-1})	First spray									Second spray								
			Mean population of thrips per plant*							Post-treatment mean	MRC (%)	Mean population of thrips per plant*							Post-treatment mean	MRC (%)
			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS		
T_1	Diafenthiuron	300	5.80 (2.60)	5.33 (2.51)	4.33 (2.30)	3.13 (2.03)	3.47 (2.11)	3.93 (2.22)	4.67 (2.38)	4.38	32.19	4.67 (2.38)	4.00 (2.23)	3.27 (2.06)	2.93 (1.98)	2.07 (1.74)	1.60 (1.60)	1.27 (1.49)	2.83	53.98
T_2	Abamectin	10	5.47 (2.54)	5.27 (2.50)	4.47 (2.33)	3.40 (2.09)	3.87 (2.20)	4.27 (2.29)	5.07 (2.46)	4.54	29.72	5.07 (2.46)	4.60 (2.36)	3.73 (2.16)	3.27 (2.05)	2.73 (1.92)	2.27 (1.79)	1.87 (1.68)	3.36	45.36
T_3	Buprofezin	250	5.67 (2.58)	4.67 (2.37)	3.67 (2.15)	2.27 (1.80)	2.87 (1.96)	3.33 (2.08)	3.60 (2.14)	3.72	42.41	3.60 (2.14)	3.13 (2.03)	2.53 (1.87)	1.73 (1.65)	1.20 (1.48)	0.80 (1.34)	0.60 (1.26)	1.94	68.45
T_4	Indoxacarb	300	5.67 (2.58)	5.33 (2.51)	4.80 (2.40)	4.00 (2.23)	4.47 (2.33)	4.60 (2.36)	5.40 (2.52)	4.90	24.14	5.40 (2.52)	4.87 (2.42)	4.20 (2.27)	3.80 (2.19)	3.40 (2.09)	3.00 (2.00)	2.87 (1.96)	3.93	36.09
T_5	Spinosad	73	5.80 (2.60)	5.33 (2.51)	4.53 (2.35)	3.40 (2.09)	3.67 (2.15)	4.00 (2.23)	4.67 (2.37)	4.49	30.49	4.67 (2.37)	4.13 (2.64)	3.27 (2.06)	2.93 (1.98)	2.40 (1.84)	1.93 (1.70)	1.53 (1.59)	2.98	51.54
T_6	Clorantraniliprole	30	5.73 (2.59)	5.53 (2.55)	4.80 (2.4)	3.67 (2.15)	4.07 (2.25)	4.33 (2.30)	5.00 (2.44)	4.73	26.78	5.00 (2.44)	4.60 (2.36)	3.93 (2.22)	3.47 (2.11)	2.87 (1.96)	2.33 (1.82)	2.20 (1.78)	3.49	43.25
T_7	Pymetrozine	200	5.60 (2.56)	5.13 (2.47)	4.20 (2.27)	2.80 (1.94)	3.40 (2.09)	3.93 (2.22)	4.33 (2.30)	4.20	34.98	4.33 (2.30)	3.47 (2.10)	2.87 (1.96)	2.13 (1.76)	1.67 (1.62)	1.20 (1.47)	1.00 (1.40)	2.38	61.30
T_8	Flonicamid	60	5.73 (2.59)	4.93 (2.43)	3.93 (2.22)	2.47 (1.85)	3.07 (2.01)	3.60 (2.14)	3.93 (2.22)	3.95	38.85	3.93 (2.22)	3.27 (2.06)	2.53 (1.87)	1.87 (1.68)	1.40 (1.54)	1.00 (1.41)	0.80 (1.34)	2.11	65.69
T_9	Lancer gold	518	5.73 (2.59)	4.47 (2.33)	3.53 (2.12)	1.73 (1.65)	2.27 (1.80)	2.67 (1.91)	3.13 (2.03)	3.36	47.98	3.13 (2.03)	2.60 (1.89)	2.07 (1.75)	1.27 (1.50)	0.73 (1.31)	0.40 (1.18)	0.27 (1.12)	1.50	75.60
T_{10}	Control	-	5.60 (2.56)	5.87 (2.62)	6.20 (2.68)	6.47 (2.73)	6.73 (2.78)	7.00 (2.82)	7.33 (2.88)	6.46	-	7.33 (2.88)	7.40 (2.89)	7.00 (2.82)	6.53 (2.74)	5.53 (2.55)	4.93 (2.43)	4.33 (2.30)	6.15	-
SEm±			0.039	0.028	0.051	0.058	0.056	0.046	0.047			0.047	0.059	0.072	0.081	0.084	0.078	0.074		
CD @ 5%			NS	0.085	0.152	0.174	0.168	0.138	0.142			0.142	0.176	0.215	0.241	0.252	0.233	0.223		

Table 5: Effect of newer molecules of insecticides against the population of spiders in tomato after first and second application during 2019-20

Treat. No.	Treatments	Dose (g a.i. ha^{-1})	First spray									Second spray								
			Mean population of spiders per plant*							Post-treatment mean	MRC (%)	Mean population of spiders per plant*							Post-treatment mean	MRC (%)
			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS		
T_1	**Diafenthiuron**	300	4.00 (2.22)	3.53 (2.12)	2.80 (1.94)	3.13 (2.02)	3.60 (2.13)	3.80 (2.18)	4.47 (2.33)	3.62	4.00 (2.22)	4.47 (2.33)	3.93 (2.21)	3.60 (2.13)	3.20 (2.03)	2.87 (1.95)	2.40 (1.83)	2.00 (1.71)	3.21	4.47 (2.33)
T_2	**Abamectin**	10	3.93 (2.22)	3.40 (2.09)	2.73 (1.92)	3.07 (2.01)	3.27 (2.06)	3.53 (2.12)	4.00 (2.23)	3.42	3.93 (2.22)	4.00 (2.23)	3.73 (2.17)	3.13 (2.03)	2.87 (1.96)	2.53 (1.88)	2.40 (1.84)	1.73 (1.65)	2.91	4.00 (2.23)
T_3	**Buprofezin**	250	3.80 (2.17)	3.53 (2.11)	3.07 (1.99)	3.27 (2.04)	3.60 (2.13)	3.87 (2.18)	4.67 (2.36)	3.69	3.80 (2.17)	4.67 (2.36)	4.33 (2.29)	3.87 (2.18)	3.87 (2.19)	3.20 (2.03)	2.93 (1.97)	2.67 (1.90)	3.65	4.67 (2.36)
T_4	**Indoxacarb**	300	3.47 (2.19)	2.07 (1.75)	1.93 (1.71)	2.53 (2.87)	2.87 (1.96)	3.47 (2.11)	3.93 (2.22)	2.90	3.47 (2.19)	3.93 (2.22)	2.93 (1.97)	2.13 (2.70)	1.73 (1.65)	1.60 (1.61)	1.33 (1.52)	0.87 (1.36)	2.08	3.93 (2.22)
T_5	**Spinosad**	73	3.80 (2.17)	3.40 (2.08)	2.93 (1.97)	3.13 (2.02)	3.47 (2.10)	3.87 (2.19)	4.40 (2.31)	3.57	3.80 (2.17)	4.40 (2.31)	3.73 (2.17)	3.13 (2.02)	2.60 (1.89)	2.13 (1.76)	1.73 (1.64)	1.47 (1.56)	2.74	4.40 (2.31)
T_6	**Clorantraniliprole**	30	3.73 (2.17)	3.20 (2.04)	2.53 (1.87)	2.80 (1.94)	3.07 (2.01)	3.40 (2.09)	4.13 (2.26)	3.27	3.73 (2.17)	4.13 (2.26)	3.27 (2.06)	2.40 (1.83)	2.13 (1.76)	1.80 (1.67)	1.73 (1.65)	1.27 (1.50)	2.39	4.13 (2.26)
T_7	**Pymetrozine**	200	3.87 (2.19)	3.53 (2.12)	2.93 (1.97)	3.07 (2.00)	3.60 (2.13)	3.80 (2.18)	4.67 (2.37)	3.64	3.87 (2.19)	4.67 (2.37)	4.07 (2.24)	3.60 (2.14)	3.27 (2.05)	2.93 (1.97)	2.60 (1.89)	2.27 (1.79)	3.34	4.67 (2.37)
T_8	**Flonicamid**	60	3.93 (2.22)	3.73 (2.17)	3.67 (2.15)	3.67 (2.15)	4.07 (2.25)	4.60 (2.36)	4.93 (2.43)	4.09	3.93 (2.22)	4.93 (2.43)	4.27 (2.29)	4.07 (2.25)	3.87 (2.20)	3.60 (2.14)	3.47 (2.11)	3.40 (2.09)	3.94	4.93 (2.43)
T_9	**Lancer gold**	518	4.07 (2.24)	3.80 (2.19)	3.47 (2.11)	3.67 (2.16)	3.93 (2.22)	4.13 (2.26)	4.47 (2.33)	3.93	4.07 (2.24)	4.47 (2.33)	4.33 (2.30)	3.93 (2.22)	3.67 (2.15)	3.40 (2.09)	3.33 (2.08)	3.07 (2.01)	3.74	4.47 (2.33)
T_{10}	**Control**	-	3.73 (2.17)	5.27 (2.50)	5.47 (2.54)	5.60 (2.56)	5.67 (2.58)	6.00 (2.64)	6.60 (2.75)	5.48	3.73 (2.17)	6.60 (2.75)	6.67 (2.76)	6.47 (2.73)	5.87 (2.61)	5.20 (2.48)	4.60 (2.36)	4.27 (2.29)	5.67	6.60 (2.75)
SEm±			0.124	0.111	0.12	0.110	0.104	0.104	0.096			0.096	0.097	0.102	0.097	0.099	0.092	0.081		
CD @ 5%			NS	0.333	0.361	0.329	0.312	0.310	0.286			0.286	0.290	0.307	0.291	0.295	0.275	0.242		

Table 6: Effect of newer molecules of insecticides against the population of coccinellids in tomato after first and second application during 2019-20

Treat. No.	Treatments	Dose (g a.i. ha^{-1})	First spray									Second spray								
			Mean population of coccinellids per plant*							Post-treatment mean	MRC (%)	Mean population of coccinellids per plant*							Post-treatment mean	MRC (%)
			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS			PTC	1DAS	3DAS	5DAS	7DAS	10DAS	15DAS		
T_1	Diafenthiuron	300	2.93 (1.98)	2.53 (1.87)	2.20 (1.78)	2.27 (1.80)	2.47 (1.86)	2.87 (1.96)	3.33 (2.08)	2.08	2.93 (1.98)	3.33 (2.08)	2.67 (1.91)	2.33 (1.82)	2.27 (1.80)	2.00 (1.73)	1.80 (1.67)	1.60 (1.61)	2.29	3.33 (2.08)
T_2	Abamectin	10	2.87 (1.96)	2.20 (1.78)	1.73 (1.65)	2.07 (1.75)	2.33 (1.82)	2.53 (1.87)	3.00 (1.99)	1.99	2.87 (1.96)	3.00 (1.99)	2.53 (1.87)	2.13 (1.76)	1.80 (1.67)	1.53 (1.59)	1.47 (1.57)	1.33 (1.52)	1.97	3.00 (1.99)
T_3	Buprofezin	250	3.80 (2.17)	2.87 (1.94)	2.53 (1.85)	2.40 (1.82)	2.67 (1.90)	3.13 (2.02)	3.87 (2.20)	2.20	3.80 (2.17)	3.87 (2.20)	3.13 (2.03)	2.73 (1.93)	2.47 (1.86)	2.27 (1.80)	2.07 (1.75)	1.87 (1.69)	2.63	3.87 (2.20)
T_4	Indoxacarb	300	2.93 (1.98)	1.87 (1.69)	1.47 (1.56)	1.60 (1.61)	1.67 (1.63)	1.73 (1.65)	2.13 (1.76)	1.76	2.93 (1.98)	2.13 (1.76)	1.67 (1.63)	1.33 (1.52)	1.27 (1.50)	1.07 (1.43)	1.00 (1.41)	0.93 (1.38)	1.34	2.13 (1.76)
T_5	Spinosad	73	3.00 (2.00)	2.00 (1.72)	1.67 (1.62)	1.93 (1.70)	2.27 (1.80)	2.53 (1.87)	3.00 (1.99)	1.99	3.00 (2.00)	3.00 (1.99)	2.33 (1.82)	1.93 (1.70)	1.67 (1.63)	1.53 (1.59)	1.40 (1.54)	1.13 (1.45)	1.86	3.00 (1.99)
T_6	Clorantraniliprole	30	2.80 (1.94)	1.93 (1.71)	1.60 (1.60)	1.67 (1.62)	1.93 (1.71)	2.20 (1.78)	2.60 (1.89)	1.89	2.80 (1.94)	2.60 (1.89)	2.00 (1.72)	1.67 (1.62)	1.47 (1.56)	1.33 (1.52)	1.20 (1.48)	1.13 (1.45)	1.63	2.60 (1.89)
T_7	Pymetrozine	200	2.80 (1.94)	2.53 (1.87)	2.20 (1.78)	2.27 (1.80)	2.53 (1.87)	3.00 (1.99)	3.53 (2.12)	2.12	2.80 (1.94)	3.53 (2.12)	2.87 (1.96)	2.47 (1.85)	2.27 (1.80)	2.07 (1.73)	2.00 (1.71)	1.93 (1.69)	2.45	3.53 (2.12)
T_8	Flonicamid	60	3.07 (2.01)	2.93 (1.97)	2.60 (1.89)	2.53 (1.87)	3.13 (2.03)	3.73 (2.17)	4.20 (2.27)	2.27	3.07 (2.01)	4.20 (2.27)	3.40 (2.09)	3.00 (1.99)	2.93 (1.97)	2.73 (1.92)	2.47 (1.85)	2.33 (1.82)	3.00	4.20 (2.27)
T_9	Lancer gold	518	3.27 (2.06)	2.87 (1.69)	2.60 (1.89)	2.47 (1.85)	2.73 (1.92)	3.13 (2.03)	3.93 (2.21)	2.21	3.27 (2.06)	3.93 (2.21)	3.20 (2.04)	2.87 (1.96)	2.73 (1.92)	2.53 (1.87)	2.40 (1.83)	2.20 (1.78)	2.84	3.93 (2.21)
T_{10}	Control	-	3.47 (2.10)	3.73 (2.17)	4.00 (2.23)	4.20 (2.27)	4.40 (2.32)	4.60 (2.36)	4.87 (2.42)	4.18	3.47 (2.10)	4.87 (2.42)	5.00 (2.44)	4.60 (2.36)	4.33 (2.30)	4.00 (2.23)	3.80 (2.19)	3.67 (2.16)	4.32	4.87 (2.42)
SEm±			0.097	0.095	0.103	0.102	0.082	0.08	0.083			0.083	0.078	0.078	0.070	0.075	0.069	0.073		
CD @ 5%			NS	0.284	0.308	0.305	0.246	0.238	0.249			0.249	0.235	0.235	0.210	0.224	0.208	0.219		

Impact of insecticides on the natural enemies present in tomato fields:

Effect of newer insecticides on the population of spiders:

The average number of spiders per plant was observed one day before the 1st spray, which non-significantly varied from 3.73 to 4.07 spiders per plant (table 5). The highest spider population (overall mean value) (irrespective of DAS) was observed in Flonicamid (4.09 spiders per plant), followed by Lancer Gold (3.39 spiders per plant), Buprofezin (3.69 spiders per plant), Pymetrozine (3.64 spiders per plant), Diafenthiuron (3.62 spiders per plant), Abamectin (3.42 spiders per plant), Spinosad (3.57 spiders per plant), Chlorantraniliprole (3.27 spiders per plant) and Indoxacarb (2.90 spiders per plant). Thus, data on the mortality of these predators showed a slight decrease in the spider population in all insecticide-treated plots than the untreated plots (5.48 spiders per plant), which are more or less at par with each other. A more or less similar trend of insecticidal effect was also observed at 2nd spray (table 5), where the highest mean population of spiders was found in Flonicamid (3.94 spiders per plant), followed by Lancer Gold (3.74 spiders per plant), Buprofezin (3.65 spiders per plant), Pymetrozine (3.34 spiders per plant), Diafenthiuron (3.21 spiders per plant), Abamectin (2.91 spiders per plant), Spinosad (2.74 spiders per plant), Chlorantraniliprole (2.39 spiders per plant) and Indoxacarb (2.08 spiders per plant).

Effect of newer insecticides on the population of coccinellids:

The average number of spiders per plant was observed one day before the 1st spray, which non-significantly varied from 2.80 to 3.80 coccinellids per plant (table 6). The highest spider population (overall mean value) (irrespective of DAS) was observed in Flonicamid (2.27 coccinellids per plant), followed by Lancer Gold (2.21 coccinellids per plant), Buprofezin (2.20 coccinellids per plant), Pymetrozine (2.12 coccinellids per plant), Diafenthiuron (2.08 coccinellids per plant), Abamectin (1.99 coccinellids per plant), Spinosad (1.99 coccinellids per plant), Chlorantraniliprole (1.89 spiders per plant) and Indoxacarb (1.76 spiders per plant). Thus, data on the mortality of these predators showed a slight decrease in the spider population in all insecticide-treated plots than the untreated plots (4.18 spiders per plant), which are more or less at par with each other. A more or less similar trend of insecticidal effect was also observed at 2nd spray, where the highest mean population of spiders was found in Flonicamid (3.00 coccinellids per plant), followed by Lancer Gold (2.84 coccinellids per plant), Buprofezin (2.63 coccinellids per plant), Pymetrozine (2.45 coccinellids per plant), Diafenthiuron (2.29 coccinellids per plant), Abamectin (1.97 coccinellids per plant), Spinosad (1.86 coccinellids per plant), Chlorantraniliprole (1.63 spiders per plant) and Indoxacarb (1.34 spiders per plant) (table 6).Thus, based on the overall mean of the natural enemies' population after 1st and 2nd spray during the investigation, Flonicamid 50% WG and Lancer Gold (50 + 1.8) % SP appeared to be the safest treatment for the spider. In contrast, Indoxacarb 14.5% SC and Chlorantraniliprole 18.5% SC produced significantly higher mortality among all of the treatments. These findings nearly agree with the previous report of Morita *et al.* (2000) who also observed Flonicamid with a very favourable toxicological, environmental, and ecotoxic profile and showed no foremost negative impact on beneficial insects and mites such as *Bombyx mori*, *Apis mellifera*, *Harmonia axyridis*, and *Phytoseiulus persimilis.*

Conclusion

Among the numerous insect pests attacking tomato from transplanting to harvesting, the sucking pests are the prime reason for reducing the fruit yield directly by feeding and indirectly transmitting the notorious plant diseases. To conclude, we can say that the findings indicate all the pesticide treatments including plant products, were more successful than the control in lowering sucking-pest populations. Lancer Gold (50 + 1.8) % SP was highly effective in controlling the whitefly and thrips population, while Flonicamid 50% WG was found to be of more effective on the aphid population than all other treatments on tomato. In case of natural enemies also, these two insecticides were found to be the safest among all. Due to various target sites, high selective toxicity towards insects, stimulation of the mechanisms of plant self-defence, and lack of cross-resistance, these two insecticides may effectively be included in the IPM strategies of tomato sucking pest complex.

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

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Growth and yield of wheat as affected by tillage practices, seed priming and nutrient management under rain fed conditions

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ABSTRACT

The present study was conducted on wheat for two years from 2020-2022 to evaluate the effect of seed priming, tillage practices and nutrient management on growth and yield studies of crop under rainfed conditions at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The experiment was laid out in factorial randomized block design with three factors. Factor I (Tillage practices) consisted of Conventional tillage (CT), Conventional tillage+ mulch (CT+M) and Zero tillage+ mulch (ZT+M); Factor II (Seed priming) consisted of Hydropriming and micronutrient (Zn, Mn) priming; Factor III (Nutrient management practices) consisted of recommended dose of fertilizers (RDF) and Integrated nutrient management. An additional treatment of control was also kept for general comparison of results. Tillage practices and nutrient management significantly effected growth and yield studies of wheat. However, seed priming had no significant effect on various parameters. Growth and yield of wheat were found to be significantly superior under conventional tillage+mulch (CT+M) as compared to other tillage practices. Maximum height (108.4 cm), dry matter accumulation (896.7 g/m^2), leaf area index (2.43) and yield (9.19 t/ha) of wheat was obtained with CT+M. Zero tillage+mulch being the second best treatment proved to be better than conventional tillage. The growth and yield of wheat was significantly enhanced with integrated nutrient management as compared to recommended dose of fertilizers. Among different treatment combinations, T_6 (CT+M, Hydro, Int) was found to be best in terms of both growth and yield studies in wheat under rainfed conditions.

Introduction

Wheat (*Triticum aestivum* L.), being a major cereal crop, accounts for 26 per cent of world cereal production (Rahman *et al.,* 2021); and plays an important role in nutritional and food security. It is most extensively grown cereal in the world from temperate dry to irrigated and high rainfall area; and from dry cold to warm humid environment. In Himachal Pradesh, wheat is mainly grown under rainfed areas. Rainfed agriculture plays a significant part in ensuring global food security. In India, rainfed agriculture covers 86 mha which is 60 percent of net cultivated area and it produces 40 per cent of food grains (Rao *et al.,* 2015). Due to erratic and uncertain rainfalls, rainfed crop production depends on moisture stored in soil (Borgomeo *et al.,* 2020). The conventional intensive tillage practices results in reduction of soil organic carbon and increased runoff; thus reducing soil moisture.

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Several improved management practices under conservation agriculture has been reported such as zero or minimum tillage in wheat and residue management that improved resource use efficiency and crop productivity (Timalsina *et al.,* 2021). Conservation tillage along with residue retention is recognized as cost effective method to enhance soil moisture conservation and maintain productivity of crops (Mukherjee 2015). According to reports, one of the main challenges in better crop growth and yield is lack of synchronized crop establishment; which is caused by unfavourable weather conditions (Singh *et al.,* 2017a). Seed priming is easy and cost effective solution under such conditions. It reduces the time gap between sowing and emergence, and enhances synchronization in plants (Sarlach *et al.,* 2013). Excessive supply of chemical fertilizers has degraded soil structure and decreased organic matter, thus reducing microbial activity in soil (Dhaliwal *et al.,* 2021). Moreover, declined use of organic manures and crop residues have resulted in micronutrient deficiencies in north-western India (Bharti and Sharma 2017). Integrated nutrient management is a feasible approach for improving soil health as well as agricultural productivity. The use of well decomposed manure is known to improve crop yield, soil organic matter, encourage microbial population and increases amount of macro and micronutrients in soil. The challenge of improving productivity and resource conservation in rainfed areas can be addressed by proper crop establishment method along with efficient utilization of nutrients. Work done on tillage and nutrient management in wheat is region specific. Hence, investigation was carried out to study the effect of seed priming, tillage and nutrient management practices on growth and yield of wheat under rainfed conditions.

Material and Methods

The present study was undertaken at the Water Management Research Farm of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The experimental site was at 32°6' N latitude, 76°32 E longitude, and 1290 m altitude. Site is located in Himachal Pradesh's sub-temperate mid-hill region. The test site's soil had a silty clay loam texture, was acidic in reaction, rich in organic carbon and phosphorus, and had a medium level of available nitrogen and potassium. . Recommended dose of fertilizers were applied as 80:40:40 kg/ha N: P_2O_5: K_2O for wheat. In case of integrated nutrient management, 50% nitrogen was provided by FYM and 50% of nitrogen, rest of phosphorus and potassium was given through chemical fertilizers. All other recommended package of practices of region were followed for variety 'HPW 236'. Five randomly selected plants in each plot were tagged for height measurement. Plant height was measured from the base of plant to the top. The average height of the five plants was calculated and expressed as plant height (cm). For recording dry matter accumulation, plant samples were taken from the sampling rows from each plot at 30 days interval up to harvest. The plants were cut close to the ground and after sun drying; they were kept in oven for 3 hours at 70 ˚C. Weight was noted when samples attained constant weight.Leaf area index was recorded using manual method. For this, leaf area was calculated as the product of the total length and breadth at the broadest point of the longest leaf on the plant i.e. Leaf Area = lamina length x maximum width x k (coefficient). Leaf area so obtained was divided by ground area to get leaf area index. Crop growth rate was calculated using the formula:

$$\mathbf{CGR} = \frac{(w2-w1)}{t2-t1}.$$

Relative growth rate was calculated as following:

$$\mathbf{RGR}= \frac{(loge\ w2-loge\ w1)}{t2-t1}$$

where, w_1 and w_2 are dry weight per unit area at t_1 and t_2 time, respectively.

The crop from each net plot was harvested and dried thoroughly for 5 days. When most of the straw in a handful bundle broke up on folding, then total produce was weighed and recorded as biological yield (grain + straw).

Results and Discussion

Plant height

Data on plant height of wheat has been depicted in table 1. Significantly higher plant height of wheat was found in conventional tillage+mulch (108.4cm),

which was followed by zero tillage+ mulch (101.8 cm) during both years of study.
The lowest plant height (95.5 cm) was observed in conventional tillage without mulch. The reason may be good soil physical conditions and more water conservation under conventional tillage and mulch treatments. The results are in close conformity with Qamar *et al.,* 2015. Higher plant height in the zero tillage than conventional tillage was observed due to the high moisture availability and higher nutrient content in zero tillage at the upper soil surface compared to conventional tillage (Hemmat and Eskandari (2006) and Lupwayi *et al.,* (2006).
Seed priming methods had no significant effect on plant height of wheat at different intervals during both years of experiment. Plant height was significantly effected by nutrient management practices in wheat at all intervals except at 60 DAS, where the effect was found to be non significant. Significantly higher plant height (104.2 cm) of wheat was obtained with Integrated nutrient management (50% N through FYM +50% N and rest of P and K through inorganic sources) as compared to RDF(recommended dose of fertilizers) during both years. This can be possibly explained by the fact that the balanced supply of nutrients from organic manures plays an important role for rapid growth and development of a crop, which results in increased plant height.
Moreover direct and rapid supply of nutrients through chemical fertilizer; and slow release and mineralization of nutrients through organic manures during the growing period of the crop might have increased plant height under this treatment. The results are in line with Kavinder *et al.,* 2019 who observed significantly higher plant height of wheat under FYM application over no FYM. In comparison of control with other treatments, it was observed that all treatments except T_1(CT,Hydro,RDF), T_2 (CT,Hydro,Int), T_3 (CT,Micro,RDF) and T_4 (CT,Micro,Int) at 60 DAS, and T_1(CT,Hydro,RDF) and T_3 (CT,Micro,RDF) at 90 DASwere found to be significantly better than control in terms of plant height in both years of wheat. The tallest plants were observed in T_6 (CT+M,Hydro,Int) at all stages (Table 2). This is relatable to positive effects of mulch and integrated nutrient management on crop.

Dry matter accumulation

The data on dry matter accumulation in wheat is presented in Table 3. Conventional tillage+mulch resulted in significantly higher dry matter accumulation (896.7 g/m^2) as compared to other tillage practices. This was followed by zero tillage+ mulch (833.3 g/m^2). This may be attributed to better weed control and enhanced moisture retaining capacity in these treatments, resulting in increased dry matter production. Lowest dry matter accumulation was noted under conventional tillage, which may be due to sub surface soil compaction that causes hindrance to plant growth, thus reducing dry matter production. Akter *et al.,* 2018 reported similar results. Seed priming had no significant effect on dry matter accumulation of wheat. It was observed that Integrated nutrient management (50% N through FYM +50% N and rest of P and K through inorganic sources) resulted in significantly higher dry matter accumulation (868.6 g/m^2) over RDF(recommended dose of fertilizers) in wheat. Higher dry matter production in integrated nutrient management may be because organic manures supply both macro and micro nutrients and also increases the availability of native nutrients in soil, resulting in increased vegetative growth of plants. Furthermore, combined application of organic and inorganic sources supply adequate amount of nutrients to plants which increases translocation of photosynthates from source to sink and enhances dry matter production (Singh et *al.,* 2017b). The results are in line with Kavinder *et al.,* 2019. It was found that all treatments except T_1 and T_3 at 60 DAS; T_3 at 90 DAS; T_1, T_2, T_3 and T_4 at 120 DAS; T_1, T_2, T_3, T_4, T_9 and T_{11} at harvest were found to be significantly better than control in terms of dry matter accumulation during both years of study. T_6 (CT+M,Hydro,Int) resulted in highest dry matter accumulation at all stages (Table 4).

Leaf Area Index

The data on leaf area index of wheat is presented in Table 5.Among different tillage practices, conventional tillage along with mulch resulted in significantly higher leaf area index (2.43) in wheat during both years of experiment. This may be attributed to the fact that mulching increased the availability of conserved moisture in the soil and significantly enhanced plant water use efficiency and leaf area. Similar results were reported by

Table 1: Effect of tillage practices, seed priming and nutrient management on plant height of wheat at periodic intervals

	Plant Height (cm)									
	60 DAS		90 DAS		120 DAS		150 DAS		Harvest	
CT	26.9	27.7	37.1	38.0	58.7	60.1	92.0	93.6	94.0	95.5
CT + Mulch	31.9	32.9	43.6	44.7	65.8	67.5	104.5	106.3	106.7	108.4
ZT + Mulch	28.5	29.4	40.9	42.0	62.5	64.1	98.2	99.9	100.2	101.8
SEm±	0.7	0.7	1.2	1.1	0.8	0.9	1.5	1.5	1.4	1.4
LSD (P=0.05)	**2.0**	**2.0**	**3.4**	**3.2**	**2.4**	**2.5**	**4.2**	**4.3**	**4.0**	**4.1**
Seed priming										
Hydro priming	29.5	30.4	41.1	42.1	63.0	64.5	99.3	101.0	101.4	103.0
Micronutrient priming	28.7	29.6	40.0	41.1	61.7	63.2	97.2	98.9	99.2	100.8
SEm±	0.6	0.6	1.0	0.9	0.7	0.7	1.2	1.2	1.1	1.1
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nutrient management										
RDF	28.4	29.3	39.1	40.2	61.1	62.7	95.9	97.6	98.0	99.6
Integrated	29.8	30.8	42.0	43.0	63.6	65.1	100.6	102.3	102.6	104.2
SEm±	0.6	0.6	1.0	0.9	0.7	0.7	1.2	1.2	1.1	1.1
LSD (P=0.05)	**NS**	**NS**	**2.8**	**2.6**	**2.0**	**2.0**	**3.5**	**3.5**	**3.3**	**3.4**
Control vs others										
Control	24.5	25.3	32.4	33.3	52.9	54.3	80.5	82.0	82.4	83.9
Others	29.1	30.0	40.5	41.6	62.3	63.9	98.2	99.9	100.3	101.9
SEm±	1.0	1.0	1.7	1.6	1.2	1.3	2.1	2.2	2.0	2.1
LSD (P=0.05)	**3.0**	**3.0**	**5.0**	**4.7**	**3.5**	**3.7**	**6.2**	**6.4**	**5.9**	**6.0**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 2: Effect of different treatments and control onplant height of wheat at periodic intervals

Treatments	Plant Height (cm)									
	60 DAS		90 DAS		120 DAS		150 DAS		Harvest	
T_1 CT,Hydro,RDF	26.6	27.5	35.7	37.1	57.7	59.1	90.6	92.2	92.3	93.9
T_2 CT,Hydro,Int	27.4	28.3	39.8	40.3	60.7	62.1	95.4	97.0	97.4	98.9
T_3 CT, Micro,RDF	26.5	27.4	34.3	35.7	56.7	58.1	88.6	90.2	90.8	92.3
T_4 CT, Micro,Int	26.8	27.8	38.4	39.0	59.6	61.1	93.5	95.1	95.4	97.0
T_5 CT+M,Hydro,RDF	30.7	31.7	42.9	44.0	65.9	67.6	102.5	104.3	105.4	107.2
T_6 CT+M,Hydro,Int	34.6	35.6	45.3	46.5	67.3	69.0	109.3	111.1	111.5	113.4
T_7 CT+M, Micro,RDF	30.3	31.3	42.1	43.2	63.4	65.0	99.6	101.3	101.7	103.5
T_8 CT+M, Micro,Int	32.1	33.1	44.2	45.3	66.6	68.2	106.5	108.3	108.1	109.6
T_9 ZT+M,Hydro,RDF	28.4	29.3	40.2	41.2	61.6	63.1	97.5	99.2	99.2	100.8
T_{10} ZT+M,Hydro,Int	29.3	30.3	42.4	43.4	64.6	66.2	100.3	102.0	102.4	104.0
T_{11} ZT+M, Micro,RDF	27.7	28.6	39.4	39.7	61.4	63.0	96.6	98.3	98.4	100.0
T_{12} ZT+M, Micro,Int	28.6	29.5	41.7	43.6	62.5	64.0	98.5	100.2	100.8	102.4
T_{13} Control	24.5	25.3	32.4	33.3	52.9	54.3	80.5	82.0	82.4	83.9
SEm±	1.0	1.0	1.7	1.6	1.2	1.3	2.1	2.2	2.0	2.1
LSD control vs others **(P=0.05)**	**3.0**	**3.0**	**5.0**	**4.7**	**3.5**	**3.7**	**6.2**	**6.4**	**5.9**	**6.0**

***CT: Conventional tillage, ZT: Zero tillage, M: Mulch, Hydro: Hydropriming, Micro: Micronutrient priming Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Int: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 3: Effect of tillage practices, seed priming and nutrient management on dry matter accumulation of wheat at periodic intervals

	Dry matter accumulation (g/m^2)									
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**		**Harvest**	
CT	95.5	97.0	207.3	209.0	503.1	504.6	752.3	761.2	774.2	797.2
CT + Mulch	106.1	107.8	249.4	251.3	626.1	628.2	866.0	878.2	881.2	896.7
ZT + Mulch	100.5	102.1	223.1	224.8	565.8	567.7	780.7	791.5	809.3	833.3
SEm±	1.1	1.3	3.7	3.8	11.0	11.6	12.6	13.1	20.2	20.6
LSD (P=0.05)	**3.3**	**3.8**	**10.9**	**11.0**	**32.2**	**33.9**	**36.7**	**38.1**	**58.8**	**60.2**
Seed priming										
Hydro priming	101.4	103.0	229.4	231.1	570.9	572.8	812.8	824.9	825.2	846.1
Micronutrient priming	100.1	101.6	223.9	225.6	559.0	560.9	786.5	795.7	817.9	838.7
SEm±	0.9	1.1	3.1	3.1	9.0	9.5	10.3	10.7	16.5	16.8
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nutrient management										
RDF	99.3	100.7	220.0	221.8	551.8	552.3	771.8	782.7	795.9	816.2
Integrated	102.1	104.0	233.2	235.0	578.1	581.4	827.5	837.9	847.2	868.6
SEm±	0.9	1.1	3.1	3.1	9.0	9.5	10.3	10.7	16.5	16.8
LSD (P=0.05)	**2.7**	**3.1**	**8.9**	**9.0**	**26.3**	**27.7**	**29.9**	**31.1**	**48.0**	**49.1**
Control vs others										
Control	90.6	92.0	186.3	187.8	474.0	475.6	717.3	727.1	732.3	752.3
Others	100.7	102.3	226.6	228.4	565.0	566.8	799.6	810.3	821.6	842.4
SEm±	1.7	1.9	5.5	5.5	16.2	17.1	18.5	19.2	29.7	30.4
LSD (P=0.05)	**4.9**	**5.6**	**16.1**	**16.2**	**47.4**	**49.9**	**54.0**	**56.1**	**86.6**	**88.6**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 4: Effect of different treatments and control on dry matter accumulation of wheat at periodic intervals.

Treatments	Dry matter accumulation (g/m^2)									
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**		**Harvest**	
T$_1$CT,Hydro,RDF	94.4	95.1	205.6	207.2	499.9	492.9	737.3	749.6	770.9	793.7
T$_2$ CT,Hydro,Int	97.5	99.9	214.5	216.1	513.6	523.5	756.2	769.0	780.0	803.2
T$_3$ CT, Micro,RDF	93.5	94.3	198.6	200.3	494.4	496.1	739.4	748.4	769.6	792.4
T$_4$ CT, Micro,Int	96.6	98.7	210.7	212.4	504.4	506.1	776.1	777.8	776.2	799.4
T$_5$ CT+M,Hydro,RDF	105.5	107.2	245.8	247.7	619.0	621.2	810.2	821.6	857.8	873.0
T$_6$ CT+M,Hydro,Int	108.5	110.3	261.5	263.5	645.6	647.9	1009.0	1022.9	920.8	936.7
T$_7$ CT+M, Micro,RDF	103.6	105.3	235.8	237.6	605.8	607.9	789.8	801.2	843.6	858.5
T$_8$ CT+M, Micro,Int	106.7	108.4	254.6	256.5	633.8	635.9	855.1	867.1	902.6	918.3
T$_9$ ZT+M,Hydro,RDF	100.4	101.9	218.7	220.4	554.0	555.8	778.0	788.8	766.9	790.0
T$_{10}$ ZT+M,Hydro,Int	101.9	103.5	230.2	231.9	593.5	595.4	786.4	797.3	855.0	880.1
T$_{11}$ ZT+M, Micro,RDF	98.6	100.1	215.8	217.5	537.8	539.7	775.9	786.6	766.6	789.6
T$_{12}$ ZT+M, Micro,Int	101.3	102.9	227.6	229.4	577.9	579.8	782.5	793.3	848.8	873.7
T$_{13}$ Control	90.6	92.0	186.3	187.8	474.0	475.6	717.3	727.1	732.3	752.3
SEm±	1.7	1.9	5.5	5.5	16.2	17.1	18.5	19.2	29.7	30.4
LSD control vs others**(P=0.05)**	**4.9**	**5.6**	**16.1**	**16.2**	**47.4**	**49.9**	**54.0**	**56.1**	**86.6**	**88.6**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Akter *et al.,* 2018 who found that higher leaf area index was obtained with straw mulching in wheat as compared to no mulching. Moreover, Scopel *et al.,* (2004) has also concluded that increased quantity of surface residue in field was found to have a significant effect on plant available water, which reduced water stress and resulted in increased LAI. The lowest leaf area index (1.97) was found in conventional tillage, which was in accordance with Meena *et al.,* 2018 . Seed priming methods had no significant effect on leaf area index in wheat during both years of study. Nutrient management practices significantly effected leaf area index of wheat at different intervals except at 60 DAS, during both years. Significantly higher leaf area index was obtained from Integrated nutrient management (50% N through FYM +50% N and rest of P and K through inorganic sources) as compared to RDF (recommended dose of fertilizers). This can be explained by the fact that FYM contain more number of nitrogen fixing, phosphate solubilising and other beneficial microbes, antibiotics, enzymes, vitamins, etc., which resulted in enhanced growth of plants thereby increasing leaf area. This may also be attributed to better integration of organic manures and chemical fertilizers which may have provided sufficient nutrients at active growth stage, thus resulting in increased leaf area (Fazily et *al.,* 2021). The results are in close conformity with Kumar *et al.,* 2017 who reported higher leaf area index of wheat with integrated application of inorganic and organic sources of nutrients. It was observed that all the treatments were significantly better than control. The highest leaf area index was observed in T_6 (CT+M,Hydro,Int), at all stages of crop growth (Table 6).

Crop growth rate

Data pertaining to crop growth rate of wheat is reported in table 7.Different tillage practices significantly effected crop growth rate of wheat upto 120 DAS in both years of study. Significantly higher crop growth rate was found under conventional tillage+ mulch (12.56 g/m^2/day) which was followed by zero tillage +mulch (11.43 g/m^2/day). This may be due to fine and loose soil structure under conventional tillage along with positive benefits of mulch (improved nutrient and moisture availability) which increased crop growth rate of crop. Moreover, residues used as mulch contain substantial amounts of plant nutrients, as reported by Das *et al.,* 2015. The results are in line with Qamar *et al.,* 2015 who noted increased growth parameters of wheat under conventional tillage and mulch. Hydropriming and micronutrient priming showed no significant difference in terms of crop growth rate of wheat. Crop growth rate was significantly effected by nutrient management practices at 60 DAS in both years of wheat. Integrated nutrient management (50% N through FYM +50% N and rest of P and K through inorganic sources) resulted in significantly higher crop growth rate over RDF(recommended dose of fertilizers). This might be explained by the fact that organic sources provide balanced supply of nutrients which increased dry matter production of crops, resulting in enhanced crop growth rate. The plants under integrated nutrient management had comparatively easily extractable and more availability of nutrients as compared to RDF which resulted in better crop growth rate. Kavinder *et al.,* 2019 also reported similar results. All treatments except T_1(CT,Hydro,RDF) and T_3 (CT,Micro,RDF) were significantly better than control in terms of crop growth rate at 60 and 90 DAS in wheat. The highest crop growth rate was found in T_6 (CT+M,Hydro,Int) (Table 8).

Relative growth rate

Data on relative growth rate of wheat has been depicted in table 9. Significantly higher relative growth rate was noted under conventional tillage+ mulch, which was followed by zero tillage+ mulch at 60 DAS. This may be due to higher dry matter production in conventional tillage and mulch as a result of improved soil conditions and uptake of nutrients by crop. Mulch creates favourable environment for enhanced nutrient uptake which could be attributed to improvement in soil biological health, thus resulting in improved growth rate of plants. The results are in line with Ijaz and Ali 2007 who reported higher dry matter of wheat in mulched plots as compared to unmulched plots. Seed priming methods had no significant effect on relative growth rate of wheat, during both years of study.Relative growth rate was found significantly higher under Integrated nutrient management (50% N through FYM +50% N and rest of P and K through inorganic sources) as compared to RDF (recommended dose of fertilizers) at 60 DAS. This can be possibly explained by the fact that the combined application of organic manuresand mineral fertilizers results in optimizing soil nutrient pool and enhances crop growth rate. The results are in close conformity with Kumar *et al.*, 2020. It was found that all treatments except T_1 (CT,Hydro,RDF), T_3 (CT,Micro,RDF) and T_4 (CT, Micro,Int) at 60 DAS resulted in significantly higher relative growth rate than control. T_5 (CT+M,Hydro,RDF), T_6 (CT+M,Hydro,Int) and T_8 (CT+M, Micro,Int)were best treatments in terms of relative growth rate (Table 10).

Table 5: Effect of tillage practices, seed priming and nutrient management on leaf area index of wheat at periodic intervals

	Leaf Area Index							
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**	
Tillage practices	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
CT	1.33	1.37	3.19	3.24	2.41	2.49	1.88	1.97
CT + Mulch	1.60	1.65	3.61	3.68	2.80	2.89	2.31	2.43
ZT + Mulch	1.45	1.49	3.40	3.46	2.59	2.67	2.10	2.20
SEm±	0.06	0.06	0.04	0.04	0.04	0.05	0.04	0.04
LSD (P=0.05)	**0.16**	**0.17**	**0.12**	**0.12**	**0.13**	**0.14**	**0.12**	**0.12**
Seed priming								
Hydro priming	1.48	1.52	3.43	3.50	2.63	2.71	2.12	2.23
Micronutrient priming	1.44	1.48	3.37	3.43	2.57	2.65	2.07	2.17
SEm±	0.05	0.05	0.03	0.03	0.04	0.04	0.03	0.03
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nutrient management								
RDF	1.44	1.48	3.35	3.40	2.55	2.62	2.04	2.15
Integrated	1.49	1.53	3.45	3.52	2.65	2.74	2.15	2.26
SEm±	0.05	0.05	0.03	0.03	0.04	0.04	0.03	0.03
LSD (P=0.05)	**NS**	**NS**	**0.10**	**0.10**	**0.11**	**0.12**	**0.09**	**0.10**
Control vs others								
Control	1.22	1.25	2.62	2.66	1.90	1.96	1.52	1.61
Others	1.46	1.50	3.40	3.46	2.60	2.68	2.10	2.20
SEm±	0.08	0.08	0.06	0.06	0.07	0.07	0.06	0.06
LSD (P=0.05)	**0.24**	**0.25**	**0.17**	**0.18**	**0.19**	**0.21**	**0.17**	**0.18**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 6: Effect of different treatments and control onleaf area index of wheat at periodic intervals

Treatments	**Leaf Area Index**							
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**	
$\mathbf{T_1}$CT,Hydro,RDF	1.35	1.38	3.18	3.22	2.35	2.40	1.85	1.94
$\mathbf{T_2}$ CT,Hydro,Int	1.36	1.40	3.28	3.33	2.53	2.62	1.95	2.05
$\mathbf{T_3}$ CT, Micro,RDF	1.30	1.33	3.08	3.13	2.36	2.42	1.79	1.88
$\mathbf{T_4}$ CT, Micro,Int	1.33	1.36	3.24	3.29	2.42	2.50	1.91	2.01
$\mathbf{T_5}$ CT+M,Hydro,RDF	1.58	1.63	3.59	3.67	2.78	2.88	2.29	2.41
$\mathbf{T_6}$ CT+M,Hydro,Int	1.64	1.69	3.69	3.77	2.88	2.98	2.38	2.51
$\mathbf{T_7}$ CT+M, Micro,RDF	1.57	1.61	3.52	3.59	2.71	2.80	2.26	2.37
$\mathbf{T_8}$ CT+M, Micro,Int	1.61	1.66	3.63	3.69	2.81	2.90	2.32	2.44
$\mathbf{T_9}$ ZT+M,Hydro,RDF	1.42	1.46	3.38	3.44	2.57	2.65	2.07	2.17
$\mathbf{T_{10}}$ ZT+M,Hydro,Int	1.52	1.56	3.48	3.54	2.68	2.76	2.19	2.30
$\mathbf{T_{11}}$ ZT+M, Micro,RDF	1.40	1.44	3.32	3.38	2.50	2.58	2.00	2.10
$\mathbf{T_{12}}$ ZT+M, Micro,Int	1.44	1.48	3.41	3.47	2.61	2.69	2.13	2.24
$\mathbf{T_{13}}$ Control	1.22	1.25	2.62	2.66	1.90	1.96	1.52	1.61
SEm±	0.08	0.08	0.06	0.06	0.07	0.07	0.06	0.06
LSD control vs others**(P=0.05)**	**0.24**	**0.25**	**0.17**	**0.18**	**0.19**	**0.21**	**0.17**	**0.18**

***CT: Conventional tillage, ZT: Zero tillage, M: Mulch, Hydro: Hydropriming, Micro: Micronutrient priming Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Int: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 7: Effect of tillage practices, seed priming and nutrient management on crop growth rate of wheat at periodic intervals

	Crop growth rate (g/m^2/day)							
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**	
Tillage practices	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
CT	1.59	1.62	3.73	3.73	9.86	9.85	8.31	8.55
CT + Mulch	1.77	1.80	4.78	4.78	12.55	12.56	8.00	8.33
ZT + Mulch	1.68	1.70	4.08	4.09	11.42	11.43	7.16	7.46
SEm±	0.02	0.02	0.14	0.14	0.40	0.41	0.52	0.51
LSD (P=0.05)	**0.06**	**0.06**	**0.40**	**0.41**	**1.16**	**1.21**	**NS**	**NS**
Seed priming								
Hydro priming	1.69	1.72	4.27	4.27	11.39	11.39	8.06	8.40
Micronutrient priming	1.67	1.69	4.13	4.13	11.17	11.18	7.58	7.83
SEm±	0.02	0.02	0.11	0.11	0.32	0.34	0.42	0.42
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nutrient management								
RDF	1.66	1.68	4.02	4.04	11.06	11.02	7.33	7.68
Integrated	1.70	1.73	4.37	4.37	11.50	11.55	8.31	8.55
SEm±	0.02	0.02	0.11	0.11	0.32	0.34	0.42	0.42
LSD (P=0.05)	**0.05**	**0.05**	**0.33**	**NS**	**NS**	**NS**	**NS**	**NS**
Control vs others								
Control	1.51	1.53	3.19	3.19	9.59	9.59	8.11	8.39
Others	1.68	1.71	4.20	4.20	11.28	11.28	7.82	8.12
SEm±	0.03	0.03	0.20	0.21	0.58	0.61	0.76	0.75
LSD (P=0.05)	**0.08**	**0.09**	**0.59**	**0.60**	**NS**	**NS**	**NS**	**NS**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 8: Effect of different treatments and control oncrop growth rate of wheat at periodic intervals

Treatments	**Crop growth rate (g/m^2/day)**							
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**	
T_1 CT,Hydro,RDF	1.57	1.59	3.71	3.74	9.81	9.52	7.91	8.56
T_2 CT,Hydro,Int	1.63	1.67	3.90	3.87	9.97	10.24	8.09	8.19
T_3 CT, Micro,RDF	1.56	1.57	3.50	3.53	9.86	9.86	8.17	8.41
T_4 CT, Micro,Int	1.61	1.65	3.80	3.79	9.79	9.79	9.06	9.06
T_5 CT+M,Hydro,RDF	1.76	1.79	4.68	4.68	12.44	12.45	6.37	6.68
T_6 CT+M,Hydro,Int	1.81	1.84	5.10	5.10	12.80	12.81	12.11	12.50
T_7 CT+M, Micro,RDF	1.73	1.76	4.41	4.41	12.33	12.34	6.13	6.45
T_8 CT+M, Micro,Int	1.78	1.81	4.93	4.94	12.64	12.65	7.38	7.70
T_9 ZT+M,Hydro,RDF	1.67	1.70	3.94	3.95	11.18	11.18	7.47	7.77
T_{10} ZT+M,Hydro,Int	1.70	1.73	4.27	4.28	12.11	12.12	6.43	6.73
T_{11} ZT+M, Micro,RDF	1.64	1.67	3.91	3.91	10.73	10.74	7.94	8.23
T_{12} ZT+M, Micro,Int	1.69	1.71	4.21	4.22	11.68	11.68	6.82	7.12
T_{13} Control	1.51	1.53	3.19	3.19	9.59	9.59	8.11	8.39
SEm±	0.03	0.03	0.20	0.21	0.58	0.61	0.76	0.75
LSD control vs others**(P=0.05)**	**0.08**	**0.09**	**0.59**	**0.60**	**NS**	**NS**	**NS**	**NS**

***CT: Conventional tillage, ZT: Zero tillage, M: Mulch, Hydro: Hydropriming, Micro: Micronutrient priming Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Int: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Biological yield

Data pertaining to biological yield of wheat is given in Table 11. Significantly higher biological yield of wheat was obtained under conventional tillage+ mulch (9.19 t/ha), which was followed by zero tillage+ mulch (8.76 t/ha). Higher biological yield under these treatments may be because of enhanced growth parameters due to positive effects of mulch such as increased nutrient availability, moisture conservation and improved soil physical, chemical and microbial properties. Similar results were reported by Ali *et al.,* 2016. Significantly higher yields in happy seeder than in conventional tillage are reported (Sip et al., 2009). Zero tillage and crop residues retention on soil surface increased the organic matter content in soil (Lal et al., 2003) and was more supportive for dry land crop production (Baumhardt and Jones, 2002). Seed priming methods had no significant effect on biological yield of wheat. It was further observed that Integrated nutrient management(50% N through FYM +50% N and rest of P and K through inorganic sources) resulted in significantly higher biological yield (8.75 t/ha) of wheat as compared to RDF(recommended dose of fertilizers) in both years. This can be explained by the fact that addition of organic manure provide balanced and continous supply of nutrient, which may have enhanced dry matter production in plants. Shah and Ahmad 2006 also reported similar results. Mohan *et al.,* 2018 also reported higher yield of wheat under integrated nutrient management because of balanced proportion and adequate amounts of nutrients supplied to crop at growth stages which improved yield attributing characters. Comparison of control with other treatments revealed that all treatments except T_1(CT,Hydro,RDF) and T_3 (CT,Micro,RDF) resulted in significantly higher biological yield of wheat as compared to control, during both years of research. The highest biological yield was obtained in T_6 (CT+M,Hydro,Int) (8.80 and 9.29 t/ha, during first and second years, respectively) in both years (Table 12).

Table 9: Effect of tillage practices, seed priming and nutrient management on relative growth rate of wheat at periodic intervals

	Relative growth rate (g/g/day)							
	60 DAS		**90 DAS**		**120 DAS**		**150 DAS**	
CT	0.076	0.076	0.026	0.026	0.030	0.029	0.013	0.014
CT + Mulch	0.078	0.078	0.028	0.028	0.031	0.031	0.011	0.011
ZT + Mulch	0.077	0.077	0.027	0.026	0.031	0.031	0.011	0.011
SEm±	0.0002	0.0002	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P=0.05)	**0.001**	**0.001**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.002**
Seed priming								
Hydro priming	0.077	0.077	0.027	0.027	0.030	0.030	0.012	0.012
Micronutrient priming	0.077	0.077	0.027	0.026	0.030	0.030	0.011	0.012
SEm±	0.0002	0.0002	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Nutrient management								
RDF	0.077	0.077	0.026	0.026	0.031	0.030	0.011	0.012
Integrated	0.077	0.077	0.027	0.027	0.030	0.030	0.012	0.012
SEm±	0.0002	0.0002	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P=0.05)	**0.0005**	**0.001**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Control vs others								
Control	0.075	0.075	0.024	0.024	0.031	0.031	0.014	0.014
Others	0.077	0.077	0.027	0.027	0.030	0.030	0.012	0.012
SEm±	0.0003	0.0003	0.001	0.001	0.001	0.001	0.001	0.001
LSD (P=0.05)	**0.001**	**0.001**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 10: Effect of different treatments and control onrelative growth rate of wheat atperiodic intervals

Treatments	Relative growth rate (g/g/day)							
	60 DAS		90 DAS		120 DAS		150 DAS	
T_1 CT,Hydro,RDF	0.076	0.076	0.026	0.026	0.030	0.029	0.013	0.013
T_2 CT,Hydro,Int	0.076	0.077	0.026	0.026	0.029	0.029	0.013	0.013
T_3 CT, Micro,RDF	0.076	0.076	0.025	0.025	0.030	0.030	0.013	0.014
T_4 CT, Micro,Int	0.076	0.077	0.026	0.026	0.029	0.029	0.014	0.014
T_5 CT+M,Hydro,RDF	0.078	0.078	0.028	0.028	0.031	0.031	0.009	0.009
T_6 CT+M,Hydro,Int	0.078	0.078	0.029	0.029	0.030	0.030	0.015	0.015
T_7 CT+M, Micro,RDF	0.077	0.078	0.027	0.027	0.031	0.031	0.009	0.009
T_8 CT+M, Micro,Int	0.078	0.078	0.029	0.029	0.030	0.030	0.010	0.010
T_9 ZT+M,Hydro,RDF	0.077	0.077	0.026	0.026	0.031	0.031	0.011	0.012
T_{10} ZT+M,Hydro,Int	0.077	0.077	0.027	0.027	0.032	0.031	0.009	0.010
T_{11} ZT+M, Micro,RDF	0.077	0.077	0.026	0.026	0.030	0.030	0.012	0.013
T_{12} ZT+M, Micro,Int	0.077	0.077	0.027	0.027	0.031	0.031	0.010	0.010
T_{13} Control	0.075	0.075	0.024	0.024	0.031	0.031	0.014	0.014
SEm±	0.0003	0.0003	0.001	0.001	0.001	0.001	0.001	0.001
LSD control vs others**(P=0.05)**	**0.001**	**0.001**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

***CT: Conventional tillage, ZT: Zero tillage, M: Mulch, Hydro: Hydropriming, Micro: Micronutrient priming Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Int: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 11: Effect of tillage practices, seed priming and nutrient management on biological yield of wheat

	Biological yield (ton/ha)	
Tillage practices	2020-21	2021-22
CT	7.66	7.95
CT + Mulch	8.70	9.19
ZT + Mulch	8.45	8.76
SEm±	0.10	0.09
LSD (P=0.05)	**0.28**	**0.27**
Seed priming		
Hydro priming	8.30	8.67
Micronutrient priming	8.23	8.60
SEm±	0.08	0.08
LSD (P=0.05)	**NS**	**NS**
Nutrient management		
RDF	8.14	8.51
Integrated	8.39	8.75
SEm±	0.08	0.08
LSD (P=0.05)	**0.23**	**0.22**
Control vs others		
Control	7.31	7.63
Others	8.27	8.63
SEm±	0.14	0.14
LSD (P=0.05)	**0.42**	**0.40**

***CT: Conventional tillage, ZT: Zero tillage, Micronutrient priming: Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Integrated: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Table 12: Effect of different treatments and control on biological yield of wheat

Treatments	Biological yield (ton/ha)	
T_1CT,Hydro,RDF	7.53	7.77
T_2 CT,Hydro,Int	7.84	8.19
T_3 CT, Micro,RDF	7.48	7.66
T_4 CT, Micro,Int	7.78	8.18
T_5 CT+M,Hydro,RDF	8.68	9.17
T_6 CT+M,Hydro,Int	8.80	9.29
T_7 CT+M, Micro,RDF	8.62	9.10
T_8 CT+M, Micro,Int	8.70	9.18
T_9 ZT+M,Hydro,RDF	8.25	8.73
T_{10} ZT+M,Hydro,Int	8.73	8.86
T_{11} ZT+M, Micro,RDF	8.32	8.63
T_{12} ZT+M, Micro,Int	8.49	8.81
T_{13} Control	7.31	7.63
SEm±	0.14	0.14
LSD control vs others**(P=0.05)**	**0.42**	**0.40**

***CT: Conventional tillage, ZT: Zero tillage, M: Mulch, Hydro: Hydropriming, Micro: Micronutrient priming Zn (0.5%), Mn (0.1%), RDF: Recommended dose of fertilizers, Int: 50% N through FYM + 50% N and rest of P and K through inorganic sources, Control: CT, no priming, RDF**

Conclusion

It was concluded from present study that better growth of wheat plants and enhanced biological yield resulted from conventional tillage+ mulch, which was followed by zero tillage+ mulch. It was further revealed that seed priming methods had no significant effect on growth and yield studies of wheat. Among nutrient management practices, integrated nutrient management proved to be superior in terms of growth parameters and biological yield of wheat. Based on these results of study, it is suggested to follow conventional tillage along with residue retention and integrated nutrient management in rainfed wheat in Himachal Pradesh.

Conflict of interest

The authors declare that they have no conflict of interest.

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Micro scale level rainfall trend analysis at Madhira, Khammam district of Telangana

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ABSTRACT

Rainfall is an important factor for agriculture in India as nearly 60 per cent net arable land lacks irrigation and water availability determines the yield of pulses, rice, wheat and sugarcane. The variation of rainfall was one of the major concerns under present climatic situation challenging the farmers to obtain optimum farm production and maintain employment. Therefore, trend analysis was examined for long-term trends from 1975-2021 for rainfall at micro scale level of Madhira in Telangana state. In this study, average monthly and seasonal rainfall, coefficient of variation (CV) was ranging from 43.91 to 300.56 per cent and 27.44 to 171.87 per cent, respectively. The annual value of kurtosis and skewness were -0.39 and 0.47 which resembles light tailed to normal distribution and positively skewed resembling right to the mean. During monsoon, post monsoon and annual rainfall trends were showing non-significantly deceasing trend while winter and post monsoon showed non-significantly increasing trends but, as per Spearman's Rho (non parametric) tests significant increasing trend for winter season. Overall, the four decades of observations showed that there is alternate increasing and decreasing trend at Madhira for monsoon season. The post-monsoon season four decades showed negative departures and annual rainfall was mostly dominated by monsoon, a similar pattern was observed in it also.

Introduction

Climate is one of the important components in the earth system for agriculture. There are many parameters such as rainfall, temperature, humidity and atmospheric pressure that constitute weather and climate of a region. In all other weather parameters, rainfall is one of the most important parameters which decides the success or failure of agriculture production and productivity. Each year

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variation in monsoon rainfall occasionally leads to extreme hydrological events such as large-scale floods and droughts, resulting in considerable reduction in agricultural production and affecting the many people (an extent of one billion) and national economy (Fasullo and Webster, 2003).A normal monsoon with widely distributed rainfall all over the country is a bonanza, while extreme flood or drought over large or a smaller region constitutes a natural hazard. The southwest (SW) monsoon brings around 80% of the total rainfall over the country. The changes in the frequency, pattern and variation of SW monsoon would have a significant impact on crop production, water availability and overall economy of most of the countries (Saha and Mooley, 1979; Sinha and Srivastava, 2000; Seoand Ummenhofer, 2017). Hence the variation in seasonal monsoon precipitation may be considered a measure to evaluate climate change/change over the Indian monsoon domain in the context of global warming (McCarthy*et al*., 2001).Here some of the studies have described trends at large scale (Kumar *et al*., 2010), regional scales (Duhan and Pandey, 2013) and at individual stations (Ranjan and Saha, 2022). Actually, local and regional scale analysis (Babar and Ramesh, 2013) was more applicable to devise-specific development and adaptation plans to mollify negative effects of climate change. Trend analysis of rainfall, temperature and other climatic parameters on different spatial scales may help in the establishment of future climate scenarios (Meshram *et al.,* 2018). Laskar *et al*. (2014) have performed trend analysis of temperature and rainfall of selected stations over north-east India and found a significantly decreasing trend of monsoon rainfall at Agartala during 1954-2012. Das *et al*. (2015) analyzed seasonal and yearly rainfall amounts for the two stations of Agartala and Passighat and no statistically significant trend was found.The major objective of the present study is to find whether there was any trend in the monthly, seasonal and annual rainfall pattern at Madhira, Khammam district of Telangana.

Material and Methods

The data was collected from the agrometeorological observatory, Agriculture Research Station (ARS), Madhira lying between 16.9182° N latitude, 80.3633° E longitude in Khammam district of Telangana state. The rainfall data was analyzed for monthly, seasonal and annual from 1975 to 2021 (47 years) using different types of statistical tools. The whole year was partitioned into four seasons by following India Meteorological Department, New Delhi norms. Winter season begins from January to February followed by summer season from March to May. Southwest monsoon starts from June to September followed by post monsoon in October and November. Trend was described as general movement of a series over an extended time period (Webber and Hawkins, 1980). The trend was determined by relationship between the received rainfall amount in mm and its temporal resolution. The mean, standard deviation (SD), coefficient of variation (CV) and Kurtosis and Skewness of rainfall were computed to analyze the relationship.

$$\text{Standard deviation } (s) = \sqrt{\frac{\sum_{i=1}^{n}(xi-\bar{x})^2}{n-1}}$$

$$\text{CV } = \frac{StandardDeviation}{Mean} \times 100$$

$$\text{Skewness (g)} = \frac{\sum_{i=1}^{n}(xi-\bar{x})^3}{(n-1)s^3}$$

$$\text{Kurtosis (K)} = n\frac{\sum_{i=1}^{n}(xi-\bar{x})^4}{(\sum_{i=1}^{n}(xi-\bar{x})^2)^2}$$

x_i= each value of the observation
$\bar{x}$= sample mean
n = total number of observations
s = standard deviation

The statistical methods like analysis of regression and coefficient of determination R^2 were used for deducing significance of rainfall trends. The trend analysis was derived and tested by Mann- Kendall (non parametric), Spearman's Rho (non parametric) and Linear regression (parametric) tests.The non parametric Mann- Kendall (M-K) test was proposed by Mann (1945) and was large-scale used with climatological time series. There are two advantages of this test. First one, it is non parametric test and does not necessarily need the data to be normally distributed. Second, the test has less sensitivity with abrupt breaks due to inhomogeneous time series. According to the test, null hypothesis

(H_0) assumes that there was no trend (data was independent and randomly ordered). It can be tested against the alternative hypothesis (H_1), which assumes that there was a trend. The Mann- Kendall statistic was computed as follows:

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} sgn\,(x_j - x_k)$$

The trend test is applied to a time series x_k, ranked from k = 1,2,3,…,n−1, and j = i+ 1,i + 2,i + 3…..n. Every data point's x_j was taken as a reference point,

$sgn\,(x_j - x_k) = 1$ if $x_j - x_j > 0$
$= 0$ if $x_j - x_j = 0$
$= -1$ if $x_j - x_j < 0$

A higher positive value of S was an indicator of an increasing trend and lower negative value indicated a decreasing trend. The presence of a statistical significance trend was evaluated using Z value.Spearman's Rho (Sneyers, 1990 and Lehmann, 1975) test was one of the rank-based nonparametric methods used for trend analysis, applied as a comparison with the M-K test. The test assumes that time series data were independent and evenly distributed, the null hypothesis (H_0) again showed no trend over time, the alternate hypothesis (H_l) was that a trend existed and that data increased or decreased. The positive values represented an increasing trend over the time period and negative values represented the decreasing trends.

Spearman rank correlation coefficient $(\rho) = S_{xy} / (S_x S_y)^{0.5}$

Where, $Sx = \sum_{i=1}^{n} (x_i - \bar{x})^2$

$$Sy = \sum_{i=1}^{n} (y_i - \bar{y})^2$$

$$Sxy = \sum_{i=1}^{n} ((x_i - \bar{x})(y_i - \bar{y})$$

x_i (time series), y_i (variable of interest), $\bar{x}$ and $\bar{y}$ refer to the ranks

The parametric test, linear regression is assumed data to be normally distributed and examined whether there was a linear trend between time series (x) and variable of interest (y).The regression gradient was computed by,

$$b = \frac{\sum_{i=1}^{n} ((x_i - \bar{x})((y_i - \bar{y})}{\sum_{i=1}^{n} (x_i - \bar{x})^2}$$

The intercept was computed as $a = \bar{y} - bx$, the test statistic S is

S = b / σ

Where, $\sigma = \sqrt{\frac{12 \sum_{i=1}^{n} (y_i - a - bx_i)}{n(n-2)(n^2-1)}}$

The test statistic S followed a Student-t distribution with n-2 degrees of freedom. The linear regression test assumed that data was normally distributed. The trend was determined by Trend analysis software (Francis and Lionel, 2005).

The decadal variation of rainfall at Madhira station was studied for the four decades 1981-90, 1991-00, 2001-10 and 2011-20.

Results and Discussion

This section describes rainfall analysis mean, SD, coefficient of variation, kurtosis and skewness of monthly and seasonal rainfall; assessment rainfall trends monthly, seasonal and annual through M-K (nonparametric), Spearman's Rho (nonparametric), linear regression (parametric) methods and decadal variation of rainfall. The descriptive statistics information of rainfall like maximum and minimum received rainfall, mean, SD, coefficient of variation, kurtosis and skewness were presented in Table 1. Here in computed table, it was identified that the average annual maximum, minimum and mean rainfall were 1609.6, 529.8 and 982.4 mm, respectively. The mean highest and lowest monthly rainfall were 226.5 mm and 6.1 mm in the month of August and February, respectively and mean seasonal highest and lowest rainfall was 720.5 mm and 71.2 mm during monsoon and pre monsoon period. The average monthly and seasonal SD range observed was13.42 to 114.30 mm and 64.02 to 269.57 mm, respectively. Average monthly and seasonal rainfall CV was ranging from 43.91 to 300.56 per cent and 27.44to 171.87 per cent, respectively.Similar results were reported by Guhathakurta *et al.* (2020) with highest contribution to the southwest monsoon rainfall was 30.5 % in the month of August followed by 29.7 %, 21.2 %, and 18.6 % in the months of July, September and June respectively. Telangana state received annual rainfall about 78.8 % from southwest monsoon. The CV of monsoon was 22.5 % and annual rainfall for Telangana state was 20.5 %.

Table 1: Historically (1975-2021) rainfall analysis mean, standard deviation, coefficient of variation (CV), kurtosis and skewness for monthly, seasonal and annual rainfall

Parameters	Maximum (mm)	Minimum (mm)	Mean (mm)	SD (mm)	CV (%)	Kurtosis	Skewness
Jan	51.4	0.0	6.2	13.42	217.95	4.56	2.34
Feb	102.7	0.0	6.1	18.19	300.56	18.73	4.13
March	67.4	0.0	10.4	19.16	184.27	2.77	2.00
April	73.6	0.0	15.8	21.88	138.52	1.56	1.55
May	268	0.0	46.0	55.18	120.03	6.28	2.28
June	277.1	1.2	120.8	81.57	67.54	-1.00	0.33
July	553.6	76.8	213.9	103.72	48.48	2.47	1.47
Aug	465.9	44.6	226.5	99.46	43.91	0.38	0.69
Sept	356	0.0	159.3	86.75	54.46	-0.55	0.45
Oct	472.6	0.0	130.8	114.30	87.36	0.74	1.19
Nov	392	0.0	39.4	69.14	175.46	14.50	3.41
Dec	87.3	0.0	8.3	19.38	234.43	8.57	2.97
Winter	102.7	0.0	12.2	20.98	171.87	6.84	2.39
Pre monsoon	335.4	0.0	71.2	64.02	89.93	6.41	2.17
Monsoon	1363.5	398.0	720.5	220.95	30.67	0.34	0.65
Post monsoon	493.4	0.0	178.5	129.81	72.73	-0.18	0.86
Annual	1609.6	529.8	982.4	269.57	27.44	-0.39	0.47

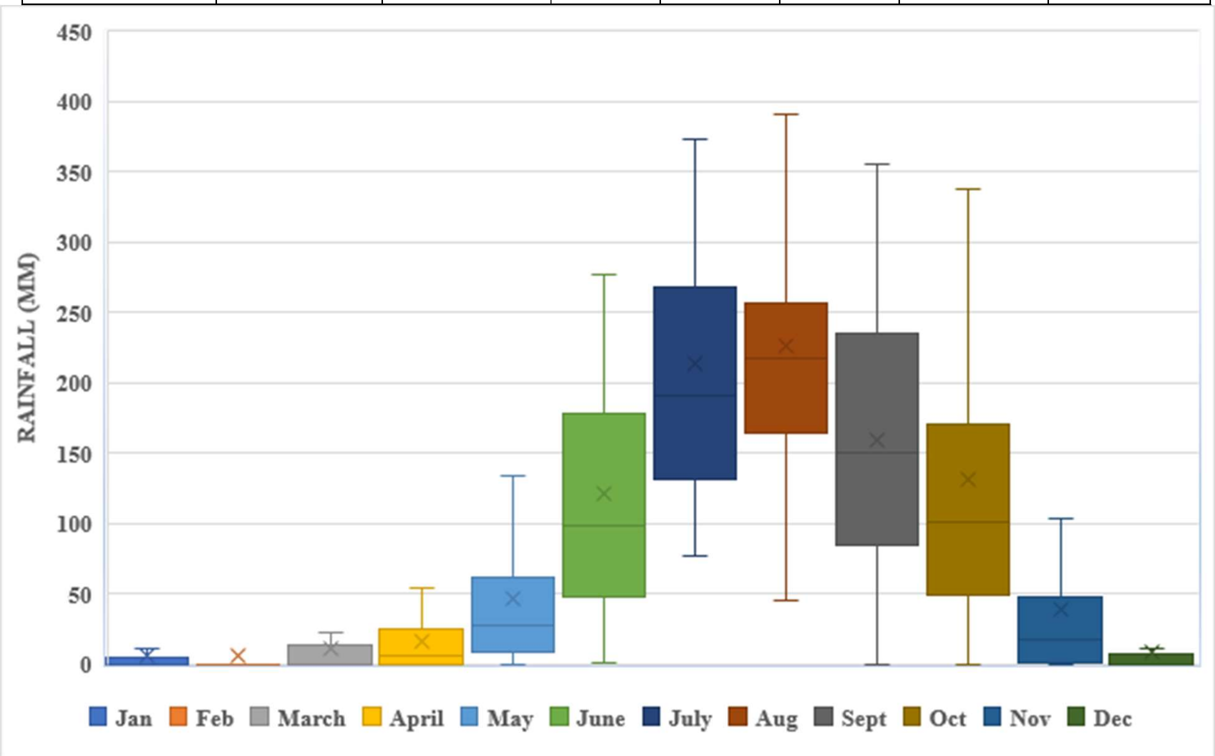

Figure 1: Monthly rainfall (mm) in Box and whisker plot for Madhira station from 1975 to 2021

Kurtosis measures of whether the rainfall data were heavy to light tailed against normal distribution. The data sets with excess kurtosis tend to have outliers or heavy tails. Data sets with lower kurtosis tend to have a lack of outliers or light tails with identical distribution being an extreme case. The highest and lowest values of coefficient of kurtosis were found as 18.73 and -1.00 for the month of February and June resembling heavy tailed and light tailed respectively. The highest and lowest values were found as 6.41 and -0.18for pre and post monsoon seasons that were heavy and light tailed respectively. The annual value of kurtosis was -0.39 which overall resembled light tailed to nearly normal distribution. Skewness measures symmetry or more accurately the lack of symmetry. A data set or distribution is symmetric whether it looks the same to the left and right from centre point. The skewness was found to be high for the month of February with4.13 representing asymmetry and low for the month of June with0.33 representing nearer to symmetry. For the season winter, skewness was high at 2.39 resembling positively skewed and the monsoon season was low at 0.65 even though it was positively skewed. The annual value was 0.47 indicated positively skewed resembling the right of the mean. Similar study analyzed by Vishnuvardhan *et al.* (2020) and observed that kurtosis was having an annual value of 0.23 in contrast to the present study, indicating slightly leptokurtic in nature. Skewness was the mean predominantly positive skewness of average annual value 0.44 representing annual rainfall in the region (YSR Kadapa district, AP) was asymmetric and it lied to the right of the mean that was right skewed which was similar to the present study.

Rainfall trend

Monthly rainfall

Figure 2 showed that the monthly rainfall pattern revealed that excess amounts of rainfall occur in SW monsoon months that were from June to September at Madhira station, monthly rainfall analysis was presented in Table 2. In these results, it was observed that January, February, March, May, June, September and December months showed non significantly increasing trend while April, July, August, October and November district showed non significantly increasing trend. In Telangana state, non-significant increasing trend was observed for the Khammam district and all other districts showed significantly decreasing trend for the rainfall month of June. Khammam district indicated non-significantly decreasing trend while all other districts indicated significantly decreasing trend in rainfall for the month of July and August (Guhathakurta *et al.*, 2020).

Seasonal rainfall trend

Winter

The rainfall trends graphically presented in Figure 2 showed an increasing trend of winter rainfall at Madhira, where the linear regression equation indicated positive slope value (b =0.035) and R^2 value was0.00. R^2 the coefficient of determination value explained that no variance in winter rainfall was elucidated by the linear regression model. In Table 2, Spearman's Rho (non parametric) tests showed that there was significance of increasing trend at 1.00per cent level and other two tests showed no significance. Krishnakumar *et al.* (2009) also reported an increasing trend in winter rain events in Karnataka state.

Pre monsoon

The rainfall trends in Figure 3 showed that there was an increasing trend of pre monsoon rainfall at Madhira and the linear regression equation showed positive slope value (b = 0.155), R^2 value of 0.001. R^2 was 0.1 % of variability in winter rainfall explained by the linear regression model. In Table 2 all three rainfall trend tests showed insignificance. Analysis of trend by Dash *et al.* (2011) represented that during the summer monsoon, short spell rain activities with high intensity increased over India as a whole.

Monsoon / Southwest monsoon

The rainfall trends in Table 2showedno significant increasing trend for M-K and Spearman's Rho tests and decreasing trends for in Linear regression test during pre monsoon rainfall at Madhira, but in the Figure 4 linear regression equation was indicating negative slope value (b = - 0.522), theR^2 value was 0.001. R^2 explained that 0.1 % of variability in monsoon rainfall was explained by this linear regression model. For the SW monsoon season, Khammam district indicated the non-significantly decreasing trend and these results were similar to the analysis of Guhathakurta *et al.* (2020).The decrease in standard precipitation index (SPI)

Table 2: Assessment rainfall of monthly, seasonal and annul through parametric and non parametric methods

Parameters	Mann- Kendall (Non parametric)	Spearman's Rho (Non parametric)	Linear regression (Parametric)
Jan	0.779	0.911	0.929
Feb	0.128	4.203	-0.650
March	1.366	3.444	0.871
April	-0.028	0.795	0.005
May	0.000	0.158	-0.043
June	0.779	0.911	0.929
July	-0.367	-0.461	-0.340
Aug	-1.376	-1.383	-0.949
Sept	0.138	0.167	0.064
Oct	-1.000	-1.141	-1.038
Nov	-0.908	-0.776	-1.426
Dec	0.202	3.172	1.074
Winter	0.569	3.085***	0.153
Pre monsoon	0.734	0.815	0.224
Monsoon	0.22	0.049	-0.217
Post monsoon	-1.623	-1.734	-1.526
Annual	-0.651	-0.718	-0.836

Note: * = significant at 0.01, ** = significant at 0.05, * = significant at 0.1 level**

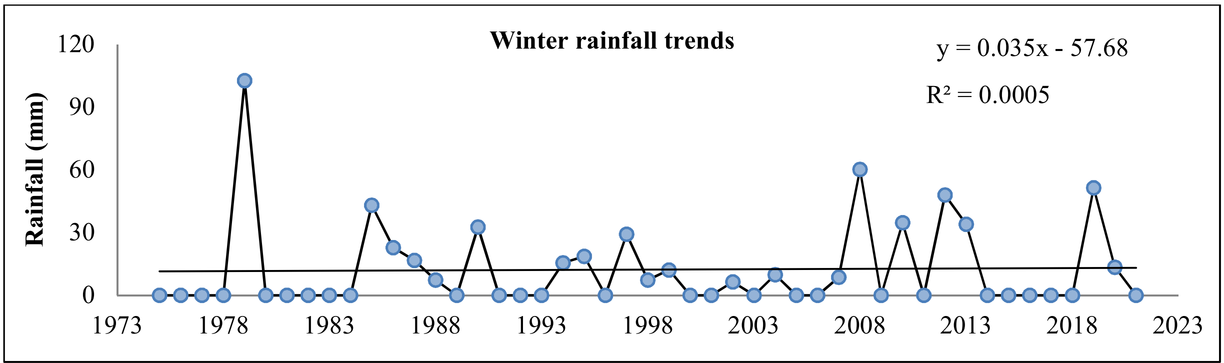

Figure 2: Winter rainfall trends at Madhira from 1975 to 2021

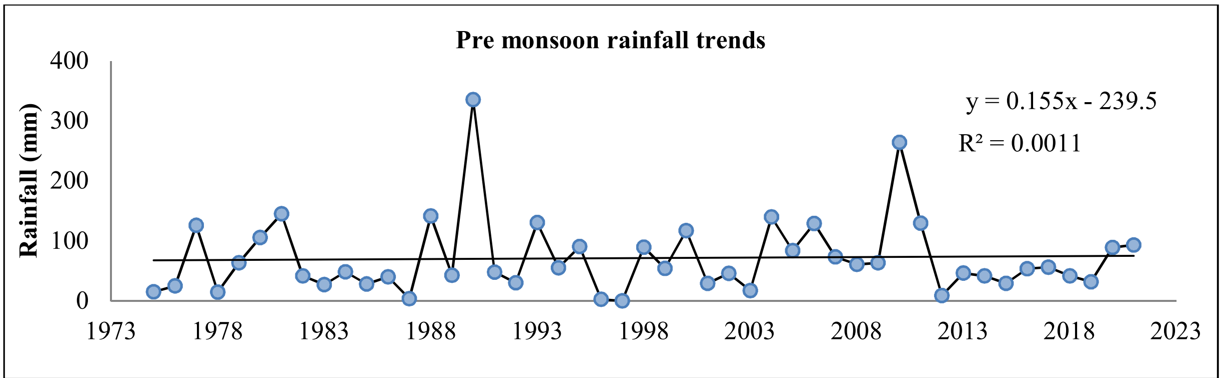

Figure 3: Pre monsoon rainfall trends at Madhira from 1975 to 2021

values were observed over most of the districts of central India and endmost south peninsular India during June, July and September months, while in the month of August it was over eastern, north-eastern and Tamil Nadu region (Guhathakurta, 2017).

Post Monsoon

In Figure 3 rainfall trend was showing general decreasing trend for all three tests during post monsoon at Madhira, where linear regression equation was indicating negative slope value (b = -2.099), R^2 value was 0.049. $R^2$was explained that 4.9% of variability in the monsoon rainfall was elucidated by the linear regression model. In over all, all three rainfall trend tests as given in Table 2 were showed insignificance. Guhathakurta *et al.* (2020) in their analysis indicated that the monthly rainfall did not indicate any significant increasing or decreasing trend while seasonal or annual rainfall indicated non-significant decreasing trend.

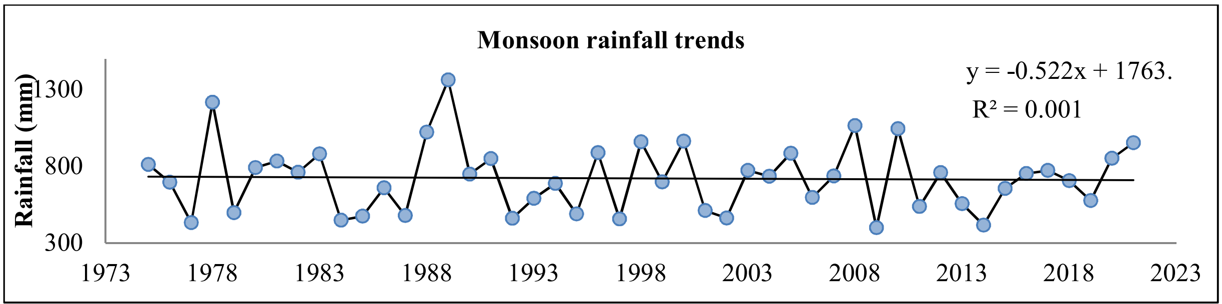

Figure 4: Monsoon rainfall trends at Madhira from 1975 to 2021

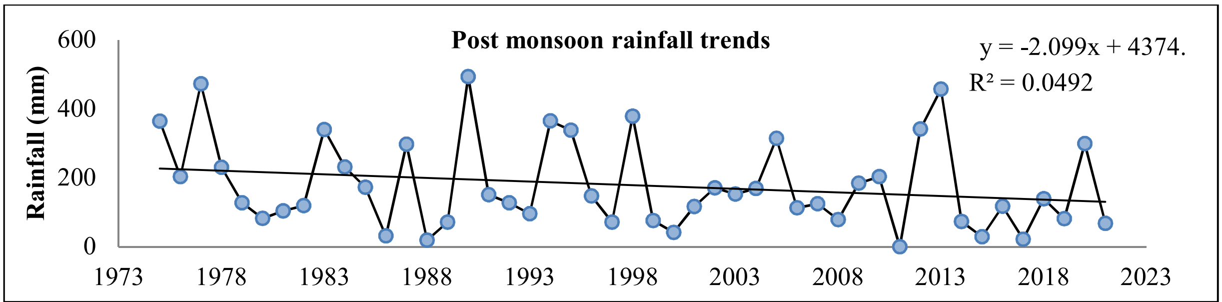

Figure 5: Post monsoon rainfall trends of Madhira from 1975 to 2021

Annual rainfall trend

Figure 6 showed the general decreasing trend of annual rainfall at Madhira, where the linear regression equation was negative slope value of b = - 2.431 and the R^2 value was 0.015. R^2 showed 1.5 % variability in the annual rainfall as shown in the linear regression model. In Table 2, all rainfall trend test methods showed that there was insignificance of decreasing trend. The analysis indicated that neither monthly or seasonal nor annual rainfall for the state as whole rainfall showed any statistically significant increasing/decreasing trend. Guhathakurta *et al.* (2020) also stated that seasonal (SW) or annual rainfall show non-significant decreasing trend for Telangana state.

Decadal variation in rainfall

Apart from the annual variation in rainfall, it was observed that southwest monsoon rainfall exhibited significant multi decadal variations in the Indian region (Guhathakurta and Rajeevan, 2008). In view of the importance of decadal variation in rainfall, many researchers have studied the same using different scales.Guhathakurta *et al.*, 2014 have studied such variation for Indian summer monsoon rainfall and stated that there was a decreasing trend in decadal rainfall during the period 1961-2010. At micro scale (station level), studies on decadal variability of rainfall were carried out by Seetharam (2003) for Jalpaiguri and Kavi (1988) for Bangalore.

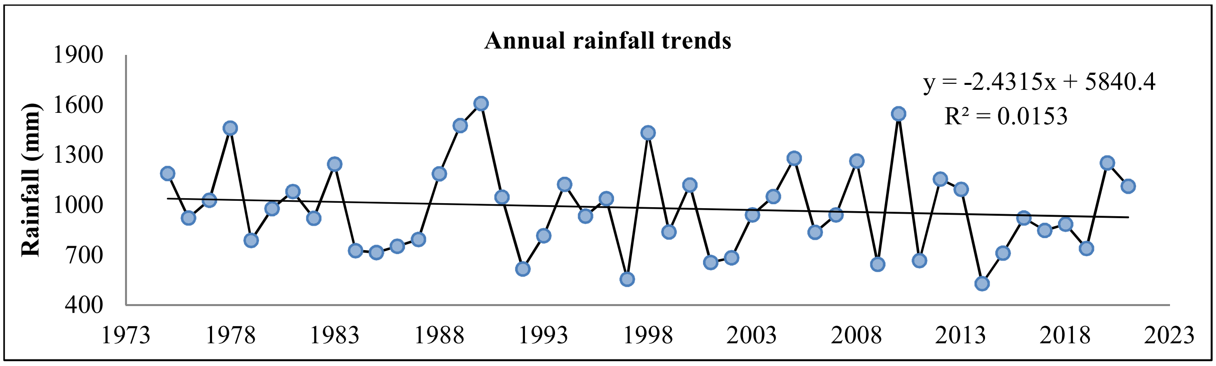

Figure 6: Annual rainfall trends of Madhira from 1975 to 2021

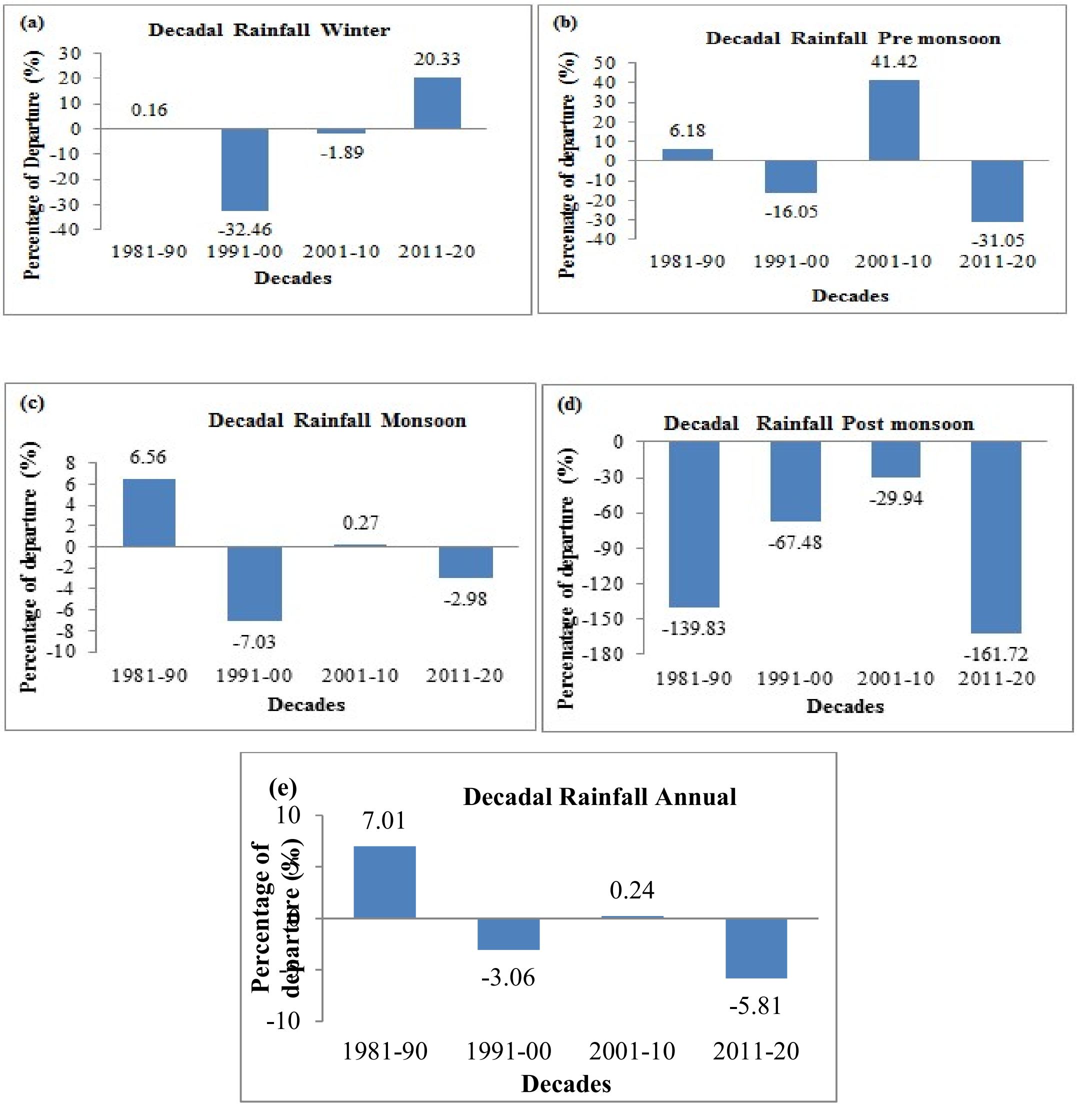

Figure 6(a-e): Plots of decadal variation in rainfall at Madhira

A similar attempt was made here at Madhira to study for the decades 1981-90, 1991-00, 2001-10 and 2011-20. It was observed from Figure 6 that during the winter season, decadal rainfall departures mostly showed deep negative departure during the decade 1991-00 and positive departure during 2011-20. However, significant positive of 41.42 per cent and negative of 31.05 per cent, departures were observed during the decades 2001-10 and 2011-20 respectively for pre monsoon season. Similar trend was observed for winter and pre monsoon season for the decades of 1981-90 and 1991-00.The monsoon season of 1991-00 showed the highest negative at Madhira station in the period of study. Similarly, *vice-versa* was observed during the decade 1981-90, which was the decade with highest positive departure for Madhira station. The overall observation of all decades showed an alternate increasing and decreasing trend at Madhira during the period of study for the monsoon season. For the post-monsoon season from 1981-90 to 2011-20 all decades were showing negative departures fall on the same side of the axis and highest negative departure (161.72 per cent) was observed for the decade of 2011-20. Since the annual rainfall is mostly dominated by monsoon so that a similar pattern is observed in annual rainfall also.Similar observations were also studied by Ranjan and Saha (2022) for rainfall trend over Tripura dominated by monsoon season that majorly influencing annual rainfall.

Conclusion

In the present rainfall analysis, an attempt has been made to find the trends at Madhira station, Khammam district of Telangana at micro scale level variations during the period 1975-2021. The CV, kurtosis and skewness were computed along with trend analysis and decadal variation of rainfall. The average monthly and seasonal rainfall CV ranged from 43.91 to 300.56 per cent and 27.44to 171.87 per cent respectively. The annual value of kurtosis and skewness were -0.39 and 0.47 which overall resembles light tailed to normal distribution and positively skewed to the right of the mean. During monsoon, post monsoon seasons and annual rainfall, the trends were showing non-significantly decreasing trends while winter and post monsoon were showing non-significantly increasing trends. But as per Spearman's Rho (non parametric) tests there was a significant increasing trend for the winter season. Overall, the decades of observations showed an alternate increasing and decreasing trend at Madhira during the period of study for the monsoon season. For the post-monsoon season all four decades showed negative departures. The annual rainfall was mostly dominated by monsoon and a similar pattern was observed in annual rainfall also.

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Conflict of interest

The authors declare that they have no conflict of interest.

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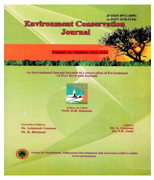

Screening germplasm lines for identification of resistant source against gray mold disease (*Amphobotrys ricini* (N.F. Buchw.) Hennebert) of castor

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ABSTRACT

The present study was aimed to screen castor germplasm lines varying in wax content against gray mold disease for the identification of resistance source under *in vitro* conditions using detached spike and detached capsule technique. Out of thirty-three lines screened against gray mold under *in vitro* conditions six lines with low wax content *viz*., RG-1754 (0.24 µg/mg), RG-1875 (0.12 µg/mg), RG-1915 (0.21 µg/mg), RG-1919 (0.24 µg/mg), RG-1972 (0.08 µg/mg) and RG-1926 (0.08 µg/mg) recorded significantly low levels of infection ranging from 10 to 20 per cent 7 days after inoculation. Whereas the susceptible cultivar DCH -519 has recorded disease severity of more than 90 %. The pearman's rank correlation analysis showed a strong positive relation between disease severity and capsule wax content, with $p < 0.01$ and $r = 0.884$.

Introduction

Castor (*Ricinus communis* L.), a predominant non-edible oilseed crop that is native to Ethiopia and North Africa, is a member of the Euphorbiaceae family (Ramanjaneyulu *et al.,* 2017). Castor seed, primarily grown in tropical and subtropical climates contains 50-55 per cent oil and plays a significant role in the Indian oil economy. India produces approximately 73 per cent of the world's castor with an annual production of around 1.19 million tonnes. India earned about 780 million USD from the sales of castor seed, castor oil and its derivatives during 2020-21 (SEA, 2021). Currently, India leads the world in both castor production and area. Castor oil has several industrial applications and is valuable as a source of therapeutic oil. In addition to being used in the production of a number of complex products such as nylon fibres, lubricants for jet engine, hydraulic fluids, polymers, and synthetic leather. Dehydrated castor oil is utilised as an antifreeze for engine fuels and lubricants used in spacecraft and rocket. (1982 by Ogunniyi, 2006). As the oil contains up to 90% of the hydroxy fatty acid ricinoleic acid, castor oil stands out from other vegetable oils. Due to the huge demand for castor oil and its derivatives, India is expected to stay as a major producer of castor seeds worldwide with China and Brazil as its only competitors. However, India has not yet fully utilised the potential of its monopoly because few value-added goods and largely raw oil are exported. (Bhavanidurga, 2013;

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Severino *et al*., 2012; Jeong and Park, 2009).Castor crop inspite of its importance as commercial crop and hardy nature is attacked by numerous bacterial and fungal diseases. At various crop growth stages there are 150 or more identified pathogens of castor plant. Among these, the most destructive disease is gray mold caused by the fungus *Amphobotrys ricini* (N.F. Buchw.) Hennebert (Prasad *et al.,* 2016). The anamorphic phase is mainly responsible for causing disease epiphytotics. Heavy yield losses were observed, as the inflorescence (raceme) and the capsules are the primary targets of the fungal infection. Heavy rains that last for a long time during the spike/capsule formation stage results in significant losses. As the development of pathogen is favored by the prevalence of high relative humidity and extended wet conditions during the flowering stage. This disease causes significant yield losses up to 100 % in India (Soares, 2012). which pose a serious hazard to the crop's commercial cultivation. As a result, there have been only a few advancements in the management of gray mold disease. Although breeding initiatives to create resistant genotypes have been unsuccessful, those with a moderate level of tolerance have been identified (Anjani, 2012). The preliminary studies of Ayesha *et al.* (2022) indicates a positive correlation between wax content and gray mold severity. The present study was conducted to identify resistant sources against gray mold disease by screening different germplasm lines varying in bloom content under *in vitro* conditions.

Material and Methods

Isolation of pathogen

The castor capsules with typical gray mold symptoms obtained from research farm, ICAR-IIOR, Hyderabad were used for isolation of the fungal pathogen. The pathogen was isolated from the pericarp of infected castor capsules The pericarp pieces along with fungal growth were picked, surface sterilized and isolated on the enriched oat meal agar media (OMA). The mycelial growth appeared 48 h after inoculation and the pathogen was further purified by single spore isolation method.

Estimation of wax content of the capsules

Wax content of the capsules was estimated using calorimetric approach. From each germplasm line with 15 day old spike, by using rapid calorimetric method (Ebercon *et al.,* 1977).

Screening against gray mold disease

Castor germplasm lines were screened against gray mold under *in vitro* conditions using detached spike and detached capsule techniques (Prasad *et al.,* 2016).

Preparation of Inoculum of the pathogen

`Pathogen inoculum for disease screening was prepared from seven day-old, highly sporulating culture of *Amphobotrys ricini* grown on oat meal agar medium. (Prasad & Bhuvaneswari, 2014).

Evaluation of castor germplasm lines using detached spike technique against *A. ricin*i infection

Thirty three germplasm lines along with resistant (ICS-324) and susceptible (DCH-519) checks were screened against gray mold disease under glass house conditions by following the standard screening protocol given by Prasad *et al* (2016). The Gray mold disease severity was recorded manually based on the visual symptoms starting from 3rd day till the entire spike of the susceptible check (DCH-519) was covered by the disease. Disease severity and host resistance were assessed based on the diagrammatic scale developed by Sussel *et al*. (2009) and the disease reaction was categorized based on the scale developed by Prasad *et al.* (2016). (Table-1)

Table 1: Disease scale for host resistance assessment against gray mold of castor

Disease scale	Intensity of infection (%)	Reaction
0	No infection	Immune
1	1 to 10% raceme area infected	Resistant
3	11 to 20% raceme area infected	Moderately resistant
5	21 to 30% raceme area infected	Moderately susceptible
7	31 to 50% raceme area infected	Susceptible
9	>51% raceme area infected	Highly susceptible

Evaluation of castor germplasm lines using detached capsule technique against *A. ricini* infection

The germplasm lines were screened against gray mold infection under *invitro* conditions by using detached capsule method (Prasad *et al.*, 2016). The disease reaction was scored on 1-4 scale as given in table 2.

Table 2: Disease scale for assessment against gray mold of castor in detached capsule technique

Disease scale	Symptoms observed
1	Browning of capsules
2	Little mycelia growth
3	Mycelium development with few sporulation
4	Entire capsule covered with high sporulation

Results and Discussion

Per cent disease severity and wax intensity on capsules

Thirty three germplasm lines varying in wax content were screened under artificial epiphytotic conditions in glass house. The initial symptoms of *A. ricini* infection appeared on third day after inoculation and the disease severity was recorded using the disease severity scale at regular intervals till maximum disease severity occurred on susceptible check cv. DCH-519. All the germplasm lines showed disease severity of >10 per cent. Of 33 germplasm lines, six lines with low wax content *viz.*, RG-1754 (0.24 µg/mg), RG-1875 (0.12 µg/mg), RG-1915 (0.21 µg/mg), RG-1919 (0.24 µg/mg), RG-1972 (0.08 µg/mg) and RG-1926 (0.08 µg/mg) recorded significantly low levels of infection ranging from 10 to 20 per cent. The germplasm lines RG-1836 (0.19 µg/mg), RG-1881 (0.27 µg/mg), RG-1839 (0.22 µg/mg), RG-155 (0.35 µg/mg), RG-1905 (0.10 µg/mg) and RG-1906 (0.25 µg/mg) recorded disease severity levels of 20-30 per cent, which were moderately resistant. The remaining lines exhibited disease severity levels greater than 30 per cent showing a susceptible reaction. (Table-3 fig-1)

Detached capsule technique (*in vitro* screening)

There was significant difference in disease severity readings among germplasm lines varying in wax content and the results were in accordance with detached spike screening. Six germplasm lines with low wax content (RG-1754, RG-1875, RG-1915, RG-1972, RG-1906, RG-1926) have shown low level of disease infection with browning observed after 7 days of inoculation. The germplasm line RG-1919, RG-1836, RG-155, RG-1881, RG-1851 have shown little mycelial growth on the capsules, which were moderately resistant. The remaining germplasm lines have shown to take up infection at greater rate with 10 lines showing profuse mycelial growth with little sporulation and 12 lines have recorded high disease infection rate shown as entire capsule covered with high sporulation, which was similar to the susceptible check DCH-519 showing a susceptible reaction (Table 3 and figure 2). The Pearman's correlation analysis between per cent intensity of infection from *in vitro* screening with biochemically extracted wax on the capsules revealed that wax content and per cent disease severity were significantly correlated at 0.01 level (2-tailed) with $p < 0.01$ and r = 0.884 representing a strong positive correlation. Gray mold is considered as the most destructive disease of castor, the mechanisms underlying the resistance to this fungal pathogen remains unclear. Understanding the interaction between the host and the pathogen and identifying the resistance sources is of great significance in plant breeding programmes. The present study aimed to identify the resistant sources against the gray mold disease from the existing castor germplasm lines. The results of the present study, indicates that among the germplasm lines screened ICS-324 having low amount of capsular wax has shown significantly less amount of disease severity under *invitro* conditions. The results are further supported by Sujatha *et al.* (2016) also screened four castor genotypes using detached capsule technique and reported that genotype DCS-9 was highly susceptible to the disease, while genotype RG-3216 R exhibited least susceptibility. Also, variable degree of tolerance to gray mold pathogen in castor genotypes has been reported (Araújo *et al.*, 2007; Anjani, 2012). The study further indicate a significant positive correlation between the gray mold severity and the amount of capsule wax content which is in accordance with earlier studies of Ayesha *et al.* (2022) indicating a possible role of wax governing the pathogenesis. Studies so far have focused on the kind of bloom (no bloom, single, double, and triple bloom) and its influence on the severity of gray mold (Prasad *et al.*, 2016), and also pathogenesis of various diseases in the presence or absence of wax in various host plants (Wang *et al.,* 2008; Bourdenx *et al.,* 2011; Hansjakob *et al.,* 2011; Zhu *et al.,* 2017) governing disease. The wax layer acting as protective barrier is playing a role in pathogenesis which is similar to the results observed by Weidenbach *et al.* (2014) where cuticular wax determined the pathogenicity of

Table 3: Evaluation of castor germplasm lines for their reaction to gray mold

Germplasm lines	Wax content (µg/mg)	Disease severity in detached spike method	Disease scale	Disease severity scale in detached capsule method
RG-1754	0.240	18.0 (25.08)*	3	1
RG-1875	0.128	13.3(21.3)	3	1
RG-1915	0.219	11.6(19.9)	3	1
RG-1919	0.242	20.0 (26.5)	3	2
RG-1836	0.194	21.6 (27.7)	5	2
RG-1843	0.387	51.3 (45.7)	9	3
RG-1972	0.083	17.3 (24.5)	3	1
RG-1853	1.030	71.6 (57.8)	9	4
RG-1859	0.520	41.6 (40.1)	7	3
RG-155	0.355	27.0 (31.2)	5	2
RG-1961	0.103	43.3 (41.1)	7	3
RG-1971	0.120	46.6 (43.0)	7	3
RG-1920	0.513	53.3 (46.8)	9	3
RG-1966	1.510	85.0 (67.18)	9	4
RG-1906	0.257	25.0 (29.9)	5	1
RG-1855	0.627	63.3 (52.7)	9	4
RG-1881	0.277	28.6 (32.3)	5	2
RG-1839	0.220	23.0 (28.6)	5	2
RG-1998	0.323	43.3 (41.1)	7	3
RG-1926	0.087	12.3 (20.4)	3	1
RG-1854	0.907	73.3 (58.9)	9	4
RG-1860	1.470	85.0 (67.2)	9	4
RG1905	0.103	29.0 (32.5)	5	2
RG-1851	0.530	48.3 (44.0)	7	3
RG-1872	0.740	51.6 (45.9)	9	3
RG-1921	1.013	42.3 (40.5)	7	3
RG-1952	0.960	75.0 (59.9)	9	4
RG-1791	0.640	58.0 (49.5)	9	3
RG-1917	1.010	70.0 (56.7)	9	4
RG-1999	1.120	76.0 (60.6)	9	4
RG-2058	0.427	53.3 (46.8)	9	4
RG-2012	1.083	71.6 (57.8)	9	4
RG-1777	0.857	66.6 (54.7)	9	4
DCH-519	1.927	93.3 (75.2)	9	4
ICS-324	0.150	7.3 (15.6)	1	1
C.D.@ 5%	0.158	2.822		
SE(m)	0.056	0.998		
SE(d)	0.079	1.412		
C.V.	16.658	3.739		

DCH-519 **RG-1919** **ICS-324**

Figure 1: Reaction of castor germplasm lines to gray mold in detached spike technique

DCH-519 **RG-1919** **ICS-324**

Figure 2: Reaction of castor germplasm lines to gray mold in detached capsule technique

powdery mildew. Similar results were observed by Ayesha (2020) who screened 26 castor genotypes varying in the morphological characters against gray mold infection using detached capsule technique. However, the focus of this investigation was to identify low waxy content germplasm lines and their reaction to gray mold disease which further help in identification of resistant sources. Considering the impact of waxy bloom levels on the severity of the gray mold disease severity helps in eliminate high wax plants at early stages saving time and improving the quality of breeding programmes. Additionally, breeding programmes aimed to develop gray mold resistant cultivars can select germplasm lines with low wax content which are expected to be least affected by fungus.

Conclusion

From the study it can be concluded that there exist a positive correlation between wax content and gray disease severity. The germplasm lines RG-1754, RG-1875, RG-1915, RG-1919, RG-1972 and RG-1926 with low level of wax serve as better source of resistance to gray mold disease of castor and the amount of wax on the castor capsule can be used as a biochemical marker to screen large number of germplasm lines or early generation breeding material with large population size.

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Conflict of interest

The authors declare that they have no conflict of interest.

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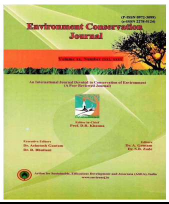

Efficiency of reactors composed of plant based absorbents in combination with sand and gravel for physicochemical parameters of different category water

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ABSTRACT

According to the world Summit of sustainable Development, the major reason for lack of safe water is either scarcity of water or contamination of water sources. Therefore this study eye on developing nations, seeks to find sustainable, yet economically and socially practical solution to the problems associated with polluted water. To fulfil the objectives of the present study, two medicinal value plants *Dhav (Anogeissuslatifolia wall.)* and *Aragvadha (Cassia fistula Linn.)* were selected and absorbents were prepared from the bark of both the plants. Then the reactors were prepared using the different compositions of sand, gravel, absorbents of *Dhav*and *Aragvadha* and cotton.Inthis way four filter reactors were prepared. The feeding rate of raw water is maintained at 0.5 litre per hour. The results revealed that *Dhav* plant absorbent was found more effective than*Aragvadha*. All reactors shows different efficiencies for different parameters suggesting that reactors should be prepared based on need or targeted parameters. Over all reactor 4 shows better efficiency for all the parameters. The main exclusivity of the present reactor is low cost with no electricity demand. The prepared reactors are environment friendly and easily implementable but further study is required toprove it on large scale.

Introduction

Water is one among the basic requirement for the survival of human race on this earth. Water is a key resource for human civilization, for human life, for our economy, agriculture and for every aspect of our existence. Water and its conservation have inevitably had a central place in the Indian ethosand in custom and culture (Ahamad *et al.,* 2022; Dineshа*et al.,* 2023). Water and water access are key not just to country's economic development but also for socio-economic equity and gender justice. Water is becoming a rare resource in the world (Tyagi *et al.,* 2020). In India alone the International water management Institute (IWMI) predicted that by 2025, one person in three will live in conditionof absolute water scarcity (IWMI, Bhutiani and Ahamad, 2018). Water as resource is under relentless pressure due to population growth, rapid urbanization, large scale industrialization and environmental concern (Ruhela *et al.,* 2022; Bojago *et al.,* 2023). The conservation of a better living environment requires fighting against all forms of environmental pollution. It is important that water is managed optimally and efficiently (Bhutiani *etal.,* 2016; Patel *et al.,* 2020; Bhutiani *et al.,* 2022).

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Wastewater is generated in almost all the human routine activities in all the sectors viz. industrial, domestic and agriculture. Most of the water supplied to a society returned as wastewater due to increased automation of life style (Singh *et al.,* 2021; Owodunni and Ismail, 2021). Therefore a huge quantity of wastewater is generated throughout the world both in developed and developing countries. In developed countries, almost all the quantity of wastewater generated is recycled or discharged after treatment due to availability of funds, skilled manpower and advanced technologies (Bhutiani *et al.,* 2017; Yin, 2010). At the same time, 70% of wastewater generated is discharged either in untreated or partially treated form in developing countries due to lack of funds, skilled manpower and advanced technologies (Bhutiani *et al.,* 2021). Wastewater discharged either in untreated or partially treated form causes the pollution of both surface water and groundwater. Drinking of this polluted surface and ground water causes different diseases in human beings (Khan *et al.,* 2013; Megersa *et al.,* 2014; Tiwari*et al.,* 2022). About 14000 deaths per day due to water pollution were reported by Anderson and Fenger (2003). The problem of ground and surface water pollution become more dangerous in developing countries such as India. Currently most of the India states are facing the problem of both quantity and quality degradation of both ground and surface water pollution. At the time India is facing the problem of wastewater generation and management. A lot of conventional and non- conventional wastewater treatment technologies are available but most of them have drawbacks like complex treatment process, requirement of skilled labour, high capital cost and high energy demand (Singh *et al.,* 2021). Biosorption is a low cost, easy to operate, no requirement of skilled labour, and environmental friendly technology of wastewater as compared to other (Singh *et al.,* 2021). A lot of literature is available using Biosorption technology for the treatment of natural as well as synthetic wastewater (Megersa *et al.,* 2014; Saleem and Bachmann, 2019; Chauhan *et al.,* 2019; Patel *et al.,* 2020; Shabaa *et al.,* 2021; Singh *et al.,* 2021; Owodunni and Ismail, 2021). But very few documents are available reporting the efficiency of plant based absorbents in combination with sand and gravel. Therefore in the present study an attempt has been made to assess the efficiency of two medicinal value plants (*Dhav* and *Aragvadha*) based absorbents in combination with sand and gravel for the treatment of tree different category water (Ganga water, Tap water and Sewage).

Material and Methods

Collection and preparations of bark ash:

The collection of selected plants part and ash preparation was performed as given below-

Step 1	*Dhava* and *Aragvadha* were collected from Chandi Devi hills Shyampur forest range and UAU, Rishikul Campus, Haridwar respectively
Step 2	They were dried in Sunlight for 7 days
Step 3	Dried bark weight of *Dhava* was 4.56 Kg and *Aragvadha* was 5.40 Kg respectively
Step 4	The calcinations of dried bark was done in Muffle furnace
Step 5	Bark was kept in *Sarav-samput* (Earthen pot with lid) and placed in furnace
Step 6	Desired temp. or set temp. of furnace was 550°C for 120 minutes
Step 7	Weight of bark after Calcination was about 2.6 and 1.8 Kg respectively

Analysis of bark absorbent: Then the prepared absorbents were analyzed for appearance, odour, taste, touch, pH value, Loss on drying at 105°C,Total ash, Acid- insoluble ash, Water soluble extractive, Alcohol soluble extractive.

Material required for the preparations of Reactors:

Following materials were required for reactor preparation-

- Bark ash coarse grinded particle
- Sand
- Gravel
- Markin Cloth
- Cotton
- Bisleri Bottles (1 liter)
- Apparatus holding Stands
- Containers fitted with tap (5 lit. Capacity)

Method of the preparations of Reactors:

Step 1	Filtration apparatus was made by using empty Bisleri water bottles
Step 2	In brief, a tiny hole was made in the cap of the bottle and bottle was cut down from the bottom
Step 3	Bottle was inverted upside down
Step 4	To fabricate the filtration apparatus, cotton was placed near the neck of bottle
Step 5	Afterwards, Gravel, sand, markin cloth, charcoal or bark ash, markin cloth, sand layer was filled respectively, after the cotton layer

Method of the preparations of Reactors: After the material arrangements, next step is reactor preparations.The description of each reactor is presented in table 1.The schematic diagram of reactor prepared is presented in figure 1.

Water sampling and analysis
Tap water sample was collected from Gurukula Kangri Vishwavidyalaya, Haridwar. Ganga water sample was collected from Kashyap ghat, Haridwar. Sewage sample was collected from 27MLD sewage treatment plant (STP) located in Jagjeetpur, Haridwar. After collection all the samples were analyzed as per the standard methods prescribed in APHA (2011) and Trivedi and Goel (1986), Khanna and Bhutiani (2011). Then the experiment was run and after the experiment all the water samples were analyzed using the same methodology.

Table 1: Description of each reactor

Reactor	Description of layer				
	I layer	**II layer**	**III layer**	**IV layer**	**Cotton layer**
1	Sand (2 cm)	Gravel layer (2 cm)	Sand (5 cm)	Gravel layer (5 cm)	Diameter=1 cm Thickness= 1 cm
2	Sand (1 cm)	*Dhava* absorbent layer (8 cm)	Sand (3 cm)	Gravel layer (3 cm)	Diameter=1 cm Thickness= 1 cm
3	Sand (1 cm)	*Aragvadha* absorbent layer (8 cm)	Sand (3 cm)	Gravel layer (3 cm)	Diameter=1 cm Thickness= 1 cm
4	Sand (1 cm)	*Dhava* and *Aragvadha* absorbent layer (8 cm)	Sand (3 cm)	Gravel layer (3 cm)	Diameter=1 cm Thickness= 1 cm

Results and Discussion

The results of organoleptic and physicochemical parameters of both the plant bark ash is given table2.

1. **Organoleptic study:** Blackish coloured coarse bark chips. *Dhava* bark ash light black whereas *Aragvadha* is dark black in colour. Brittle and crisp in touch.

2. **Physicochemical parameters-**

- **pH Value:** The pH value of an aqueous liquid may be defined as the common logarithm of reciprocal of the hydrogen ion concentration expressed in grams per liter. The aqueous solution of the sample was used for pH measurement by pH meter. The pH of the *Dahva* plant ash was 8.27 and *Aragvadha* plant ash was 8.01.

- **Loss on drying:** The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified condition. The loss on drying value of the *Dahva* plant ash was 2.90 and *Aragvadha* plant ash was 3.74.

- **Total ash:** The ash limit test is designed to measure the amount of the residual substance when sample is ignited under specified conditions. The total ash value of the *Dahva* plant ash was 23.11 and *Aragvadha* plant ash was 20.91.

- **Acid-insoluble ash:** The acid insoluble ash content was conducted to assess the % of

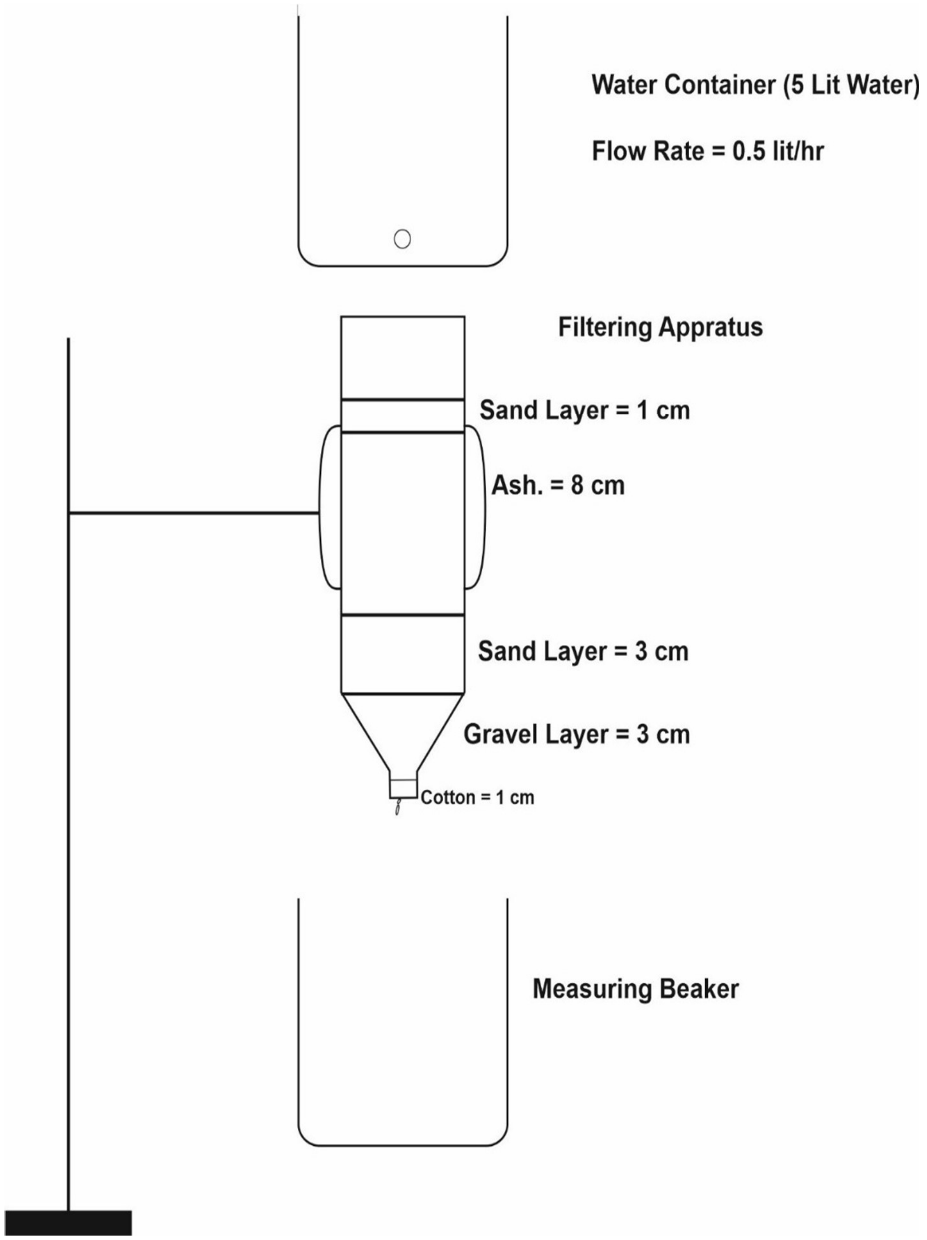


Figure 1: Schematic diagram of reactor

inorganic content of the sample, which is insoluble in dilute acid. The acid insoluble ash value of the *Dahva* plant ash was 1.05 and *Aragvadha* plant ash was 1.43.

- **Water soluble extractive:** This test was carried out to evaluate the water soluble extractive of the test drug. The water soluble extractive value of the *Dahva* plant ash was 2.15 and *Aragvadha* plant ash was 2.17.

- **Alcohol soluble extractive:** This test was carried out to evaluate the alcohol soluble extractive of the test drug. The alcohol soluble extractive value of the *Dahva* plant ash was 0.26 and *Aragvadha* plant ash was 0.34.

Table 2: Analytical description of *Dhava* and *Aragvadha*ash

SN	Test Parameters	*Dhava*	*Aragvadha*	Method reference
1.	Appearance	A light blackish coloured coarse bark chips.	A blackish coloured coarse bark chips.	Visual
2.	Odour	Odourless	Odourless	Smell
3.	Taste	Tasteless	Tasteless	Taste
4.	Touch	Brittle solid	Brittle solid	Touch
5.	pH	8.27	8.01	API
5.	Loss on drying	2.90	3.74	API Part II, Vol-1, Appendices-2.2.10
6.	Total ash	23.11	20.91	API Part II, Vol-1, Appendices-2.2.3
7.	Acid insoluble ash	1.05	1.43	API Part II, Vol-1, Appendices-2.2.4
8.	Water soluble extractive	2.15	2.17	API Part II, Vol-1, Appendices-2.2.8
9.	Alcohol soluble extractive	0.26	0.34	API Part II, Vol-1, Appendices-2.2.7

The results of all type of water i.e. Ganga water (GW), Tap water (TW) and Sewage (S) before and after the treatment with prepared reactors are given in table 3, 4 and 5. The percentage reduction is given table 6.

Turbidity

Turbidity is a measure of the light-transmitting properties of water and is comprised of suspended and colloidal material. Ideal value is below 1 NTU. It is important for health and aesthetic reasons. Minimum reduction of turbidity was observed in case of tap water (0%) by reactor 1 while maximum in case of sewage (67.6%) by reactor 4. The significant difference was found in mean turbidity among various treatments reactors {F(TW)=10.99, F(GW)=5.37, F(SW)=38.44, p<0.001). Turbidity decreased in each treated sample due to adsorption property of plant charcoal (ash) and slow sand intermittent filtration mechanism.

Total Solids (TS)

Total Solids (TS) is the amount of dissolved and suspended solids present in water. Minimum reduction of TS was observed in case of tap water (4.0%) by reactor 1 while maximum was also observed in case of tap water (39.5%) by reactor 4. The significant difference was found in mean TSamong various treatments reactors {F(TW) =23.35, F(GW)=27.41, F(SW)=10.71, p<0.001).

Total Suspended Solids (TSS)

Total Suspended Solids (TSS) is the amount of suspended solids present in water. Minimum reduction of TSS was observed in case of sewage (1.7%) by reactor 1 while maximum in case of Ganga water (79.0%) by reactor 2 and 4. The significant difference was found in mean TSS among various treatments {F(TW)=33.35, F(GW)=180.58, F(SW)=1.25, p<0.001).

Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS) is the measure of salt dissolved in a water sample after removal of suspended solids. TDS is the residue remaining after evaporation of the water. Many dissolved substance are undesirable in water. Dissolved minerals, gases and organic substances may produce colour, taste and odour which are aesthetically displeasing. Some chemicals may betoxic and carcinogenic. Minimum reduction of TDS was observed in case of tap water (2.5%) by reactor1 while maximum in case of sewage (34.6%) by reactor 4. The significant difference was found in mean TDS among various treatments {F(TW)=5.58, F(GW)=7.58, F(SW)=44.76, p<0.001). These parametric values decreased in each treated filter due to adsorption of organic and inorganic matter.

Electrical Conductivity (EC)

The Electrical Conductivity (EC)measures the capacity of water to transmit electric current. The concentration of total dissolved solid is related to electrical conductivity. The conductivity increases as the concentration of TDS increases.The corrosiveness of the water increases as TDS and EC increases. Minimum reduction of EC was observed in case of tap water (2.5%) by reactor 1 while maximum in case of sewage (34.6%) by reactor 4. The significant difference was found in mean EC among various treatments {F(TW)=5.58, F(GW)=7.58, F(SW)=44.80, p<0.001). EC also decreased because of decreased amount of dissolved organic compound or salt due to adsorption.

pH

Measurement of pH is one of the most important and frequently used tests, as every phase of waterand waste water treatment and waste quality management is pH dependent. The intensity of the acidic and basic character of a solution is indicated by pH or hydrogen ion concentration. pH valuesfrom 0 to 7 are diminishing acidic, 7 to 14 increasingly alkaline and 7 is neutral. Minimum gain of pH was observed in case of Ganga water (6.0%) by reactor 3 while maximum in case of sewage (34.9%) by reactor 2. Reduction was observed only in tap water (5.8%) by reactor 1. The significant difference was found in mean pH among various treatments {F(TW)=19.24, F(GW)=34.77, F(SW)=34.77, p<0.001).

Biochemical Oxygen Demand (BOD)

The amount of oxygen required by microorganisms to decompose organic matter present in water and waste water is known as biochemical oxygen demand (BOD). The BOD of raw water indicates the extent of organic matter present, thus indicating the extent of treatment required for purifying this water to make it safe and wholesome.Minimum reduction of BOD was observed in case of Ganga water (5.9%) by reactor 1 while maximum in caseof Ganga water (47.7%) by reactor 2. The significant difference was found in mean BOD among various treatments {F(TW)=15.12, F(GW)=33.81, F(SW)=13.93, p<0.001).

Table 3: Physicochemical characteristics of Ganga water before treatment and after treatment with reactor 1, 2, 3, and 4

Parameters	Untreated Water	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Turbidity	2.0	1.6±0.2	1.4±0.2	1.5±0.3	1.3±0.3
TS	545.0	470.0±1.7	389.0±32.4	433.7±15.0	389.0±32.4
TSS	225.0	73.3±12.7	47.3±12.7	67.0±8.7	47.3±12.7
TDS	403.0	371.3±11.0	341.7±21.0	366.7±6.4	341.7±21.0
EC	620.0	571.2±16.9	525.6±32.4	564.1±9.8	525.6±32.4
pH	7.8	6.5±0.2	8.4±0.2	8.3±0.2	8.4±0.2
BOD	2.6	2.4±0.2	1.4±0.4	1.4±0.3	1.3±0.3
COD	76.0	74.2±1.9	62.7±7.6	62.7±8.3	62.4±8.3
Hardness	120.0	110.7±6.1	85.3±14.0	100.7±11.0	76.0±14.4
Calcium	40.1	40.1±0.0	26.0±3.5	30.7±4.6	24.0±0.0
Magnesium	19.5	17.2±1.5	14.5±2.7	17.1±1.8	12.7±3.5
Acidity	125.0	178.3±2.9	86.7±15.3	103.3±5.8	78.3±20.2

Table 4: Physicochemical characteristics of Tap-water before treatment and after treatment with reactor 1, 2, 3, and 4

Parameters	Untreated Water	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Turbidity	0.2	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0
TS	372.0	357.2±5.2	307.0±39.2	320.4±32.0	225.1±48.6
TSS	19.0	12.9±1.9	9.4±1.6	10.1±1.7	9.1±1.5
TDS	353.0	344.3±3.8	297.6±37.6	310.3±30.5	294.0±38.3
EC	543.1	529.7±5.9	457.8±57.9	477.4±46.9	452.3±58.9
pH	7.4	6.9±0.2	8.2±0.4	7.9±0.3	8.6±0.2
BOD	3.8	2.9±0.2	2.4±0.4	2.9±0.3	2.3±0.5
COD	68.0	62.7±1.2	40.7±18.6	54.7±8.3	44.0±15.1
Hardness	286.0	248.0±8.0	197.3±26.6	239.3±9.0	190.7±32.3
Calcium	242.0	229.7±6.1	186.1±23.2	223.7±5.1	184.0±31.4
Magnesium	10.7	4.5±0.6	2.7±1.0	3.8±1.0	1.6±0.2
Acidity	155.0	198.3±7.6	126.7±15.3	126.7±5.8	120.0±20.0

Table 5: Physicochemical characteristics of Sewage water before treatment and after treatment with reactor 1, 2, 3, and 4

Parameters	Untreated Water	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Turbidity	29.0	17.0±4.6	9.9±1.8	12.9±4.5	9.4±2.3
TS	877.0	746.3±6.7	732.3±4.5	734.7±4.0	720.7±7.5
TSS	519.0	510.0±5.3	496.7±9.5	490.0±6.0	489.3±17.5
TDS	358.0	244.3±14.0	235.7±5.0	244.7±2.1	234.0±14.1
EC	550.8	375.9±21.6	362.6±7.7	376.4±3.2	360.0±21.7
pH	6.2	6.8±0.2	8.4±0.2	8.3±0.3	8.3±0.1
BOD	129.0	114.7±4.6	100.7±8.3	105.3±9.9	96.7±4.6
COD	1280.0	832.0±128.0	306.7±16.7	538.7±218.4	304.0±154.3
Hardness	320.0	265.3±12.9	215.3±5.0	246.0±35.0	178.7±20.1
Calcium	120.2	76.1±5.6	34.7±6.1	48.1±17.5	31.7±8.5
Magnesium	48.7	47.1±1.5	43.7±0.3	48.3±4.8	36.1±3.5
Acidity	200.0	286.7±5.8	116.7±7.6	116.7±7.6	58.3±14.4

Table 6: Percentage reduction in different physicochemical parameters of Ganga water, Tap-water and sewage after treatment with reactor 1, 2, 3, and 4

Parameters	Reactor 1			Reactor 2			Reactor 3			Reactor 4		
	GW	TW	S	GW	TW	S	GW	TW	S	GW	TW	S
Turbidity	18.7	0.0	41.4	30.0	50.0	66.0	26.7	50.0	55.6	33.3	50.0	67.6
TS	13.8	4.0	14.9	28.6	17.5	16.5	20.4	13.9	16.2	28.6	39.5	17.8
TSS	67.4	32.3	1.7	79.0	50.5	4.3	70.2	47.0	5.6	79.0	52.1	5.7
TDS	7.9	2.5	31.8	15.2	15.7	34.2	9.0	12.1	31.7	15.2	16.7	34.6
EC	7.9	2.5	31.8	15.2	15.7	34.2	9.0	12.1	31.7	15.2	16.7	34.6
pH	16.2	5.8	-9.1	-7.7	-11.0	-34.9	-6.0	-7.8	-33.9	-7.7	-16.8	-34.2
BOD	5.9	22.8	11.1	47.7	36.8	22.0	45.5	24.6	18.3	48.5	40.4	25.1
COD	2.4	7.8	35.0	17.5	40.2	76.0	17.5	19.6	57.9	18.0	35.3	76.3
Hardness	7.8	13.3	17.1	28.9	31.0	32.7	16.1	16.3	23.1	36.7	33.3	44.2
Calcium	0.0	5.1	36.7	35.0	23.1	71.1	23.4	7.6	60.0	40.0	24.0	73.6
Magnesium	11.7	58.5	3.3	25.8	74.5	10.3	12.5	64.4	0.9	35.0	84.8	25.9
Acidity	-42.7	-28.0	-43.3	30.7	18.3	41.7	17.3	18.3	41.7	37.3	22.6	70.8

***GW=Ganga Water, TW= Tap Water, S= Sewage *Negative value = Increase in the value after the treatment**

Chemical Oxygen Demand (BOD)

The amount of oxygen needed to oxidize all the organic matter in waste water sample, a measure of level of organic pollution. Ideal Cod of water is 0. Minimum reduction of COD was observed in case of Ganga water (2.4%) by reactor 1 while maximum in case of Sewage (76.3%) by reactor 4. The significant difference was found in mean COD among various treatments {F(TW)=7.50, F(GW)=6.96, F(SW)=49.05, $p<0.001$). Due to *Vishaghna* and anti-microbial property of bothplants, BOD and COD of treated water might be decreased.

Total Hardness (TH)

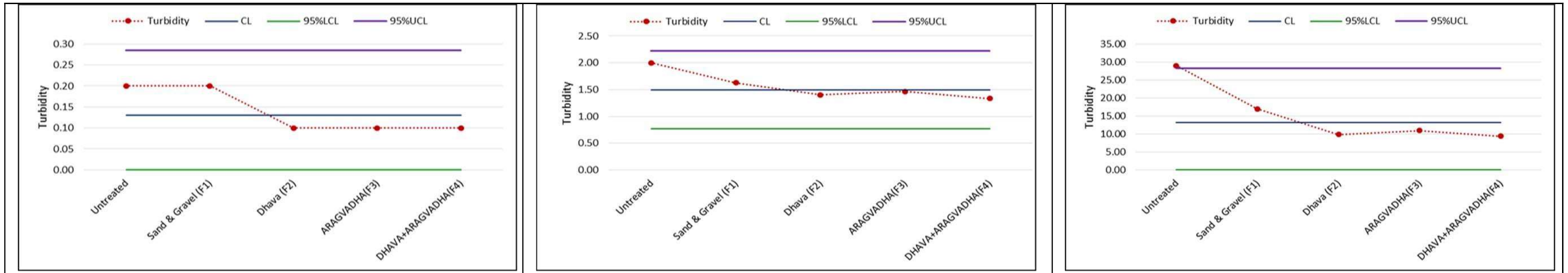
Water hardness is a traditional measure of the capacity of water to precipitate soap. Total hardness of water is defined as the sum of calcium and magnesium concentration. Hardness is temporary if it is associated with carbonates and bicarbonates and permanent if associated with sulphate and chlorides. Minimum reduction of TH was observed in case of Ganga water (7.8%) by reactor 1 while maximum in case of Sewage (44.2%) by reactor 4. The significant difference was found in mean TH among various treatments {F(TW)=20.85, F(GW)=17.97, F(SW)=40.36, $p<0.001$). Decreased in hardness of all treated water might be due to dissociation of Carbonate and Bicarbonate from calcium and magnesium compound as well as their adsorption.

Calcium and Magnesium

Minimum reduction of calcium was observed in case of Ganga water (0.0%) by reactor 1 while maximum in case of Sewage (73.6%) by reactor 4. The significant difference was found in mean calcium among various treatments {F(TW)=13.58, F(GW)=52.15, F(SW)=68.27, $p<0.001$). Minimum reduction of magnesium was observed in case of Sewage (3.3%) by reactor 1 while maximum in case of tap water (74.5%) by reactor 2. The significant difference was found in mean magnesium among various treatments {F(TW)=121.81, F(GW)=8.99, F(SW)=20.56, $p<0.001$).

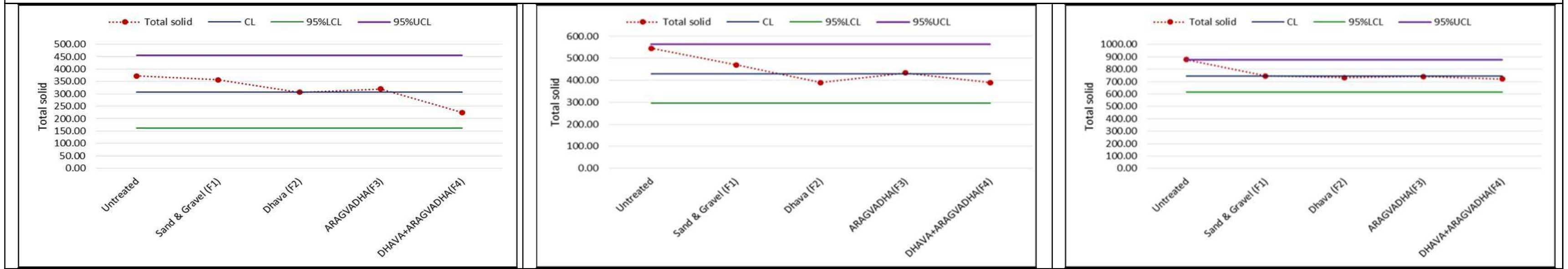
Acidity

Increased acidity of water causes Cancer, Cardiac and kidney diseases. Normal range= 150 mg/l. It is the sum of all titrable acid present in water sample. Minimum reduction of acidity was observed in case of Ganga water (17.3%) by reactor 3 while



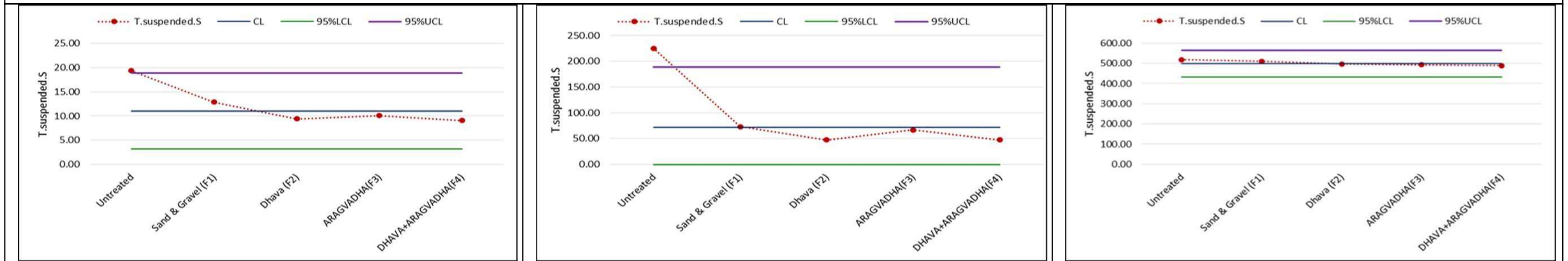
(a) Tap water | (b) Ganga water | (c) Sewage water

Figure 2: Effect on Turbidity in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



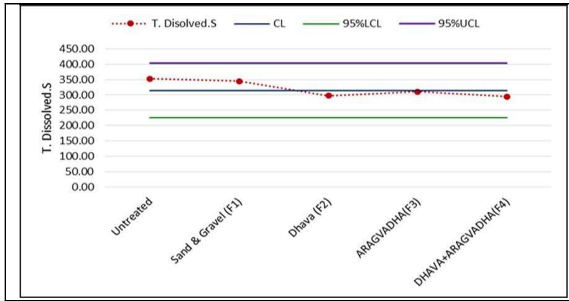
(a) Tap water | (b) Ganga water | (c) Sewage water

Figure 3: Effect on TS in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4

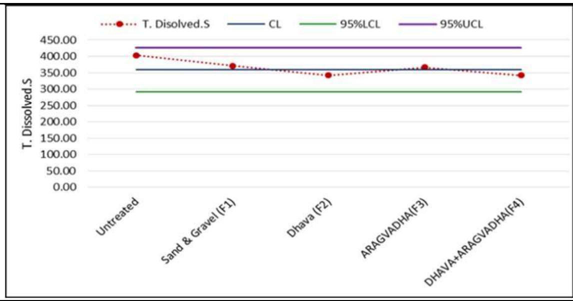


(a) Tap water | (b) Ganga water | (c) Sewage water

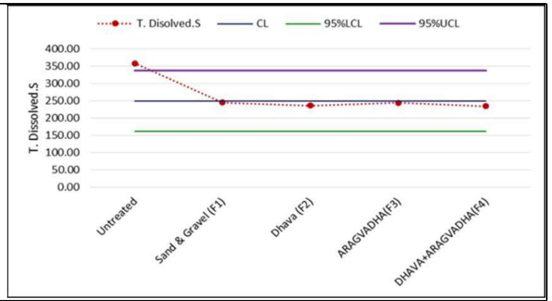
Figure 4: Effect on TS in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



(a) Tap water

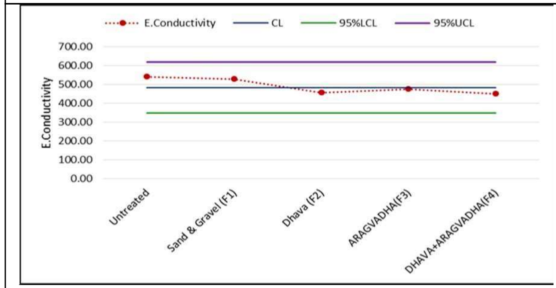


(b) Ganga water

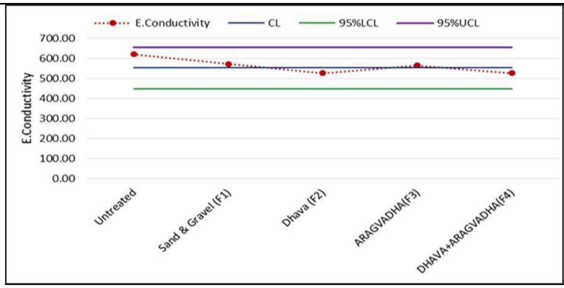


(c) Sewage water

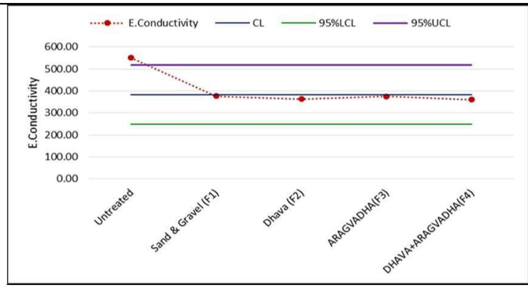
Figure 5: Effect on TDS in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



(a) Tap water

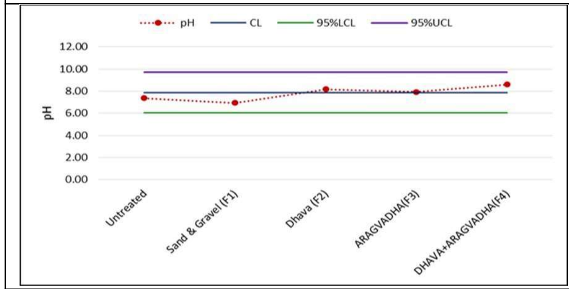


(b) Ganga water

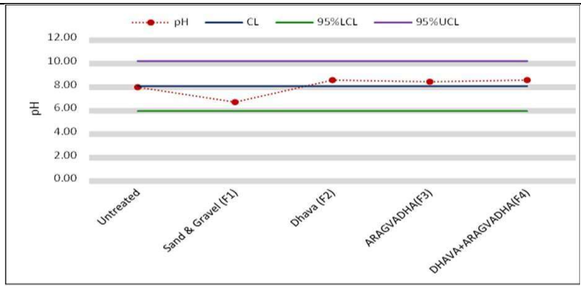


(c) Sewage water

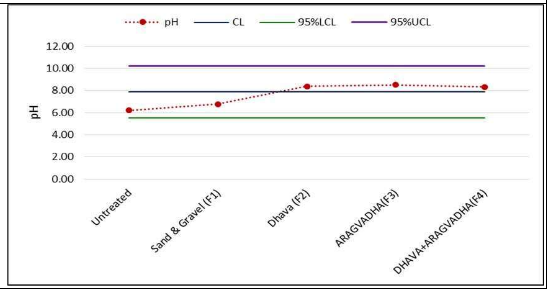
Figure 6: Effect on EC in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



(a) Tap water

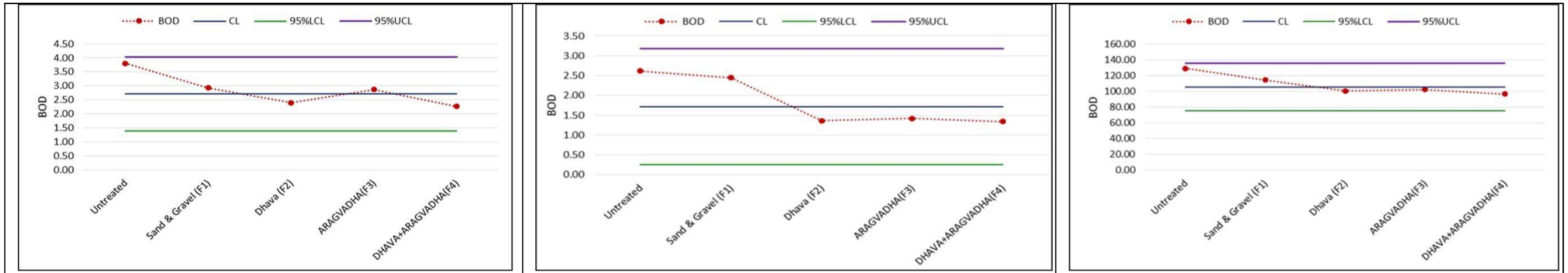


(b) Ganga water



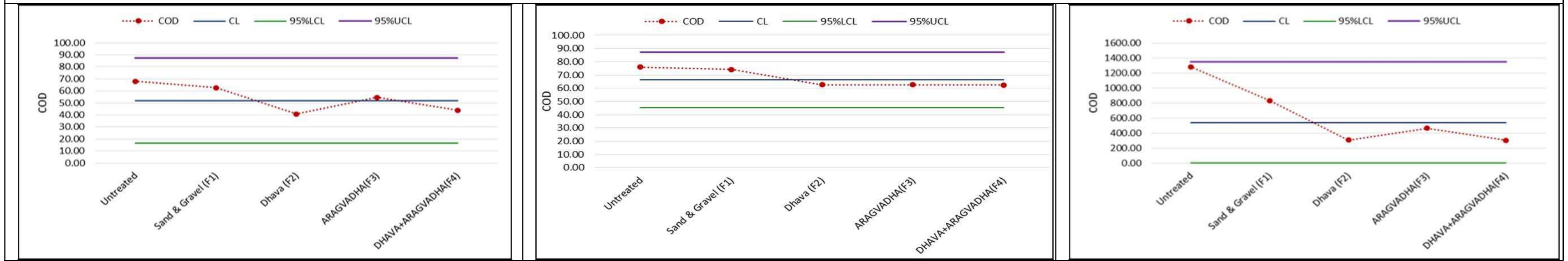
(c) Sewage water

Figure 7: Effect on pH in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



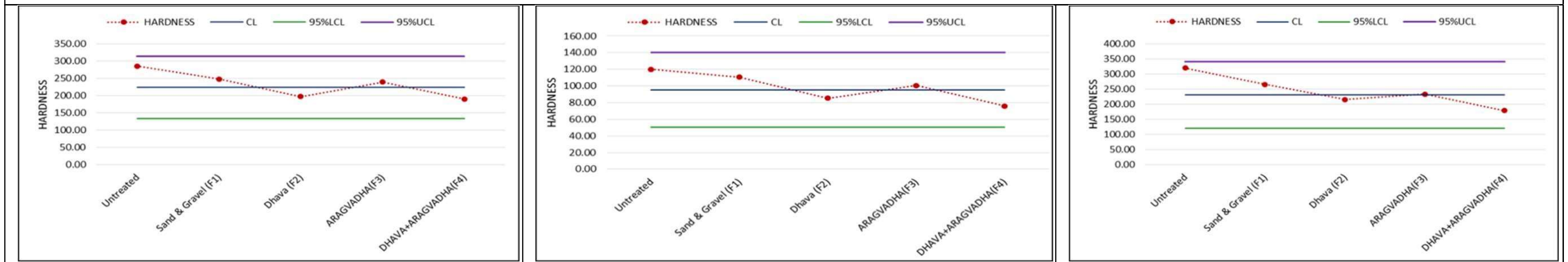
(a) Tap water **(b) Ganga water** **(c) Sewage water**

Figure 8: Effect on BOD in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



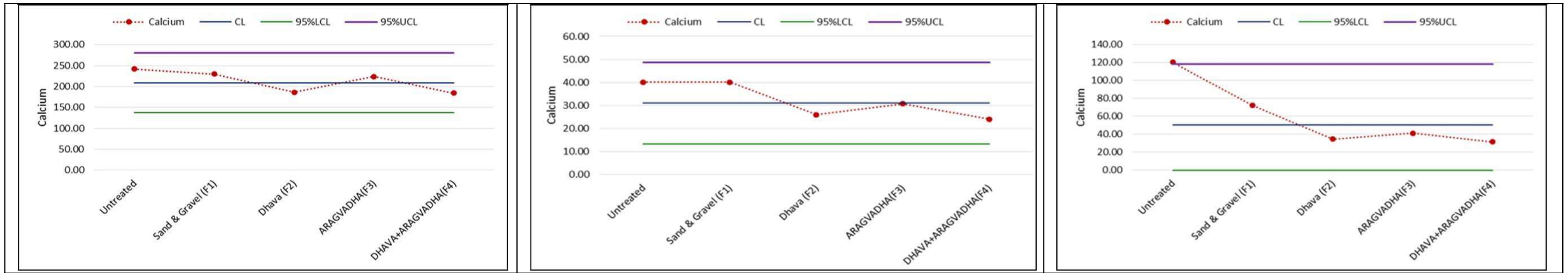
(a) Tap water **(b) Ganga water** **(c) Sewage water**

Figure 9: Effect on COD in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



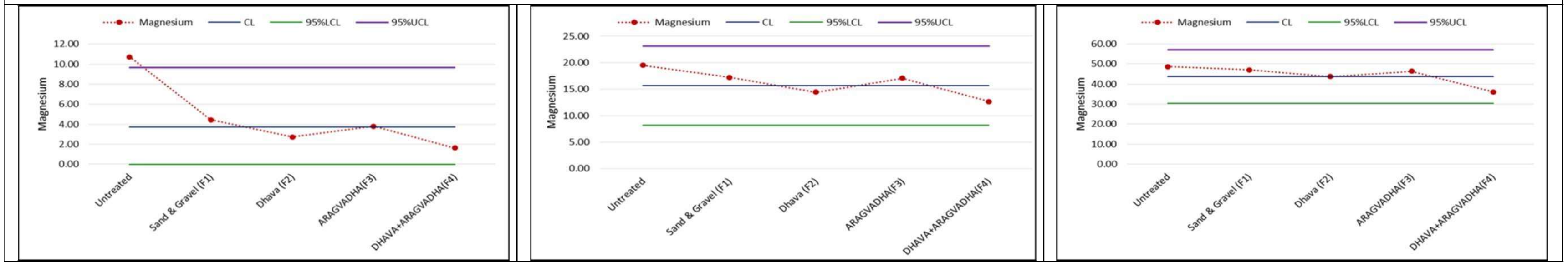
(a) Tap water **(b) Ganga water** **(c) Sewage water**

Figure 10: Effect on TH in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



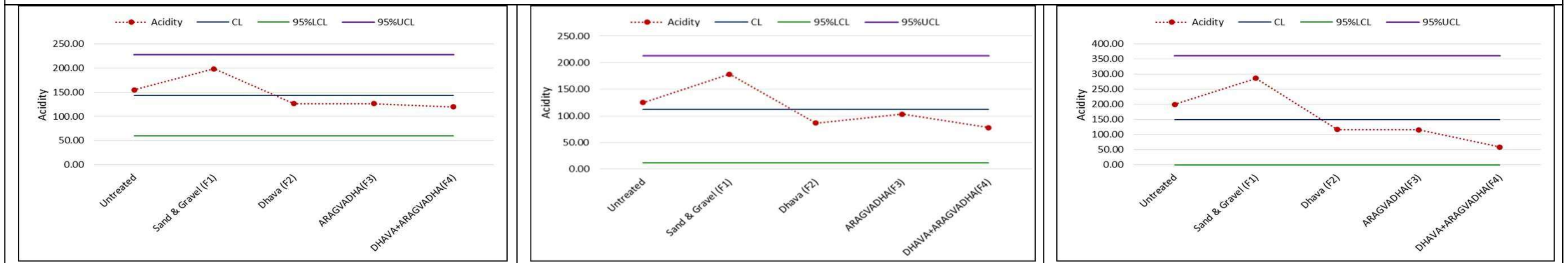
(a) Tap water (b) Ganga water (c) Sewage water

Figure 11: Effect on calcium in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



(a) Tap water (b) Ganga water (c) Sewage water

Figure 12: Effect on magnesium in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4



(a) Tap water (b) Ganga water (c) Sewage water

Figure 13: Effect on TH in (a) Tap water (b) Ganga water (c) Sewage water after treatment with Filter reactor F1, F2, F3, and F4

maximum in case of Sewage (70.8%) by reactor 4. Gain in reactor 1 was found in case of all types of water. The significant difference was found in mean acidity among various treatments {F(TW)=55.94, F(GW)=95.78, F(SW)=554.79, $p<0.001$). Increased acidity in reactor 1 might be due to silica while decreased in acidity value in reactor 2, 3, 4 may be due to neutralization process.

Conclusion

Dhava and *Aragvadha* are abundantly and easily available plants having medicinal properties. In our study we focused to establish their water purifying potential. Acharya sushruta described 9 plants and many other processes for purification of *Dushitjala, Dhava* and *Aragvadha* are two of them. In thisstudy we selected stem bark of both the plant as these parts are used for medicinal purpose. An 8 cm thick layer of ash of both the plants were used separately and in combination to prove their efficacy on total 20 different modern monitoring parameters of water. Efficiency of the reactors increased as the value of parameters in raw water increased. The effect of *Dhava* stem bark are better than *Aragvadha*stem bark ash while the combined effect of both the plants are much better than individual plants on almost all parameters. This study also proves the statement of *Acharya Sushruta* about *jalashodhana* properties of *Dhava* and *Aragvadha.* Many techniques such as chlorination, distillation, boiling, sedimentation and use of high tech filter have been utilized to purify water. These methods however, face major barriers such as high price, maintenance and conservationof fossil fuels. Our proposed solution to this problem involves production of low cost and effective water filter, which requires no electricity; it is environment friendly and easily implementable but further study is required to prove it on large scale.

Conflict of interest

The authors declare that they have no conflict of interest.

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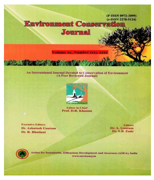

Potential of *Melia dubia*-wheat based agroforestry system to cope up with climate change

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ABSTRACT

Climate change has impact on agricultural production, because it raises CO_2 levels in the atmosphere, which leads to higher temperatures. Agroforestry systems aid in climate change adaptation and mitigation by providing relatively lower temperature beneath the tree canopy. Therefore, diversified agricultural systems are needed to be identified and studied throughout the world that can help annual crops in providing better survival conditions with least effects on yield. Although food crop output in agroforestry systems is lower than in open regions, agroforestry is seen to be capable of supporting food security, soil and water conservation, land use diversification, and micronutrient adequacy and most importantly climate change mitigation. The present study showed that all the tree parameters (*i.e.,* tree DBH, tree height and canopy spread) recorded in the intercropped conditions (*Melia dubia* based agroforestry system) were found higher compared to the trees devoid of intercrops. Trees with intercrops showed more canopy spread, tree height and DBH (6.9 m, 16.3m, 56.6 m) as compared to pure stand (6.6 m, 16.2 m, 55.6 m) of *Melia dubia* trees. The canopy temperature depression (CTD) of wheat crop grown with *Melia dubia* trees was -5.58 ^{0}C whereas; -5.27 ^{0}C CTD was recorded in non-shaded conditions. Significantly higher biological yield was observed in wheat variety HD 3086 in open and intercropped conditions (134.9 q/ha and 100.5 q/ha respectively). Study revealed that *M. dubia* based agroforestry provides trees to perform better in intercropped conditions and provides favourable environment for the crop growing beneath in terms of lowering temperature and maintaining apt moisture to the crop raised beneath.

Introduction

Agroforestry is an intersection between agriculture and forestry as a promising and sustainable land use activity because of their potential to absorb atmospheric CO_2 and store it in plant biomass and soil; some agroforestry practises have attracted increased attention for their net carbon sequestration impact. As a coping mechanism against the negative effects of climate change, agroforestry offers a unique opportunity to combine the twin goals of climate change adaptation and mitigation (Yirefu *et al.*, 2019). Climate change has the greatest impact on agricultural systems. Trees play an important role in reducing vulnerability, increasing farming system resilience, and protecting households from climate-related risks (Arya *et al.,* 2018). It has emerged as a promising way to improve resistance to current and

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future climate change. Agroforestry systems have a win-win situation by serving as carbon sinks while also assisting to food security, increasing farm profits, improving soil quality, and reducing deforestation (Nanda *et al*., 2021b).The number of agroforestry-related research in the Asia is rapidly increasing; India and China are hotspots for agroforestry studies with supportive policies and institutes (Shin *et al.,* 2020) that have possibly assisted in increase of forest cover share in the world by both the countries. Traditional agroforestry activities promote species diversity and serve as proof of biodiversity reserves that need additional research and development attention (Saikia *et al*., 2017). In the current climate change situation, agroforestry activities are emerging as a promising solution for mitigating climate change's negative effects. Agroforestry has a lot of potential in India. This method can be implemented on a broad hectare of land that is accessible in the form of borders, bunds and wastelands. This method allows appropriate tree species to be grown in fields. Agroforestry systems comprises different models used in various parts of the world increasing tree-crop diversification, which leads to greater carbon storage capacity than solely cultivating agricultural crops (Toppo and Raj 2018). *Melia dubia* generally known as Malabar neem is a fast-growing tree species that could help to deal with rising global temperature. *Melia dubia* tree is well adapted to various climatic zones hence, can be successfully integrated in agroforestry systems all over the country. *Melia* tree creates very suitable environmental conditions for the crop raised beneath its canopy (K. N., *et. al.* 2021). *Melia dubia* tree could be one such alternate indigenous fast growing multipurpose tree species highly suitable to agroforestry systems in India with immense potential to serve the mankind by wide range of products and environmental services (Chauhan *et al.,* 2019, Nanda *et. al,* 2019).This paper consists of findings on tree-crop (*i.e., Melia dubia*–wheat) interaction concentrating on the possibilities of agroforestry systems to combat climate change demonstrating how agroforestry programmes can easily combine mitigation and adaptation methods, providing a variety of options for ensuring food security for poor farmers while also helping to mitigate climate change.

Material and Methods

Description of the study area

The research was carried out in village Gillan Khera of district Fatehabad, Haryana, India located at 29˚50' latitude and 75˚30' longitude in Haryana of north-western India. The mean annual rainfall is 360-400 mm, 70-80 per cent rainfall occurs during July to September in this area. Data collection for the study was done in a 5-year-old plantation (planted during August 2013) of *Melia dubia* planted at a spacing of 3m × 3m. Five wheat varieties were raised in randomized block design taking four replications for each variety of wheat in the interspaces of the trees during *rabi* season on 15th November 2018.

Data collection

Growth studies of *Melia dubia* Cav. at initial and harvest stages of experiment

The DBH, tree height and canopy spread were recorded following the standard rules from *Melia dubia* plantation prior and after harvest of wheat varieties. Circumference (C) of *Melia dubia* trees was measured at 1.37 m height from the ground level and converted into DBH by using the relationship between two *i.e.* (dbh = C/π). Measurements were carried out with measuring tape (cm). Height of the trees was calculated with the help of clinometer and expressed in meter. The average values of crown spread in north-south and east-west directions were calculated and expressed in meter at sowing time and after harvesting of agricultural crop (Hangarge *et al*., 2012).

Canopy temperature depression (CTD, ^{0}C):

Canopy temperature (CT) was recorded with Hand held infrared thermometer (IRT) using the model AG-42, Tele temp Corp, Fullerton. Measurements were taken between 12:00 to 14:00 (IST) on bright, cloudless days three recordings per plot at about 0.5 m from the edge of the plot and approximately 0.5 m over the canopy with an approximately 30-60^o from horizontal (Hojjat *et al*., 2012). The following formula was used to calculate Canopy temperature depression:

CTD (^{0}C) = Canopy Temperature (CT) – Ambient Temperature (AT)

Biological yield (q/ha)

After harvesting of wheat varieties sun dried weight of all plants in each net plot was recorded and calculated for biological yield of crop later converted into q/ha. Biological yield was calculated for both the environments *i.e.,* wheat varieties growing under trees in intercropping and wheat varieties growing under trees devoid of wheat varieties. The mean monthly values of weather parameters obtained from the meteorological observatory situated nearest to the research site during research are depicted in Fig. 1.

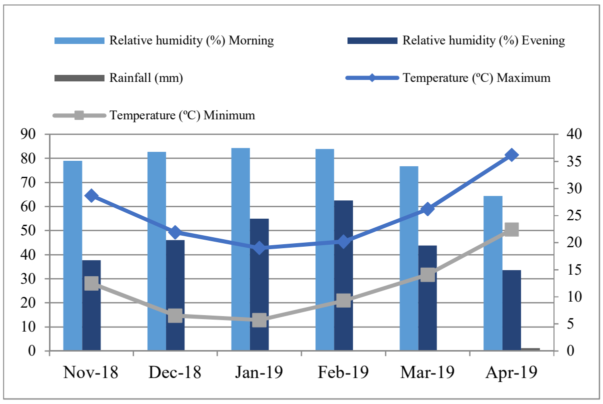

Figure 1: Monthly weather data of site of experiment from November 2018 to April 2019

Fresh and dry matter accumulation at 30, 60, 90, 120 DAS and at maturity

Fresh samples of plants of 0.5 m^2 from each replication for each variety were taken and weighed immediately after cutting them from base of the ground at 30, 60, 90, 120 DAS and at maturity. Dry weight from same samples was weighed for dry matter accumulation. Samples were put in open for sun drying and then were put in oven at 60 ^{0}C till a stable weight was obtained.

Results and Discussion

Tree parameters

At both the beginning and end of the experiment, the mean diameter at breast height was observed to be greater in trees intercropped with different wheat varieties than in trees without wheat varieties as an intercrop. In intercropped conditions, the mean DBH measured at the start of the experiment (November 2018) was 56.2 cm and increased to 56.6 cm at the end of the experiment (April 2019). Trees growing without wheat varieties underneath them, on the other hand, had a mean DBH of 55.3 cm at the start and 55.6 cm at the end. As a result of the data (Table 1) it appears that trees grown with wheat as an intercrop have a higher DBH increase. At the beginning and end of the trial, the mean tree height (Table 1) found in trees standing with wheat varieties was 16.1 m and 16.3 m, respectively. However, the average height of trees growing without wheat varieties was 16.1 m before wheat varieties were planted (November 2018) and 16.2 m after wheat varieties were harvested (April 2019). Chaudhry *et al.* (2003) in their study found that *Populus deltoides* stand raised as agroforestry trees (having annual crops beneath) yielded more timber than the trees growing alone without any crops. The mean canopy spread (6.9 m) observed in trees with intercropped conditions was greater than that of standing trees without wheat varieties as intercrop, *i.e.*, 6.6 m in the end of season, according to the data provided in Table 1. As a result, evidence shows that intercropped environments result in a greater increase in canopy growth. According to Singh and Oraon (2017), agroforestry systems have a dynamic relationship between tree and crop components that benefits both the systems. The mean of DBH, tree height, and canopy spread of *Melia dubia* trees, as seen in, shows that DBH, tree height, and canopy spread were all higher in intercropped conditions than in pure *Melia dubia* stands. These findings may be attributed to soil management techniques used in intercropping situations, which resulted in improved root growing conditions through increasing aeration and decreasing soil compactness. Similar remarks have been given by Nandal and Kumar (2010).

Canopy temperature differences in shaded and non-shaded environment

The data of Canopy temperature depression (CTD) revealed more CTD under shaded conditions as compared to open conditions as can be seen in (Table 2). Among both the environment highest CTD was observed in WH 1105 followed by DBW 88, WH 711, HD 3086 and HD 2967 in shaded conditions. The decreased values of Canopy temperature depression (CTD) under open conditions might be due to higher rate of transpiration than shaded conditions that leads to water deficit and cause warmer canopy in open conditions. Similar results were also observed by Roohi *et al.*, 2015 and Chaudhari *et al.*, 2017 in wheat genotypes.

Table 1: Tree growth parameters at initial and harvest stage of experiment in intercropped and pure stand of *Melia dubia*

	Trees with intercrops						Tree without intercrops					
	DBH (cm)		Tree height (m)		Canopy spread (m)		DBH (cm)		Tree height (m)		Canopy spread (m)	
SN	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
1.	45.8	45.9	13.8	13.9	5.6	5.7	70.6	70.8	13.9	14.1	8.5	8.8
2.	63.4	63.6	15.3	15.6	7.3	7.6	70.5	70.6	18.9	19.0	5.0	5.1
3.	70.8	70.9	18.5	18.6	8.2	8.4	61.1	61.2	16.9	17.0	4.5	4.7
4.	60.2	60.8	18.3	18.5	6.1	6.4	70.3	70.8	16.6	16.7	6.2	6.4
5.	70.3	70.9	15.1	15.4	7.4	7.8	44.4	44.6	14.9	14.9	5.8	6.0
6.	45.4	45.7	14.2	14.3	6.4	6.5	58.3	58.4	18.6	18.9	7.4	7.7
7.	58.1	59.3	16.2	16.3	6.0	6.3	32.5	32.8	18.2	18.2	6.0	6.3
8.	34.8	34.9	14.0	14.1	5.1	5.3	40.8	41.0	15.5	15.6	7.8	7.9
9.	42.5	42.8	17.2	17.5	5.8	6.0	59.2	60.2	14.0	14.1	7.0	7.1
10	70.8	70.9	18.6	18.9	9.0	9.2	44.9	44.9	13.5	13.7	5.7	5.8
Mean	56.2	56.6	16.1	16.3	6.7	6.9	55.3	55.6	16.1	16.2	6.4	6.6

Other physiological parameters such as photosynthetic rate, stomatal conductance and transpiration rate, decreased significantly in shaded conditions as compared to open conditions. In both environments, high CTD values were found in the intercropped condition. As compared to open conditions, the temperature under the canopy of trees in an agroforestry environment is significantly lower. As a result, for a planet dealing with climate change, agroforestry can be a viable alternative for growing crops that can provide relief to plants as intercrops from high temperatures. However, since the agroforestry environment is a dynamic ecosystem, smart crop choice will also be an essential criterion for obtaining optimal yields, whether visible or intangible. High canopy temperature depression (CTD) or low CT, according to Reynolds (1994), can indicate a high demand for photosynthates during the rapidly grain filling stage in resistant lines. Agroforestry has been suggested as a possible solution for reducing climate change vulnerability. It's a resource management scheme that incorporates trees on a farm that's dynamic and ecologically sound. On the field, trees provide development and security.

Biological yield

The biological yield of a crop determines its productivity in large part. The biological yield (Table 3) was significantly influenced by environment *i.e.,* higher biological yield in without tree (121.9 q/ha) was observed in comparison to under tree plantation (89.5 q/ha). The interaction effect between crop environment and variety were also found significant. According to Gill *et al.* (2009), the biological yield of wheat declined under tree plantation. HD 3086 (117.7 q/ha) had the highest biological yield of wheat varieties. However, wheat variety WH 711 (91.8 q/ha) had the lowest biological yield. Low biological yield could be attributed to competition for growth resources, especially sunlight, between *Melia dubia* trees and wheat varieties. Similar reasons have been stated by Mishra *et al.* (2010) and Kumar *et al.* (2013) in their research. According to Chauhan *et al.* (2015) wheat and barley yields were lower under the poplar canopy than in the open however, they stated that the poplar-based agroforestry system with conventional wheat crop offers a better potential for farm diversification and income than a single cropping system. When compared to single cropping, Bisht *et al.* (2017) found a decline in the biological yield of wheat growing under poplar. They discovered that sunlight, moisture, and nutrients were the most important major constraints in intercropping wheat crop growth and yield (Nanda *et. al*., 2021a).

Fresh and dry matter accumulation at 30, 60, 90,120 DAS and at maturity

The accumulation of fresh and dry matter of plant is an important parameter which influences the production of yield attributes and yield of plant by adequate transfer of assimilates to the sink. Dadhwal and Narayan (1984) reported more DMA of in wheat growing without tree (*i.e.,* in open) in comparison to under tree conditions because translocation of photosynthates was happening to both *i.e.,* trees as well as wheat varieties. Roots of trees are deep rooted as compared to wheat so uptake of nutrients and water were more by trees as compared to open conditions.

Table 2: Effect of environment on canopy temperature depression at flag leaf in wheat varieties

Canopy temperature depression (CTD, ^{0}C)			
Variety	**Under tree**	**Without tree**	**Mean**
WH 1105	-6.07	-5.37	-5.72
WH 711	-5.70	-5.63	-5.67
DBW 88	-5.87	-5.60	-5.73
HD 3086	-5.13	-4.80	-4.97
HD 2967	-5.13	-4.97	-5.05
Mean	-5.58	-5.27	
C. D. at 5%	Variety =0.29; Environment =0.18; Variety × Environment = NS		

Table 3: Biological yield (q/ha) of wheat varieties in intercropped and pure stand of *Melia dubia.*

Variety	**Under tree**	**Without tree**	**Mean**
WH 1105	95.2	129.3	112.2
WH 711	77.3	106.2	91.8
DBW 88	80.4	109.1	94.8
HD 3086	100.5	134.9	117.7
HD 2967	94.2	130.1	112.2
Mean	89.5	121.9	
C. D. at 5%	Variety=1.68; Environment=1.07; Variety X Environment=2.38		

The effect of environment on wheat varieties was well pronounced on growth attributes such as plant fresh and dry matter accumulation. The fresh and dry matter accumulation in different wheat varieties differed significantly among plantation and in control (without tree) from 30 DAS to maturity (Fig. 2 and 3). At 120 DAS and at harvest significantly higher fresh matter accumulation was recorded in HD 3086 which is at par with HD 2967. However minimum fresh matter accumulation recorded in WH 711 in all the growth stages. At harvest significantly higher dry matter accumulation was recorded in HD 3086 which is at par with WH 1105 and HD 2967 in both the environment. However, significantly lesser dry matter accumulation was observed in WH 711 in under tree and open conditions at maturity. The dry matter accumulation of wheat varieties was recorded higher in without tree condition than the varieties growing under trees. Reduced amount of dry matter accumulation may be ascribed to competition between tree and wheat varieties for light, moisture and nutrients in *Melia dubia* based agroforestry system. Similar results were obtained by Alebachew *et al.* (2015). Result of the present study support the findings of Bargali *et al.* (2009), Datta and Singh (2007), Bhati *et al.* (2008), Osman *et al.* (1998) and Evensen *et al.* (1995). Wassinck (1954) revealed that photosynthetic efficiency of crops increased because of presence of more light intensity in control that resulted in better growth performance of crop. Crop yield was reduced mostly due to the decrease in light intensity underneath the trees; this could be mitigated by increasing plantation width and proper training and pruning of trees on a regular basis. Agri-silvicultural systems based on poplars produce a lot of litter, which increases the amount of organic matter in the soil Arya and Toky (2017). Similarly, *Melia dubia* is a deciduous tree, it contributes a significant amount of organic matter to the soil. If handled properly, agroforestry systems can help to not only deal with environmental problems, but also increase their profits by diversifying their sources of income. Since the majority of India's arable land is cultivated, agroforestry on agricultural fields would have a significant proportion of the ability to store carbon by afforestation. The overall carbon storage capacity of an agroforestry system varies from region to region and is determined by the growth and function of the individual tree species (Basu 2014).

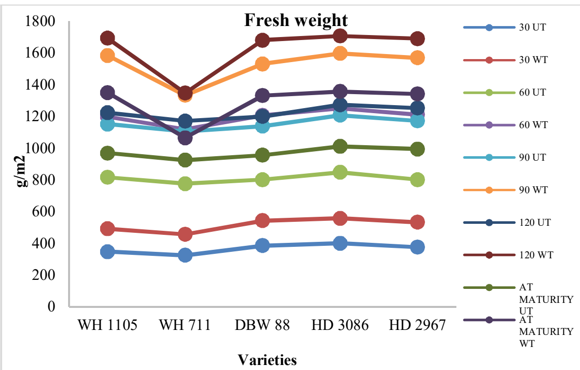

Figure 2: Fresh matter accumulation (g/m^2) at 30, 60, 90, 120 days and at maturity of wheat varieties

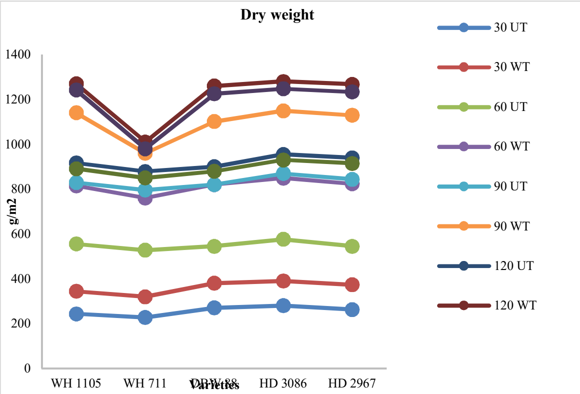

Figure 3: Dry matter accumulation (g/m^2) at 30, 60, 90, 120 days and at maturity of wheat varieties

of *Melia dubia*. This demonstrates that trees have better growth in agroforestry environment. Planting trees on farmland expands the tree canopy outside of forests and eventually adding to total tree cover of the country. Also trees in *Melia dubia* based agroforestry system provides cooler environment to the wheat crops growing under them. The research discovered a number of beneficial environmental properties of trees in relation to wheat production. *Melia dubia* can be suggested as a possible tree in an agroforestry system in provinces with similar climatic conditions to cope with shifting climatic conditions and to increase farmer profits, according to the current report.

Conclusion

In order to slow the rise in temperature, immediate action is needed in the face of climate change. Trees, forests and agriculture are key to reducing carbon emissions and combat climate change. Farmers can better prepare for climatic effects by planting the appropriate tree species in combination with appropriate crop species. In the present study five wheat varieties were raised under *Melia dubia* plantation. Various growth parameters studied such as DBH, tree height, and canopy spread were found higher in agroforestry system (wheat varieties growing under trees) compared to a pure stand (trees not having any annual crop growing beneath)

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Conflict of interest

The authors declare that they have no conflict of interest.

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Assessment of block-wise status of micro nutrients in some soils of Shivalik hills of Himachal Pradesh

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ABSTRACT

The investigation was undertaken and micro nutrients status of the area was assessed. During February-March 2020, 50 soil sampling locations from tomato growing areas were selected at random and 100 representative surface (0-15 cm) and sub-surface (15-30 cm) soil samples were collected. Various micronutrients, *i.e.* DTPA-extractable Zn, Cu, Mn and Fe were examined. The soils were neutral to slightly alkaline, and the EC values of all the soil samples were within normal limits. The general state of the soil organic carbon concentration was medium to high. The DTPA- Mn, Fe, Cu and Zn in soils of the studied area were found to be in medium category. Positive correlations between the DTPA-extractable iron, copper, zinc and manganese with the organic carbon content was observed.

Introduction

Agriculture plays a vital role in Indian economy and over 70 per cent of rural households depend upon it (Arjun, 2013). Soil is an important factor of crop production and the nutrients in a soil determine its fertility and the crop output. The availability of nutrients in soil varies naturally from soil to soil and some nutrients may be sufficient while others may be insufficient (Rajendiran *et al.,* 2020; Ruhela *et al.,* 2022), so the evaluation of a region's nutrient status helps farmers to develop a plan for proper fertiliser applications and soil management measures accordingly that will result in economically better returns. One of the most important factors affecting agricultural productivity is the reduction in soil fertility caused by negligence of micronutrients supply. The importance of micronutrients in well-balanced plant nutrition is widely known. Despite the fact that they are required in much lesser quantities than main nutrients, they have a significant influence in crop development and post green revolution, they have shown a negative impact on human nutrition as well as crop productivity (Das *et al.,* 2020; Bhardwaj *et al.,* 2020).It is difficult to get the most out of NPK fertilisers and cultivate high yielding types without an appropriate supply of micronutrients. Micronutrient availability is determined by their distribution in soil as well as other physicochemical characteristics of the soil (Yadav, 2011). Information on micronutrient status for various soil types, districts, and regions, as well as for the entire country, is critical for determining the nature and degree of deficiencies/toxicities and formulating strategies for correcting them in order to improve crop output. Therefore, an attempt has been made to generate information regarding the DTPA extractable Fe, Cu, Zn and Mn status of soils of Sirmour district of HP.

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Material and Methods

The survey was carried out during the months of February-March 2020 in the tomato growing areas of Sirmour districts(Nahan, Pachhad, Rajgarh, Sangrah and Shillai block)of Himachal Pradesh. District Sirmour is located in outer Himalayas, which is commonly known as Shivalik range. The district lies between 30^0 22' 30" to $31^{.0}$ 01'20" north latitudes and 77^0 01' 12" to 77^0 49' 40" east longitudes. The RiverGiri, a tributary of the river Yamuna, is the biggest river in Sirmour district. The district's climate is sub-tropical to moderate, depending on elevation. The region receives about 1405 mm of yearly rainfall on average (Anonymous, 2013). The monsoon season accounts for 90% of total precipitation. The district's average maximum and lowest temperatures are roughly 35°C and 5°C. The district's soil ranges from thin, barren dirt found high in the mountains to rich, deep alluvial soil found in lowlands. In the months of February and March 2020, one hundred representative surface (0-15 cm) and subsurface (15-30 cm) soil samples were taken from fifty different locations around the Sirmour area. Two soil samples were taken from each site/location, at a depth of 0-15 cm (surface) and 15-30 cm (deep) (sub-surface). The samples were obtained with a stainless steel auger and spade to avoid contamination. The soil samples were dried in the shade and pulverised with a wooden pestle and mortar before passing through a 2 mm sieve. The processed samples were then placed in cloth bags fortesting.

Table 1: Critical limits used for available micronutrients (Lindsay and Norvell, 1978)

Micronutrients (mg/kg)	Availability				
	Very low	Low	Medium	High	Very high
Zn	<0.5	0.5-1.0	1.0-3.0	3.0-5.0	>5.0
Cu	<0.1	0.1-0.3	0.3-0.8	0.8-3.0	>3.0
Fe	<2.0	2.0-4.0	4.0-6.0	6.0-10	>10
Mn	<0.5	0.5-1.2	1.2-3.5	3.5-6.0	>6.0

The pH and electrical conductivity of the soil was measured using a pH metre and an EC meter on a 1:2 soil/water ratio (w/v) solution(Jackson, 1973). The rapid titration method was used to quantify organic carbon in soil(Walkley and Black, 1934). DTPA-extractable micronutrients (Zn, Fe, Cu, and Mn) were estimated using an atomic absorption spectrophotometric technique(Sharma *et al.,* 2017).

Results and Discussion

Soil pH

A perusal of data in table 2 revealed that soil pH of the soils of different blocks of Sirmour district. The soil pH value in Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks for surface soils ranged from 6.93 to 7.41, 7.12 to 7.43, 6.62 to 7.44, 6.62 to 7.45 and 6.61 to 7.44 with the mean value of 7.20, 7.37, 7.05, 7.27 and 7.09, respectively. Whereas, the soil pH varied from 6.99 to 7.48, 7.32 to 7.48, 6.66 to 7.48, 6.63 to 7.49 and 6.63 to 7.48 with mean values of 7.27, 7.44, 7.11, 7.33 and 7.15, respectively in sub-surface soils. The highest mean soil pH values were recorded in Pachhad block in both surface (7.37) and sub-surface (7.44) depths. While, lowest mean value was recorded in Rajgarh block in both surface (7.05) and sub-surface (7.11) depths. The soil pH increased with increase in the soil depth which was also reported by Chandel (2020) and Salve and Bhardwaj (2020)which may be due to soil pH was less at upper layer due to the continuous use of farm yard manure in agriculture soil result decrease soil pH value at the surface layer. The reduction in soil pH was mainly due to the release of organic acids in the soil upon decomposition of organics. These results are similar as the findings of Suri (2018) who also observed the identical trends in the soils of the Sirmour district where pH was found to be neutral to alkaline in reaction.

Electrical conductivity

A scrutiny of data presented in table 2 revealed that in different blocks of Sirmour district the electrical conductivity of soils were in normal range. The soil EC value in Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks for surface soils ranged from 0.14 to 0.25, 0.11 to 0.25, 0.13 to 0.27, 0.14 to 0.20 and 0.12 to 0.21 dS/m with the mean value of 0.18, 0.17, 0.18, 0.16 and 0.16dS/m, respectively. While, it ranged from 0.11 to 0.19, 0.10 to 0.20, 0.11 to 0.22, 0.11 to 0.18 and 0.11 to 0.20dS/m with mean values of 0.15, 0.15, 0.15, 0.13 and 0.14dS/m, respectively in sub-surface soils.The data on EC(Table 2) of the studied soils revealed that all the sites do not have any salinity problem and decreased with increase in the

Table 2: Soil reaction, electrical conductivity(dS/m) and organic carbon (g/kg)content of the soils of Sirmour district

Blocks		Soil pH		EC (dS/m)		OC (g/kg)	
		0-15	15-30	0-15	15-30	0-15	15-30
1	Nahan	6.93-7.41 (7.20)	6.99-7.48 (7.27)	0.14-0.25 (0.18)	0.11-0.19 (0.15)	7.35-22.50 (16.68)	7.20-21.00 (14.81)
2	Pachhad	7.12-7.43 (7.37)	7.32-7.48 (7.44)	0.11-0.25 (0.17)	0.10-0.20 (0.15)	5.70-21.80 (13.41)	5.10-19.80 (11.95)
3	Rajgarh	6.62-7.44 (7.05)	6.66-7.48 (7.11)	0.13-0.27 (0.18)	0.11-0.22 (0.15)	6.80-22.10 (13.30)	6.00-19.50 (11.78)
4	Sangrah	6.62-7.45 (7.27)	6.63-7.49 (7.33)	0.14-0.20 (0.16)	0.11-0.18 (0.13)	9.50-15.30 (12.33)	8.60-13.50 (11.30)
5	Shillai	6.61-7.44 (7.09)	6.63-7.48 (7.15)	0.12-0.21 (0.16)	0.11-0.20 (0.14)	6.80-16.40 (10.24)	6.50-14.80 (8.99)

(Values in parenthesis are the mean value)

depth. The main reason of low EC of soil might be due to high leaching of soluble salts takes place from surface to sub surface because of the high permeability. Loria *et al.* (2016) also revealed similar normal range (0.134 to 0.137) of EC in vegetable growing soils of HP.

Organic carbon

The data in the table 2 showed that the organic carbon content of the soils of the study area was medium to high. The data showed that the OC values in the surface layers ranged from 7.35 to 22.50, 5.70 to 21.80, 6.80 to 22.10, 9.50 to 15.30 and 6.80 to 16.40 g/kg with the mean value of 16.68, 13.41, 13.30, 12.33 and 10.24 g/kg in Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks, respectively. Whereas, in sub-surface layer the value varied from 7.20 to 21.00, 5.10 to 19.80, 6.00 to 19.50, 8.60 to 13.50 and 6.50 to 14.80 g/kg with mean values of 14.81, 11.95, 11.78, 11.30 and 8.99 g/kg, respectively. The highest OC mean value was found to be in Nahan block in both surface (16.68) and sub-surface (14.81) depths. While, lowest mean value was recorded in Shillai block in both surface (10.24) and sub-surface (8.99) depths. The OC content was found to be high on the surface layer as compared to lower layers. The higher organic carbon content of surface soils as compared to sub-surface soils may be attributed to accumulation of root, plant biomass and increased microbial activity. The accumulation of organic matter on the surface may have increased the organic carbon content in the upper surface as compared to sub-surface soils. Also, the addition of organic manures is also done on the surface which might have resulted in increased OC content. The findings collaborated with the findings of Chandel (2013).

DTPA-extractable micronutrients

DTPA-extractable Fe, Cu, ZnandMnwere analyzed and found to be in medium category in all the soils of the study area. The DTPA-extractable Fe content in the different blocks of the studied area, *i.e.* Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks for surface soils ranged from 4.54 to 7.76, 4.24 to 6.72, 4.59 to 7.81, 5.23 to 5.88 and 4.45 to 5.82mg/kgwith the mean value of 5.53, 5.22, 5.64, 5.49 and 5.49mg/kg, respectively. While, it ranged from 4.15 to 6.51, 4.02 to 6.36, 4.32 to 6.65, 4.65 to 5.52 and 4.19 to 5.01mg/kg with mean values of 4.95, 4.79, 5.11, 4.99 and 4.90mg/kg, respectively in sub-surface soils (Table 3).The highest mean value for DTPA-extractable Fe was recorded in Rajgarh block in both surface (5.64) and sub-surface (5.11) depths. While, lowest mean value was recorded in Pachhad block in both surface (5.22) and sub-surface (4.79) depths. The results of the study were also collaborated with the results of Chauhan (2018). The decrease in DTPA-extractable iron with the increase in the depth of soil might be due to reduction of OC in the sub-surface layers. Similarly, surface horizons had higher concentration of DTPA-extractable Fe due to higher organic carbon content in surface horizons. Also, solubility of Fe decreases by approximately 1000-fold for each unit increase of soil p, which may be also the reason. This trend was also reported by Arshad (2020). Among various sites selected for the study, the DTPA-extractable copper in the surface layer (0-15 cm) varied from0.38 to 1.93, 0.39 to 1.65, 0.36 to 2.32, 0.52 to 1.27 and 0.42 to 0.74mg/kgwith the mean value of 0.73, 0.78, 0.97, 0.75 and 0.61mg/kgin Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks, respectively. Whereas, in sub-surface layer (15-30 cm) the value varied from 0.32 to 1.82, 0.35 to 1.52, 0.31 to 2.18, 0.45 to 1.17 and 0.37 to 0.69 mg/kgwith mean values of 0.63, 0.68, 0.87, 0.67 and 0.54mg/kg, respectively. The results get strength from the findings of Mahajan*et al.* (2007) and Fayed and Rateb (2013). Further, analysis of the result revealed that the trend of decrease in copper content with increase in depth may be due to the fact of their positive and significant correlation with organic carbon as the OC content decreased with increase in the soil depth in these soils.Also, Also, solubility of Cu decreases by approximately 100-fold for each unit increase of soil pH.

Table 3:Status of DTPA-extractable Iron (mg/kg) and Copper (mg/kg) of the soil of Sirmour district

Blocks		DTPA-extractable Iron (mg/kg)		DTPA-extractable Copper (mg/kg)	
		0-15	15-30	0-15	15-30
1	Nahan	4.54-7.76 (5.53)	4.15-6.51 (4.95)	0.38-1.93 (0.73)	0.32-1.82 (0.63)
2	Pachhad	4.24-6.72 (5.22)	4.02-6.36 (4.79)	0.39-1.65 (0.78)	0.35-1.52 (0.68)
3	Rajgarh	4.59-7.81 (5.64)	4.32-6.65 (5.11)	0.36-2.32 (0.97)	0.31-2.18 (0.87)
4	Sangrah	5.23-5.88 (5.49)	4.65-5.52 (4.99)	0.52-1.27 (0.75)	0.45-1.17 (0.67)
5	Shillai	4.45-5.82 (5.49)	4.19-5.01 (4.90)	0.42-0.74 (0.61)	0.37-0.69 (0.54)

(Values in parenthesis are the mean value)

Critical analysis of the data in table 4 revealed that the DTPA-extractable zinc in the surface layer (0-15 cm) ranged from 1.17 to 3.51, 1.59 to 3.29, 1.29 to 3.52, 1.54 to 2.94 and 1.27 to 3.12mg/kgwith the mean value of 2.49, 2.18, 2.14, 2.17 and 1.69mg/kgin Nahan, Pachhad, Rajgarh, Sangrah and Shillai blocks, respectively. Whereas, in sub-surface layer (15-30 cm) the value varied from 1.04 to 3.23, 1.13 to 3.01, 1.15 to 3.41, 1.29 to 2.48 and 1.15 to 2.93 mg/kg with mean values of 2.20, 1.86, 1.90, 1.77 and 1.51mg/kg, respectively.Therefore, the analysis revealed that all samples were in medium category and the decreased zinc content with increase in the soil depth was may be due to the positive relation of Zn with the OC content. Also, the solubility of Zn in the soil decreases 100-fold for each unit increase in soil pH, which may be also the reason for the decrease content in lower depths. Similar trend has been reported by Meena *et al.* (2006).The DTPA-extractable manganese in the surface layer of the study area soils varied from 1.39 to 3.84, 1.75 to 3.96, 1.62 to 3.48, 1.77 to 3.73 and 1.84 to 2.52 mg/kg with the mean value of 2.92, 2.68, 2.56, 2.64 and 2.20 mg/kg in Nahan, Pachhad,Rajgarh, Sangrah and Shillai blocks, respectively. While, in sub-surface layer the manganese content varied from 1.21 to 3.52, 1.28 to 3.59, 1.25 to 2.95, 1.42 to 3.52 and 1.29 to 2.12mg/kgwith mean values of 2.36, 2.10, 1.90, 2.18 and 1.65 mg/kg, respectively (Table 4). The lowest mean value for DTPA-extractable Mn was found to be in Shillai block in both surface (2.20) and sub-surface depths (1.65). While, highest mean values were recorded in Nahan block in surface (2.92) and sub-surface (2.36) depths.The trend is in line with the findings of Sharma *et al.* (2003)who showed the higher content of Mn on surface soils which might be due to the chelating of organic compounds released during the decomposition of organic matter left after the harvest of the crop. Correlation relationships of different properties were determined in which (**) and (*) was attributed to significant at the 0.01 level and significant at the 0.05 level, respectively.

Table 4: Status of DTPA-extractable Zinc (mg/kg) and Manganese (mg/kg) of Sirmour district

Blocks		DTPA-extractable Zinc (mg/kg)		DTPA-extractable Manganese (mg/kg)	
		0-15	15-30	0-15	15-30
1	Nahan	1.17-3.51 (2.49)	1.04-3.23 (2.20)	1.39-3.84 (2.92)	1.21-3.52 (2.36)
2	Pachhad	1.59-3.29 (2.18)	1.13-3.01 (1.86)	1.75-3.96 (2.68)	1.28-3.59 (2.10)
3	Rajgarh	1.29-3.52 (2.14)	1.15-3.41 (1.90)	1.62-3.48 (2.56)	1.25-2.95 (1.90)
4	Sangrah	1.54-2.94 (2.17)	1.29-2.48 (1.77)	1.77-3.73 (2.64)	1.42-3.52 (2.18)
5	Shillai	1.27-3.12 (1.69)	1.15-2.93 (1.51)	1.84-2.52 (2.20)	1.29-2.12 (1.65)

(Values in parenthesis are the mean value)

Table 5: Correlation coefficient (r) values of important soil parameters

Dependent variable		Soil pH		EC(dS/m)		OC(g/kg)	
		0-15	15-30	0-15	15-30	0-15	15-30
1	Fe	0.014	-0.028	0.077	0.088	0.665**	0.651**
2	Cu	-0.320*	-0.322*	-0.044	-0.057	0.364**	0.367**
3	Zn	-0.091	-0.112	-0.018	-0.034	0.405**	0.452**
4	Mn	0.083	-0.008	-0.360**	-0.270	0.288*	0.309*

****. Significant at the 0.01 level**
***. Significant at the 0.05 level**

These values correspond to the probability of observing such an extreme value by chance. Correlation studies showed that iron and manganese showed a negative relationship with soil pH at 15-30 cm depth, however, positive relationship at 0-15 cm depth, but it did not achieve statistical significance. But, DTPA-extractable copper showed a negative and significant correlation with soil pH for both surface (-0.320*) and sub-surface depth (-0.322*). In case of EC, DTPA-extractable Mn showed a significant and negative relationship (-0.360**). Positive correlations between the DTPA-extractable iron, copper, zincand manganese with the organic carbon content was observed. Increased organic matter content improved micronutrient availability because (i) organic matter improves soil structure and aeration, (ii) organic matter protects micronutrients from oxidation and precipitation into unavailable forms, and (iii) organic matter supplies soluble chelating agents that increase the solubility of micronutrient contents. Mahajan *et al.* (2007) also showed a positive relationship between the DTPA-extractable copper, zinc and manganese with the OC content which shows that DTPA-extractable micronutrients have significant and positive effect of OC content in the soil and OC readily affects the availability of the micronutrients in the soil.

Conclusion

The assessment of available nutrients is crucial for the proper use of fertilizers and the assurance of higher crop yields which helps in maximizing the profits. The study area was found to be neutral to slightly alkaline in reaction and the electrical conductivity values of all the soil samples were under normal range. The organic carbon content was under medium to high range. The soils of the study area fall in the medium category with respect to the DTPA-extractable micronutrients viz., iron, copper, zinc and manganese contents. Correlation studies showed that increase in the organic matter content improved the micronutrient availability.

Conflict of interest

The authors declare that they have no conflict of interest.

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Effect of integrated nutrient management in brinjal (*Solanum melongena* L.) on micronutrient uptake and physical properties of soil

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Soil properties

ABSTRACT

A field experiment was conducted at the experimental site of Department of Soil Science and Water Management, College of Horticulture and Forestry, Neri, Hamirpur (Himachal Pradesh). The experiment consisted of eleven treatments which were carried out in randomized block design with three replications. Observations for micronutrient uptake and soil physical properties were recorded and it was found that the lowest bulk density of the soil was achieved with treatment T_6 (100 per cent RDN through vermicompost) and T_{11} (100 per cent RDN through vermicompost + *Azotobacter*). Particle density, porosity and water holding capacity were also slightly improved with the application of different treatments, however the differences were not significant. Further, application of chemical fertilizer or organic manure or both with or without biofertilizer positively influenced the micronutrient uptake (Zn, Cu, Fe and Mn) and the maximum uptake was recorded in treatment T_{11} (100 per cent RDN through vermicompost + *Azotobacter*), while minimum was observed under treatment T_1 (control).

Introduction

India is regarded as a horticultural paradise owing to extensive commercial production of a wide variety of vegetable crops (Saravaiya and Patel, 2005). Brinjal is the second most important vegetable crop next to the tomato. It is also known as eggplant and originates originally from India (Kiran *et al.,* 2010). It is a crop that is quite productive and is frequently referred to as "poor man's crop." The treatment of diabetes, asthama, cholera, pneumonia, and diarrhoea may be among egg plant's medical uses. Its fruit and leaves are also reported to lower specific blood cholesterol levels. As productivity has either stagnated or decreased, there is currently growing worry about sustainability. In order to ensure sustainability in the quantity and quality of produce, it is crucial to use nutrient sources properly. These sources should be freely accessible, contain an acceptable level of nutrients, maintain soil fertility, and create an environment that is conducive to a higher yield. Application of high input technology like chemical pesticides, herbicides, and fertilizers boosts production, but there is rising worry about the negative impacts of chemical use on soil quality, human health, and the environment (Sharma *et al.,* 2012). Therefore, it is the need of the hour to use different biocontrol agents (Kumar *et al.*, 2009) not

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only for sustainable plant disease management but also judicious use of biofertilizers for sustainable development of crops (Srivastava *et al.,* 2009).
Exploiting organic sources has become necessary due to the decline in soil health brought on by the indiscriminate application of chemical fertilizers and the rising cost of chemical fertilizers. The presence of organic carbon and growth-promoting elements like enzymes and hormones makes organic amendments a preferential choice for improving soil fertility and productivity further ensuring high level of crop productivity and also protecting the soil from deterioration, thereby ensuring sustainable crop production. Organic amendments contain major nutrients in small quantities compared to chemical fertilizers. In addition to having a high yield, it also takes a lot of nutrients out of the soil. In order to improve soil health, crop production, and crop quality over the long term, it is now essential to establish an adequate nutrient management package that includes the use of inorganic, organic, and biofertilizer additions (Srivastava *et al*., 2009). Therefore, an effort has been made to research how integrated nutrient management affects soil physical characteristics and micronutrient uptake in brinjal (*Solanum melongena* L.).

Material and Methods

The field experiment was conducted at the Department of Soil Science and Water Management's Experimental Farm at Neri, Hamirpur (Himachal Pradesh). With eleven treatments and three replications of each, a Randomized Block Design (RBD) was set up. The treatments comprised of control [T_1], 100 % RDF [T_2], 75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost) [T_3], 50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost) [T_4], 25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost) [T_5], 100 % RDN (Vermicompost) [T_6], 100 % RDF + *Azotobacter* [T_7], 75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost) + *Azotobacter* [T_8], 50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost) + *Azotobacter* [T_9], 25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost) + *Azotobacter* [T_{10}] and 100 % RDN (Vermicompost) + *Azotobacter* [T_{11}]. As a source of nitrogen, phosphorus, and potassium, respectively, recommended dose of fertilizer (RDF) was administered in the form of urea, single super phosphate and muriate of potash. Vermicompost was used for substituting RDN through inorganic sources and the amount applied was calculated based on its nitrogen content. No manure or fertilizer of any type was applied to control plots. All of the treatments subjected to standard cultural practices advised for brinjal. The initial soil properties of the experimental site are presented in table 1.

Calculation for nutrient uptake

Nutrient uptake on dry weight basis by brinjal plant was determined by multiplying the respective nutrient content in per cent with the obtained dry matter yield of fruit and stover (q/ha). The uptake of nutrients by stover and fruit was added up to obtain the total amount of nutrients removed by brinjal.

Nutrient uptake (kg/ha) = Nutrient content (%) x Yield (q/ha)

Total uptake = Uptake by fruit + Uptake by stover

Table 1: Initial soil properties of experimental site (0-15 cm)

SN	Soil property	Value
1	Bulk density (Mg/m^3)	1.41
2	Particle density (Mg/m^3)	2.53
3	Porosity (%)	44.33
4	Water holding capacity (%)	33.87
5	Texture	Sandy loam

Results and Discussion

Zinc uptake

The total zinc uptake in the brinjal crop ranged from 116.69 g/ha in treatment receiving no fertilizer, manure or biofertilizer (T_1) to 306.27 g/ha in treatment receiving 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}). The increase in total uptake was more when 100 per cent RDF along with *Azotobacter* (T_7) was applied in comparison to application with 100 per cent RDF alone (T_2). It was further noticed from the data that the treatments where *Azotobacter* was applied additionally (T_7-T_{11}) increased the total zinc uptake and maximum total zinc uptake was obtained with the application of 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) followed by 25 per cent RDN through inorganic fertilizer + 75 per cent RDN through vermicompost + *Azotobacter* (T_{10}) while minimum was obtained with the application of 100 per cent RDF along with *Azotobacter* (T_7). Treatments where RDN was substituted by vermicompost but *Azotobacter* was not applied (T_3-T_6) also showed increase in total zinc uptake over 100 per cent RDF and uptake of 219.87, 245.10, 270.19 and 293.21 g/ha was obtained with the use of 75 per cent RDN through inorganic fertilizer + 25 per cent RDN through vermicompost (T_3), 50 per cent RDN through inorganic fertilizer + 50 per cent RDN through vermicompost (T_4), 25 per cent RDN through inorganic fertilizer + 75 per cent RDN through vermicompost (T_5) and 100 per cent RDN through vermicompost (T_6), respectively (Figure 1).

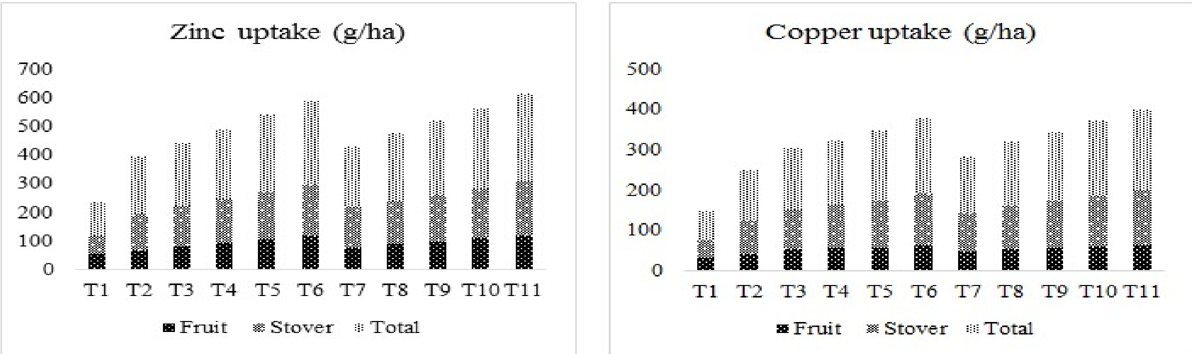

Figure 1: Effect of integrated nutrient management practices on zinc and copper uptake.

Poor biomass production in control lead to lower uptake of zinc by plant. Application of RDF helps in direct supply of nutrients to the plants leading to better root development and therefore resulting in increased uptake of nutrients by the plant. The application of organic manures has solubilizing effect on plant nutrients and chelating effect on metal ions resulting in their increased availability and thus improve its uptake. Organic manures also increase the availability of nutrients for longer period as the nutrients are slowly released into the soil. The increase in uptake with biofertilizers might be due to its synergistic effect with other fertilizers in making availability of plant nutrients more readily and by solubilizing the nutrients in the soil. These results of the study are corroborated with previous reports of Chavan (2003), Rashid *et al*. (2008), Dhiman (2012) and Hemalatha *et al*. (2018).

Copper uptake

The total copper uptake in the brinjal crop ranged from 73.64 g/ha in treatment T_1 (control) to 199.83 g/ha in treatment T_{11} (100 per cent RDN through vermicompost + *Azotobacter*). Conjunctive use of vermicompost and *Azotobacter* resulted in increased uptake. Application of 75 per cent RDN through inorganic fertilizer + 25 per cent RDN through vermicompost + *Azotobacter* (T_8), 50 per cent RDN through inorganic fertilizer + 50 per cent RDN through vermicompost + *Azotobacter* (T_9), 25 per cent RDN through inorganic fertilizer + 75 per cent RDN through vermicompost + *Azotobacter* (T_{10}) and 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) recorded uptake of 159.41, 171.90, 185.40 and 199.83 g/ha, respectively. However, treatments T_{10} and T_{11} were found to be at par with each other. Application of RDN by using vermicompost as a substitute (T_3-T_6) was observed to increase total copper uptake over 100 per cent RDF (T_2) and among these treatments, maximum uptake was obtained with the application of 100 per cent RDN through vermicompost (T_6) whereas minimum was observed with 75 per cent RDN through inorganic fertilizer + 25 per cent RDN through vermicompost (T_3) (Figure 1).

Low copper uptake in control might be due to lower yield in control plots ascribed to the lower nutrient content in the soil causing starvation of plants for nutrients. The increased uptake with application of RDF might be due to the better proliferous root system developed that resulted in better absorption of water and nutrients by the plants thus increasing the copper uptake. Organic compounds in the soil solutions are capable of chelating solution Cu^{2+}, which increases the concentration of Cu^{2+} in soil solution (Raut, 2017). Vermicompost release the nutrient slowly and steadily into the soil system and enables the plant to absorb nutrients for a longer period of time thereby enhancing the yield and uptake by the plants. This might be the reason for the increase in copper uptake by the plants upon substitution of RDN with vermicompost. The increase in copper uptake with the additional application of *Azotobacter* might be due to better root development resulting in increased uptake of water and nutrient by the plant. The results are in authentication with the conclusions of Chavan (2003), Rashid *et al*. (2008) and Dhiman (2012).

Iron uptake

The total iron uptake in the brinjal crop varied from 279.61 to 727.94 g/ha. The lowest values obtained from control (T_1) and highest values from 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}). Application of RDF alone (T_2) or in conjunction with *Azotobacter* (T_7) was found to be superior over control. Substitution of RDN by vermicompost alone (T_6) or with the additional application of *Azotobacter* (T_{11}) increase the total iron uptake over 100 per cent RDF by 51.15 and 56.69 per cent, respectively. Further, it was observed that among all the treatments comprising application of RDN by vermicompost (T_3-T_6), the highest uptake was obtained with the use of 100 per cent RDN through vermicompost (T_6) followed by 25 per cent RDN through inorganic fertilizer + 75 per cent RDN through vermicompost (T_5), 50 per cent RDN through inorganic fertilizer + 50 per cent RDN through vermicompost (T_4) and 75 per cent RDN through inorganic fertilizer + 25 per cent RDN through vermicompost (T_3). Similar trend was followed by the treatments where *Azotobacter* was applied along with recommended doses of nitrogen by vermicompost as by hundred per cent substitution of recommended doses of nitrogen by vermicompost (T_6) registered higher total uptake compared to no substitution of recommended doses of nitrogen (T_2) (Figure 2). Lower uptake in control treatment is attributed to lower yield in these plots due to poor nutritional status. The increase in uptake due to addition by vermicompost might be probably due to release of iron during its decomposition and by preventing loss of iron through chelation, thereby increasing its uptake by the plant. Inorganic fertilizer helps in direct availability of nutrients to the plants thereby increasing their uptake. Application of RDN by organic manure in combination with RDN by chemical fertilizers showed positive effect on iron uptake. Higher uptake in treatments with the application of biofertilizer might be attributed due to the synergistic effect of biofertilizer with organic manure and chemical fertilizer. The results are in testimony with the findings of Chavan (2003), Dhiman (2012) and Hemalatha *et al*. (2018).

Manganese uptake

Application of 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) was found to be superior in terms of total manganese uptake by brinjal crop over other treatments. The increase in total uptake was more when applied with 100 per cent RDF + *Azotobacter* (T_7) in comparison to application with 100 per cent RDF alone (T_2). Similar, trend was also followed when substitution of 100 per cent RDN by vermicompost was done. It was further obvious from the data that treatments applied with *Azotobacter* (T_7-T_{11}) increased the total manganese uptake by the plant and maximum total manganese uptake was achieved with the application of 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) while, minimum was obtained with application 100 per cent RDF + *Azotobacter* (T_7). Treatments where RDN was substituted by vermicompost but *Azotobacter* was not additionally applied (T_3-T_6) also showed a significant increase in total manganese uptake over 100 per cent RDF and uptake of 266.58, 295.19, 317.60 and 343.13 g/ha was obtained with the use of 75 per cent RDN through 85 inorganic fertilizer + 25 per cent RDN through vermicompost (T_3), 50 per cent RDN through inorganic fertilizer + 50 per cent RDN through vermicompost (T_4), 25 per cent RDN through inorganic fertilizer + 75 per cent RDN through vermicompost (T_5) and 100 per cent RDN through vermicompost (T_6), respectively (Figure 2).The increased uptake due to treatment combinations of inorganic, organic and biofertilizers over the control might be attributed to the increased yield of crop with their addition which may be explained on the basis that synergistic effect of these combinations improved the physical conditions of the soil and supported better aeration to plant root, sustained availability of nutrients and there by the uptake of manganese by the plants. Substitution of

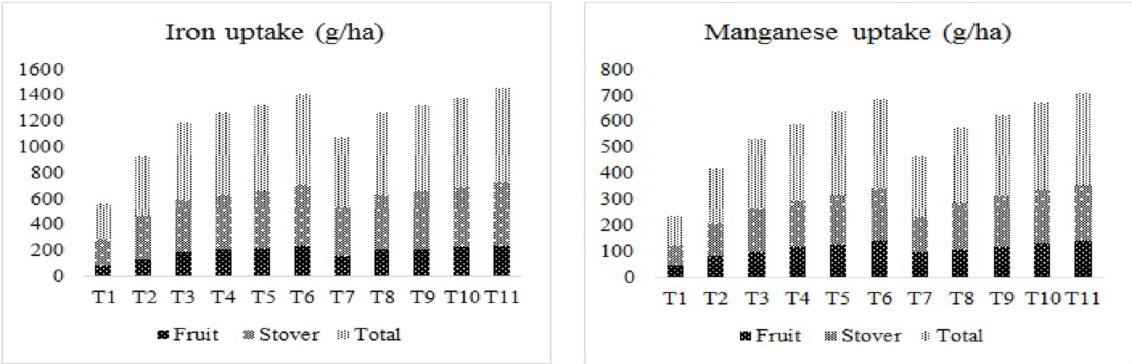

Figure 2: Effect of integrated nutrient management practices on iron and manganese uptake.

RDN by vermicompost increased the uptake over 100 per cent RDF due to the release of manganese during its decomposition thereby increasing its availability and uptake.No fertilizer or manure or biofertilizer application in control might be the reason for its low uptake. Increase in uptake with the application of biofertilizer may be ascertained due to better root development, better transportation of water uptake and deposition of nutrients. These results are in line with those of Chavan (2003), Rashid *et al*. (2008), Dhiman (2012) and Sharma *et al*. (2020).

Bulk density

The data presented in the table 2 revealed that the bulk density of the soil varied from 1.41 to 1.36 Mg/m^3 after the harvest of brinjal crop. The bulk density of the soil was observed to decrease with the increase in substitution rate of RDN by vermicompost and lowest bulk density (1.36 Mg/m^3) was achieved with the application of 100 per cent RDN through vermicompost alone (T_6) or with the addition of *Azotobacter* (T_{11}). The bulk density of the plots receiving no fertilizer, manure or biofertilizer did not undergo any change from the initial value which might be due to no application of organic manure and compaction of soil in those treatments. Higher bulk density in control might be due to no application of organic manure in those treatments. Treatment receiving vermicompost recorded decrease in the bulk density of soil as compared to control treatment which might be attributed to the fact that the application of organics might have caused better aggregation thereby increasing soil aeration. Decrease in bulk density with the application of biofertilizer might be ascertained to synergistic effect of organic manure and bio-fertilizers in improving the soil physical conditions.The results are also in authentication with the conclusions of Salvi *et al*. (2015), Batabyal *et al*. (2017) and Lakra *et al*. (2017). Comparing a well-aggregated soil to a dispersed and poorly organised soil, the former has a lower bulk density. It might be because the soil's microbial production of gum and polysaccharides increased significantly as a result of the addition of organic materials. Due to its resistance to further deterioration, the microbial breakdown product serves as a binding substance. This could aid in soil aggregation, resulting in a decrease in soil bulk density.

Particle density

An inquisition of data presented in table 2 showed that the effect different nutrient management practices on the particle density of soil was found to be non-significant. The initial value of the particle density did not change as compared to control (T_1). The plots receiving substituted doses of RDN by vermicompost with or without *Azotobacter* marginally declined the particle density over initial value. The slight change in soil particle density might be attributed to the increase in organic carbon content in soil with the application of organic manure. Understanding and determining other physical parameters, such as bulk density and porosity, depend heavily on particle density. Application of nutrient management practices affects both bulk density and porosity, but it has no effect on particle density. Because nutrient management and other short-term modifications do not influence the overall quantity or the chemical makeup of the soil mineral particles and the particle

Table 2: Effect of integrated nutrient management practices on soil bulk density and particle density

Treatment		Bulk density (Mg/m^3)	Particle density (Mg/m^3)
T_1	Control	1.41	2.53
T_2	100 % RDF	1.40	2.52
T_3	75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost)	1.39	2.52
T_4	50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost)	1.38	2.52
T_5	25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost)	1.37	2.52
T_6	100 % RDN (Vermicompost)	1.36	2.52
T_7	100 % RDF + *Azotobacter*	1.40	2.52
T_8	75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost) + *Azotobacter*	1.39	2.52
T_9	50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost) + *Azotobacter*	1.38	2.52
T_{10}	25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost) + *Azotobacter*	1.37	2.52
T_{11}	100 % RDN (Vermicompost) + *Azotobacter*	1.36	2.52
CD (P=0.05)		NS	NS

density does not change. The results recorded are in testimony with the work done by Salvi *et al.* (2015) and Lakra *et al.* (2017).

Porosity

The data on effect of integrated nutrient management on porosity is furnished in table 3 and it revealed that maximum (46.23%) value of porosity was observed with the application of 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) whereas minimum (44.04%) was obtained under control (T_1), however the differences were found to be non significant. Application of 100 per cent RDF with and without the use of *Azotobacter* (T_7 and T_2, respectively) showed slight increase in porosity of the soil compared to initial value. Substitution of RDN by vermicompost showed minor increase in the porosity of the soil compared to initial soil value. The slight decrease in bulk density of soil with the increase in substitution of RDN by vermicompost with and without the use of *Azotobacter* showed a favourable change in porosity of the soil which might be attributed to the rise in soil polysaccharide and microbial gum production. These microbial breakdown products may also have worked as soil particle binding agents, assisting in soil aggregation and enhancing porosity. In addition, the inclusion of organic matter improved aggregate stability and by reducing the bulk density, the addition of organic matter increases the volume of pore space and hence increases the soil's porosity. Bio inoculants are also found to be useful as it increases soil porosity. These results are in accordance with those obtained by Kumari (2017) and Lakra *et al.* (2017).

Water holding capacity

An examination of data presented in table 3 depicts that water holding capacity of the soil varied from 33.94 per cent in control (T_1) to 35.69 per cent where application of 100 per cent RDN through vermicompost + *Azotobacter* (T_{11}) was done. However, it was found to be non significant. Application of RDF alone (T_2) or in addition with *Azotobacter* (T_7) showed increase in water holding capacity of the soil over control and initial value. Substitution of RDN by vermicompost showed marginal increase in the water holding capacity of the soil over 100 per cent RDF alone. Similarly, when *Azotobacter* was applied along with the substituted RDN by vermicompost also increased the water holding capacity. The increase in water holding capacity of the soil with the increase in the substitution rate of RDN by vermicompost might be due to the addition of organic matter which promote aggregation, improve the quantity of pores, their distribution in size and the specific surface area of soils and hence soil structure which positively affect the water holding capacity. Moreover, the use of bio inoculants is useful as it improves water holding capacity of the soil. Also, soils with more organic matter have a larger capacity to hold water than soils with a similar texture but less organic matter. This might also be the reason for the increase in water holding capacity of the soil with the application of *Azotobacter*. The findings are in conformity with those of Salvi *et al*. (2015) and Dhiman *et al*. (2018).

Table 3: Effect of integrated nutrient management practices on soil porosity and water holding capacity

Treatment		Porosity (%)	Water holding capacity (%)
T_1	Control	44.04	33.94
T_2	100 % RDF	44.35	34.02
T_3	75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost)	44.94	34.33
T_4	50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost)	45.23	34.64
T_5	25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost)	45.47	34.86
T_6	100 % RDN (Vermicompost)	46.09	35.37
T_7	100 % RDF + *Azotobacter*	44.55	34.21
T_8	75 % RDN (Inorganic fertilizer) + 25 % RDN (Vermicompost) + *Azotobacter*	45.01	34.49
T_9	50 % RDN (Inorganic fertilizer) + 50 % RDN (Vermicompost) + *Azotobacter*	45.31	34.79
T_{10}	25 % RDN (Inorganic fertilizer) + 75 % RDN (Vermicompost) + *Azotobacter*	45.61	35.08
T_{11}	100 % RDN (Vermicompost) + *Azotobacter*	46.23	35.69
CD (P=0.05)		NS	NS

Conclusion

Application of inorganic fertilizer or organic fertilizer or both with or without biofertilizer positively influenced micronutrient uptake *viz.,* Zn, Cu, Fe and Mn. Maximum nutrient uptake was observed in treatment T_{11} (100 per cent RDN through vermicompost + *Azotobacter*), while minimum was observed under treatment T_1 (control). Treatments where *Azotobacter* was used in addition with the dose of RDN supplied through inorganic fertilizer or vermicompost or both showed increased uptake against the respective treatments where it was not used. Physical properties of soil were also slightly improved with the application of different treatments, however the differences were not significant.

Conflict of interest

The authors declare that they have no conflict of interest.

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Nutrient use efficiency indices as influenced by nutrient management practices under cotton-green gram intercropping system in vertisols

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ABSTRACT

A field study was conducted during 2021-22 at the Research field of All India Coordinated Research Project for Dryland Agriculture (AICRPDA), Dr. Panjabrao Deshmukh Krishi Vidyapeeth, (Dr. PDKV) Akola, Maharashtra (India), on an ongoing long-term experiment initiated in 1987-88 under cotton + green gram (1:1) intercropping system in Vertisols. The eight treatments comprised of a control, sole use of organics and chemical fertilizers, and integration of organics with chemical fertilizers to partially substitute Nitrogen (N). The results after 35th cycle revealed that the uptake of N, Phosphorus (P), and Potassium (K) was significantly higher in Integrated Nutrient Management (INM) treatments, particularly the treatments T_6 and T_7 where 50 percent N was substituted by either gliricidia or Farm Yard Manure (FYM). Further, the various parameters of nutrient use efficiency of N, P, and K were also found to be significantly higher in INM treatments followed by T_2 (100% RDF). Therefore, the present investigation concludes that under the cotton + green gram intercropping system in Vertisols, INM that involves conjoint use of different nutrient sources appears to be a promising strategy for improvement in fertilizer use efficiency as a whole including Nutrient Use Efficiency (NUE).

Introduction

Cotton being an important cash crop plays a key role in the Indian economy. China and India represent around 58 percent of the world's cotton consumption. Several factors influence cotton growth, including genotype, environmental conditions, and management practices. Fertilizer is a major input in cotton production, particularly Nitrogen (N), which is one of the yield and quality limiting factors and is required more than other nutrients (Zuluaga and Sonnante, 2019). As a result, farmers tend to use a lot of N fertilizer to boost growth and productivity and ensure a high yield (Dong *et al*., 2012). Excessive N application, on the other hand, causes not only excessive cotton vegetative growth, delayed maturity, and yield and quality reductions, but also increases N release and environmental pollution (Rochester and Peoples, 2001). Nitrogen is one of the most important mineral nutrients because the production of high yields depends on its adequate supply (Zuluaga and Sonnante, 2019). The Phosphorus (P) in plants is important for root development, while Potassium (K) controls water balance, increases water uptake, and strengthens plant resistance to pests and diseases (Hartman *et al*., 2011). The efficiency of applied fertilisers is low, and the overall application efficiency has been around or less than 50 percent for N, 10–15 percent for P, and around 40 percent for K. The lower efficiencies under field conditions are associated with nutrient losses such as leaching, runoff, fixation, volatilization, etc. Furthermore, the choice of crops and cultivars also affects the use efficiency of nutrients. The Nutrient Use Efficiency (NUE) depends on the nutrient uptake, transport, assimilation, storage, remobilization, and synthesis of storage compounds during plant growth and

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development (Weih *et al*., 2018). In the literature, several site-specific management interventions have been recommended to increase the farm output per unit of input used in terms of fertilizers. A better NUE would result in lowering the need of fertilisers and associated costs. By optimizing nutrients for crop nourishment while minimizing nutrient loss to the field, nutrient use strives to improve the overall performance of cropping systems and support agricultural sustainability through contributions to soil fertility or other soil quality components (Fixen *et al*., 2015). Integrated Nutrient Management (INM) aims to improve soil health and crop productivity to make farming more sustainable (Das *et al*., 2014). A positive effect is seen on the soil properties when INM is used over other methods of nutrient management. In addition to providing nutrients to crops, organic sources of plant nutrients can improve the physical and chemical properties of soil (Sandhu *et al*., 2020). Furthermore, organic manures help increase the efficiency with which inorganic fertilizers are used. Therefore the present study has been undertaken to study the long-term effect of different nutrient management practices on nutrient use efficiency under the cotton + green gram intercropping system of Vertisol of Vidarbha region of Maharashtra.

Material and Methods

Site description

A long-term field experiment on cotton + green gram intercropping system was initiated in 1987-88 on the research field of All India Coordinated Research Project for Dryland Agriculture (AICRPDA), Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. PDKV), Akola. The experimental site (latitude of 20° 32' N and longitude of 77° 7' E at an elevation of 325 m above Mean Sea Level (MSL) has a hot, semi-arid ecoregion. Rainfall during *Kharif* 2021 (June-September) amounted to 741.8 mm. Most of the rainfall is received from the southwest monsoon. The maximum summer temperature is around 42°C and the winter temperature dips to 11°C. Experimental soil belongs to the Vertisols order (classified as Typic Haplusterts) with clay loam to clay texture as well as calcareous and lime concretions at varying depths. Soils have a high Available Water Holding Capacity (AWC) (180–200 mm) and are subject to drought occurrence once every 10 years. The initial soil sample analysis data (1987-88) indicate that the soil was moderately alkaline (pH 8.2), Electrical Conductivity (EC) 0.30 dS/m, Organic Carbon (OC) 4.6 g/kg, Available Nitrogen (AN) 214 kg/ha, Available Phosphorus (AP) 12.97 kg/ha, and Available Potassium (AK) 316 kg/ha (Anonymous, 1988).

Experimental design, treatments, and crop management

The experiment is a part of a long-term experiment that was taken on the same site since 1987-88 without changing randomization, under the rainfed condition on the farm of AICRPDA, Dr. PDKV, Akola, Maharashtra (India) in Randomized Block Design (RBD) with eight treatments replicated thrice. The eight treatments comprising organic and inorganic sources of fertilizer were : T_1- Control; T_2- 100 percent RDF; T_3- 50 percent RDF; T_4 -50 percent N/ha through gliricidia; T_5 -50 percent N/ha through FYM; T_6 -50 percent $N_{gliricidia}$ + 50 percent $N_{\text{Fertilizers (F)}}$ + 100 percent P_2O_5 $_{\text{Fertilizers (F)}}$ + 100 percent K_2O $_{\text{Fertilizers (F)}}$; T_7 -50 percent N_{FYM} + 50 percent $N_{\text{Fertilizers (F)}}$ + 100 percent P_2O_5 $_{\text{Fertilizers (F)}}$ + 100 percent K_2O $_{\text{Fertilizers (F)}}$; T_8 -100 percent $N_{gliricidia}$ + 100 percent P_2O_5 $_{\text{Fertilizers (F)}}$ + 100 percent K_2O $_{\text{Fertilizers (F)}}$. The Recommended Dose of Fertilizer (RDF) was 60:30:30 kg/ha N, P_2O_5, K_2O respectively. Treatment-wise basal dose of FYM and chemical fertilizers were applied at the time of sowing and the remaining half dose of N was applied through chemical fertilizers and gliricidia, 30 days after sowing (DAS) as per treatments. Every year, the same treatments were imposed on cotton (60 x 30 cm) + green gram (60 x 10 cm) intercrop. The gross plot size was 9.0 x 9.9 m^2 while the net plot size was 8.4 x 9.0 m^2. Both the crops (Cotton var. “AKH 9916”; Green gram var. “Greengold”) were sown in June or July depending upon the onset of the monsoon. All other agronomic practices were performed as per standard packages of practice recommended by the university.

Plant analysis and calculations of nutrient use efficiency

The plant samples were air-dried in shade and digested by using a di-acid and Tri-acid mixture. Total N was analysed by Micro-Kjeldahl method (Parkinson and Allen, 1975), total P by

Vanadomolybdate yellow colour method (Jackson, 1973), total K by Flame photometry (Chapman and Pratt, 1961). The uptake of major nutrients was worked out by multiplying total dry matter and nutrient concentration. Various use efficiency of nutrients N, P, and K was calculated as per the formulae given in Table 1.

Statistical Analysis

The data were statistically analysed by the technique of analysis of variance (ANOVA) as suggested by Gomez and Gomez, (1984) using the WASP statistical package (https://ccari.icar.gov.in/wasp/index.php). The least significant difference (*lsd*) at $p \leq 0.05$ was used for multiple comparisons of treatment means.

Table 1: Calculations of various indices of nutrient use efficiency

NUE indices	Formulae	Unit
Agronomic Efficiency (AE)	$AE = \frac{(EYf - EYc)}{F}$	kg grain yield increase per kg nutrient applied
Physiological Efficiency (PE)	$PE = \frac{(BYf - BYuf)}{(NUf - NUc)}$	Biological yield obtained per unit of applied nutrients.
Apparent Recovery Efficiency (ARE)	$ARE = (\frac{NUf - NUc}{F}) \times 100$	Quantity of nutrient uptake per unit of nutrient applied (%)
Internal Utilization Efficiency (IUE)	$IUE = \frac{EY}{NU}$	Kg grain per kg nutrient applied

Where, EYf- Economic Yield of crops in the fertilized plot; EYc- Economic Yield of crops in control plot; F- Amount of fertilizer input applied; BYf- Biological Yield of crops in the fertilised plot; BYuf- Biological Yield of crops in unfertilized plot; NUf-Nutrient Uptake in the fertilized plot; NUc-Nutrient Uptake in the control plot; EY-Economic Yield; NU: Nutrient Uptake.

Results and Discussion

Nutrient uptake by cotton and green gram

The uptake of Nitrogen (N), Phosphorus (P), and Potassium (K) by cotton and green gram were significantly affected by different nutrient management practices. The N uptake by cotton and green gram were significantly highest in Integrated Nutrient Management (INM) treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_7 (50% N_{FYM} + 50% N_F + 100% P_F + 100% K_F) followed by treatment T_8 (100% $N_{gliricidia}$ + 100% P_F + 100% K_F) and T_2 (100% RDF) (Figure 1). The uptake of N in cotton and green gram was higher by about 110 and 114 percent in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) or T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F) as compared to control (T_1). The P uptake by cotton and green gram was significantly higher in INM treatments, particularly T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F) (Figure 2). The P uptake by cotton and greengram were higher by about 195 and 131 percent in treatments T_6 (50%$N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) or T_7 (50%N_{FYM} + 50% N_F+100% P_F + 100% K_F) as compared to control (T_1). Similar result was also found for the K uptake by cotton and green gram, where K uptake in T_6 (50%$N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) or T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F) were higher by 154 and 200 percent as compared to control (T_1) (Figure 3). The higher nutrient uptake in INM treated plot is mainly because of improved soil properties resulting from the application of organics and inorganics, which helped in maintaining sufficient moisture in the soil for the better uptake of nutrients. These organics further help in reducing the P fixation in soil and thus increased the availability, mobility, and uptake of P in INM treated plots. The release of organic acids from the decomposition of gliricidia or FYM in INM treated plot may have resulted in the solubilisation of native potash-bearing minerals, as well as supplied a large amount of K. Further, the nutrient supply from both organic and chemical sources tended to increase nutrient content when compared to nutrient supply from only chemical fertilizers. This could be attributed to a balanced supply of plant nutrients from both organic and chemical sources (Panigrahi *et al.*, 2014). It could also be attributed to increased microbial activity as a result of the integrated use of organic manure, and chemical fertilizer which aided in improving nutrient supply and better nourishment of

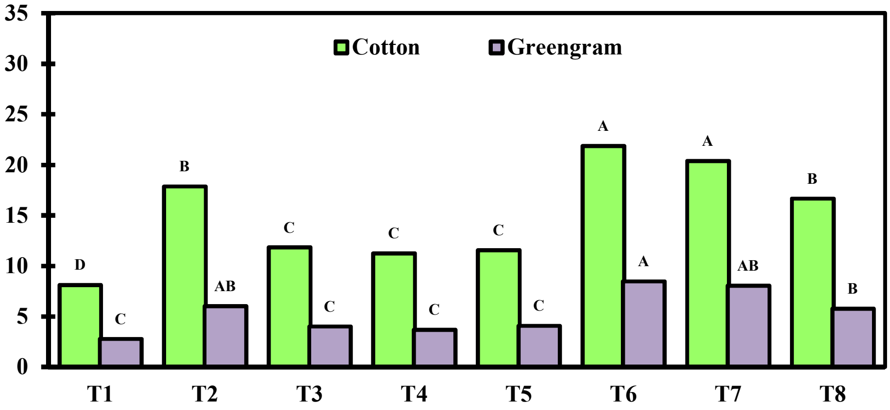

Figure 1: Nitrogen uptake (kg/ha) by cotton and green gram.

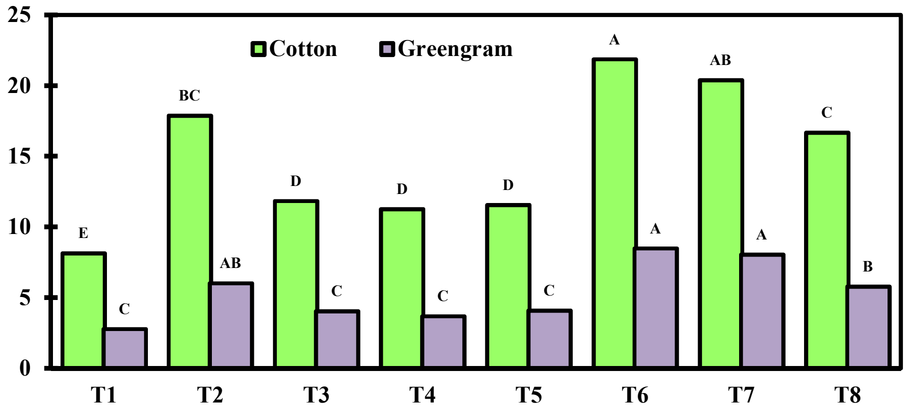

Figure 2: Phosphorus uptake (kg/ha) by cotton and green gram.

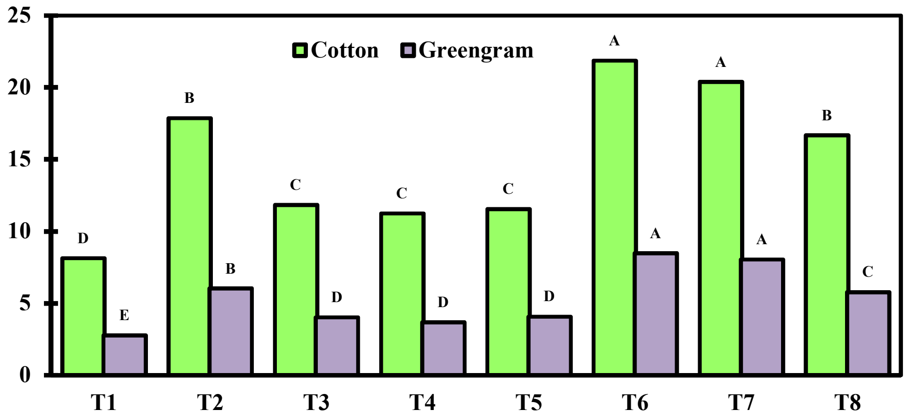

Figure 3: Potassium uptak (kg/ha) e by cotton and green gram.

Bars followed by a different letter are significantly different at $P < 0.05$ according to Tukey's HSD. T_1- Control; T_2- 100 percent RDF; T_3- 50 percent RDF; T_4 -50 percent N/ha through gliricidia; T_5 -50 percent N/ha through FYM; T_6 -50 percent $N_{gliricidia}$ + 50 percent N $_{Fertilizers\ (F)}$ + 100 percent P_2O_5 $_{Fertilizers\ (F)}$ + 100 percent K_2O $_{Fertilizers\ (F)}$; T_7 -50 percent N_{FYM} + 50 percent N $_{Fertilizers\ (F)}$ + 100 percent P_2O_5 $_{Fertilizers\ (F)}$ + 100 percent K_2O $_{Fertilizers\ (F)}$; T_8 -100 percent $N_{gliricidia}$ + 100 percent P_2O_5 $_{Fertilizers\ (F)}$ + 100 percent K_2O $_{Fertilizers\ (F)}$.

crops throughout their growing period, resulting in increased uptake of nutrients. All of these processes resulted in higher K uptake in INM treated plots. Similar results were also reported by Jadhao *et al.,* 2018; Ramakrishna *et al*., 2017; Khambalkar *et al.,* 2017.

Nitrogen use efficiency Indices

The various Nitrogen Use Efficiency (NiUE) indices in cotton were significantly affected by different nutrient management practices (Table 2). The Agronomic Efficiency (AE) in cotton ranged from 0.84 to 4.52 kg/kg. AE was significantly higher in the treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F), followed by treatment T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F). The treatments with 50 percent RDF (T_3) and sole organics (T_4 and T_5) treatments had lower AE. The Physiological Efficiency (PE) ranged between 37.86 to 77.78 kg/kg. It was highest in treatment T_4 (50% N through gliricidia) which was statistically at par with treatment T_5 (50% N through FYM). Similarly, treatment T_3 (50% RDF) also had higher PE. Further, it was observed that the INM treatments (T_6, T_7, and T_8) as well as 100 percent RDF treatment (T_2), had significantly lower PE over the rest of the treatments. Likewise, the Internal Utilization Efficiency (IUE) was highest in control (T_1) treatment followed by 50% RDF (T_3) and sole organics treatments (T_4 and T_5). Significantly lowest IUE was observed in the INM treatments (T_6, T_7, and T_8), however, INM treatments were not significantly different among themselves. The Apparent Recovery Efficiency (ARE) was significantly highest in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) while the lowest ARE was observed in sole organics treated plots (T_4 and T_5). Similar to cotton, in green gram too, AE and ARE were significantly highest in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) followed by treatment T_7 (50% N_{FYM} + 50% N_F + 100% P_F + 100% K_F), while the lowest AE and ARE were observed in 50 percent RDF (T_3) and sole organics treated plots (T_4 and T_5) (Table 3). The PE was lowest in treatment T_8 (100% $N_{gliricidia}$ + 100% P_F + 100% K_F), while IUE was significantly lowest in all the three INM treatments, as compared to other treatments.AE is used as a short-term indicator of the impact of applied nutrients on productivity. Significantly higher values of AE in INM treated plots were mainly due to the direct effect integration of organics and chemical fertilizers which suggests that the N mineralization from FYM/gliricidia was in synchronization with crop requirement during various growth stages. FYM/gliricidia used in the present investigation also contains a sufficient amount of NPK, thus enriching the nutrient pool of soil. Moreover, organics enhance the use efficiency of native as well as applied fertilizers. Combined use of organic manure and N fertilizer maintains a continuous N supply, checks losses and thus helps in more efficient utilization of the applied N. The higher values of PE in treatments except INM (T_6, T_7, and T_8) and T_2, suggest the deficiency of nutrients in those treatments. The lower PE in INM and T_2 indicates that the nutrient uptake was much better in these treatments due to improvement in soil properties. A higher value of IUE indicates the nutrient deficiency while a lower value suggests a poor internal nutrient conversion mechanism depending upon the stresses, management, and genotype environment (Dobermann, 2007; Fixen *et al.,* 2015). IUE was higher in control (T_1) and sole organics (T_4 and T_5) and inorganics treated plots (T_2 and T_3) indicating the deficiency of nutrients, while it was significantly lowered in INM treatments (T_6, T_7, and T_8). Better synchronization and slow-release nature of organics and immediate availability of fertilizers in INM treatments might have enhanced the IUE. ARE is a measure of the potential for nutrient loss from a cropping system and the effectiveness of management practices. Lower levels of ARE indicate that management changes could improve efficiency or that nutrients are accumulating in the soil (Dobermann, 2007; Fixen *et al.,* 2015). The ARE was higher under INM treatments because in these treatments N was supplied by both organic and inorganic sources thus help maintained the steady and continuous supply of N throughout the plant growth. Since only organics do not meet all the N requirements of a crop, ARE was found to be lowest under organic amended treatments. Several other long-term experiments also observed similar results (Swain *et al.,* 2006; Huang *et al.,* 2008; Singh, *et al.,* 2012; Mondal *et al.,* 2016).

Phosphorus use efficiency Indices

Similar to NiUE indices, the Phosphorus Use Efficiency (PUE) indices in cotton were also significantly affected by different nutrient management practices (Table 4). The AE and ARE in cotton ranged from 2.6 to 8.12 and 9.51 to 35.81% respectively. The significantly higher AE was observed in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) followed by T_2 (100% RDF) and treatment T_7 (50% N_{FYM} + 50% N_F + 100% P_F + 100% K_F) while ARE was highest in treatment T_4 (50% N through gliricidia). It was followed by treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_2 (100% RF). Furthermore, in case of PE and IUE, significantly higher PUE was

Table 2: Effect of nutrient management practices on nitrogen use efficiency indices in cotton

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	23.21^{a}	-
T2	3.51^{c}	37.86^{c}	19.91^{d}	20.30^{c}
T3	1.30^{e}	49.64^{bc}	20.62^{c}	12.32^{d}
T4	0.84^{f}	77.78^{a}	21.86^{b}	8.06^{e}
T5	1.12^{e}	63.35^{ab}	21.48^{b}	8.74^{e}
T6	4.52^{a}	35.33^{c}	19.32^{e}	30.98^{a}
T7	4.13^{b}	35.76^{c}	19.39^{e}	26.12^{b}
T8	2.34^{d}	37.57^{c}	19.66^{de}	18.71^{c}
lsd ($p \leq 0.05$)	0.18	18.7	0.45	2.5

Table 3: Effect of nutrient management practices on nitrogen use efficiency indices in greengram

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	34.39^{a}	-
T2	1.71^{b}	14.81^{b}	24.69^{d}	12.47^{c}
T3	0.56^{c}	20.42^{a}	29.73^{b}	6.51^{f}
T4	0.11^{d}	12.42^{d}	29.00^{b}	6.33^{f}
T5	0.53^{c}	12.88^{cd}	27.77^{c}	8.43^{e}
T6	2.23^{a}	13.85^{bcd}	22.67^{e}	18.36^{a}
T7	2.15^{a}	14.62^{bc}	23.80^{d}	15.24^{b}
T8	0.80^{c}	8.10^{e}	24.14^{d}	11.11^{d}
lsd ($p \leq 0.05$)	0.30	1.90	1.11	1.20

Means followed by the same letter in a column are not significantly different at $P < 0.05$ according to Tukey's HSD AE: Agronomic Efficiency, PE: Physiological Efficiency, IUE: Internal Use Efficiency, ARE: Apparent Recovery Efficiency. T_1- Control; T_2- 100 percent RDF; T_3- 50 percent RDF; T_4 -50 percent N/ha through gliricidia; T_5 -50 percent N/ha through FYM; T_6 -50 percent $N_{gliricidia}$ + 50 percent $N_{\text{Fertilizers (F)}}$ + 100 percent $P_2O_{5\ \text{Fertilizers (F)}}$ + 100 percent $K_2O_{\text{Fertilizers (F)}}$; T_7 -50 percent N_{FYM} + 50 percent $N_{\text{Fertilizers (F)}}$ + 100 percent $P_2O_{5\ \text{Fertilizers (F)}}$ + 100 percent $K_2O_{\text{Fertilizers (F)}}$; T_8 -100 percent $N_{gliricidia}$ + 100 percent $P_2O_{5\ \text{Fertilizers (F)}}$ + 100 percent $K_2O_{\text{Fertilizers (F)}}$.

Table 4: Effect of nutrient management practices on phosphorus use efficiency indices in cotton

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	115.09^{a}	-
T2	7.02^{ab}	103.01^{b}	70.97^{d}	15.03^{c}
T3	2.60^{c}	125.13^{a}	85.35^{c}	9.51^{e}
T4	7.34^{ab}	142.25^{a}	89.37^{b}	35.81^{a}
T5	3.19^{c}	138.45^{a}	88.58^{b}	11.93^{d}
T6	8.12^{a}	100.96^{b}	68.49^{e}	17.91^{b}
T7	6.11^{b}	99.50^{b}	68.29^{e}	13.61^{cd}
T8	3.82^{c}	90.27^{b}	68.12^{e}	11.57^{de}
lsd ($p \leq 0.05$)	1.39	22.5	2.4	2.15

observed under INM treated plots (T_6, T_7, and T_8). However, the INM treatments were statistically at par with each other. Similar results were also observed for the various PUE indices in green gram too (Table 5). It was observed that the range of the AE and ARE was highest in cotton while IUE was dramatically higher in green gram crop. AE and ARE in green gram were highest in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F), whereas, the same treatment also had significantly lower PE and IUE. Higher AE in INM treated plots (T_6, T_7, and T_8) reflects the use of P at lower rates and because, while yield was increased, P was less efficiently utilized, whereas lower AE of P indicates less P being mobilized during the crop growth period, resulting in an increased availability as well as higher yield. This was also supported by the findings of Mitran and Mani, (2017). The higher value of PE in the sole organic treated plos (T_4 and T_5) and also in 50 percent RDF (T_3) suggest the deficiency of nutrients in these plots. The INM treated plots (T_6, T_7, and T_8) have significantly improved the soil conditions resulting in better uptake of nutrients. Further, lower PE in organics treated plot may also be ascribed to the fact that P released from FYM/gliricidia during crop growth season is less mobile in nature. Under INM, recovery of P was invariably greater compared with sole organics and sole inorganics treatments. The treatments that received organics in combination with mineral fertilizers (INM treatments) had lower IUE than the treatments that received only mineral fertilizers (T_2 and T_3) or sole organics (T_4 and T_5). High IUE in the sole inorganic treated plot (T_2 and T_3) indicated nutrient deficiency and suboptimal utilization of P, resulting in higher values although the nutrients were used at reasonably higher rates. ARE of P was higher in treatment T_4 (50% N through gliricidia) and in INM treatment T6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F).

This is because in this treatment lower rate of P was applied resulting in a more recovery at a lower rate of application. This could be also because long-term application of gliricidia altered surface soil properties, optimized soil P status, and maximized P recycling. These results are in conformity with the findings of (Singh *et al.,* 2012.) ARE was higher in the treatment T_4 (50% N through gliricidia) may be due to the mobilization of P from the organic pool to the inorganic pool from gliricidia and also that the release was not in congruent between nutrient supply and crop demand. Similar results were also reported by Das *et al.,* (2015). Similar to our findings, Vats *et al.*, (2001) found that using organic materials in conjunction with fertilizer was very beneficial for improving the efficiency of fertilizer P. This could be because long-term application of organic materials modified surface soil properties, optimised soil P status, and maximized P recycling from organic and mineral fertilizers. Further, Das *et al.,* (2015) and Dwivedi *et al.,* (2017) also reported similar results.

Potassium Use Efficiency

In case of Potassium (K), all the Potassium Use Efficiency (KUE) indices were significantly affected by different nutrient management practices except for the PE of cotton (Table 6). The AE in cotton ranged from 1.17 to 7.02 kg/kg and was maximum in treatment T_2 (100% RDF) followed by treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F). The IUE was significantly lowest in INM treatments (T_6, T_7, and T_8), while highest IUE was observed in control (T_1) treatment. AE was highest for the treatment T_2 (100% RF), followed by treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_7 (50% N_{FYM} + 50% N_F + 100% P_F + 100% K_F). Significantly lowest ARE was observed in treatment T_5 (50% N through FYM). In green gram, KUE indices were smaller as compared cotton, except for the IUE which was more in green gram. AE in green gram ranged from 0.16 to 3.42 kg/kg and was significantly highest in T_2 (100% RDF), followed by INM treatments T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) and T_7 (50%N_{FYM} + 50% N_F + 100% P_F + 100% K_F) (Table 7). The PE was lower in INM treatments (T_6 and T_7) and sole organics treatments (T_4 and T_5) as compared to sole inorganics treatments (T_2 and T_3) indicating that during the crop growth period, K was not properly mobilized from organics. This might also be the probable reason for higher PE in the sole inorganic fertilized plots. The IUE was highest in control (T_1) and reduced significantly in all other treatments, with the lowest IUE was observed under INM treatments (T_6 and T_7). Higher IUE in control (T_1) plots also showed nutrient mining over the years resulting in a deficiency of nutrient to plants and thus lower yield and uptake. This may also be attributed to farmers' indifferent and non-judicious use of fertilizers and manures, which results in reduced available nutrients in the soil and lower uptake. The ARE ranged from 4.50 to 11.68 percent. The significantly lowest ARE was observed in treatment T_6 (50% $N_{gliricidia}$ + 50% N_F + 100% P_F + 100% K_F) might be due to the fact that gliricidia has more K content and continuous mineralization of K over the years has led to increased K in soil and ultimately resulting in higher uptake by the crop and thus ARE. The soils of the present investigation are inherently higher in available K content, therefore previously K

Table 5: Effect of nutrient management practices on phosphorus use efficiency indices in green gram

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	270.68^{a}	-
T2	3.42^{ab}	115.72^{b}	193.66^{e}	3.28^{c}
T3	1.12^{c}	107.77^{bc}	214.70^{d}	2.52^{d}
T4	1.00^{c}	140.78^{a}	240.46^{b}	5.01^{a}
T5	1.51^{c}	119.56^{b}	226.62^{c}	2.77^{d}
T6	4.00^{a}	98.37^{c}	168.45^{f}	4.35^{b}
T7	3.18^{b}	96.05^{c}	169.87^{f}	3.55^{c}
T8	1.30^{c}	72.02^{d}	199.03^{e}	1.83^{e}
lsd ($p \leq 0.05$)	0.73	17.12	8.96	0.37

Table 6: Effect of nutrient management practices on potassium use efficiency indices in cotton

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	40.82^{a}	-
T2	7.02^{a}	50.29	30.34^{c}	32.82^{a}
T3	2.60^{d}	49.24	31.31^{b}	24.77^{c}
T4	1.17^{f}	50.45	31.70^{b}	16.58^{e}
T5	1.22^{f}	50.05	31.60^{b}	11.98^{f}
T6	5.28^{b}	43.50	27.57^{e}	27.74^{b}
T7	4.31^{c}	45.41	28.40^{d}	21.13^{d}
T8	1.93^{e}	42.76	28.32^{de}	12.21^{f}
lsd ($p \leq 0.05$)	0.27	NS	0.81	1.31

Table 7: Effect of nutrient management practices on potassium use efficiency indices in green gram

Treatments	AE (kg/kg)	PE (kg/kg)	IUE (kg/kg)	ARE (%)
T1	-	-	105.54^{a}	-
T2	3.42^{a}	34.18^{a}	65.63^{d}	11.04^{b}
T3	1.12^{c}	31.04^{a}	76.91^{c}	8.56^{c}
T4	0.16^{d}	23.21^{b}	80.37^{b}	4.86^{d}
T5	0.58^{cd}	25.75^{b}	75.72^{c}	4.50^{d}
T6	2.60^{b}	24.98^{b}	50.26^{f}	11.68^{a}
T7	2.24^{b}	25.47^{b}	52.33^{f}	9.03^{c}
T8	0.66^{cd}	15.07^{c}	58.88^{e}	4.39^{d}
lsd ($p \leq 0.05$)	0.55	3.32	3.40	0.54

Means followed by the same letter in a column are not significantly different at $P < 0.05$ according to Tukey's HSD
AE: Agronomic Efficiency, PE: Physiological Efficiency, IUE: Internal Use Efficiency, ARE: Apparent Recovery Efficiency. T_1- Control; T_2- 100 percent RDF; T_3- 50 percent RDF; T_4 -50 percent N/ha through gliricidia; T_5 -50 percent N/ha through FYM; T_6 - 50 percent $N_{gliricidia}$ + 50 percent $N_{Fertilizers (F)}$ + 100 percent P_2O_5 $_{Fertilizers (F)}$ + 100 percent K_2O $_{Fertilizers (F)}$; T_7 -50 percent N_{FYM} + 50 percent $N_{Fertilizers (F)}$ + 100 percent P_2O_5 $_{Fertilizers (F)}$ + 100 percent K_2O $_{Fertilizers (F)}$; T_8 -100 percent $N_{gliricidia}$ + 100 percent P_2O_5 $_{Fertilizers (F)}$ + 100 percent K_2O $_{Fertilizers (F)}$.

supplementation through fertilizer was not recommended. Further, the organic sources such as gliricidia and FYM used in this experiments are also rich sources of K. All these resulted in better KUE indices in INM treated plots as compared to others. Furthermore, several researchers (Singh *et al*., 2010; Singh *et al*., 2018; Dhillon *et al*., 2019) also reported that crop K requirements could be improved by use of different organics further improving KUE. Such improvements in KUE were also supported by the findings of Singh *et al*., (2004); Oborn *et al*., (2005); Yadav and Sidhu, (2016).

Conclusion

Apart from the loss of productive potential of soils as a result of unscientific land management practices, the NUE is one of the most important yield constraints in crop production in almost all the agro-ecological regions of the world. The present investigation revealed that under the cotton + green gram intercropping system in Vertisols, INM that involves conjoint use of different nutrient sources appears to be a promising strategy for improvement in fertilizer use efficiency as a whole including NUE. Further, it was observed that the overall NUE of N, P, and K was better under different INM treatments. All the NUE indices of N, P, and K were higher during cotton except for IUE which was higher during green gram. Further, in the sole organic and inorganic plots, the nutrients either do not synchronize the crop requirement or are lost in the soil-plant system resulting in comparatively lower NUE indices.

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Conflict of interest

The authors declare that they have no conflict of interest.

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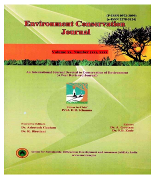

Variability and correlation studies in bacterial wilt resistant advanced tomato lines (*Solanum lycopersicum* L.)

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ABSTRACT

Twenty bacterial wilt resistant lines including recently developed lines at CSKHPKV, Palampur (08) were studied to ascertain the extent of genetic variability, the type and magnitude of correlation between component characters & marketable yield, as well as the direct and indirect impacts of every character on marketable yield. For all variables except plant survival, analysis of variance exhibited significant differences across genotypes. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values for lycopene content (80.581%, 80.148%), titrable acidity (73.666%, 71.219%), marketable yield / plant (53.953%, 52.598%), marketable fruits / plant (34.994%, 34.050%), gross yield / plant (34.094%, 30.553%), average weight of fruit (30.558%, 29.13%), locules / fruit (28.549%, 25.050%), and ascorbic acid (23.641%, 20.919%) respectively, were found high across different parameters of variability. Marketable yield / plant (95.043%, 45.633%), marketable fruits /plant (94.68%, 68.252%), titrable acidity (93.467%, 48.838%), average weight of fruit (90.877%, 57.206%), gross yield / plant (80.305%, 56.402%), ascorbic acid (78.295%, 38.130%), and locules / fruit (76.985%, 45.276%) were found with high heritability and high genetic advance. Marketable yield / plant was significantly and positively associated with average weight of fruit (0.734, 0.795), gross yield / plant (0.774, 0.853), marketable fruits / plant (0.734, 0.742), pericarp thickness (0.693, 0.806), ascorbic acid (0.381, 0.469), titrable acidity (0.347, 0.364) and locules/ fruit (0.284, 0.345) at both phenotypic as well as genotypic levels, respectively. Path coefficient analysis showed that marketable fruits / plant (0.589, 0.608), gross yield / plant (0.278, 0.617) and average weight of fruit (0.382, 0.182) are the three main parameters that had the greatest positive direct impact on marketable yield / plant at the phenotypic and genotypic levels, respectively.

Introduction

Tomato is the third most widely grown vegetable crop in the world, after potato followed by sweet potato, but it ranks first among vegetables as a processing crop. Cultivation of off-season tomato crops during the peak rainy season has increased in Himachal Pradesh during the last three decades, particularly in the mid and low Hills. It is currently farmed on around 13794.98 hectares with an output of 577004.5 metric tonnes (Anonymous, 2020-21). Himachal Pradesh has emerged as a major supplier of fresh produced tomatoes to plain areas because increased temperatures and regular rains hamper production in these areas during the rainy season. The frequency of bacterial wilt caused by *Ralstonia solanacearum* has severely harmed tomato farming in numerous areas of state (low and mid hills). Because it is soil-borne, chemical management is neither efficient nor effective. As a result, genetic

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resistance in cultivars is important and appears to be a great option for successful tomato cultivation in wilt-prone areas. Some bacterial wilt resistant and high yielding lines have recently been developed at CSKHPKV, Palampur, appropriate for growing in the local conditions of low and mid hill areas of the state receiving considerable rainfall, particularly during the rainy season. Continuous researchon different parameters of variability alongwith interrelationship in newly developed genotypes are important for selecting superior genotypes for future crop improvement programme.

Material and Methods

The data were analyzed using OPSTAT 6.8 software for variability, heritability, genetic advance, correlation and path analysis. Twenty bacterial wilt resistance tomato genotypes including two bacterial wilt resistant checks were tested in summer-rainy season, 2020 in Randomized Block Design with three replications. The observations were recorded on ten competitive plants on various parameters viz. plant survival/bacterial wilt disease incidence (%), days to 50 per cent flowering, days to first harvest, average weight of fruit (g), fruit shape index, thickness of pericarp (mm), locules / fruit, plant height (cm), harvest duration, total number of fruits / plant, marketable fruits / plant, gross yield /plant (kg), marketable yield / plant (kg), total soluble solids (°Brix), lycopene content (mg/100g), titrable acidity (%) and ascorbic acid content (mg/100g). For fruit shape index, standard protocol as suggested by Roy and Choudhury (1972) was followed. For lycopene content, acetone- ether extraction method suggested by Ranganna (2000); for titrable acidity, official method 942.15 (AOAC 2000); for ascorbic acid, 2,6-dichlorophenol-indophenol visual titration method as described by Ranganna (1979) were used.

Study Area

The research was conducted at Department of Vegetable Science and Floriculture Research Farm, CSK Himachal Pradesh Krishi Vishvavidyalaya Palampur (Figure 1). This Farm is located at 32°6' N latitude and 76°3' E longitude, at an elevation of 1,290.8 metres above mean sea level. It is located in mid-hill zone of Himachal Pradesh. The location has a humid climate, a sub-temperate climate, and a high rainfall of about 2332 mm per year, the majority of which (70-80 percent) falls between June and September.

Figure 1: Experimental location (32°6' N latitude, 76°3' E longitude)

Results and Discussion

For all variables except plant survival, analysis of variance (ANOVA) exhibited significant differences across genotypes (Table-1). PCV, GCV, heritability, and genetic advance were estimated for fruit yield and other traits in tomato genotypes (Table-2). PCV and GCV ranged from 5.110 (days to first harvest) to 80.581 percent (lycopene content) and 4.265 (days to first harvest) to 80.148 percent (lycopene content), respectively. Both PCV and GCV estimates were found high for lycopene content, titrable acidity, marketable yield / plant, marketable fruits / plant, gross yield / plant, average weight of fruit, number of locules / fruit and ascorbic acid content among different components of variability. High GCV and PCV defines greater variability among the genotypes and thus, better improvement is possible by selection. Harvest duration, thickness of pericarp, days to 50% flowering, fruit shape index, and plant height exhibited moderate level of PCV and GCV indicating that a moderate level of genetic variability is present in these traits, however days to first harvest had low PCV and GCV values indicating limited scope for improvement through this trait. The findings are consistent with previous findings for lycopene content (Rai *et al.*, 2016), locules per fruit (Rai *et al.*, 2016), average fruit weight and total fruits per plant (Al-Aysh *et al.*, 2012), and plant height (Shweta *et al.*, 2016). High

Table 1: Analysis of variance (ANOVA) for various characters in tomato genotypes

Sr. No.	Traits		Mean sum of squares		
			Replications	Treatments	Error
		degree of freedom	2→	19	38
1.	Plant survival percentage		0.066	1.911	0.277
2.	Days to 50 per cent flowering		18.200	72.052*	3.375
3.	Days to first harvest		5.217	31.575*	4.006
4.	Average fruit weight (g)		39.200	626.540*	20.288
5.	Fruit shape index		0.003	0.049*	0.005
6.	Pericarp thickness(mm)		0.977	2.599*	0.314
7.	Locules per fruit		0.253	2.122*	0.192
8.	Plant height (cm)		180.469	377.543*	114.750
9.	Duration of fruit harvest (days)		397.850	136.438*	28.411
10.	Total fruits / plant		22.467	47.596*	7.835
11.	Marketable fruits / plant		2.017	59.118*	1.087
12.	Gross yield / plant (kg)		0.012	0.310*	0.023
13.	Marketable yield / plant (kg)		0.015	0.348*	0.006
14.	Total soluble solids (˚Brix)		0.996	43.669*	19.757
15.	Titrable acidity (%)		0.001	0.090*	0.002
16.	Lycopene content (mg/100g)		0.113	8.462*	0.030
17.	Ascorbic acid (mg/100g)		0.263	10.422*	0.882

*level of significance at 5%

PCV was also found for marketable yield per plant, marketable fruits per plant (Chadha and Walia, 2016) and ascorbic acid (Shankar *et al*., 2013). Similarly, days to first harvest was observed to have low PCV estimates (Chadha and Walia, 2016). PCV estimations for pericarp thickness (Khapte and Jansirani, 2014),fruit shape index (Khapte and Jansirani, 2014), and days to 50% flowering (Khapte and Jansirani, 2014) were moderate (Chadha and Walia, 2016).Researchers previously discovered comparable results for lycopene content (Rai *et al.*, 2016), average fruit weight (Al-Aysh *et al*., 2012), and locules per fruit (Rai *et al*., 2016). For marketable fruits per plant, (Chadha and Walia, 2016) found comparable results, as did (Sehgal, 2017) for marketable yield per plant. (Shankar *et al*., 2013) observed high GCV estimations for titrable acidity. (Shweta *et al*., 2016) found moderate GCV estimates for days to 50% flowering, and (Khapte and Jansirani, 2014) reported moderate GCV estimates for pericarp thickness.The heritability estimates alone fail to indicate the response to selection. Therefore, estimations of heritability seem to have more significance when combined with estimates of genetic advance. For different traits, heritability and genetic advance estimates varied from 43.291 (plant height) to 98.127 percent (lycopene content) and 7.331 (days to first harvest) to 68.252 percent (number of marketable fruits / plant), respectively. Marketable yield / plant, number of marketable fruits / plant, titrable acidity, average weight of fruit, gross yield / plant, ascorbic acid, and locules / fruit had high heritability and high genetic advance demonstrating that these traits are under the strong influence of additive gene action and hence simple selection based on phenotypic performance of these traits would be more efficient. Days to 50% flowering, fruit shape index, pericarp thickness, and total fruits / plant showed high heritability with moderate level of genetic advance indicating the influence of non-additive gene action and considerable influence of environment on the expression of these traits. This trait could be exploited through expression of dominance and epistatic components through heterosis. Fruit harvesting period, TSS, and plant height exhibited moderate level of heritability and genetic advance. Plant height, harvest duration and total soluble solids indicated moderate level of heritability with low genetic advance. High heritability estimates for marketable fruits per plant were reported (Chadha and Bhushan, 2013); for marketable yield per plant; for gross yield per plant (Chadha and Walia, 2016); and for average fruit weight (Meena *et al.*, 2015). The characters with high heritability indicated that these traits are least influenced by the environmental effects, the selection for improvement of these traits may not be useful because broad sense heritability is

Table 2: PCV, GCV, heritability, and genetic advance estimates for fruit yield and other characters in tomato genotypes

Sr. No.	Characters	PCV (%)	GCV (%)	Heritability (%)	GA
1	Days to 50 per cent flowering	12.926 (M)	12.067(M)	87.150(H)	23.206(M)
2	Days to first harvest	5.110 (L)	4.265(L)	69.640(H)	7.331(L)
3	Average fruit weight (g)	30.558 (H)	29.13(H)	90.877(H)	57.206(H)
4	Fruit shape index	14.715(M)	12.64(M)	73.778(H)	22.365(M)
5	Pericarpthickness (mm)	17.446(M)	14.682(M)	70.822(H)	25.453(M)
6	Locules / fruit	28.549(H)	25.050(H)	76.985(H)	45.276(H)
7	Plant height (cm)	16.589(M)	10.915(M)	43.291(M)	14.794(M)
8	Duration of fruit harvest (days)	19.271(M)	14.408(M)	55.897(M)	22.190(M)
9	Total fruits / plant	21.850(H)	17.322(M)	62.847(H)	28.289(M)
10	Marketable fruits / plant	34.994(H)	34.050(H)	94.68(H)	68.252(H)
11	Gross yield / plant (kg)	34.094(H)	30.553(H)	80.305(H)	56.402(H)
12	Marketable yield / plant (kg)	53.953(H)	52.598(H)	95.043(H)	45.633(H)
13	Total soluble solids (°Brix)	22.030(H)	16.079(M)	53.275(M)	24.177(M)
14	Titrable acidity (%)	73.666(H)	71.219(H)	93.467(H)	48.838(H)
15	Lycopene content (mg/100g)	80.581(H)	80.148(H)	98.927(H)	50.217(H)
16	Ascorbic acid (mg/100g)	23.641(H)	20.919(H)	78.295(H)	38.130(H)

PCV range = {Low level (L) : < 10%, Moderate level (M): 10-20%, High level (H) :>20%}
GCV range= {Low level (L) : <10%, Moderate level (M): 10-20%, High level (H) :>20%}
Heritability = - {Low level (L) : <30%, Moderate level M): 30-60%, High level (H): >60%}
GA = - {Low level (L) :< 10%, Moderate level (M): 10-30%, High level (H) :>30%}

based on total genetic variance which includes both fixable (additive) and non-fixable variance (dominance and epistasis). (Rai *et al.*, 2016) found high genetic advance for traits viz. average fruit weight, gross yield per plant, and locules per fruit. Correlation studies are useful for genetic breeding because they allow researchers to identify and quantify the proportion of phenotypic correlation that is due to genetic causes, confirm whether selection for one trait influences another, quantify indirect gains due to selection on correlated traits, and assess the traits complexity (Tiwari and Upadhyay, 2011). Correlation between various tomato traits at the phenotypic (P) and genotypic (G) levels was estimated (Table-3). Genotypic correlations were higher as compare to phenotypic exhibiting high degrees of genetic association among traits under consideration. Marketable yield / plant was found significantly and positively correlated with average weight of fruit, gross yield/plant, marketable fruits/ plant, thickness of pericarp, ascorbic acid content, titrable acidity, and number of locules / fruit at both phenotypic as well as genotypic levels which illustrated that marketable yield could be increased through selection in the component characters like average weight of fruit, gross yield / plant, marketable fruits / plant, thickness of pericarp, ascorbic acid content, titrable acidity, and number of locules / fruit. The present results are comparable with Chadha and Walia, 2016. Direct and indirect effects of different tomato characters on marketable yield were estimated(Table-4). At the phenotypic level, analysis of path coefficient showed number of marketable fruits / plant withhighestpositive direct impact on marketable yield / plant, afterwards average weight of fruit, gross yield per plant, pericarp thickness, titrable acidity, days to 50% blooming, and number of locules / fruit. On the other hand, TSS had the maximum negative direct effect which suggested that selection on the basis of this trait might lead to the loss in terms of fruit yield. The highest positive direct impact on marketable yield / plant at the genotypic level was of total yield / plant, marketable fruits / plant, average weight of fruit, titrable acidity, harvest duration, days to first harvest, fruit shape index and days to 50% blooming. Since yield is a polygenic and complex attribute that is influenced by many other factors, direct selection based solely on the association pattern between two variables may occasionally lead the breeder misled; as a result, it is split into direct and indirect effects

Table 4: Direct and indirect effects of component characters on marketable yield of tomato at both phenotypic as well as genotypic levels

Traits		Days to 50 % flowering	Days to first harvest	Average fruit weight (g)	Fruit shape index	Pericarp thickness (mm)	Locules per fruit	Plant height (cm)	Duration of fruit harvest (days)	Total fruits/ plant	Marketable fruits / plant	Gross yield/ plant (kg)	Total soluble solids (°Brix)	Titrable acidity (%)	Lycopene content (mg/100g)	Ascorbic acid (mg/100g)	Correlation with Marketable yield / plant
Days to 50% flowering	P	**0.028**	-0.009	-0.002	0.000	-0.006	-0.002	0.001	0.000	0.007	0.067	0.006	-0.002	-0.006	-0.011	-0.001	0.070
	G	**0.013**	0.043	-0.006	0.007	0.010	0.001	-0.041	-0.001	0.023	0.089	-0.015	-0.006	-0.015	-0.024	-0.003	0.076
Days to first harvest	P	0.018	**-0.013**	0.007	0.000	0.001	0.002	0.000	0.000	-0.006	0.098	0.020	-0.001	0.001	-0.006	0.000	0.249
	G	0.014	**0.040**	-0.016	0.001	-0.007	-0.003	-0.029	-0.006	-0.056	0.124	0.073	-0.005	0.001	-0.019	-0.006	0.303*
Average fruit weight (g)	P	0.000	0.000	**0.382**	0.000	0.031	0.005	0.000	0.001	0.036	0.111	0.222	-0.001	0.015	-0.002	-0.006	0.792**
	G	0.000	-0.003	**0.182**	-0.002	-0.047	-0.005	-0.008	-0.024	0.078	0.122	0.524	-0.004	0.041	-0.005	-0.053	0.795**
Fruit shape index	P	0.005	0.001	-0.025	**-0.001**	-0.019	0.001	0.000	0.000	0.027	-0.196	-0.033	0.002	-0.021	0.007	0.001	-0.249
	G	0.002	0.001	-0.010	**0.039**	0.031	-0.001	-0.029	-0.008	0.083	-0.227	-0.110	0.007	-0.060	0.018	0.005	-0.257*
Pericarp thickness (mm)	P	-0.003	0.000	0.209	0.000	**0.056**	0.007	0.000	0.000	-0.025	0.267	0.172	0.001	0.012	-0.001	-0.002	0.693**
	G	-0.002	0.004	0.121	-0.017	**-0.070**	-0.006	0.019	-0.006	-0.058	0.294	0.517	0.002	0.034	-0.002	-0.025	0.806**
Locules per fruit	P	-0.002	-0.001	0.071	0.000	0.014	**0.026**	0.000	0.000	-0.032	0.106	0.098	0.000	0.008	-0.005	-0.001	0.284*
	G	-0.001	0.005	0.044	0.001	-0.021	**-0.020**	0.012	-0.012	-0.083	0.126	0.289	-0.001	0.025	-0.011	-0.008	0.345**
Plant height (cm)	P	0.007	-0.001	0.037	0.000	-0.005	-0.004	**0.002**	0.000	0.050	0.051	-0.025	-0.001	-0.005	0.001	0.002	0.108
	G	0.008	0.016	0.020	0.016	0.019	0.004	**-0.070**	0.014	0.142	0.012	-0.074	-0.005	-0.018	0.002	0.002	0.089
Duration of fruit harvest (days)	P	-0.002	0.001	-0.138	0.000	0.004	-0.005	0.000	**-0.001**	-0.024	0.128	-0.072	0.002	-0.005	-0.006	0.004	-0.116
	G	0.000	-0.005	-0.081	-0.006	0.007	0.004	-0.019	**0.053**	-0.048	0.127	-0.191	0.004	-0.021	-0.017	0.033	-0.159
Total fruits / plant	P	-0.001	0.000	-0.078	0.000	0.008	0.005	-0.001	0.000	**-0.174**	0.213	0.109	0.001	-0.005	-0.001	0.001	0.076
	G	-0.001	0.008	-0.048	-0.011	-0.014	-0.006	0.034	0.009	**-0.291**	0.285	0.155	-0.002	-0.016	-0.001	0.013	0.113
Marketable fruits /plant	P	0.003	-0.002	0.072	0.000	0.026	0.005	0.000	0.000	-0.063	**0.589**	0.101	-0.003	0.003	0.005	0.000	0.734**
	G	0.002	0.008	0.036	-0.015	-0.034	-0.004	-0.001	0.011	-0.137	**0.608**	0.267	-0.008	0.006	0.010	-0.007	0.742**
Gross yield / plant (kg)	P	0.001	-0.001	0.304	0.000	0.035	0.009	0.000	0.000	-0.068	0.215	**0.278**	-0.001	0.011	-0.004	-0.005	0.774**
	G	0.000	0.005	0.154	-0.007	-0.059	-0.009	0.008	-0.016	-0.073	0.263	**0.617**	-0.004	0.032	-0.009	-0.048	0.853**
Total soluble solids(°Brix)	P	-0.007	0.001	-0.060	0.000	0.005	0.000	0.000	0.000	-0.013	-0.214	-0.021	**0.009**	-0.010	0.007	0.003	-0.299*
	G	-0.005	-0.011	-0.047	0.017	-0.007	0.001	0.022	0.012	0.030	-0.312	-0.168	**0.016**	-0.033	0.021	0.036	-0.428**
Titrable acidity (%)	P	-0.005	-0.001	0.160	0.000	0.019	0.006	0.000	0.000	0.024	0.042	0.083	-0.002	**0.036**	-0.012	-0.004	0.347**
	G	-0.002	0.000	0.082	-0.026	-0.027	-0.006	0.014	-0.012	0.052	0.042	0.216	-0.006	**0.091**	-0.025	-0.031	0.364**
Lycopene content (mg/100g)	P	0.008	-0.002	0.020	0.000	0.002	0.003	0.000	0.000	-0.003	-0.070	0.028	-0.002	0.011	**-0.039**	0.000	-0.045
	G	0.004	0.010	0.011	-0.009	-0.002	-0.003	0.002	0.011	-0.002	-0.074	0.071	-0.004	0.028	**-0.080**	-0.003	-0.040
Ascorbic acid content (mg/100g)	P	0.001	0.000	0.204	0.000	0.012	0.002	0.000	0.001	0.013	0.020	0.132	-0.003	0.012	-0.002	**-0.011**	0.381**
	G	0.000	0.003	0.116	-0.003	-0.021	-0.002	0.002	-0.021	0.045	0.050	0.357	-0.007	0.035	-0.003	**-0.083**	0.469**

*** indicates significance at $P \leq 0.05$** **** indicates significance at $P \leq 0.01$** **Residual effect (P): 0.00868; (G): 0.00343**

for successful selection. It turned out that traits with a direct impact on marketable yield per plant were a major factor in yield. Therefore, should be regarded as a crucial selection criterion for increasing tomato yield.

Conclusion

For all characters except plant survival, analysis of variance exhibited significant differences across genotypes. The presence of high PCV and GCV estimates indicated that the provided genetic stock had a lot of variability for that trait. This information will aid in the development of an effective breeding programme. The majority of the characters made contribution towards marketable yield / plant via gross yield / plant, marketable fruits / plant and average weight of fruit as per path coefficient analysis. All of these characters are positively correlated with marketable yield / plant. As a result, these characters must be considered in order to increase marketable yield / plant. The residual effect (0.00343) in this investigation was extremely small, implying that all of the primary yield components had been taken into account.

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Conflict of interest

The authors declare that they have no conflict of interest.

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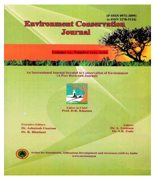

Morpho-physiological and biochemical attributes as tools to screen tolerance and susceptible rice cultivars for drought stress

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ABSTRACT

Present research work was aimed to observe possible changes in the metabolism of rice plants (*Oryza sativa* L.) through drought stress. Rice belongs to the family *Poaceae*. It is considered as a vital food crop across all the major countries worldwide. Rice is prone to be affected by drought stress. Therefore, developing the drought tolerant cultivars of cereal crops assumed considerable importance. This work was carried out with an objective to study the Screening of rice cultivars against water stress and compare biochemical characteristic among different drought tolerant and sensitive rice cultivars. A set of 25 cultivars of rice were screened against drought stress at vegetative stage through various morpho-physiological characters such as moisture, relative water content (RWC), membrane stability index(MSI), membrane injury(MI), seedling length and seedling weight. The RWC is a best criterion for plant water status. The osmotic adjustment is a influential mechanism of conserving cellular hydration under water stress and RWC expression also affects osmotic adjustment in this respect. Thus, it can be considered that the higher RWC having cv. GAR-13 and NWGR-16026 were tolerant and lower RWC having cv. NWGR-16009 and NWGR-16019 were susceptible. Hence, cv. GAR-13 & NWGR-16026 was used as tolerant and NWGR-16009 & NWGR-16019 were used as susceptible. On the basis of first experiment total four cultivars (Two tolerant NWGR-16026 & GAR-13, two susceptible NWGR-16009 & NWGR-16019) were selected for various biochemical analysis. The results indicated that total soluble sugars (TSS), glycine betaine and ascorbic acid content were found significantly higher in cultivar NWGR-16026. The proline content was found significantly higher in cultivar GAR-13. So, RWC and some biochemical parameters are best indicators for selection regarded as potentially useful for drought tolerant rice cultivars and targets for development through transgenic approaches.

Introduction

Rice (*Oryza sativa* L.) belongs to the family *Poaceae*. The basic chromosome number of rice is n=12. Rice is considered as a main food crop across worldwide. As a food crop, it forms the staple food and which is more than three billion people accounting for about 50-80% of their daily calorie intake (Khush, 2005). In India rice production was 116.48 million tons in area of 43.80 million ha (United States Department of Agriculture, 2018-19). It yields about one third of the total carbohydrates source. The usage of the crop diverges widely ranging from its use as food in cereals, snacks, brewed beverages, flour, rice bran oil to its use in religious occasions across India. It is the second most important crop in the world after wheat, covering almost 90% of area across Asia alone.Water deficiency is one of the major environmental constraints of plant growth and crop

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productivity (Chaves and Oliveira, 2004). The crop is subjected to soil moisture scarcities of varying degree and duration, which occasionally outcome in substantial loss of yield. Plant water status is disturbed due to water stress, resulting in disruption of important metabolic processes. A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Farooq *et al*., 2009). Drought stress leads to biochemical response such as transient decrease in photochemical efficiency, accumulation of stress metabolites (Reddy *et al*., 2004). Drought increases the formation of reactive oxygen species (ROS) resulting in lipid peroxidation, protein denaturation (Hansen *et al*., 2006).The effect of drought reduces in growth rates and nutritional quality of crops (Mahajan and Tuteja, 2005, Verslues *et al*., 2006). Therefore, to develop the drought tolerant varieties of cereal crops assumed considerable importance.The present work aims to govern the effect of water stress on various morpho-physiological parameters like moisture, RWC, membrane injury, MSI, seedling length and seedling dry weight. Some biochemical parameters like proline, glycine betaine, TSS and ascorbic acid.The comparison of these responses will be beneficial in identifying the similarities and the differences associated to the relative ability of the rice seedlings to cope with diverse pattern of responses to drought stress and useful as potential targets for upgrading of rice cultivars by conventional and transgenic approaches.

Material and Methods

There were total 25 cultivars of rice procured from Main Rice Research Station, Nawagam, Anand Agricultural University, Nawagam for investigation. All twenty-five rice cultivar's seed grown in pot. Then, after twenty-five days of germination, given treatment of water stress by five days holding of water in all rice cultivars. Then, taken seedlings of all cultivars and measured all morpho-physiological parameters. For biochemical attributes, from all twenty-five rice cultivar's we selected total four cultivars (Two drought tolerance and two drought sensitive) on the basis of morpho-physiological parameters total four cultivars seed grown in pot. Then, after 25 days of germination taken seedlings of all cultivars and measured all biochemical attributes. The experiment was conducted under completely randomized block design

Morpho-physiological parameters

Moisture

Moisture content of rice seedlings at vegetative stage were estimated as per procedure developed by A.O.A.C. (2000).

Relative water content (RWC)

The method described by Jayaweera *et al.* (2016) was used for the determination of relative water content of rice cultivars under control as well as water deficit stress conditions.

Membrane stability index (MSI)

Membrane stability index (MSI) was determined by using the method of Al-Ashkar *et al*. (2016).

Membrane injury (MI)

Membrane injury (MI) was determined by using the method of Atarzyna *et al.* (2010) with some modification in Caraway (*Carum Carvi* L.) Genotype in water deficit conditions.

Seedling length (cm)

Five randomly selected seedlings were taken from each growing treatment to calculate the length of seedling. It was measured with a measuring scale and stated in centimeters.

Seedling dry weight (mg)

Five randomly selected seedlings were occupied from each growing treatment to calculate the weight of shoot. Weight of seedling were measured by three-digit balance and expressed in milligram.

Biochemical attributes

Osmolytes

Total soluble sugars were determined using the phenol-sulphuric acid method as described by Mostajeran and Rahimi-Eichi (2009) with some modification.

Proline

The proline content in the rice leaves samples were analyzed at vegetative stage by the method suggested by Mostajeran and Rahimi-Eichi (2009).

Glycine betaine
Glycine-betaine extraction and estimation was done in dried leaf powder of rice as per the method of Khattab *et al*. (2014).

Ascorbic acid
Ascorbic acid content was estimated from rice leaves by 2-4 dinitrophenylhydrazine (DNPH) method as described by Anthon and Barrett (2003).

Statistical analysis
The mean values were taken from measurements of total four replications and standard error (SE) of the means was calculated. Data obtained by biochemical constituents and enzymes determination were subjected to simple completely randomized design for study in the significance of various data using "F" test.

Results and Discussion
Morpho-Physiological parameters
Moisture
The moisture content is a supreme element in determining the distribution of species and the responses and adaptation of species to drought stress are severe for their success in any environmental niche and for their usage and productivity in agricultural systems.The moisture content of 25 cultivars of rice leaves were analyzed at vegetative stage, the data us presented in Table 1. The moisture content was found significantly varied among the rice cultivars and it was varied from 50.90 % to 77.86 % (Table 1). However, significantly the highest moisture content was recorded for NWGR-12015 (77.86%). The non-significant moisture difference was recorded among NWGR-16022 (72.27 %), NWGR-16026 (71.63 %), GAR-13 (68.87 %) and NWGR-14071 (68.78 %). Significantly lower moisture content was recorded for cultivar NWGR-12002 (50.90%) which was significantly at par with NWGR-16003 (54.62%), GR-6 (54.99 %) and GR-7 (55.21 %). Kumari *et al.* (2019) studied the physiological, biochemical and molecular screening of ten rice (*Oryza sativa* L.) genotypes. They observed that the moisture content between 43.7 to 49.9 among all the rice genotypes. With increasing the drought condition moisture content was also decreasing. Similar trend was also recorded by various researchers (Aly and Latif, 2011; Noorka and Silva, 2012) in wheat. The present experimental data of moisture content indicated that the cv. NWGR-12015, NWGR-16022, NWGR-16026, GAR-13 and NWGR-14071 are considered as tolerant and cv. NWGR-12002, NWGR-16003, GR-6 and GR-7 are considered as susceptible as compared to other cultivars.

Relative water content(RWC)
The osmotic adjustment is a commanding mechanism of preserving cellular hydration under water deficit stress. The RWC is a relevant tool for screening drought tolerance,it expresses the effect of osmotic adjustment in the drought condition.
The relative water content of 25 cultivars of rice leaves were analyzed at vegetative stage and the data are showed in Table 1.The relative water content was found knowingly differed among the rice cultivars and it was varied between 25.07 % to 58.06 %. However, significantly higher RWC was found in cultivar GAR-13 (58.06%), which was at par with cultivar NWGR-16026 (56.30%). Significantly lower RWC was recorded for cultivar NWGR-16019 (25.07%). The non-significant difference was recorded among NWGR-16009 (30.88 %), NWGR-16003 (32.24 %) and NWGR-15017 (32.46 %).The RWC is considered as the best integrated measurement of plant water status, and it represents the variations in water potential, turgor potential, and the osmotic adjustment (OA) of the plant (Bhushan *et al*., 2007) though RWC vary due to differences in OA. Previously reported studies on different crop species like rice, wheat and foxtail millet have also shown wide genotypic variations in RWC under water stressed conditions (Garg *et al.* 2012, Jayaweera *et al.* 2016). This study showed highly tolerant cultivars have the ability to maintain high RWC. The susceptible cultivar may not maintain higher RWC. Thus it can be concluded that the cv. GAR-13 and NWGR-16026 are considered as tolerant and cultivar NWGR-16019 and NWGR-16009 are considered as susceptible as compared to all other cultivars in water deficit stress condition.

Membrane injury and Membrane stability index
Membrane stability index (MSI) is a physiological index widely used for the estimate of drought tolerance and it is the finest indicator for screening different varieties for water deficit stress. The higher

Table 1: Effect of Water stress on mopho-physiological parameters

Sr. No.	Cultivars	Moisture %	RWC %	MI %	MSI	Seedling length (cm)	Seedling dry wt. (gm)
1	**NWGR-9081**	60.60	34.54	19.74	0.80	17.50	0.020
2	**NWGR-12002**	50.90	37.07	9.26	0.91	17.40	0.017
3	**NWGR-12015**	77.86	35.03	9.76	0.90	15.20	0.021
4	**NWGR-14071**	68.78	47.17	64.35	0.36	20.53	0.062
5	**NWGR-15017**	62.57	32.46	47.73	0.52	17.43	0.018
6	**NWGR-15024**	66.36	39.66	54.31	0.46	17.93	0.035
7	**NWGR-15038**	64.97	49.60	72.83	0.27	22.93	0.041
8	**NWGR-16003**	54.62	32.24	14.69	0.85	16.93	0.018
9	**NWGR-16009**	62.47	30.88	26.23	0.74	12.30	0.010
10	**NWGR-16019**	56.50	25.07	72.93	0.27	17.10	0.025
11	**NWGR-16022**	72.27	44.85	41.77	0.58	14.20	0.027
12	**NWGR-16026**	71.63	56.30	3.66	0.96	17.77	0.043
13	**NWGR-16031**	56.75	34.63	52.05	0.48	20.03	0.039
14	**NWGR-16033**	64.45	38.58	62.55	0.37	18.57	0.052
15	**Gurjari**	62.52	34.66	77.78	0.22	20.40	0.042
16	**Mahisagar**	55.87	36.72	13.45	0.87	15.83	0.029
17	**GAR-1**	64.42	55.20	39.58	0.60	18.27	0.031
18	**GAR-13**	68.87	58.06	40.66	0.59	17.44	0.041
19	**GR-4**	58.82	40.44	27.92	0.72	14.47	0.015
20	**GR-5**	64.86	39.15	18.34	0.82	23.77	0.066
21	**GR-6**	54.99	51.25	15.27	0.85	19.63	0.059
22	**GR-7**	55.21	33.71	38.61	0.61	17.27	0.030
23	**AAUDR-1**	65.08	38.54	48.05	0.52	28.97	0.090
24	**Dandi**	56.38	54.93	35.78	0.64	16.57	0.032
25	**SLR-51214**	60.33	39.00	26.55	0.73	19.00	0.045
	S.Em. ±	**1.69**	**0.91**	**1.56**	**0.02**	**0.68**	**0.001**
	C.D.@ 5%	**4.80**	**2.60**	**4.44**	**0.04**	**1.92**	**0.003**
	C.V. %	**4.70**	**3.88**	**7.24**	**4.31**	**6.39**	**5.503**

and lower values of MI indicate that the susceptible and tolerant condition of cultivar. The membrane injury was analyzed for leaves of rice cultivars and the data are presented in Table 1. Significantly the highest and the lowest membrane injury were recorded for cultivars Gurjari (77.78 %) and NWGR-16026 (3.66 %), respectively. The minimum MI was recorded for NWGR 16026 (3.66 %) which, was followed by NWGR 12002 (9.26 %). NWGR 12015 (9.76 %) and Mahisagar (13.45 %).While, the non-significant difference was observed between cv. NWGR-16019 (72.93 %) & NWGR-15038 (72.83 %) and NWGR 12002 (9.26 %), NWGR 12015 (9.76 %).The result of membrane stability index indicated that MSI was varied from 0.22 to 0.96 of 25 rice cultivars (Table 1). Significantly maximum and minimum MSI was recorded for NWGR-16026 (0.96) and Gurjari (0.22), respectively. Whereas, the cv.GR-7(0.61), GAR-1 (0.60), GAR-13 (0.59) and NWGR-16022 (0.58) were recorded significantly at par with each other. Our results are supported by Al-Ashkar *et al*. (2016). They observed that MSI reduced with increasing water stress and there were significant differences among the cultivars. The tolerant cultivarshave higher membrane stability as compared to susceptible cultivars of wheat crop (Hasheminasab *et al*., 2012).Thus from the above results it can be determined that the cv. NWGR-16026 and Gurjari are considered as a tolerant and susceptible respectively, as compared to all other cultivars in water deficit stress condition.

Seedling length and seedling dry weight

Seedling length was found significantly differed among the rice cultivars (Table 1). However, the seedling length of 25 cultivars of rice was recorded between 12.30 to 28.97 cm. significantly the highest seedling length was recorded for AAUDR-1 (28.97 cm). The lower seedling length was recorded for cultivar NWGR-16009 (12.30 cm) which was at par with NWGR-16022 (14.20 cm).Seedling length was also affected by the water stress treatment and it is a one type of indicator which was use for the screening of various types of cultivars. Our results are concurrence with Zain and his coworkers (2014). They have concluded that plant height reduced with increased duration of water stress cycle in rice. Sokoto and Muhammad (2014) have recorded the significant difference of plant height at 6, 9, 12 and 15 weeks after planting at tillering stage in rice. Seedling dry weight was found significantly differed among the rice cultivars (Table 1). However, significantly the highest seedling dry weight was recorded in cultivar AAUDR-1 (0.09 gm). None of cultivars at par with AAUDR-1. Significantly lower seedling dry weight was recorded for cultivars NWGR-16009 (0.01 gm). Seedling dry weight is affected under the water deficit stress condition, which is a one type of indicator which is used for the screening of various types of cultivars. Our results are accordance with Surapornpiboon *et al.* (2008). Among all morpho-physiological characters such as moisture, relative water content, membrane injury, membrane stability index, seedling length and seedling dry weight, the relative water content is a best criterion for plant water status. Then osmotic adjustment is a powerful mechanism of preserving cellular water under drought stress and relative water content expresses also the effect of osmotic adjustment in this respect. Hence, RWC is an suitable estimation of plant water status in term of cellular hydration under the promising effect of both leaf water potential and osmotic adjustment. The GAR-13 and NWGR-16026 were registered with higher RWC, while NWGR-16009 and NWGR-16019 were registered with lower RWC. Hence, cv. GAR-13 and NWGR-16026 are used as tolerant and NWGR-16009 and NWGR-16019 are used as susceptible for further experiment.

Comparison of drought tolerant and drought susceptible cultivars in response tobiochemical attributes at vegetative stage

Moisture

Moisture is important to make photosynthesis possible. In the case of rice, higher moisture content around the plant is more important. If the plant has less amount of moisture the stomata will close with the result that photosynthesis process can be inhibited.The maximum and minimum moisture content was recordedinNWGR-16026 (78.02%) and NWGR-16019 (70.88%), respectively. However, the non-significant difference was recorded between NWGR-16026 and GAR-13 as well as between NWGR-16019 and NWGR-16009 (Fig.1).

Our results are in agreement with the results of Kumari *et al.* (2019). They have analyzed ten rice cultivars and found that moisture content was varied between 43.70 to 49.90 %. Thus on the basis of moisture content it was concluded that the cv. NWGR-16026 is considered as a tolerant and cv. NWGR-16009 and NWGR-16019 both are considered susceptible.

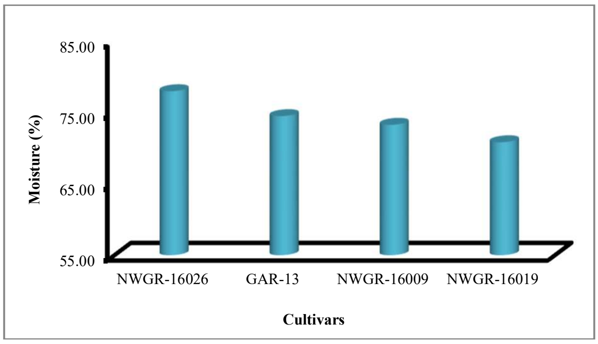

Figure 1: Effect of water stress on moisture content in rice cultivars

Osmolytes

The osmoregulation is one of the core mechanisms preserving turgor pressure in most plants against water loss from plant so, it causes plant to continue water absorption and preserve metabolic activities. Then osmotic adjustment is a powerful mechanism of conserving cellular hydration under drought stress.

Total soluble sugars

Mostly all soluble sugars play a dual role with respect to ROS, either promoting ROS production or contributing indirectly in ROS scavenging mechanisms. Under water stress, carbohydrate concentrations tended to rise as the level of stress increased, though the rise was greater in drought resistant than in the drought susceptible cultivars. The transformations in sugar concentrations between tolerant and susceptible genotypes were significant only for severe osmotic stress treatment. The total soluble sugars were significantly differed among all rice cultivars. However, it was recorded significantly higher and lower in cv. NWGR-16026 (1.52 %) and NWGR-16019 (1.24 %), respectively in Fig. 2(A).

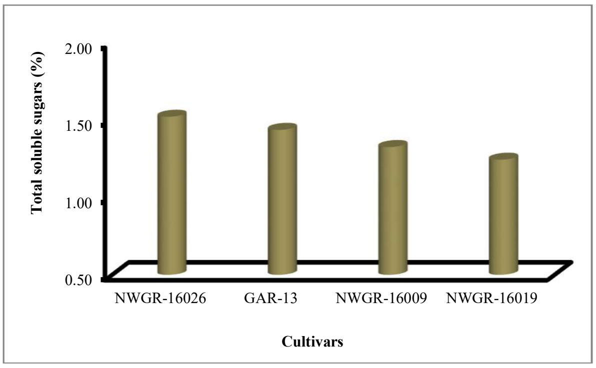

Figure 2(A): Effect of water stress on total soluble sugars in rice cultivars

Mostajeran and Rahimi-Eichi (2009) recorded that Zayandeh-Rood cultivar had significantly higher TSS content as compared to other cultivars in control condition. On the basis of their experiment they have concluded that tolerant cultivar has higher TSS content in control condition. Thus the present experimental data suggest that the cv. NWGR-16026 is considered as a tolerant and cv. NWGR-16019 is considered as a susceptible.

Proline

Proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions. Rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages. The proline content was varied between 32.59 to 49.69 mg/100g. However, the maximum and minimum proline accumulation was found in cv. GAR-13 (49.69 mg/100g) and NWGR-16009 (32.59 mg/100g), respectively in Fig. 2(B). Significantly NWGR-16026 &NWGR-16019 was found at par with each other. Various scientist has (Mostajeran and Rahimi-Eichi, 2009 and Shamsul *et al*., 2012) suggested that proline plays three major roles during drought stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule. Al-Ashkar *et al*. (2016) studied six different rice genotypes and found that proline

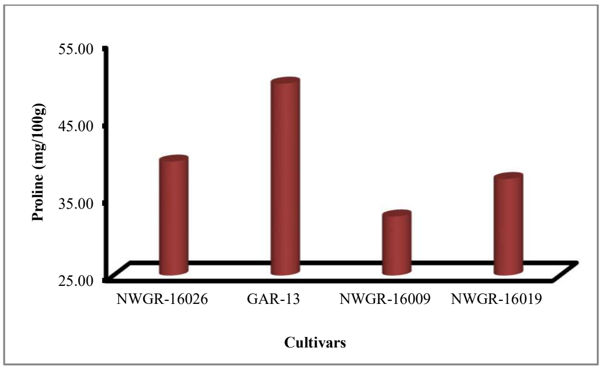

Figure 2(B): Effect of water stress on proline content in rice cultivars

content was significantly higher in IRAT 170 compared to other rice genotypes. Thus the result indicated that GAR-13 is considered as a tolerant and cultivar NWGR-16009 is considered as a susceptible.

Glycine betaine

Glycine betaine is rich mainly in chloroplast where it plays a vital role in adjustment and guard of thylakoid membrane, thereby conserving photosynthetic efficiency. In higher plants, glycine betaine is produced in chloroplast from serine via ethanolamine, choline and betaine aldehyde.The data presented in Fig. 2(C) recorded that significantly the highest glycine betaine content was found in cv. NWGR-16026 (289.54 mg/100g).

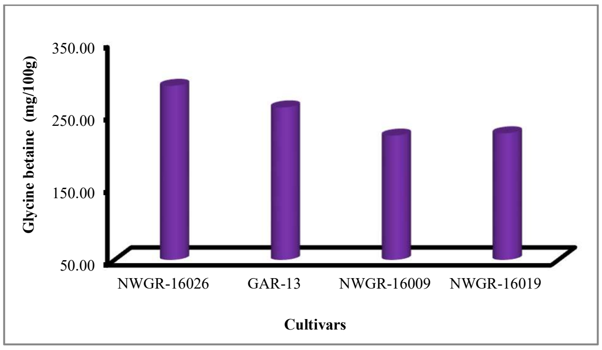

Figure 2(C): Effect of water stress on glycine betaine content in rice cultivars

Glycine betaine content was recorded lower in cv. NWGR-16009 (221.25 mg/100g) which was at par with NWGR-16019 (224.41 mg/100g). Khattab and his co-workers (2014) observed that variety Giza 177 has higher glycine betaine content compared to variety IET 1444 in control condition. These results are also agreement with Kumari and Sairam (2013) in Foxtail millet. They observed that glycine betaine content was significantly higher in variety PBD-343 followed by HD-2987 and C-306 in control condition.

Ascorbic acid

The ascorbic acid plays very crucial role as antioxidant in plant system. It is influences many enzyme activities and reduces the damage caused by oxidative process. The ascorbic acid content was found higher in cultivar NWGR-16026 (54.64 mg/100g) which was at par with GAR-13 (53.97 mg/100g) in Fig. 2(D).

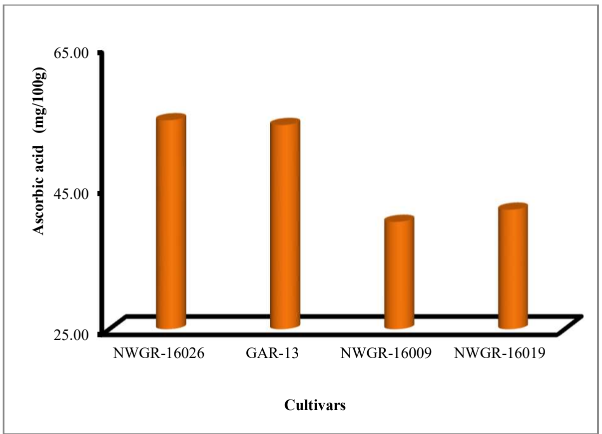

Figure 2(D): Effect of water stress on ascorbic acid content in rice cultivars.

Non-significant and minimum ascorbic acid content was recorded in cultivar NWGR-16009 (40.27 mg/100g) which was at par with NWGR-16019 (41.99 mg/100g). The results are in good concurrence with Hameed and his coworkers (2013). They have recorded that the tolerant variety FD-83 (275 µg/g) had meaningfully higher ascorbic acid as compared to sensitive variety Nesser (205 µg/g) in wheat under control condition. This higher accumulation of ascorbic acid by tolerant genotype may be in part responsible for its better performance under drought stress. Thus on the basis of ascorbic acid content it was concluded that the cultivars NWGR-16026 and GAR-13 both are considered as tolerant cultivar and NWGR-16009 and NWGR-16019 both are considered as susceptible cultivar.

Conclusion

From the morpho-physiological characters the relative water content was found to be the best indicator to identify the tolerant or susceptible characteristics of crop. Cultivars NWGR-16026 and GAR-13 were found tolerant and NWGR-16009 and NWGR-16019 as susceptible. The NWGR-16026 having higher accumulation of all biochemical characters except proline. The maximum proline accumulation occurs in GAR-13. So, on the basis of all biochemical attributes we can also identify the tolerant or susceptible cultivars. These morpho-physiological and biochemical characters are very use full for the further studies to identify the tolerant or susceptible cultivars.

Conflict of interest

The authors declare that they have no conflict of interest.

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Performance of Quinoa (*Chenopodium quinoa* Willd.) under varied sowing windows and planting patterns

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ABSTRACT

The experiment on "Performance of quinoa (*Chenopodium quinoa* Willd.) under varied sowing windows and planting patterns" was carried out at Main Research Station, Hebbal, UAS, GKVK, Bengaluru, during *Kharif*-2019. The experiment constituted four sowing dates (D_1: July second fortnight, D_2: August first fortnight, D_3: August second fortnight and D_4: September first fortnight) and four planting patterns (S_1: 30 × 15 cm, S_2: 45 × 15 cm, S_3: 60 × 15 cm and S_4: 75 × 15 cm) and replicated thrice was laid out in split-plot design. The results revealed that, increase in AGR and CGR with advancement of age of quinoa and was peak at 60 DAS and showed decreasing trend towards harvest. Sowing during July second fortnight showed significantly, higher AGR and CGR between 30-60 DAS (0.367 and 5.11, respectively) and grain and stover yield (2051 and 2439 kg/ha, respectively) as compared to other sowing windows. Similarly, between 60 DAS-harvest, Absolute Growth Rate and Crop Growth Rate (0.195 and 2.65, respectively) were significantly higher under July second fortnight sowing window, yet was found to be on par with sowing on August first fortnight and August second fortnight. In contrary, September first fortnight sown crop reached days to 50 per cent flowering (43.90) and days to maturity (97.36) early, which was significantly lower compared to other sowing windows and found on par with August first fortnight sown crop (41.16 and 95.53, respectively). Among the varied planting patterns, 45 × 15 cm spacing was found to be optimum and recorded significantly higher grain and stover yield (1941 and 2346 kg/ha, respectively) as compared to other spacings.

Introduction

Food security of world in coming days relies on the sustained accomplishment of cereals production in Asia. Since 2002, production of cereals has shown a steady increase (Anon., 2019), the road to food security faces major hurdles *viz*., with increasing demand versus declining yield and area harvested; soil fertility and decline in productivity of intensive cereal-based cropping systems (Bell *et al.,* 2019); exhaustion and or limitations of natural resources for production; stabilization of yield potential of recently released varieties/hybrids; biotic stresses, abiotic stresses (low temperature, drought and salinity); low income from major cereal crops production and changing socio-economic situations (Van and Ferrero, 2006). Quinoa (*Chenopodium quinoa* Willd.), an herbaceous annual plant belongs to *Amaranthaceae* family. Quinoa cultivation is one of the main livelihoods of Andean farmers in South America where it is known to be cultivated since ages, but no longer restricted to them as it spread to

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different parts of the world *viz*., Bolivia, Chile, Ecuador and Peru (Jaikishun *et al.,* 2019). In recent years, North America and Europe have taken up quinoa farming in sizeable area. India has recently joined the list of countries cultivating quinoa. The quinoa life cycle is approximately 6 months, but it varies depending on the region, which determines the sowing and harvest months (Sajjad *et al.,* 2014). Being a quantitatively short-day species, quinoa has wider adaptability to varied climatic conditions (Miguel *et al.,* 2020). It can be grown well at the altitude of about 3,900 m from mean sea level, soil p^H ranging from 6 to 8.5 and temperature varying from humid to sub-tropical and tropical areas. It is a hardy plant and can thrive well under moisture stress conditions of marginal soils as well. However, the most suited soil for quinoa farming is sandy loam. September to May is the optimum period for quinoa in the Andean region *i.e*., during the austral spring–summer time, with mean temperatures between 10 to 25 ^{0}C. However, the most adequate range of mean temperature for its growth is 15-20 ^{0}C (Garcia *et al.,* 2019).Quinoa required almost 70 to 200 days to complete its entire growth period and maturity of some entries is location specific. The results reported from the experiment conducted for evaluation of quinoa entries in America, Europe and Africa were – it is observed that growing period of quinoa in Kenya was 65-98 days with 100 per cent cultivars maturity with seed yield of 4000 kg/ha. Whereas, it is varied between 70 to 200 days and some entries did not mature in some locations. But it is 120 to 160 days in countries like Denmark and Sweden, the yield observed is also lower with maturity of only few entries of quinoa. The growing period in Greece was 110-160 days and the yield was 2000 kg/ha (Jacobsen, 2017).In India, it grows naturally in Himalayan region where temperature ranges between 0-20 °C. Performance of quinoa varieties varies with the latitude and altitude of a region (Jacobsen, 2017) in terms of important phenological changes with respect to duration of the growth stages needed to complete their life cycle and differing in their canopy morphology and inflorescence levels. In Karnataka, as a part of research programme in All India Co-ordinated Research Network on Potential crops, Bangalore who initiated adoptability studies and evaluation of some quinoa germplasms for semiarid plains region. Although, growth and develo pment are vital in developing continuous information to back-up the agronomical research and breeding program, till today no/limited information is available on how and where to grow quinoa. Hence, there is a need to standardize the optimum sowing time and plant spacing, which could help the farmers to cultivate this crop for higher productivity with economic benefits. The yield is mainly dependent on the growth parameters, with increase in leaf area, photosynthesis will be augmented which in order leads to higher synthesis and partitioning of photosynthates into the economic parts of the crop. Physiological growth indicators depict the crop growth progress at various phenological stages of the plant. Hence, the growth components not only play vital role in plant's development which is a criterion of yield attributes. In this regard various growth indices (*viz.*, Leaf Area Index, Crop Growth Rate, Relative Growth Rate, Net Assimilation Rate and Leaf Area Duration) are often used in evaluating the plant productive capability and environmental efficiency (Anzoua *et al.,* 2010).

Material and Methods

The experiment was carried out at Main Research Station, Hebbal, UAS, GKVK, Bengaluru, during *Kharif*-2019. The experiment constituted four sowing dates (D_1: July second fortnight, D_2: August first fortnight, D_3: August second fortnight and D_4: September first fortnight) and four planting patterns (S_1: 30 × 15 cm, S_2: 45 × 15 cm, S_3: 60 × 15 cm and S_4: 75 × 15 cm) and replicated thrice was laid out in split-plot design. Totally there were sixteen treatments combinations with three replications. This site comes under 5th Agro-climatic zone (i.e, Eastern Dry Zone) of Karnataka at 13° 04' North latitude, 77° 58' East longitude and 904 m above mean sea level. The variety used was EC 507744. The monthly mean temperature during crop growth period was 27.7 °C (maximum) and 16.1 °C (minimum) with an average relative humidity varying between the 58.3-91.4 per cent and rainfall occurred during the crop growth period (July-December) was 786.4 mm. The soil texture was red sandy loam with acidic pH, low in organic carbon (0.25%), low in available N (254.14 kg/ha) and medium in available P_2O_5 (28.32 kg/ha) and K_2O (186.04 kg/ha). The recommended dose of fertilizer supplied was 60:40:40 kg $N:P_2O_5:K_2O$ ha^{-1} through urea, Di-ammonium phosphate (DAP) and Muriate of Potash (MOP), respectively. Complete dose of P, K and half the dose of N was applied at the time of sowing as basal application. 50 per cent of N was top dressed later before inter cultivation at 30 DAS. Protective irrigations were given when there was no rainfall for more than 8-10 days, only two irrigations were given during the month of July and August especially during germination stage to ensure after crop establishment. July 26th, August 9th, August 26th and September 16th sown crop were harvested at 97, 95, 93 and 90 DAS, respectively. Five plants from each net plot were randomly selected to record the data on growth and yield attributes and the mean values were represented in the tabular form. Data recorded was subjected to statistical analysis by following the analysis of variance as suggested by Panse and Sukhatme, (1978). Critical difference was calculated wherever F test was found significant at 5 percent probability level and the values were furnished.

Absolute growth rate (AGR): It is the dry matter produced per plant per unit time. It is expressed in g/day was worked out from the below mentioned formula (Watson, 1952).

$$\mathrm{AGR} = \frac{W_2 - W_1}{t_2 - t_1}$$

Where, AGR = Absolute growth rate expressed (g/day), W_1= Dry weight of hill at time t_1 and W_2= Dry weight of hill at time t_2

Relative growth rate (RGR): It is expressed as the gram of dry weight increased per gram of initial dry matter per unit time and expressed as g/g/day.

$$\mathrm{RGR} = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)}$$

Where, W_1 and W_2- plant dry weigh at time t_1 and t_2, respectively.

Crop growth rate (CGR): Is the amount of dry matter produced per unit land area per unit time and expressed in g/m^2/day (Watson, 1952).

$$\mathrm{CGR} = \left(\frac{1}{P}\right) \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where, W_1 = Dry weight of hill at time t_1, W_2 = Dry weight of hill at time t_2 & P = Land area in cm^2

Net Assimilation Rate (NAR): The rate of increase in dry weight per unit leaf area of the plant over a period of time. It is expressed in g/dm^2/day and worked out from the below formula (Gregory, 1926).

$$\mathrm{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

Where, W_1 and W_2 = total plant dry weight at the time t_1 and t_2, respectively

L_1 and L_2 = leaf area at time t_1 and t_2, respectively

Results and Discussion

Crop Growth Rate (CGR, g/m^2/day)

The crop growth rate of quinoa increased with the increasing age of the crop and it is maximum at 60 DAS, later slightly declined during harvest without considering sowing date and spacing. Sowing during July second fortnight showed significantly higher CGR between 30-60 DAS (5.11 g/m^2/day) and 60 DAS-harvest (2.65 g/m^2/day) which was found on par with August first fortnight and August second fortnight date of sowing during the 60 DAS-harvest. However, sowing during September first fortnight recorded significantly lower CGR at all the crop growth phases of quinoa. This difference in CGR is due to availability of sufficient solar radiation during early sowing date (July second fortnight) that accelerated the rate of photosynthesis and higher translocation of photosynthates to various sinks. Whereas, insufficient solar radiation and scarcity of water observed under later dates of sowing which leads to lower crop growth rate of quinoa at all growth stages as reflected in the varied dry matter per unit area. Christiansen *et al*. (2010), Hirich *et al.* (2014) and Ramesh *et al*. (2017) also reported that sowing during October 15th showed higher CGR between 30-60 DAS (5.4), 60-90 DAS (11.3) and 90 DAS-harvest (7.0) and at par with sowing during first fortnight of November at all crop growth stages of quinoa. Among the varied crop geometry, narrow spacing (30 × 15 cm) produced significantly higher CGR (g/m^2/day) between 30-60 DAS (6.07) and 60 DAS-harvest (3.17). However, lower CGR was recorded with 75 × 15 cm spacing. Higher CGR values can be ascribed to more plants and higher

Table 1: Effect of different sowing windows and varied crop geometry on absolute growth rate (g/day) and crop growth rate (g/m^2/day) of quinoa

Treatments	AGR		CGR	
	30-60 DAS	60- harvest	30-60 DAS	60-harvest
Main: Sowing windows				
D_1: Second fortnight of July (July 26)	0.367	0.195	5.116	2.65
D_2: First fortnight of August (August 09)	0.323	0.192	4.501	2.63
D_3: Second fortnight of August (August 26)	0.293	0.169	4.038	2.31
D_4: First fortnight of September (September 16)	0.262	0.117	3.660	1.59
F – test	*	*	*	*
S.Em.±	**0.012**	**0.009**	**0.149**	**0.11**
C.D. (p=0.05)	**0.043**	**0.027**	**0.517**	**0.37**
Sub: Crop geometry				
S_1: 30 × 15 cm	0.273	0.143	6.078	3.17
S_2: 45 × 15 cm	0.317	0.173	4.700	2.56
S_3: 60 × 15 cm	0.322	0.175	3.579	1.95
S_4: 75 × 15 cm	0.333	0.183	2.957	1.51
F – test	*	*	*	*
S.Em.±	**0.009**	**0.008**	**0.107**	**0.16**
C.D. (p=0.05)	**0.026**	**0.024**	**0.312**	**0.47**
Interaction (D × S)				
D_1S_1	0.329	0.200	7.308	4.45
D_1S_2	0.367	0.180	5.432	2.67
D_1S_3	0.380	0.177	4.222	1.96
D_1S_4	0.394	0.223	3.500	1.54
D_2S_1	0.284	0.155	6.302	3.44
D_2S_2	0.339	0.198	5.021	2.93
D_2S_3	0.329	0.207	3.659	2.30
D_2S_4	0.340	0.210	3.022	1.87
D_3S_1	0.249	0.129	5.533	2.86
D_3S_2	0.297	0.187	4.395	2.77
D_3S_3	0.296	0.190	3.284	2.11
D_3S_4	0.331	0.169	2.939	1.50
D_4S_1	0.233	0.088	5.168	1.95
D_4S_2	0.267	0.127	3.951	1.88
D_4S_3	0.284	0.127	3.153	1.41
D_4S_4	0.266	0.128	2.367	1.14
F - test	**NS**	**NS**	**NS**	**NS**
S.Em.±	**0.018**	**0.017**	**0.214**	**0.32**
C.D. (p=0.05)	**-**	**-**	**-**	**-**

dry matter output per unit area under close spacing. As a result, during all growth phases, wider spacing resulted in significantly decreased CGR. Despite the fact that the individual plant canopy was raised in these spacings, CGR was reduced because the plant population and dry matter production per unit area were lesser. These results are in accordance with the findings of Ramesh *et al*. (2017), that at different crop growth stages, narrow spacing (15 × 10 cm) resulted considerably greater CGR (g/m^2/day) during 30-60 DAS (6.7), 60-90 DAS (14.9) and 90 DAS - harvest (11.1), followed by 30 × 10 cm spacing (3.9, 10.2, and 8.0, respectively). The interaction effect on crop growth rate as influenced by date of sowing and varied crop geometry was found non-significant with respect to CGR.

Absolute Growth Rate (AGR, g/day)

Among different sowing dates, at 30-60 DAS (0.36) and 60 DAS-harvest (0.19) the absolute growth rate (AGR g/day) was substantially higher when sown during second fortnight of July which was on par with the August first fortnight sown crop at all growth stages. Lower AGR was recorded in September first fortnight sown crop. Among the varied crop geometry, wider spacing of 75 × 15 cm recorded substantially higher AGR during 30-60 DAS (0.33) and 60 DAS-harvest (0.18) which was on par with 60 × 15 cm and 45 × 15 cm, while lower AGR was recorded with 30 × 15 cm spacing. This could be due to greater availability of growth resources in widely spaced plants as compared to narrow spaced ones which lead to greater expansion of leaves *i.e.*, increased leaf area per unit area causing increased photosynthetic efficiency of plants reflecting in increased dry matter accumulation and in-turn the crop growth. The above findings were in line with the results of Hirich *et al.* (2014) and Ramesh *et al.* (2017). The interaction effect of sowing date and different spacings was found non-significant with respect to AGR.

Relative Growth Rate (RGR, g/g/day)

The rate of dry matter increase per unit dry matter over a unit time period is measured by relative growth rate. Relatively lower RGR was observed during early growth phase and increased between 30-60 DAS. Effect of sowing windows and varied crop geometry on RGR was non-significant. In general, among different sowing windows, July second fortnight sowing date recorded higher RGR at 30-60 DAS and 60 DAS-harvest (0.0146 and 0.0046 g/g/day, respectively). However, lower RGR recorded in September first fortnight sowing. The per cent increase in RGR under July second fortnight sown crop was up to 20.61 and 53.33 per cent during 30-60 DAS and 60 DAS-harvest, respectively over delayed sowings. This is attributed to the environmental conditions *viz.*, sufficient rainfall, solar radiation and optimum temperature which favours better crop growth and development finally resulting in increased dry matter production per unit area within the given time period. Similar results were also recorded by Ramesh *et al.* (2017).Among the varied crop geometry, plant spacing of 75 × 15 cm recorded higher relative growth rate during 30-60 DAS (0.0134 g/g/day) and 60 DAS-harvest (0.0045 g/g/day) as compared to other plant spacings. However, narrow spacing of 30 × 15 cm recorded lower RGR. This could be due to individual plant performance in terms of dry matter production being better under wider spacing due to better utilization of available resources such as sunlight, water, nutrients, and space which improved physiological activities of the plants resulting in a higher RGR. The higher inter-plant competition in closed spaced crops due to higher plant population per unit area causing reduced access to resources in sufficient quantity to individual plants hindering the potential growth and development of the crop. Ramesh *et al.* (2017) also found that wider spacing of 60 × 15 cm resulted in higher RGR than closer spacing of 15 × 10 cm however, the difference between the geometries was non-significant during 90 DAS-harvest growth phase of the crop. In comparison to other crop geometries, greater RGR was reported during 30-60 DAS under 15 × 10 cm spacing and during 60-90 DAS with wider spacing of 60 × 10 cm.

Net Assimilation Rate (NAR, g/dm^2/day)

The net gain of assimilates per unit of leaf area and over a unit time period is known as the NAR or unit leaf rate. The capacity of a crop's net assimilation rate (NAR) and leaf area determine its yield. NAR as influenced by different sowing windows was found significant only during 60 DAS-harvest. Whereas, varied crop geometry could not produce significant difference. Among the different sowing windows, higher NAR was recorded in July second fortnight sowing during 30-60 DAS and 60 DAS-harvest (0.090 and 0.0233 g/dm^2/day, respectively). However, lower NAR was recorded during September first fortnight sowing. The above findings were in line with the results of Ramesh *et al.* (2017). The interaction effect of different date of sowing and varied spacing on net assimilation rate was found non-significant.

Days to 50 % blooming and days to maturity

Data pertaining to days to 50% blooming as influenced by different planting windows and crop geometry presented in table 3. Significant results were observed with respect to different dates of sowing of quinoa in case of days to attain 50 per cent flowering and maturity. Among the dates of sowing, sowing during July second fortnight had taken more days to attain 50 per cent blooming (43.90) which was on par with sowing during August first fortnight (41.16) as compared to other sowing dates. Significantly, early flowering was observed in September first fortnight sowing. However, July second fortnight sowing had taken more days to maturity (97.36) which was superior over other dates of sowing. Whereas, early maturity was observed under crop sown in September first fortnight. Quinoa is a cool season crop and with decrease in temperature from July second fortnight to September first fortnight, number of days to 50 per cent blooming and days taken to maturity reduced significantly. The above results were similar with the findings of Sajjad *et al*. (2014) who reported that quinoa is a short-day plant and exhibited a positive relation with photoperiodism and it is a function of sowing dates and time taken to complete its phenology and its development phases. As the temperature and photoperiod limits the plant life cycle which depicts the late planting is responsible for yield reduction which was stated by Parvin *et al.* (2013). Days taken to 50 per cent blooming and for maturity of quinoa were found non-significant to different inter row spacings. This was similar to findings of Belmonte *et al.* (2018) and Rishi and Galwey (1991). Interaction effect for above traits was found non-significant in quinoa with respect to different sowing windows and varied spacings.

Table 2: Effect of different sowing windows and varied crop geometry on relative growth rate (g/g/day) and net assimilation rate (g/dm^2/day) of quinoa

Treatments	RGR		NAR	
	30-60 DAS	60- Harvest	30-60 DAS	60- Harvest
Main: Sowing windows				
D_1: Second fortnight of July (July 26)	0.0146	0.0046	0.090	0.0233
D_2: First fortnight of August (August 09)	0.0133	0.0044	0.077	0.0230
D_3: Second fortnight of August (August 26)	0.0129	0.0042	0.074	0.0218
D_4: First fortnight of September (September 16)	0.0121	0.0033	0.073	0.0119
F - test	**NS**	**NS**	**NS**	*
S.Em.±	**0.0006**	**0.0003**	**0.010**	**0.0021**
C.D. (p=0.05)	-	-	-	**0.0072**
Sub: Crop geometry				
S_1: 30 × 15 cm	0.0128	0.0039	0.070	0.0172
S_2: 45 × 15 cm	0.0134	0.0041	0.076	0.0206
S_3: 60 × 15 cm	0.0133	0.0042	0.078	0.0209
S_4: 75 × 15 cm	0.0134	0.0045	0.090	0.0212
F - test	**NS**	**NS**	**NS**	**NS**
S.Em.±	**0.0005**	**0.0002**	**0.008**	**0.0018**
C.D. (p=0.05)	-	-	-	-
Interaction (D × S)				
D_1S_1	0.0151	0.0049	0.092	0.0295
D_1S_2	0.0142	0.0039	0.086	0.0216
D_1S_3	0.0143	0.0037	0.088	0.0204
D_1S_4	0.0147	0.0036	0.093	0.0217
D_2S_1	0.0127	0.0040	0.067	0.0177
D_2S_2	0.0137	0.0045	0.082	0.0204
D_2S_3	0.0134	0.0046	0.078	0.0243
D_2S_4	0.0134	0.0046	0.082	0.0297
D_3S_1	0.0119	0.0037	0.063	0.0142
D_3S_2	0.0132	0.0046	0.074	0.0264
D_3S_3	0.0127	0.0046	0.072	0.0262
D_3S_4	0.0139	0.0039	0.085	0.0202
D_4S_1	0.0114	0.0029	0.058	0.0076
D_4S_2	0.0125	0.0035	0.063	0.0140
D_4S_3	0.0129	0.0034	0.075	0.0129
D_4S_4	0.0116	0.0034	0.101	0.0132
F - test	**NS**	**NS**	**NS**	**NS**
S.Em.±	**0.0010**	**0.0004**	**0.015**	**0.0037**
C.D. (p=0.05)	-	-	-	-

Table 3: Effect of different sowing windows and spacings on days to 50 % flowering and days to maturity of quinoa

Treatments	Days to 50% flowering	Days to maturity
Main: Sowing windows		
D_1: Second fortnight of July (July 26)	43.90	97.36
D_2: First fortnight of August (August 09)	41.16	95.53
D_3: Second fortnight of August (August 26)	40.72	93.56
D_4: First fortnight of September (September 16)	38.38	90.34
F - test	*	*
S.Em.±	**0.81**	**0.52**
C.D. (p=0.05)	**2.79**	**1.80**
Sub: Crop geometry		
S_1: 30 × 15 cm	41.68	95.11
S_2: 45 × 15 cm	41.28	94.46
S_3: 60 × 15 cm	40.52	93.92
S_4: 75 × 15 cm	40.69	93.30
F - test	**NS**	**NS**
S.Em.±	**0.62**	**0.58**
C.D. (p=0.05)	-	-
Interaction (D × S)		
D_1S_1	45.35	98.31
D_1S_2	44.55	97.45
D_1S_3	42.56	97.15
D_1S_4	43.15	96.52
D_2S_1	43.25	96.23
D_2S_2	39.69	95.98
D_2S_3	40.47	95.24
D_2S_4	41.25	94.68
D_3S_1	40.85	94.25
D_3S_2	41.30	93.85
D_3S_3	40.52	93.26
D_3S_4	40.23	92.87
D_4S_1	37.29	91.65
D_4S_2	39.58	90.56
D_4S_3	38.52	90.03
D_4S_4	38.12	89.12
F - test	**NS**	**NS**
S.Em.±	**1.24**	**1.15**
C.D. (p=0.05)	-	-

Grain and stover yield (kg/ha)

The effect of sowing windows and spacings on grain yield, stover yield and interaction effect was found significant. Among sowing windows, July second fortnight recorded significantly higher grain yield (2051 kg/ha). Nevertheless, sowing during August first fortnight and August second fortnight were found on par with each other. However significantly lower grain yield was recorded in September first fortnight sown crop. The superiority of July second fortnight sowing date with respect to grain and stover yield might be due to the higher vegetative growth with optimum plant population made it maximum utilization of natural resources very effectively and efficiently. The increased leaf area helps in more absorption of carbon dioxide causing accelerated photosynthetic activity and effective translocation of photosynthates from source to all plant parts that reflected in the higher growth (plant height, number of tillers, leaf area and dry matter production) and yield attributes (number of panicles, panicle length, panicle weight and grain yield per plant etc). Similar results were also noticed by Hakan *et al*. (2014) in quinoa, Parvin *et al*. (2013)

and Sajjad *et al*. (2014) in grain amaranth. Thus, lower yield observed under late planted crop largely suffered limitation of temperature and photoperiod throughout the plant life cycle. Among varied spacings, grain and stover yield obtained with the spacing of 45 × 15 cm (1941 and 2346 kg/ha) was significantly higher compared to narrow spacing (30×15 cm) (1695 and 2065 kg/ha) and 60 × 15 cm (1648 and 2049 kg/ha) of wider spacing. The grain yield and stover yield are on par in spacing of 30 × 15 cm (1695 and 2065 kg/ha) and 60 × 15 cm (1648 and 2049 kg/ha) and both were superior over the wider spacing of 75 × 15 cm. The per cent increase in grain yield in 45 x 15 cm was to the tune of 14.5 per cent. Further increase in the row spacing to 60 x 15 cm and 75 x 15 cm shows negative to the tune of 16 and 17 per cent, respectively as compared to 30 x 15 cm narrow spacing. This clearly indicates that wider spacing could not compensate in the grain yield mainly due to lesser plant density and more density in narrow spacing could compensate with grain yield due to higher growth and yield parameters. Hence, 45 x 15 cm is found to be optimum for higher grain yield of quinoa crop. This could be due to lesser competition in wider spacing which results in improved growth and development in all phases of crop in higher row spacing compared to lesser row spacing. Though, more vegetative growth per plant was observed under wider spacing, stover yield was more in narrow spacing due to higher plant density per unit area. The results of Pourfarid *et al*. (2014), Olofintoye *et al.* (2015) and Sangul *et al*. (2020) also support the present findings. Row spacing greatly influence the grain and stover yield and hence, optimum row spacing is crucial for achieving higher yield levels as reported by Yarnia *et al*. (2010), Prommarak (2014) and Malligawad and Patil (2015). The interaction effect of sowing date and spacing on grain and stover yield was found significant. Interaction of date of sowing and spacing showed that July second fortnight date of sowing at a spacing of 45 × 15 cm recorded significantly higher grain yield (2392 kg/ha) followed by sowing during July second fortnight at a spacing of 30 × 15 cm (2083 kg/ha). Significantly lower grain yield was attained by September first fortnight date of sowing with 75 × 15 cm spacing. This increased grain and stover yield in combination of early sowing and optimum spacing (45 × 15 cm) might be due to higher growth of crop during early sown crop due to favorable environment conditions coupled with lesser competition for various natural resources in wider spacing due to optimum plant stand which enhanced the grain and stover yield. These results are in line with the findings of Parvin *et al*. (2013) and Sajjad *et al*. (2014).

Harvest index

Effective partitioning of assimilates from source to sink portion is represented by harvest index. Harvest index of quinoa is high and at par with most of the cereal crop (like Finger millet, Bajra, sorghum). Among different sowing windows, significantly higher harvest index was recorded in July second fortnight (46.03%) sowing, which was superior over other sowing dates. Significantly lower harvest index was recorded in September first fortnight sowing. Among varied crop geometry, significantly higher harvest index was recorded with the spacing 45 × 15 cm (45.59 %), which was superior over other spacings. 75 × 15 cm spacing recorded significantly lower harvest index. These results are in line with the findings of Olofintoye *et al*. (2015). On the contrary, Carlos and Juliana (2008) who reported non-significant harvest index between plant densities of 1,00,000 to 6,00,000 plants ha^{-1}. Interaction effect of sowing windows and spacing on harvest index of quinoa was found significant. Among different combinations, July second fortnight date of sowing with 45 × 15 cm spacing recorded significantly higher harvest index (47.83 %) which was significantly superior over other treatment combinations. Significantly lower harvest index was recorded in September first fortnight sowing with 75 × 15 cm spacing.

Conclusion

As per the results obtained, it can be concluded that July second fortnight is the optimum date of sowing for quinoa during *Kharif* season under eastern dry zone of Karnataka. It is due to the fact that, the July sown crop was in synchrony with the onset of monsoon in the present study zone. Thus, leisure availability of soil moisture coupled with sufficient solar energy have accelerated the physiological processes reflecting in higher yield level as compared to delayed crop wherein, crop suffered moisture shortages at the grain filling stages as the

Table 4: Effect of different sowing windows and spacings on grain yield, stover yield and harvest index (%) of quinoa

Treatments	Grain yield (kg/ha)	Stover yield (kg/ha)	Harvest index (%)
Main: Sowing windows			
D_1: Second fortnight of July (July 26)	2051	2439	4.60
D_2: First fortnight of August (August 09)	1688	2098	4.45
D_3: Second fortnight of August (August 26)	1517	1916	4.41
D_4: First fortnight of September (September 16)	1398	1799	4.36
F - test	*	*	*
S.Em.±	**31.34**	**45.22**	**0.16**
C.D. (p=0.05)	**108.46**	**156.49**	**0.56**
Sub: Crop geometry			
S_1: 30 × 15 cm	1695	2065	4.49
S_2: 45 × 15 cm	1941	2346	4.55
S_3: 60 × 15 cm	1647	2049	4.45
S_4: 75 × 15 cm	1371	1791	4.32
F - test	*	*	*
S.Em.±	**19.18**	**38.64**	**0.12**
C.D. (p=0.05)	**55.99**	**112.79**	**0.35**
Interaction (D × S)			
D_1S_1	2083	2389	4.65
D_1S_2	2392	2789	4.78
D_1S_3	1985	2389	4.53
D_1S_4	1744	2189	4.43
D_2S_1	1620	2029	4.44
D_2S_2	2064	2476	4.54
D_2S_3	1710	2119	4.46
D_2S_4	1361	1768	4.35
D_3S_1	1587	1978	4.45
D_3S_2	1755	2165	4.47
D_3S_3	1498	1879	4.43
D_3S_4	1230	1645	4.27
D_4S_1	1492	1867	4.44
D_4S_2	1554	1954	4.43
D_4S_3	1398	1810	4.35
D_4S_4	1150	1565	4.23
F - test	*	-	*
S.Em.±	**38.36**	**77.28**	**0.24**
C.D. (p=0.05)	**111.97**	**NS**	**0.70**

rains were withdrawing with simultaneous aging of crop. Among plant geometry, spacing of 45× 15 cm is optimum for quinoa as it is evidenced with higher grain yield and higher monitory returns of quinoa as compared to other spacing. It is found that July second fortnight sowing with the spacing of 45 × 15 cm is ideal for higher grain yield of quinoa during *Kharif* season under eastern dry zone of Karnataka.

Conflict of interest

The authors declare that they have no conflict of interest.

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Forecasting area, productivity and prices of mango in Valsad District of Gujarat: Time series analysis

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ABSTRACT

Mango on an average account approximately 75 per cent of total production quantity. India is the largest mango producer, accounting for about half of the world-wide mango production. Forecasting of area, production and price fluctuations are the key to provide support in decision making and proper planning for sustainable growth of farmers and other people who are dependent on horticulture. The prices of mango are affected by cultivated area and yield of mango but in other ways pre or post-harvest management also affects it. The problems regarding the price fluctuations arise due to seasonality in arrival and its perishable nature. Therefore, the present study was carried out with time series intervention modelling in forecasting area, productivity and prices of mangoes. In the current investigation, simple exponential smoothing (SES) implemented to develop the forecasting models for area and productivity of mango. Under the SES, the error measurements at different values of alpha (α) for forecasting of area and productivity were observed that the value 0.8 and 0.9 of alpha (α) showed minimum Mean Absolute Percentage Error (MAPE) error i.e. 3.11 per cent, and 12.73 per cent, respectively. The study also developed time series ARIMA models for forecasting the prices of the mango (Keshar and Alphonso) for Valsad markets of Gujarat. It was showed that ARIMA (6, 1, 2) and ARIMA (1, 1, 2) were found good models for forecasting the prices of the Keshar and Alphonso, respectively in Valsad district of Gujarat.

Introduction

Mango is referred to as the "*King of fruits*" because of its overall rich eating characteristics. India is the main producer and consumer of mangoes and it ranks first among world's mango producing countries accounting for about 50 per cent of the world's mango production. Fruits and vegetables account for nearly 90 per cent of the total horticulture production in the country which plays an important role in horticulture, agriculture and Indian economy. It is consumed as a fresh fruit, preserved, in the frozen, dried forms, processed into juices, purees, chutneys and pickles etc. In the horticulture industry, fruit productivity and area consecration under mango stand at the top position and it covers 21.83 per cent of total fruits crops area and holds the second rank in total fruits production 35.53 per cent (Singh *et al.* 2018). The Gujarat is fifth largest mango producing state in India and Valsad is first

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largest mango producing district in Gujarat with a production of 237,203 million tons with 36,890 ha area. (Anonymous, 2021). In India, it has good scope for increasing the area and productivity of mango due to demand for mango fruit is growing per annum. The procedure, time and place efficacies adjust production, consumption and which help making efficient marketing decisions. Time series forecasts are statistical methods designed to identify patterns in series data that can be predicted in the future. But it also concluded that very often, the future will not look like the past, and we need insight into how, and why, the future will look different and that is the role of market intelligence (Moon, 2013). Considering the facts, present study swotted the several researches on forecasting. Yusuf and Sheu (2007), studied trend analysis for forecasting of future production of citrus and mango in Nigeria using various forecasting techniques up to the year 2010. Khan *et al.* (2008) predicted the production of Mango in Pakistan using a log linear model and ARIMA modelling approach. Pradhan (2012) utilised ARIMA model for forecasting agricultural productivity in India. Qureshi *et al.* (2014), Hamjah (2014) and Pardhi *et al.* (2017) developed Box-Jenkins ARIMA model to forecast production of Mango. Rathod and Mishra (2017) developed weather based models by using methods of stepwise regression analysis and ARIMA model to forecast area and production of mango. Kumar and Gupta (2020) have obtained forecast values for the production and area of Mango by using Autoregressive, Exponential and Gompertz models.

Material and Methods

The study area Valsad is located at Latitude 20.63^{0}N, Longitude 72.93^{0}E having an average elevation of 13 meter above sea level. Major horticultural crops that are produced in the Valsad district are mango, sapota, papaya, guava, cucurbits and banana. Valsad is famous for Alphonso (*Valsadi Haafus*) and *Keshar*. Therefore it is also called a horticulture hub of Gujarat and Mango capital of Gujarat. The study utilised the time series secondary data on area and productivity of the mango starting from the year 2002-03 to 2019-20 which was collected from Directorate of Horticulture, Gandhinagar, Gujarat. The price data of mango for different markets under Valsad was collected from website of Directorate of Marketing & Inspection (DMI), Ministry of Agriculture and Farmers Welfare, Government of India (https://agmarknet.gov.in/). The major markets of Valsad district viz. Valsad, Pardi, Dugari and Chikli were selected purposively based on availability of the data. The price forecasting was carried out for major growing varieties of mango i.e. Keshar and Alphonso. The weekly average of all market prices under the Valsad districts were utilized for forecasting. The weekly missing price data were interpolated.

Statistical techniques

The analytical tool adopted are Simple Exponential smoothing (SES) and Box-Jenkins Autoregressive models (ARIMA) techniques are discussed below. The software EViews 9.1 was used for time series data analysis.

Simple Exponential smoothing (SES)

The simple exponential smoothing (SES) technique is based on averaging series data of a series in a decreasing (exponential) manner. This particular method is generally utilized when forecasting data has no clear trend or seasonal pattern. Exponential Smoothing assigns exponentially increasing or decreasing weights (smoothing constant) to the data series over time. The smoothing constant value is higher for most recent value and lesser for the older data points. The value of smoothing constant i.e. alpha is always taken between 0 & 1 because if the value of smoothing constant is greater than 1, then the expression of single exponential smoothing acquires negative value which denotes the failure of the method. The forecast of area and productivity, for the period *t+1* is given by Box *et al.* (1994).

$$F_{t+1} = F_t + \alpha\,(Y_t - F_t)$$

$$F_{t+1} = \alpha\, Y_t + (1-\alpha)\, F_t$$

(*on simplification*)

Where, F_{t+1} = Forecast value for period $t+1$
F_t = Forecast value for period t
α = Smoothing constant
Y_t = Actual value for period t (Area and productivity)

The value of α lies between 0 and 1. The large value of α (say 0.9) gives very little smoothing in the forecast, whereas a small value of α (say 0.1) gives acceptable smoothing. Alternatively, it can be chosen (α) from a set of values (say α = 0.1, 0.2, 0.3,…, 0.9) and finally choose the value that yields the minimum MSE value (Kumari *et al.*, 2017, Garde *et al*, 2022).

Box-Jenkins Autoregressive models (ARIMA)

The stationarity of the data was carried out with Augmented Dickey-Fuller (ADF) Test (Unit root test) before initializing the operational steps of ARIMA. The model is usually stated as ARIMA (*p, d, q*), where, *p* denotes orders of auto-regression, *d* means integration (differencing) and *q* represents moving average. The Box and Jenkins proposed a practical four-stage procedure for finding a good model. A) Identification b) Estimation of parameters c) Diagnostic checking and d) Forecasting (Garde *et al*, 2022). The forecasting through ARIMA model was carried by using E-Views 9.0 statistical software, viz., checking the stationarity through Augmented Dickey-Fuller (ADF) test, identification of tentative models based on scrutiny of the parameters of the selected models were estimated by maximum likelihood Estimation (MLE) method. The adequacy of the model was judged based on the significance of Ljung-Box 'Q' Statistic using residual diagnostics (Box and Jenkins 1994, Brockwell and Davis 1996).

The performance of developed models

The identification of the suitable forecasting models for area, productivity and prices of mango were done using different goodness of fit techniques viz. Adj.R^2, Forecast error (%), Mean Absolute Percentage Error (MAPE), Root mean square error (RMSE), and Thiel's inequality coefficient (U). The forecasting performance of the developed model is excellent, when $U = 0$. Also when $U = 1$ that means the predicting performance is not improved by just using the last observed value as a forecast. (Friedhelm, 1973).

Results and Discussion

Forecasting of the area and productivity of mango using exponential models

In the present study, the simple exponential smoothing (SES) was adopted to forecast the value of area (ha) and productivity (mt ha^{-1}) for the Valsad district. The below Table 1, showed the error measurements at different values of alpha (α) for forecasting of area (ha) and productivity (mt ha^{-1}) in the Valsad district. Here values of α were selected from a grid of values viz. 0.2, 0.4, 0.6, 0.8, 0.9. Depending upon these values, the forecast with minimum error measurement and corresponding smoothing constant were selected. It was observed from Table 1 that for the value of alpha (α) 0.8 and 0.9 showed minimum MAPE error i.e. 2.56 per cent and 12.73 per cent which was low among all other values of alpha for area and productivity, respectively. Also for other values of alpha, Root Mean Error Percentage (RMSE) was high. The graphical representation of actual and forecast values of area and productivity of mango in Valsad district is given in Figure 1.

Forecasting of the prices (Rs./q) of mango using ARIMA models

The detailed analysis of forecasting of mango prices (Rs./q) for the variety of Keshar and Alphonso in Valsad district are discussed separately under following sub-heads.

KESHAR

Stationarity check

Table 2 revealed that Augmented Dickey-Fuller (ADF) unit root test statistic at level (no difference) was accepted the null hypothesis i.e. prices data of Keshar mango has a unit root (non-stationary). The probability value were more than rejection values at 1 per cent level of the significance (*p*-0.0181) thus analysis was proceeding further by taking 1st differencing and again tested stationarity. Table 2 showed that at 1st differencing the null hypothesis for test statistic was rejected which indicated prices data of Keshar mango had stationarity (*p*-0.0000). Therefore ARIMA model identification was proceed with taking value, d= 1.

Identification of the model

The tentative models were first identified based on the Auto-Correlation Function (ACF) and Partial Auto-Correlation Function (PACF) plots shown in Figure 2. Based on numbers of spike outside the confidence level in the correlogram, the all possible combination of the *p* and *q* values were carried out for identification of the best model. The method of ARMA maximum likelihood was applied for model development. The tentatively identified five models for forecasting prices of Keshar mango and are presented in Table 3. It has also indicated the values of Akaike Information Criteria (AIC) and Schwarz Bayesian Information Criteria (SIC) along with adjusted R^2 and SIGMASQ. Based on the lower the value of AIC, SIC and SIGMASQ with higher value of Adjusted R^2 the selected model was

Table 1: Forecast validation using different values of weight (α) using SES method

Valsad	α	Damping factor (1-α)	MSE	RMSE	MAPE (%)
Area	0.9	0.1	1885340.51	1373.08	3.26
	0.8	**0.2**	1447874.41	1203.28	**3.11**
	0.6	0.4	1508140.42	1228.06	3.37
	0.4	0.6	3853917.55	1963.14	5.45
	0.2	0.8	24176012.96	4916.91	13.74
Productivity	**0.9**	**0.1**	2.09	1.44	**12.73**
	0.8	0.2	2.14	1.46	13.67
	0.6	0.4	2.30	1.51	16.91
	0.4	0.6	2.39	1.54	19.77
	0.2	0.8	2.16	1.47	19.90

Table 2: Stationarity (ADF) test

ADF test at level

Null Hypothesis: KESHAR_PRICE has a unit root				
Exogenous: Constant				
Lag Length: 0 (Automatic - based on AIC, maxlag=12)				
			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-3.281056	0.0181
Test critical values:	1% level		-3.489117	
	5% level		-2.887190	
	10% level		-2.580525	

ADF test at 1st differencing

Null Hypothesis: D(KESHAR_PRICE) has a unit root				
Exogenous: Constant				
Lag Length: 1 (Automatic - based on AIC, maxlag=12)				
			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-8.650301	0.0000
Test critical values:	1% level		-3.490210	
	5% level		-2.887665	
	10% level		-2.580778	

Table 3: Identification of ARIMA model

ARIMA Model →	(6, 1, 2)	(2, 1, 6)	(2, 1, 2)	(0, 1, 2)	(2, 1, 0)
AIC	15.380	15.401	15.413	15.409	15.425
SIC	15.482	15.503	15.516	15.486	15.501

Table 4: Estimation of coefficients of the ARIMA model through residual diagnostics

Dependent Variable: D(KESHAR_PRICE)				
Method: ARMA Maximum Likelihood (OPG - BHHH)				
Sample: 3 105				
Included observations: 103				
Convergence achieved after 17 iterations				
Variable	**Coefficient**	**Std. Error**	**t-Statistic**	**Prob.**
C	24.01931	24.27641	0.989410	0.3249
AR(6)	-0.261532	0.172739	-1.514032	0.1333
AR(3)	-0.143158	0.129989	-1.101314	0.2735
MA(2)	-0.328782	0.142659	-2.304675	0.0233
MA(13)	-0.176468	0.140555	-1.255512	0.2123
SIGMASQ	241607.0	23897.69	10.11006	0.0000
R-squared	0.179801	Mean dependent var		30.67867
Adjusted R-squared	0.137523	S.D. dependent var		545.3983
S.E. of regression	506.5094	**Akaike info criterion**		15.36126
Sum squared resid	24885522	**Schwarz criterion**		15.51474
Log likelihood	-785.1051	Hannan-Quinn criter.		15.42343
F-statistic	4.252806	Durbin-Watson stat		2.037531
Prob(F-statistic)	0.001539			

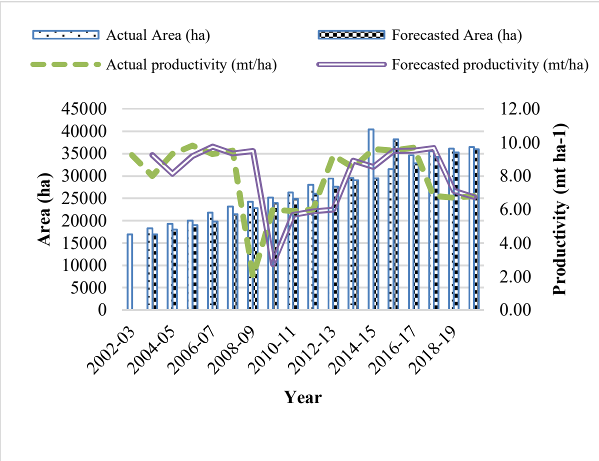


Figure 1: Graphical representation of actual and forecast values of area and productivity of mango

Sample: 1 115
Included observations: 113

Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	0.080	0.080	0.7339	0.392
		2	-0.196	-0.204	5.2496	0.072
		3	-0.086	-0.053	6.1170	0.106
		4	-0.001	-0.030	6.1172	0.191
		5	-0.065	-0.095	6.6231	0.250
		6	-0.200	-0.211	11.497	0.074
		7	0.011	0.006	11.512	0.118
		8	-0.004	-0.113	11.514	0.174
		9	-0.065	-0.106	12.040	0.211
		10	0.047	0.016	12.320	0.264
		11	0.090	0.008	13.344	0.271
		12	0.098	0.047	14.571	0.266
		13	-0.116	-0.120	16.308	0.233
		14	-0.170	-0.167	20.090	0.127
		15	-0.029	-0.086	20.202	0.164
		16	0.023	-0.049	20.276	0.208
		17	0.080	0.038	21.151	0.220
		18	0.006	-0.023	21.157	0.272
		19	-0.033	-0.090	21.310	0.320
		20	0.069	0.019	21.972	0.342

Figure 2: Correlogram at first differencing

Sample: 1 105
Included observations: 103
Q-statistic probabilities adjusted for 2 ARMA terms

Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	-0.022	-0.022	0.0507	
		2	0.002	0.002	0.0513	
		3	-0.177	-0.177	3.4374	0.064
		4	-0.031	-0.040	3.5430	0.170
		5	-0.102	-0.107	4.6877	0.196
		6	0.002	-0.037	4.6881	0.321
		7	-0.038	-0.057	4.8481	0.435
		8	-0.019	-0.065	4.8888	0.558
		9	-0.107	-0.134	6.2075	0.516
		10	0.063	0.022	6.6693	0.573
		11	0.024	-0.004	6.7367	0.665
		12	0.090	0.037	7.7044	0.658
		13	-0.176	-0.191	11.441	0.407
		14	-0.100	-0.147	12.646	0.395
		15	-0.066	-0.077	13.184	0.434
		16	0.073	-0.012	13.842	0.462
		17	0.048	-0.015	14.132	0.516
		18	0.099	0.015	15.375	0.497

Figure 3(a): Residual diagonostic AR(6), MA(2)

Sample: 1 105
Included observations: 103
Q-statistic probabilities adjusted for 4 ARMA terms

Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	-0.028	-0.028	0.0817	
		2	-0.003	-0.004	0.0826	
		3	-0.037	-0.038	0.2346	
		4	-0.041	-0.043	0.4190	
		5	-0.114	-0.117	1.8505	0.174
		6	-0.019	-0.029	1.8906	0.389
		7	-0.050	-0.058	2.1757	0.537
		8	-0.082	-0.100	2.9475	0.567
		9	-0.129	-0.155	4.8562	0.434
		10	-0.025	-0.067	4.9279	0.553
		11	-0.011	-0.047	4.9430	0.667
		12	0.092	0.049	5.9463	0.653
		13	-0.036	-0.082	6.1055	0.729
		14	-0.043	-0.106	6.3263	0.787
		15	0.021	-0.023	6.3782	0.847
		16	0.057	0.021	6.7864	0.871
		17	0.051	0.026	7.1173	0.896
		18	0.051	0.007	7.4507	0.916

Figure 3(b): Residual diagnostic AR(6), AR(3), MA(2), MA(13)

Sample: 1 141
Included observations: 139

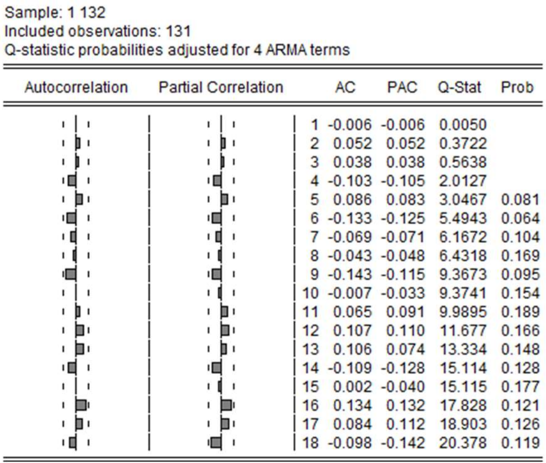
Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	0.318	0.318	14.333	0.000
		2	-0.197	-0.332	19.910	0.000
		3	-0.254	-0.081	29.217	0.000
		4	-0.090	-0.027	30.381	0.000
		5	-0.012	-0.075	30.400	0.000
		6	-0.110	-0.169	32.199	0.000
		7	-0.113	-0.059	34.094	0.000
		8	-0.125	-0.184	36.422	0.000
		9	-0.154	-0.213	40.004	0.000
		10	-0.043	-0.076	40.290	0.000
		11	0.145	0.014	43.495	0.000
		12	0.244	0.051	52.700	0.000
		13	0.072	-0.090	53.513	0.000
		14	-0.182	-0.202	58.698	0.000
		15	-0.028	0.091	58.825	0.000
		16	0.230	0.133	67.240	0.000
		17	0.177	0.001	72.292	0.000
		18	-0.087	-0.067	73.519	0.000
		19	-0.174	0.018	78.450	0.000
		20	-0.138	-0.087	81.606	0.000

Figure 4: Correlogram at first differencing

Sample: 1 132
Included observations: 131
Q-statistic probabilities adjusted for 2 ARMA terms

Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	0.020	0.020	0.0541	
		2	0.026	0.026	0.1465	
		3	-0.205	-0.206	5.8637	0.015
		4	-0.027	-0.020	5.9660	0.051
		5	-0.036	-0.025	6.1453	0.105
		6	-0.127	-0.175	8.4031	0.078
		7	-0.094	-0.105	9.6551	0.086
		8	-0.102	-0.117	11.138	0.084
		9	-0.135	-0.222	13.752	0.056
		10	-0.043	-0.124	14.019	0.081
		11	0.102	0.022	15.528	0.077
		12	0.154	0.029	18.984	0.040
		13	0.100	0.012	20.453	0.040
		14	-0.130	-0.186	22.988	0.028
		15	0.015	-0.038	23.023	0.041
		16	0.151	0.144	26.486	0.022
		17	0.136	0.077	29.303	0.015
		18	-0.085	-0.099	30.419	0.016

Figure 5(a): Residual diagnostics AR(1), MA(2)



Sample: 1 132
Included observations: 131
Q-statistic probabilities adjusted for 4 ARMA terms

	AC	PAC	Q-Stat	Prob
1	-0.006	-0.006	0.0050	
2	0.052	0.052	0.3722	
3	0.038	0.038	0.5638	
4	-0.103	-0.105	2.0127	
5	0.086	0.083	3.0467	0.081
6	-0.133	-0.125	5.4943	0.064
7	-0.069	-0.071	6.1672	0.104
8	-0.043	-0.048	6.4318	0.169
9	-0.143	-0.115	9.3673	0.095
10	-0.007	-0.033	9.3741	0.154
11	0.065	0.091	9.9895	0.189
12	0.107	0.110	11.677	0.166
13	0.106	0.074	13.334	0.148
14	-0.109	-0.128	15.114	0.128
15	0.002	-0.040	15.115	0.177
16	0.134	0.132	17.828	0.121
17	0.084	0.112	18.903	0.126
18	-0.098	-0.142	20.378	0.119

Figure 5(b): Residual diagnostics AR(1), AR(3), MA(2) MA(3)

stated better for forecasting. From Table 3, it was found that ARIMA (6,1,2) was good model for forecasting the prices of the mango (Keshar).

Diagnostic check and estimation of parameters

The residual diagnostics (Correlogram-Q-statistics) was carried out on ARIMA (6,1,2) and estimated the coefficients for good forecast model. Table 4 showed that based on residual diagnostics ARIMA (6,1,2) model was modified by introducing the variables AR(3), MA(13) with existing AR(6) and MA(2). The graphical representation of the residuals of the correlogram are shown in Figure 3 (a) & (b).

Validation of model and Forecasting

The cross validation of the selected model was done based on RMSE, MAPE and Theil inequality coefficient. From Table 5, it was observed that the value of forecast error per cent varies from the -60.558 per cent to 17.535 per cent. The value of RMSE and MAPE were observed low 887.504 and 20.245, respectively. It was also observed the Theil inequality coefficient was 0.099 which indicated that the predictive performance of the model was good. Thus study revealed that the model AR(6), AR(3), MA(2), MA(13) found fitted well and further forecasted the Keshar mango price (Rs./q) for the year 2020 was computed (Table 6). It was observed that Actual prices were high in start of the season and goes decreasing at end of the season but the forecasted values also showed same trend. Similarly Pardhi *et al.* (2018) made efforts on forecasting the prices of mango using ARIMA model in Varanasi market of Uttar Pradesh.

ALPHONSO

Similar steps were followed as explained above for forecasting prices of Alphonso mango in Valsad district and results were discussed hereunder;

Stationarity check

Table 7 revealed that Augmented Dickey-Fuller (ADF) unit root test statistic at level (no difference) was accepted the null hypothesis i.e. prices data of Alphonso mango has a unit root (non-stationary). The probability value were more than rejection values at 1 per cent level of the significance (*p*-0.0453). The analysis was proceeding further by taking 1st differencing and again tested stationarity. Table 7 showed that at 1st differencing the null hypothesis for test statistic was rejected which indicated prices data of Alphonso mango had stationarity (*p*-0.0000). Therefore ARIMA model identification was proceed with taking value, d= 1.

Identification of the model

The tentative models were first identified based on the Auto-Correlation Function (ACF) and Partial Auto-Correlation Function (PACF) plots shown in Figure 4. Based on numbers of spike outside the confidence level in the correlogram, the all possible combination of the *p* and *q* values were carried out for identification of the best model. The Method of ARMA Maximum Likelihood was applied for model development. The tentatively identified five best models for forecasting prices of Alphonso mango and are presented in Table 8. It also indicated the values of Akaike Information Criteria (AIC) and Schwarz Bayesian Information Criteria (SIC) along with adjusted R^2 and SIGMASQ. Based on the lower the value of AIC, SIC and SIGMASQ with high value of Adjusted R^2 the selected model was stated better for forecasting. From Table 8, it was found that ARIMA (1, 1, 2) was good model for forecasting the prices of the Alphonso mango.

Diagnostic check and estimation of parameters

The residual diagnostics (Correlogram-Q-statistics) was carried out on ARIMA (1, 1, 2) and estimated the coefficients for good forecast model. Table 9 showed that based on residual diagnostics ARIMA (1, 1, 2) model was modified by introducing the variables AR(3) and MA(3) with existing AR(1) and MA(2). The graphical representation of the residuals of the correlogram are shown in Figure 5 (a) & (b).

Validation of model and Forecasting

The validation of the selected model was done based on RMSE, MAPE and Theil inequality coefficient. From Table 10, it was observed that the value of forecast error per cent varies from the 7.575 per cent to 16.653 per cent. The value of RMSE and MAPE were observed low 742.529 and 14.325, respectively. It was also observed the Theil inequality coefficient was 0.079 which indicated that the predictive performance of the model was good. Thus study revealed that the model AR(1), AR(3), MA(2), MA(3) found fitted well and further forecasted the Alphonso mango price (Rs./q) for the year 2020 was computed (Table 11). It was observed that forecasted prices were high in start of the season and goes decreasing, but at end of the season increased upto some extent.

Table 5: Validation of the ARIMA model AR(6), AR(3), MA(2), MA(13)

Week	Actual Price (Rs./q)	Forecasted Price (Rs./q)	Forecast Error (%)
28-04-2019 - 04-05-2019	5220	4888	06.365
05-05-2019 - 11-05-2019	5375	4460	17.018
12-05-2019 - 18-05-2019	5375	4432	17.535
19-05-2019 - 25-05-2019	5000	4778	04.445
26-05-2019 - 01-06-2019	5000	4273	14.535
02-06-2019 - 08-06-2019	4600	4226	08.134
09-06-2019 - 15-06-2019	4300	4128	04.003
16-06-2019 - 22-06-2019	3500	4326	-23.587
23-06-2019 - 29-06-2019	2959	4328	-46.271
30-06-2019 - 06-07-2019	2732	4387	-60.558
RMSE	887.504		
MAE	753.412		
MAPE (%)	20.245		
Theil inequality coefficient	0.099		

Table 6: Forecasted Keshar mango prices (Rs./q) for the year 2020 in Valsad market

Week	Forecasted Price (Rs./q)
30-04-2020 - 06-05-2020	4530
07-05-2020 - 13-05-2020	4508
14-05-2020 - 20-05-2020	4442
21-05-2020 - 27-05-2020	4403
28-05-2020 - 03-06-2020	4440
04-06-2020 - 10-06-2020	4467
11-06-2020 - 17-06-2020	4469
18-06-2020 - 24-06-2020	4504
25-06-2020 - 01-07-2020	4551

Table 7: **Stationarity (ADF) test**

ADF test at level

Null Hypothesis: ALPHONSO_PRICE has a unit root				
Exogenous: Constant				
Lag Length: 2 (Automatic - based on AIC, maxlag=13)				
			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-2.922830	0.0453
Test critical values:	1% level		-3.478547	
	5% level		-2.882590	
	10% level		-2.578074	

ADF test at 1st differencing

Null Hypothesis: D(ALPHONSO_PRICE) has a unit root				
Exogenous: Constant				
Lag Length: 8 (Automatic - based on AIC, maxlag=13)				
			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-6.673096	0.0000
Test critical values:	1% level		-3.481217	
	5% level		-2.883753	
	10% level		-2.578694	

Table 8: Identification of ARIMA model

ARIMA Model →	(1, 1, 2)	(12, 1, 1)	(2, 1, 1)	(11, 1, 1)	(0, 1, 1)
SIGMASQ	264500.300	267311.300	269970.200	271832.800	275623.200
Adjusted R^2	0.169	0.160	0.152	0.146	0.141
AIC	15.390	15.397	15.407	15.415	15.412
SIC	15.478	15.485	15.495	15.503	15.478

Table 9: Estimation of coefficients of the ARIMA model through residual diagnostics

Dependent Variable: D(ALPHONSO_PRICE)				
Method: ARMA Maximum Likelihood (OPG - BHHH)				
Sample: 2 132				
Included observations: 131				
Convergence achieved after 36 iterations				
Variable	**Coefficient**	**Std. Error**	**t-Statistic**	**Prob.**
C	18.53893	11.88809	1.559453	0.1214
AR(1)	0.287674	0.073739	3.901220	0.0002
AR(3)	0.095160	0.131689	0.722609	0.4713
MA(2)	-0.417808	0.068186	-6.127502	0.0000
MA(3)	-0.431281	0.106071	-4.065967	0.0001
SIGMASQ	241206.2	28109.93	8.580818	0.0000
R-squared	0.259521	Mean dependent var	17.69589	
Adjusted R-squared	**0.229902**	S.D. dependent var	572.9303	
S.E. of regression	502.7764	**Akaike info criterion**	**15.33201**	
Sum squared resid	31598010	**Schwarz criterion**	**15.46370**	
Log likelihood	-998.2467	Hannan-Quinn criter.	15.38552	
F-statistic	8.761925	Durbin-Watson stat	2.012147	
Prob(F-statistic)	0.000000			

Table 10: Validation of the ARIMA model AR(1), AR(3), MA(2), MA(3)

Week	Actual Price (Rs./q)	Forecasted Price (Rs./q)	Forecast Error (%)
28-04-2019 - 04-05-2019	4728	4370	7.575
05-05-2019 - 11-05-2019	5000	4256	14.885
12-05-2019 - 18-05-2019	5000	4277	14.461
19-05-2019 - 25-05-2019	5125	4311	15.876
26-05-2019 - 01-06-2019	5125	4322	15.672
02-06-2019 - 08-06-2019	5050	4338	14.094
09-06-2019 - 15-06-2019	5150	4358	15.384
16-06-2019 - 22-06-2019	5250	4376	16.653
23-06-2019 - 29-06-2019	--	4394	--
RMSE	742.529		
MAE	727.575		
MAPE	14.325		
Theil inequality coefficient	0.079		

Table 11: Forecasted Alphonso mango prices (Rs./q) for the year 2020 in Valsad market

Week	Forecasted Price (Rs./q)
30-04-2020 - 06-05-2020	4976
07-05-2020 - 13-05-2020	4901
14-05-2020 - 20-05-2020	4880
21-05-2020 - 27-05-2020	4870
28-05-2020 - 03-06-2020	4871
04-06-2020 - 10-06-2020	4881
11-06-2020 - 17-06-2020	4894
18-06-2020 - 24-06-2020	4910
25-06-2020 - 01-07-2020	4927

As discussed above Pardhi *et al.* (2017) used similar approach of ARIMA for price forecasting of mango in Lucknow of Uttar Pradesh. Areef *et al.* (2020) to studied price behaviour and forecasting of onion prices in Kurnoo market by applying ARIMA approach. Similarly ARIMA model methodology was adopted by Pardhi *et al.* (2018) for forecasting the prices of mango for Varanasi market of Uttar Pradesh. Time series forecasts are almost accurate and take less effort in execution. No model is permanent to forecast the area, productivity and prices, therefore it needs update timely in frequent interval. In the current study ARIMA models to forecast prices were limited to available data for specific market.

Conclusion

Based on the current study it is anticipated that the identification of the best forecast model may help to the producers, consumers as well as dealers in making right decisions during marketing of these produces. The scientist or researcher can make their forecasts more valuable in concerned to demand, policy making, export etc. only when the challenge is to figure out how to get it, and what they need to do to acquire and use forecast. The present study was carried out to develop forecasting models for area (ha), productivity (mt ha^{-1}) and also forecasting the prices (Rs/q.) of Mango (Keshar and Alphonso) in Valsad district of south Gujarat. The forecast value of mango indicated an increasing trend of prices in selected market of Valsad. For getting better prices it needs a specialised marketing infrastructure, spatial market intelligence and post-harvest loss reduction technology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Expressed sequence tag-based prediction of putative genes responsive to drought tolerance in rice (*Oryza sativa*) using *in silico* approach

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ABSTRACT

In present genomic era, rapid genetic gains can be achieved by exploitation of novel genes associated with the trait of interest employing molecular breeding and genetic engineering. In the present study genes responsible for drought stress in rice 10746 expressed sequence tags (ESTs), expressed under drought stress condition were retrieved from the NCBI. The downloaded ESTs were clustered and assembled into 1120 contigs and 5559 singletones using CAP3 programme. The contigs were further subjected to identification of transcription factor, a total of 62 putative transcription factors were identified and sorted into 17 putative TF families. The contigs were subjected to BLASTX in NCBI to identify unique sequence which were further aligned to *Oryza sativa* Indica Group (ASM465v1) in gramene database using BLAT to retrieve the upstream and downstream sequences for putative gene identification. The retrieved sequences were analysed for transcription start site, PolyA tails and coding sequences which are essential features of gene using online tool fsgene. The present study found that, 46 contigs out of 1120 contigs has key gene structure and was considered as putative novel genes which may contribute to the drought tolerance in indica rice. These genes may be useful in development of drought tolerant varieties through smart breeding.

Introduction

Rice is a major cereal crop and a staple food for 1/3rd of the world's population, accounting for 76 percent of calorie intake in Southeast Asia. (Miura *et al.*, 2011). In relation to production, more than 90% of the rice is being produced from china, India, Bangladesh, Indonesia, Vietnam and Thailand. China being the largest population country, it has produced 146.7 million metric tons of milled rice for the 2019-2020. India fallowed the second place with 118.9 million metric tons of milled rice (Statista, 2021). The rice yield are being significantly affected by biotic and abiotic factors among which drought stress is one of the most serious threat, resulting in a considerable yield drop (Swamy and Kumar, 2013; Sahebi *et al.*, 2018). The drought situation may be depicted as low rainfall or higher evaporation rates leading to stunted crop growth and reduced yield (Rollins *et al.*, 2013). The extent of yield loss depends on the drought intensity which intern depends on frequency and duration of rainfall, evaporation and soil moisture content (Oladosu *et al.*, 2019). It is estimated that, about 42 million ha of rice cultivation is under water scarcity (Yang *et al.*, 2019) and this raised the yield gap between the actual (4 t/ha) and potential (10 t/ha) (Oladosu*et al.*, 2019). To support the needs of growing population by 2050, it is prerequisite to develop high yielding rice varieties with multiple beneficial traits along

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with drought stress (Chukwu *et al., 2019*). Breeding rice cultivars tolerant to drought is a cost-effective and long-term solution for increasing rice yields in marginal lands (Pandey and Shukla, 2015). Several researchers have attempted to transfer drought tolerant traits in the past through traditional breeding methods, but the success has been slow due to a lack of acceptable donors with high levels of tolerance, trait's polygenic nature, and lack of feasible screening procedures (Pandey and Shukla, 2015). This stagnant improvement has paved the way for exploitation of genetic engineering, which involves introduction of novel foreign genes into popular cultivar, thereby increasing the tolerance level (Yamaguchi and Blumwald, 2005). For this, identification of critical genes and pathways involved in the tolerance is pre requisite (Sanchez *et al*., 2011). Plants being sessile developed various tolerance mechanisms at genic, biochemical, anatomical and phenotypic level which are under genetic control to overcome the menace of drought stress (Gupta *et al*., 2020). Generally, drought stress involves up regulation and down regulation of hundreds to thousands genes which can be categorized into membrane transport genes, signalling genes and transcriptional regulatory genes (Upadhyaya and Panda, 2019). At molecular level, gene expression fallows ABA-dependent and ABA-independent regulatory mechanisms (Gupta *et al*., 2020). Out of variable gene products, transcription factors (TFs) are prime and has significant role in expression of drought tolerance genes (Nuruzzaman *et al*., 2013). TFs control gene expression by interacting with cis-regulatory elements in the promoter region of stress genes (Wang *et al*., 2016). Due to this, stress responsive TFs have got lot of attention and genetic engineering of crop plants with overexpression of a single TF may lead to over expression of multiple downstream genes.

With the advent of next generation DNA sequencing technologies, the complete genome sequence of japonica (Nipponbare) and indica cultivars (93-11) were successfully sequenced in 2002 (Goff *et al*., 2002; Yu *et al*., 2002). More importantly, the sequence of expressed genes were generated from cDNA libraries in the form of short nucleotide sequences (200–800 bases) known as expressed sequence tags (ESTs) which were deposited in the repository. This breakthrough has led to the access for complete gene sequences, discovery of genes and their interactions across tissues as well as development stages. ESTs are prime choice of researchers due to following reasons *viz.*, gene discovery, genome annotation, mapping, polymorphism analysis, gene prediction, gene structure identification, and expression. The ESTs were utilised to find novel genes in wheat that were responsive to drought and salt stress and also salt stress responsive genes in rice (Nahas *et al.,* 2019; Bhati *et al*., 2016). The comprehensive analysis of drought tolerance ESTs would give a clearer picture of transcriptional mechanisms to drought stress, aids in identification of critical genes and discovery of stress responsive promoters and the cis elements. The above utilities can be used in genetic engineering/cisgenic approach for improving/incorporating drought resistance genes into popular rice cultivars. In view of this, the present study was attempted to identify putative candidate genes expressed under drought conditions based on ESTs.

Material and Methods

A total of 10,746 ESTs or cDNA of *Oryza sativa*as on date 5.4.2021 that have been expressed under drought conditions were downloaded from the EST database of NCBI (www.ncbi.nlm.nih.gov). Later, redundant ESTs, low complexity sequence, vector sequence, genomic repeats were masked by EGassembler(Guo *et al*., 2015,Masoudi-Nejad *et al*., 2006). The CAP3 programme was used to aggregate the processed EST sequences into clusters (Huang & Madan 1999). The clustering yields singletons and contigs in which singletons are the ESTs havinglownucleotide similarity with other ESTs. Whereas, contigs are formed due to overlapping of the ESTs with similar sequences. All assembled contigs were further analyzed to find the transcription factors using the PlantTFcat online tool (http://plantgrn.noble.org/PlantTFcat).

ESTcontigs annotation and identification of putative genes

The nucleotide sequence of EST contig werecompared with the nr protein database of NCBI by using BLASTX programme with default options. Here, the nucleotide sequences were first translated into reading frames and compared with the proteins to assign function. The EST contigs with no BLAST hit were aligned on the reference sequence of *Oryza sativa* Indica Group (ASM465v1) in gramene data base using BLAT with 97% similarity (Kent, 2002). The length of EST contigs aligned on the rice genome increased by 1 kb upstream and downstream to predicting the structure of genes with transcription initiation sites (TSS), extreme polyA tails, and coding sequences (CDS). The FGENESH program is used for the above analysis (Salamov & Solovyev, 2000).

Promoter analysis of candidate genes

The above candidate genes were validated by examining the promoter region of the putative predictive gene for cis regulatory elements using PlantPAN website (http://PlantPAN2.itps.ncku.edu.tw). The server contains the information regarding the experimentally validated transcription factor binding sites which will be used for prediction of cis regulatory sequence in the putative genes (Chow *et al.*, 2015).

Results and Discussion

In our study, A total of 10746 EST sequences related to indica rice drought tolerance were downloaded from Gene Bank of NCBI. The online server EGassembler was used to process the EST into contigs and singletons for further analysis (Bhati *et al.*, 2016). Out of total ESTs, 149 ESTs with low quality, short sequences are eliminated in the first step of EG assembler. The remaining 10597 EST sequences were then further analysed to look for repetitions and low-complexity sequences (Bhati *et al.*, 2016). As a result, the total elements masked and cut are 139711 bp (2.50 percent) of the query sequence's overall length. The remaining EST sequences were then assembled into 1120 contigs and 5559 singletones using the CAP3 programme, which is part of the same programme(Table 1). The clustering of EST sequences reveals the number of genes, their content, and the number of gene families implicated in stress reactions (Bhati *et al.*, 2016; Nahas *et al.*, 2019). Assembled ESTs account for only 10.42% of the total ESTs utilized which are in accordance with earlier reports (Bhati *et al.*, 2016; Nahas *et al.*, 2019). Singleton represents expressed transcripts that cannot be assembled into larger contigs to absence of overlapping sequences (Nahas *et al.*, 2019). These singletons are the product of only one mRNA of a gene therefore not considered for further analysis (Nahas *et al.*, 2019). Remaining 1120 contigs were submitted to online tool PlantTFcat (http://plantgrn.noble.org/PlantTFcat) to find the transcription factors (TF) responsible for activation of downstream genes responsible for drought tolerance (Nahas *et al.*, 2019) . From the above analysis, Sixty two contigs were found to be putative transcription factors and sorted into 17 putative TF families. Among the 17 TF families, TF Hap3/NF-YB constitute nearly 20 % followed AUX-IAA (8 %,) C2H2 (8 %), Znf-LSD (6 %), C3H (5%), CCHC(Zn) (5%)and GRAS (5%) (Figure 1)TF plays important role in gene expression of downstream genes upon signal perception under abiotic stress (Yuan *et al.*, 2016).

Because transcription factors operate as master regulators directing the expression of multiple target genes, they are the most promising candidates for unravelling the molecular mechanism underpinnings of abiotic stress responses (Nakashima *et al.*, 2009). The present study has found AUX-IAA transcription factor involved in drought stress. A recent study has revealed that auxin play major role in abiotic stress response by signal transduction and modulating the expression of multiple abiotic stress-related genes through interactions with auxin responsive factor and altered antioxidant enzyme activities (Shani *et al.*, 2017; Luo *et al.*, 2018). It was also found that the Aux/IAA rice OsIAA6 and OsIAA20 gene is regulated under water stress and its overexpression in transgenic rice improved drought tolerance through up regulation of auxin biosynthesis (Zhang *et al.*, 2021).

The utilization of these genes will be of prime importance in breeding drought stress tolerance in rice. The present study also found genes encoding for NUCLEAR FACTOR Y (NF-Y) transcription factors which are found in higher eukaryotes and belong to the CCAAT-binding factor (CBF) family and also known as the heme-activating protein (HAP) family. Evidence suggests that NFY subunits are important regulators of abiotic stress in plants (Nelson *et al.*, 2007).The overexpression of gene *GmNF-YA3* and *NFYA5* in *Arabidopsis thaliana* enhanced drought tolerance

Table 1: Summary of EST analysis conducted with EGassembler

Feature	Numbers
Total number of ESTs	10746
EST total nucleotides (nt)	5580409 bp
Singleton	5559
Contig	1120
Average GC content (%)	47.95

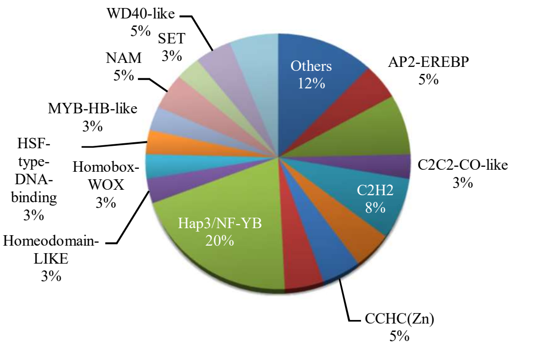

Figure 1: Distribution of TFs in the EST-contigs

(Ni *et al*., 2013). Similarly, in another study, transgenic maize and Arabidopsis expressing the gene NFYB1 showed significantly improved drought tolerance and yield under drought stress conditions (Nelson *et al*., 2007). Recently, Su *et al*. (2018) identified that interaction of ZmNF-YA3 to the promoter region of ZmMYC4, ZmbHLH92 and ZmFAMA in maize improves temperature and drought tolerance. C2H2 zinc finger proteins play a role in plant response low temperatures, salt, drought, and oxidative stress through ABA-dependent and ABA-independent pathways (Kim *et al*., 2013, Muthamilarasan *et al*., 2014). SCOF-1, a soybean cold-inducible C2H2 zinc finger transcription factor operates underlow-temperature tolerance (Kim *et al*., 2001). Furthermore, C2H2 proteins improved rice drought resistance by modulating ROS-scavenging activities, proline, H_2O_2 and other cellular components.The ZFP245 a C2H2-type zinc finger protein was expressed in the roots, stems, leaves and panicles of rice under drought stress and believed to play big role in tolerance (Huang *et al*., 2005). In soybean over expression of *GmGRAS37* in root hairs has improved the resistance to drought and salt stresses (Wang *et al*., 2020). PAT1, a GRAS member from wild grape, improved Arabidopsis abiotic stress tolerance (Czikkel and Maxwell, 2007). Likewise, GRAS23 regulates stress related gene expression in rice,resulting in drought resistance and oxidative stress tolerance, (Xu *et al*., 2015).The transcription factors found in present study could be be an excellent candidates for breeding transgenic rice cultivars tolerant to abiotic stress. Moreover, genetic engineering of multiple stress regulatory TF genes would be more rewarding for enhancement of stress tolerance in plants compared to focusing on a single individual gene.

Candidate gene prediction from EST-contigs:

Of the 1120 ESTcontigs, 37contigs with low nucleotide number i.e less than 300 were discarded from analysis. The remaining 1083 contigs were subjected to BLASTX in NCBI database of non-redundant proteins for functional annotation. The analysis showed that 63 contigs have no BLASTX hit and which were used for prediction of candidate genes responsible drought tolerant in *oryza sativa.*, these 63 contigs were aligned against the genome sequence of *Oryza sativa* Indica Group (ASM465v1) in gramene database using BLAT at 97% similarity to find the genomic position and chromosomal location. The results showed that, six contigs could not be aligned onto the reference genome were excluded from further analysis. The remaining 57contigs that were aligned with the reference genome and 1 kb upstream and downstream of the aligned sequence were retrieved for gene prediction and promoter analysis. Most of the contigs were located on chromosome 5 followed by chromosome 2, 7, 9 and least were present on chromosome 11 followed by 10 (Figure 2). Bhati *et al*. (2016) also found the similar results where in the chromosome 5 has more number of genes. The online tool FGENESH was used to predict the gene structure with transcription start site, PolyA tails at the extremes and coding sequence. The analysis showed 11 contigs viz., 118, 234, 248, 347, 381, 396, 429, 441, 841, 893 and 937 had no potential gene features and which were discarded from further analysis. Rest forty six contigs out of 1120 contigs were considered as putative novel genes which may contribute to the drought tolerance in indica rice. The coding position of new genes was presented in Table. 2. These identified genes my be mined for presence of cis regulatory elements and after which the genes are validated by designing new primers.

Table 2: The coding position of new genes

Contig Id	Chr. No.	Start (bp)	End (bp)	Length	Strand
Contig_2	4	23978692	23983123	4431	-
Contig_21	8	26499274	26501606	2332	-
Contig_74	7	17603491	17606469	2978	+
Contig_104	2	2142088	2144469	2381	-
Contig_137	5	5775957	5778539	2582	+
Contig_203	6	2645280	2647846	2566	+
Contig_205	9	17328039	17330067	2028	+
Contig_288	7	27754522	27757192	2670	+
Contig_294	6	23347486	23349696	2210	-
Contig_296	12	13766817	13769009	2192	+
Contig_324	5	8054078	8056647	2569	+
Contig_420	6	2180471	2183793	3322	+
Contig_464	2	6289181	6291445	2264	+
Contig_467	2	24254842	24256863	2021	+
Contig_481	9	15833257	15835862	2605	+
Contig_549	5	27764216	27766696	2480	+
Contig_571	4	32070044	32072546	2502	+
Contig_613	5	8053336	8055955	2619	-
Contig_620	3	1037582	1040151	2569	+
Contig_629	2	867386	869776	2390	+
Contig_678	3	4747269	4749892	2623	+
Contig_686	2	3849536	3851979	2443	-
Contig_710	2	26687427	26689869	2442	+
Contig_717	5	24238623	24241051	2428	-
Contig_718	4	27115876	27118418	2542	+
Contig_753	2	10305179	10307640	2461	+
Contig_776	6	17705156	17707616	2460	+
Contig_808	7	16778769	16781223	2454	+
Contig_812	5	549129	551455	2326	-
Contig_819	2	1197427	1199789	2362	+
Contig_823	2	1880223	1882592	2369	+
Contig_875	1	35680354	35682715	2361	+
Contig_880	5	3523583	3525971	2388	+
Contig_886	5	24047816	24050142	2326	-
Contig_888	12	20813518	20815830	2312	-
Contig_913	3	34335432	34337804	2372	-
Contig_920	9	17365261	17367637	2376	-
Contig_941	10	6254250	6256940	2690	+
Contig_950	1	3021484	3023781	2297	+
Contig_963	9	863254	865573	2319	+
Contig_967	8	26472569	26474872	2303	+
Contig_973	9	3131850	3134173	2323	+
Contig_976	5	5805432	5807745	2313	-
Contig_1063	5	22565074	22567427	2353	+
Contig_1080	3	37596768	37599101	2333	+
Contig_1082	7	20368825	20371141	2316	+

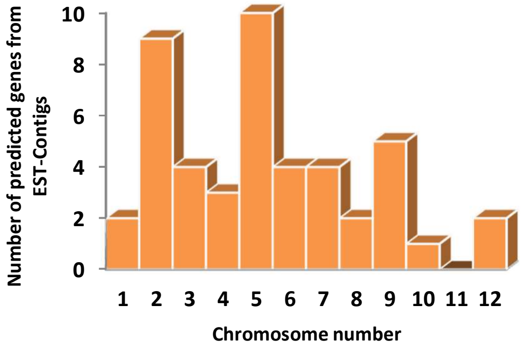

Figure 2: Number of predicted gene on different chromosome of rice using FGENESH.

Conclusion

Rice being a model crop, large sets of data is being generated on various omic approaches like genomics and proteomics. The generated data is being maintained in various databases like Gramene, NCBI, Phytozome etc., which can further utilized for in silico mining to discover new putative genes and so on for understanding the gene regulatory network and also for development of stress tolerant crops. The current investigation was focused on identification of novel putative drought responsive genes in rice using in-silico approach.The present study identified forty six putative genes with unknown functions which may directly and indirectly involved in the drought stress tolerance mechanisms. There is a need to find the functions of the newly identified putative genes with knockout technology to have comprehensive knowledge about the candidate genes and to understand the molecular mechanisms underlying the stress tolerance which in turn helps in breeding rice crop tolerant to drought stress through genetic engineering.

Conflict of interest

The authors declare that they have no conflict of interest.

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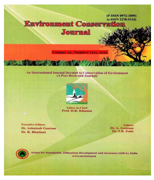

Production and characterization of briquettes made from rice straw and sawdust under high pressure and high temperature conditions

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ABSTRACT

Briquettes offer good value in terms of energy density. Briquetting of sawdust with paddy straw is a ray of hope for paddy straw waste utilization, which has been a burning issue in India for a long time. A commercial briquetting machine was used to make briquettes of sawdust with paddy straw in two different forms—shredded and chopped, in different ratios, separately. The ratio of paddy straw in briquettes was increased until the produced briquettes had an acceptable firmness. The maximum ratio of shredded paddy straw for which briquetting was possible was 40:60 with sawdust, whereas for chopped paddy straw it was 60:40 with sawdust. The briquettes were then analysed for different properties to assess their quality and durability. The shatter index of shredded straw briquettes and chopped straw briquettes varied from 0.10–10.12% and 0.26–4.54%, respectively. The friability index of shredded straw briquettes was in the range of 93.54-99.85%, whereas for chopped straw briquettes it was in the range of 89.89-99.90%. The volatile matter of shredded straw briquettes ranged from 79.60-83.89%, whereas for chopped straw briquettes it ranged from 76.69-86.07 %. The ash content of shredded straw briquettes was in the range of 9.83-13.07%, whereas for chopped straw briquettes, it was in the range of 6.60-16.44%. The fixed carbon content of shredded straw briquettes varied from 0.09 to 0.87%, whereas for chopped straw briquettes it varied from 0.11 to 0.59%. The results suggested that the densification experiments were successful and the briquettes produced were of good quality.

Introduction

There are increasing concerns about pollution and climate change through the use of fossil fuels. Coupled with their exhaustible nature, there is a pressing need to shift focus towards renewable sources of energy. A study has estimated that the reserves of coal, oil, and gas will be depleted by 2112, with coal remaining as the sole fossil fuel source after 2042 (Shafiee & Topal, 2009). Fossil fuels contribute heavily to air pollution, leading to increased mortality risks. It is estimated that fossil fuel-related emissions account for 65% of excess mortality. They are also shown to contribute to 70% of climate cooling (Lelieveld *et al.*, 2019). Biomass energy is seen as an alternative and is an attractive renewable resource. Currently, it contributes to 10–14% of the world's primary energy needs, with the potential to contribute up to 30-40% by 2050 (Rosillo-Calle, 2016). Agricultural residues are a major source of energy production. On a global scale, residue production of the six most important crops—barley, maize, rice, soybean, sugarcane, and wheat—is estimated to be 3.7 Pg. dry matter y^{-1}. The theoretical energy production from this amount is estimated to be 65 EJ y^{-1}(Bentsen *et al.*, 2014). In

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India itself, the potential for the utilisation of agricultural residues is huge. The Ministry of New and Renewable Energy estimates that India generates 500 million tonnes of crop residue per year, of which 92 million tonnes are burned (NPMCR, 2014). Currently, a lot of agricultural residue produced goes to waste, and farmers find burning the residue a convenient method to deal with it.

Stubble burning is a major problem in India as it is a significant source of gaseous pollutants like CO_2, CO, NO_x, SO_x, methane and particulate matter (Sain, 2020). This causes all sorts of health hazards, such as chronic obstructive pulmonary disease (COPD), bronchitis, cancer, etc. Utilizing this waste residue effectively is essential towards alleviating these problems (Anonymous, 2019). There are myriad ways to develop fuels from this waste, including the production of bioethanol, biodiesel (liquid fuels), bio-methane (gaseous fuel) and pellets and briquettes (solid fuels). Biomass densification into pellets or briquettes is used for improved utilisation of agri-residue waste. Densification confers multiple advantages such as improved handling, storage, and lower cost of transportation along with a reduction in particulate matter emission, higher calorific value, and uniform rate of combustion (Kaliyan & Vance Morey, 2009; Purohit *et al.*, 2006). Thus, briquettes are an attractive fuel source for the utilisation of agri-residue. Considering these advantages, this study aims to produce briquettes from paddy straw and analyses the properties to determine their attractiveness as alternative sources of fuel.

Material and Methods

The briquettes were prepared from sawdust in different combinations with paddy straw in different forms. Sawdust was procured from a local market and cleaned via sieving (sieve pore size of 1 mm) before making briquettes. The paddy straw was selected in two forms: shredded straw and chopped straw. The shredded straw had a thread-like structure. The chopped straw was prepared from full-sized paddy straw. The whole paddy straw was cut into smaller pieces by a regular chaff cutter. The briquettes were prepared in a commercially available briquetting machine (Model: EcoStan 40; Fig. 2). Briquettes were produced using this machine (Fig. 2) using rice straw (shredded and chopped) separately (Fig. 1.a & 1.b) with sawdust in various combination ratios (Table 3 and 4). The die temperature, an important parameter in briquetting, is also recorded. The proportion of straw is increased until the briquettes are made. The briquettes were analysed preliminarily for their firmness by hand. The following tests on briquettes samples were performed:

Figure 1a: Raw Material for Briquettes- Chopped Straw
Figure 1b: Raw Material for Briquettes- Shredded Straw).
Note:The smallest graph paper square size- 1mm x 1mm.

Figure 2: Briquetting machine

Moisture content: The briquette samples were powdered, and 1 g of each was baked for 1 hour at 105 °C in a hot air oven (ASTM D3173). Moisture content was calculated by a formula:

Moisture content, % = 100 * (W_1- W_2) / W_1

where,
W_1= weight of sample before placing into the oven
W_2= weight of sample after placing into the oven

True density: The briquettes were weighed and dipped into the distilled water contained in a graduated cylinder (Stamm, 1928). The increase in volume (ΔV) was immediately noted. The true density was measured by the following formula:

$$\text{True density} = 100 * W / \Delta V$$

where, W= weight of briquette sample before dipping into water

Shatter index: The Shatter Index is used to assess the hardness and friability of the briquettes. The briquette samples were dropped on a concrete floor from a height of one meter. The weight of the largest portion of the briquette after chipping out of material due to impact was noted. The percentage loss of material is calculated by formula (Rajaseenivasan *et al.*, 2018):

$$\text{Shatter index} = 100 * (W_1 - W_2) / W_1$$

Where,
W_1, W_2 = weight of briquette before and after shattering, respectively, g.

Friability index: The Friability index is a drop test for assessing the durability of the briquettes. The Friability index test is used for determining the strength and hardness of briquettes. Briquette samples were dropped from a height of 1.86 m onto a concrete floor, and the weight of the largest piece of briquettes remaining was recorded (Henning *et al.,* 2018).

$$\text{Friability index} = 100 * W_2 / W_1$$

where, W_2= weight of largest piece of briquette after impact, g
W_1 = weight of briquette before impact, g

Water resistance: To measure water resistance, the briquette samples were immersed in water maintained at the atmospheric temperature for 30 s to determine the percentage of water resistance to penetration (Davies & Davies, 2013). The water resistance indicates how the briquettes will respond with respect to moisture absorption. Water resistance was calculated with the formula:

$$\text{Water resistance} = 100 - \{(M_2 / M_1) * 100\}$$

where, M_2= Mass of briquette after 30 s water dip, g
M_1= Mass of briquette before 30 s water dip, g

Volatile matter: The powdered 1 g fresh sample of briquettes in a crucible was placed in a muffle furnace for 950±20 °C for 7 minutes (ASTM D3175-07). The volatile matter was calculated from the formula:

$$\text{Volatile matter} = \{100 * (W_2 - W_1 / W_1)\} - MC\ (\%)$$

where,
W_2= weight of briquette sample before placing into furnace, g
W_1 = weight of briquette sample after placing into furnace, g

Ash Content: The powdered 1 g fresh sample of briquettes in a crucible was placed in a muffle furnace for 950±20 °C for 2 hours (ASTM D3174-02). The samples were then taken out and placed into the desiccator to cool down. The volatile matter was calculated from the formula:

$$\text{Ash content} = 100 * (W_2 - W_1 / W_1)$$

where, W_2= weight of briquette sample before placing into furnace, g
W_1= weight of briquette sample after placing into furnace, g

Fixed Carbon: Fixed carbon is calculated by formula (ASTM D3172-07a):

Fixed Carbon, % = 100 – (Moisture content, % + Ash Content, % + Volatile matter, %)

Results and Discussion

Physical properties of the raw material

The physical properties of the raw material were studied and presented in Table 1. Chopped paddy straw has a higher bulk density than shredded straw due to its better uniformity in particle size. Saw dust has a bulk density similar to the research reported by Retana *et al.* (2019) but less than reported by Stasiak *et al.* (2019) and Trzciski *et al.,* (2021). It is quite obvious that sawdust made from different woods has a different bulk density. The paddy straw in shredded and chopped form has a maximum ash content of 14.8%. Similar results were also reported by El-Sayed and El-Samni, 2006 (15%); Ngi *et al.*, 2006 (18.1%); and Sarnklong *et al.*, 2010 (12.1%). As ash content is a material property, it is not affected by different sizes of paddy straw. The end results of different combinations are presented in Table 2 (sawdust + shredded paddy straw) and in Table 3 (sawdust + chopped paddy straw). It can be concluded from Tables 2 and 3 that paddy straw in chopped form can easily be blended with sawdust up to a 60% concentration, which offers good utilisation of paddy straw with sawdust. Similar results were reported by Alo *et al.* (2017) for sawdust with bagasse mixture. Shredded straw could be added in a 40% concentration to sawdust for the preparation of acceptably firm briquettes. The briquettes made were analysed for their different properties. The end results of the experiments are discussed below:

Table 1: Physical properties of raw material

	Shredded straw	Chopped straw	Sawdust
Bulk density, g/cc	0.04-0.05 ± 0.4	0.06-0.07 ± 0.4	0.24-0.27 ± 0.4
Moisture content, %	8.3-8.7 ± 0.1	7.0-7.3 ± 0.1	8.5-8.8 ± 0.1
Ash content, %	11.8-14.8 ± 0.1	13.0-14.2 ± 0.1	10.7-11.7 ± 0.1

Table 2: Output of different combinations of shredded straw and sawdust

Sr. No.	Sawdust, %	Shredded Rice Straw, %	Die temperature, °C	Result of the manual inspection for produced briquettes
1.	100	0	105	Acceptable firmness
2.	90	10	104	Acceptable firmness
3.	80	20	102	Acceptable firmness
4.	70	30	104	Acceptable firmness
5.	60	40	104	Acceptable firmness
6.	50	50	103	Acceptable firmness

Table 3: Output of different combinations of chopped straw and sawdust

Sr. No.	Sawdust, %	Chopped Rice Straw, %	Die temperature, °C	Result of the manual inspection for produced briquettes
1.	100	0	105	Acceptable firmness
2.	90	10	101	Acceptable firmness
3.	80	20	101	Acceptable firmness
4.	70	30	104	Acceptable firmness
5.	60	40	103	Acceptable firmness
6.	50	50	102	Acceptable firmness
7.	40	60	103	Acceptable firmness
8.	30	70	104	Not Good. Got crumbled when taken into hand (fluffy nature)

Moisture content

The moisture content of different types of briquettes made is given in Table 4 and 5. The comparison of the average moisture contents of shredded straw + sawdust and chopped straw + sawdust briquettes is given in Fig. 3. The moisture content of briquettes made from shredded material plus sawdust had a lower moisture content than the chopped straw plus sawdust combinations. It is due to the structure of the paddy straw in two forms. The chopped straw retained more moisture due to its open pore structure (Mesa & Arenghi, 2019). The shredded straw had thread-like filaments and hence offered little moisture-holding capability. The little higher moisture content of some of the briquettes than raw material can be attributed to the humidity of the environment during experiments as the briquetting machine was placed in an open area. The pure sawdust briquettes are more affected due to this effect (Glass *et al.,* 2010).

True density

The true density of different types of briquette samples is given in Table 4 and 5. The comparison of the average true density of shredded straw +

sawdust and chopped straw + sawdust briquettes is given in Fig. 4. The true density of briquettes made from shredded straw was greater than those made from chopped straw. This can be attributed to the threaded structure of the shredded straw. In the case of chopped straw briquettes, the density first decreases and then increases. The first decrease might be due to the decrease in the proportion of the sawdust, and the second increase is due to the better embodiment of chopped straw in the sawdust. In comparison to pure sawdust, the density was on the higher side due to the better packing of paddy straw in sawdust.

Based on the experimental results, it can be easily interpreted that the density of all briquettes lay between 0.79 and 1.35 g/cc, which is much denser than raw materials. Jittabut (2015) found the density of briquettes made from rice straw and sugarcane leaves with molasses as the binding agent in the range of 0.53-0.58 g/cc, whereas Urbanoviová *et al.* (2017) found the density of briquettes produced from energy plants in the range of 0.80-0.90 g/cc. The higher density of the briquettes in the present study may be attributed to the high pressure applied by the machine (Mani *et al.,* 2006). Also, the briquettes prepared were about 6–9 times denser than

Table 4: Properties of briquettes made from Shredded Paddy Straw + Sawdust

SN	Shredded straw, %	Sawdust, %	Shatter index, %	Friability Index, %	True Density, (g/cc)	Water resistance, %	Moisture content (%)
1	0	100	0.16-0.68	98.76-99.32	0.89-1.14	83.96-87.45	8.75-9.26
2	10	90	0.13-0.20	99.54-99.66	1.27-1.35	85.18-93.43	4.60-6.56
3	20	80	0.41-2.04	99.63-99.80	1.05-1.06	76.39-84.09	5.64-7.33
4	30	70	0.10-0.45	94.78-99.36	1.14-1.16	78.10-89.18	3.40-6.34
5	40	60	0.13-10.12	93.54-99.85	0.75-0.98	71.46-87.30	5.34-7.36

Table 5: Properties of briquettes made from Chopped Paddy Straw + Sawdust

SN	Shredded straw, %	Sawdust, %	Shatter index, %	Friability Index, %	True Density, (g/cc)	Water resistance, %	Moisture content (%)
1	10	90	0.26-1.22	97.89-99.66	1.09-1.21	88.51-92.50	5.27-6.55
2	20	80	0.47-0.73	97.89-99.18	0.79-1.00	76.34-83.40	6.27-7.57
3	30	70	0.21-0.49	99.04-99.90	0.77-0.85	76.37-78.44	8.67-8.73
4	40	60	0.85-4.54	96.64-99.61	0.80-0.96	89.62-93.51	6.86-8.37
5	50	50	0.63-0.76	89.89-99.34	0.86-1.02	85.44-87.73	7.15-8.42

Table 6: Proximate analysis of briquettes made from Shredded Paddy Straw + Sawdust

SN	Shredded straw, %	Sawdust, %	Volatile Matter, %	Ash Content, %	Fixed Carbon, %
1.	0	100	86.34	4.39	0.11
2.	10	90	80.92	13.39	0.11
3.	20	80	82.73	10.51	0.28
4.	30	70	82.32	12.00	0.81
5.	40	60	82.10	10.96	0.60

Table 7: Proximate analysis of briquettes made from Shredded Paddy Straw + Sawdust

SN	Shredded straw, %	Sawdust, %	Volatile Matter, %	Ash Content, %	Fixed Carbon, %
1.	10	90	78.89	15.04	0.16
2.	20	80	82.77	10.00	0.31
3.	30	70	83.75	7.26	0.30
4.	40	60	84.23	7.76	0.40
5.	50	50	84.92	6.94	0.36
6.	60	40	82.94	10.52	0.57

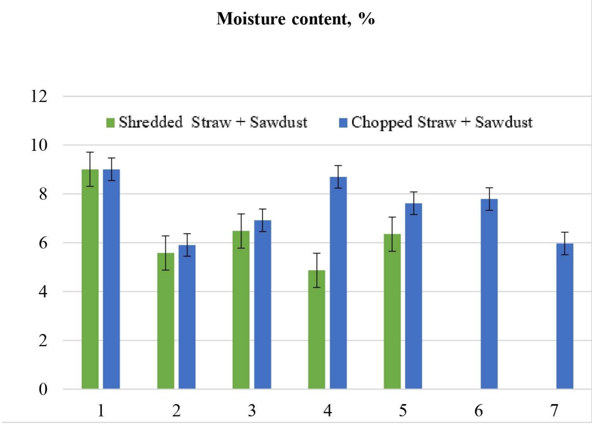

Fig. 3: Moisture content variation of different briquettes samples

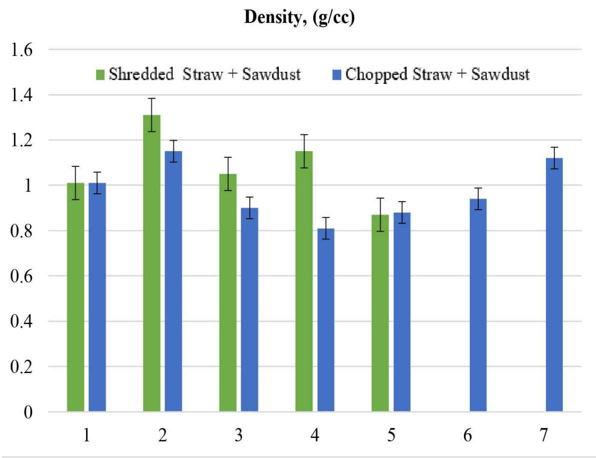

Fig. 4: Density variation of different briquettes samples

sawdust; 19–29 times denser than shredded paddy straw; and 14–18 times denser than chopped paddy straw, all three of which were used as raw materials.

Shatter Index

The shatter index of different briquette samples is given in Table 4 and 5. The comparison of the average shatter index of shredded straw + sawdust and chopped straw + sawdust briquettes is given in Fig. 5. The shatter index of different briquettes doesn't depict any definitive trend. The shatter index of the briquettes of all combinations was in the range of 0.21-1.26, which was better than that found for sawdust and rice husk combinations, having a 9.6-27.6 (Tembe *et al.,* 2014). The shatter index of the 40:60 combination of both forms of paddy straw showed a high shatter index. It might be due to the looseness caused by the paddy straw in the briquette structure.

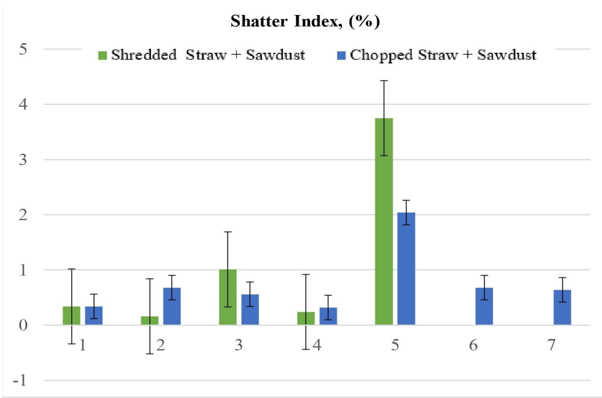

Fig. 5: Shatter index variation of different briquettes samples

Friability index

The friability index of different briquette samples is presented in Tables 4 and 5. The comparison of the average friability index of shredded straw + sawdust and chopped straw + sawdust briquettes is given in Fig. 6. The friability index of all samples was above the recommended minimum threshold friability index value of 80% (Richards, 1990). This demonstrated the good durability of the briquettes. The friability index of all briquettes decreased as the proportion of straw increased. This could be due to the looseness of paddy straw in comparison to sawdust. The decrease is more pronounced in comparison to chopped straw briquettes.

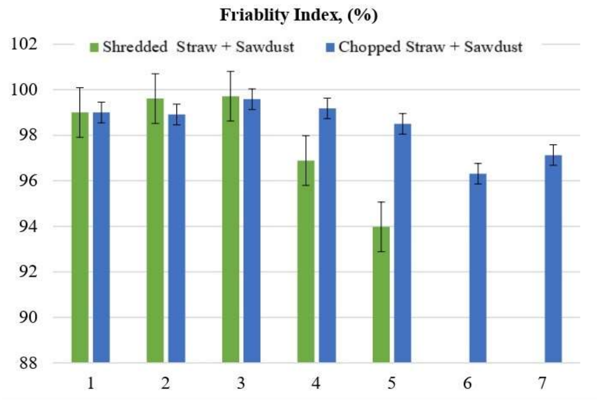

Fig. 6: Friability index variation of different briquettes samples

Water resistance

The water resistance of different briquette samples is shown in Table 4 and 5. The comparison of the average water resistance of shredded straw + sawdust and chopped straw + sawdust briquettes is given in Fig. 7. The water resistance of all briquette samples was below the recommended minimum water resistance of 95% (Richards, 1990) but similar to values obtained by Rajaseenivasan *et al.*, 2018.

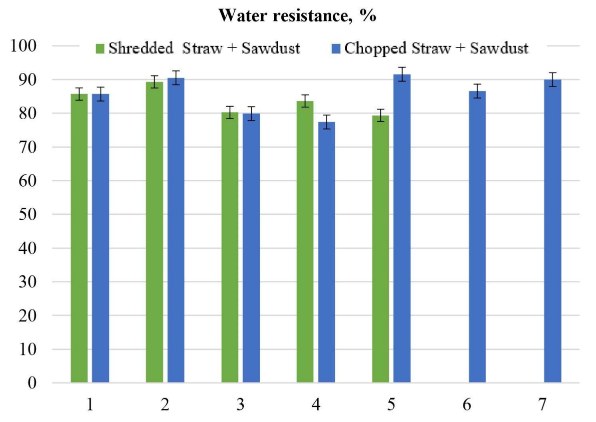

Fig. 7: Water resistance of different briquettes samples

Volatile matter, ash content and fixed carbon content

The average values of volatile matter, ash content, and fixed carbon content of different briquettes samples are shown in Tables 6 and 7P. The volatile matter of shredded straw briquettes ranged from 79.60-83.89%, whereas for chopped straw briquettes, it ranged from 76.69-86.07%. The ash content of shredded straw briquettes ranged from 9.83-13.07%, whereas for chopped straw briquettes it ranged from 6.60-16.44%. The increased ash content is due to the increased proportion of the paddy straw, which contains higher inorganic matter (El-Sayed *et al.,* 2006). The fixed carbon content of shredded straw briquettes ranged from 0.09-0.87%, whereas for chopped straw briquettes it ranged from 0.11-0.59%.

Conclusion

The briquettes formed by the commercial briquetting machines had a density of between 0.87 and 1.31 g/cc, which is much denser than raw material and hence offers a good method for energy densification of biomass. Due to the lower moisture content of the briquettes formed in comparison to raw material, they offered improved net heat content. Briquettes also had good strength and durability, as evidenced by their low shatter index and high friability index. Water resistance of most of the briquettes from different combinations was also in an acceptable range but showed scope for improvement. Tests on compressive strength, calorific value, and emission characteristics will be needed to characterize the briquettes further and determine their suitability in commercial combustion operations as a replacement for traditional fuel.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Genetic variability studies in field pea (*Pisum sativum* L.) for yield and associated characters

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ABSTRACT

The prime and foremost objective of the study was to estimate the genetic variability for yield and its associated characteristics among the 23 genotypes of field pea and also to engender information regarding genetic parameters like range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as a percentage over means for the18 traits under consideration. It was disclosed from the results of the analysis of variance that there exists a difference among the 23 genotypes for all the 18 traits under consideration. The trait including primary branches per plant, secondary branches per plant, plant height, swelling capacity, swelling index, biological yield per plant, and seed yield recorded high amounts of GCV and PCV. High heritability combined with high genetic advance was documented for the trait's plant height and seeds per plant, whereas high heritability coupled with low genetic advance, was recorded for the trait's days to fifty % flowering, days to maturity, primary branches per plant, secondary branches per plant, pod length, 100 seed weight, percent disease incidence and seed yield per plant. The existing variability identified can be further exploited in crop improvement of field pea.

Introduction

Pisum sativum L. (2n=2x=14) could be a diploid annual autogamous crop that belongs to Leguminosae or Fabaceae family, taxon rosid, dicot family, and tribe vicieae. Field pea is native to southwest Asia and is extensively cultivated in temperate climates. It is an atmospheric condition crop that may withstand light-weight frost. The peas under cultivation are of two types i. e field pea (*Pisum sativum* L. var. arvense) which is additionally referred to as dry pea and the other is garden pea (*Pisum sativum* L. var. hortense) which is additionally referred to as table pea. Field peas are grown for a variety of uses, including seed, feed, silage, and green compost. It contains a lot of high-quality protein. It is a low-cost source of proteins (especially tryptophan and lysine; 21–25 percent), complex carbohydrates, high fiber (soluble and insoluble), B vitamins, folate, and mineral content, including calcium, iron, and potassium, with 86–87 percent total digestible nutrients and very low sodium and fat content (Tiwari and Singh, 2012). Peas are also thought to be a good source of biologically active components with possible health benefits such as reducing the prevalence of colon cancer, coronary diseases, and type-2-diabetes (Kour *et al.*, 2020). Peas are an essential agricultural crop because of their beneficial characteristics such as high-value nutrient composition due to the presence of carbohydrates, proteins, vitamins, and minerals. Field pea is also having other desirable characteristics due to the presence of phytochemicals, antioxidants, anti-nutritional factors, and in-vitro digestibility (Kumari and Deka, 2021). But due to the restricted genetic base and low

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variability utilized for the improvement of the yield; the productivity of peas was low when compared to the world productivity. The existing degree of variability in the germplasm will have a significant impact on the progress of the crop improvement program. Genetic variability studies are required to properly utilize the existing variability and explore the unknown variability in various forms. Heritability is the measure of trait transmission from the parents to their progeny. It is used to determine the amount of the genetic component of total variability and the approach necessary for crop improvement through character selection. Heritability has a vital role in the selection of genotypes in which the resemblance of characters yields in their progeny. Once the study has been completed the genetically diverse genotypes are included in the breeding programs for yield enhancement.

Material and Methods

The study was steered at a research farm, department of genetics and plant breeding, Birsa agricultural university, kanke, Ranchi, in the Kharif season of the year 2020-2021. The material to be analyzed i.e., 23 genotypes was collected from various agricultural universities and research stations located throughout India (Table-1). The experiment was carried out in three replications in a randomized block design. The genotypes were grown in a plot having 6 rows each of 4meter length. The Row to row spacing was 45cm, while plant-to-plant spacing was 15cm. During the crop growth, all agronomic measures were followed to ensure a healthy crop. During the period of the experiment, observations were documented for 18 traits, i.e., Days to fifty percent flowering, days to pod initiation, days to maturity, primary branches per plant, Secondary branches per plant, plant height, pods per plant, pod length, seeds per pod, seeds per plant,100 seed weight, swelling capacity, swelling index, protein content, harvest index, biological yield per plant and seed yield per plant. The mean values of 5 randomly selected plants from each treatment were utilized for statical analysis such as analysis of variance. The magnitude of genetic variability was estimated following the standard procedures and the phenotypic (PCV) and genotypic (GCV) coefficients of variation were estimated as suggested by Singh and Chaudhary (1985) whereas heritability (broad sense) as followed by Allard (1960) and genetic advance were estimated by the methods given by Johnson *et al.*. (1955). The modified Micro-Kjeldahl technique was used to determine the quantity of protein present in field pea seeds of all samples. The distilled samples were titrated against 0.1N hydrochloric acid until a violet tint appeared as the endpoint. The titration value was used to calculate the percent Nitrogen, which was then used to estimate total protein concentration using a conversion factor of 6.25 percent.

$$\textbf{Nitrogen}\ \% = \frac{\text{Normality} \times (\text{Volume of hydrochloric acid} - \text{Volume of blank}) \times 14 \times 100}{\text{Sample weight (gm)} \times 1000}$$

Table 1: Name and Source of 23 Genotypes of Field pea (*Pisum sativum* L.)

S. No.	ENTRY	SOURCES
1	HFP 1607	CCS HAU, Hisar
2	HFP 1702	CCS HAU, Hisar
3	Pant P 498	Pantnagar, GPUAT
4	Pant P 501	Pantnagar, GPUAT
5	Pant P 497	Pantnagar, GPUAT
6	VL 72	VPKAS, Almora
7	RFPG 180	RARS, Durgapura
8	RFPG 181	RARS, Durgapura
9	KPMR 937	CSAUA&T, Kanpur
10	RFP 2010-1	Raipur
11	RFP 2010-4	Raipur
12	IPF 20-17	IIPR, Kanpur
13	IPF 20-21	IIPR, Kanpur
14	IPF 20-11	IIPR, Kanpur
15	TRCP-11	Agartala
16	RFP 12-08	BAU, Ranchi
17	TRCP 8	Agartala, Tripura (ICAR)
18	VL 42	VPKAS, Almora
19	SKNP 04-09	SDAU, SK Nagar
20	IPF 16-13	IIPR, Kanpur
21	HUDP-15 (ZC)	BHAU, Varanasi
22	Dantewada FP-1(LC)	SDAU, SK Nagar
23	Aman (NC)	IIPR, Kanpur

Results and Discussion

The existence of significant differences among the 23 genotypes of field pea for all the traits under consideration had been revealed from the results of the analysis of variance (Table2). The existing substantial quantity of genetic variability can be utilized in crop improvement programs.

The values of PCV, GCV, Heritability, Genetic advance, and Genetic advance as % of the meanare represented in the table 2.

Table 2: ANNOVA showing means squares for 18 characters in 23 genotypes of Field pea (*Pisum sativum* L.)

Source	D.f	Days to 50% flowering	Days to Pod initiation	Days to Maturity	Plant height (cm)	Primary Branches Per Plant	Secondary branches per plant	Pods Per Plant	Plant Height (cm)	Pod Length (cm)	Seeds Per Pod
		1	2	3	4	5	6	7	8	9	10
Replication	2	0.17	0.88	40.43**	0.095	0.036	0.17	22.50*	237.82	0.38**	0.22
Genotype	22	12.12**	3.17**	12.26**	0.57**	0.72**	12.12**	18.85**	1351.79**	1.30**	1.77**
Error	44	0.003	0.54	2.18	0.04	0.04	0.003	5.52	98.36	0.082	0.25

Table-2. (Contd.)

Source	D.f	Seeds per Plant	100 Seed Weight (gm)	Swelling Capacity (ml/seed)	Swelling Index (%)	Biological Yield per Plant (gm)	Seed Yield per Plant (gm)	Harvest Index	Protein Content (%)
		11	12	13	14	15	16	17	18
Replication	2	35.10	1.79	0.002*	0.03	12.02	3.34*	0.02*	0.09
Genotype	22	739.94**	15.39**	0.003**	0.13**	176.48**	17.49**	0.01**	3.21**
Error	44	91.89	0.93	0.001	0.02	15.14	0.75	0.003	0.67

Phenotypic and Genotypic Coefficient of Variability:

Among the various traits considered for investigation, the traits like primary branches per plant (54.47), secondary branches per plant (49.63), pods per plant (23.711), plant height (24.96), swelling capacity (25.47), swelling index (36.63), harvest index (22.93), biological yield per plant (35.75), seed yield per plant (33.30) are having high values of phenotypic coefficient of variation. The characters like pod length (12.23), and 100 seed weight (14.36) recorded a moderate amount of phenotypic coefficient of variation. The phenotypic coefficient of variation was low in the traits like days to 50% flowering (4.65), days to maturity (3.83), seed per pod (8.79), and protein content (4.70). Higher values of genotypic coefficient of variation are recorded for traits like primary branches per plant (49.44), secondary branches per plant (45.81), plant height (22.46), swelling capacity (20.89), swelling index (34.22), biological yield per plant (31.57), seed yield per plant (31.27).The characters like Pods per plant (15.84), pod length (11.15), 100 seed Weight (13.15), and Harvest Index (17.88) had a moderate amount of genotypic coefficient of variation. In the company of various traits days to 50% Flowering (4.36), days to maturity (3.57) seed per pod (6.37), and protein content (3.51) had a low amount of genotypic coefficient of variation. The characters primary branches per plant, secondary branches per plant, plant height, percent disease incidence, swelling capacity, swelling index, biological yield per plant, and seed Yield per plant recorded a high amount of GCV and PCV. The above traits are mostly under genetic control, which makes their phenotypic selection more reliable. These findings were in accordance with Sharma *et al.* (2003), Mehta *et al.* (2005), Kumar *et al.* (2013) and Saxesena *et al.* (2014), Ranjan *et al.* (2006). The traits pod length and 100 seed weight recorded a moderate amount of PCV and GCV but pods per plant and harvest index showed a moderate amount of GCV only. These results were in accordance with Lal *et al.* (2011). In the company of various traits days to 50% Flowering (4.3558), days to maturity (3.5720) seed per pod (6.3741), and protein content (3.5090) had a low amount of genotypic coefficient of variation. Thus, the selection for these traits may not be fruitful. These results were in acceptance by Singh *et al.* (2011), Patel (2012), and Yadav (2013).

Table 3: Genetic Parameters of 18 characters in 23 Genotypes of field pea

Characters	Genotypic Coefficient of variation	Phenotypic Coefficient of variation	Heritability in broad sense	Genetic Advance	Genetic advance as % of mean
Days to 50% flowering	2.54	3.011	71.09	3.276	4.41
Days to Pod initiation	1.07	1.36	62.08	1.520	1.73
Days to Maturity	3.26	3.53	85.37	6.796	6.120
Plant height (cm)	49.44	54.47	82.39	0.79	92.45
Primary Branches Per Plant	45.81	49.63	85	0.91	87.08
Secondary branches per plant	2.54	3.011	71.09	3.276	4.41
Pods Per Plant	15.84	23.71	44.6	2.90	21.78
Plant Height (cm)	22.46	24.96	80.94	37.88	41.62
Pod Length (cm)	11.15	12.23	83.04	1.188	20.93
Seeds Per Pod	14.99	18.28	67	1.21	25.34
Seeds per Plant	21.67	25.87	70.16	25.36	37.38
100 Seed Weight (gm)	13.15	14.36	83.86	4.13	24.81
Swelling Capacity (ml/seed)	20.89	25.47	67.22	0.05	35.27
Swelling Index (%)	17.42	22.67	59.17	0.31	27.59
Biological Yield per Plant (gm)	31.574	35.75	78.02	13.34	57.45
Seed Yield per Plant (gm)	31.271	33.30	88.17	4.57	60.49
Harvest Index	17.875	22.93	60.79	0.10	28.71
Protein Content (%)	3.509	4.702	55.69	1.41	5.40

Heritability, Genetic advance, and genetic advance as % of mean:

The traits possessing a high amount of heritability are days to 50% flowering (0.88), days to maturity (0.87), primary branches per plant (82), secondary branches per plant (85), plant height (0.81), pod length (0.83), 100 seed weight (0.84), swelling index (0.87), biological yield per Plant (0.78), seed yield per Plant (0.88). The traits with a moderate amount of heritability are seed per pod (0.53), swelling capacity (0.67), protein content (0.56), and harvest index (0.61). Finally, the only trait with a low amount of heritability is pods per plant (0.45). The genetic advance recorded among the various characters under consideration are as days to 50% flowering (6.31), days to maturity (7.53), primary branches per plant (0.80), secondary branches per plant (0.91), days to pod initiation(1.52), pods per plant (2.90), plant height (37.89), pod length (1.19), seed per pod (0.11), seeds per plant (25.36), 100 seed weight (4.13), swelling capacity (0.05), swelling index (0.66), protein content (1.41), harvest index (90.10), biological yield per plant (13.34), seed yield per plant 94.57). It was evident from the results of the analysis represented in table-3, that a high magnitude of Genetic advance as a percentage of mean was documented for the characters like primary branches per plant (92.45), secondary branches per plant (87.08), pods per plant (21.78), plant height (41.61), pod length (20.93), 100 seed weight (24.81), percent disease incidence (67.39), swelling capacity (35.27), swelling index (35.27), harvest Index (28.71), days to 50% flowering (8.40), days to pod initiation, days to maturity (6.87), biological yield per plant (57.45), seed yield per plant (60.49). There was no character with a moderate amount of genetic advance as a percentage of the mean among the considered ones. A low amount of genetic advance as a percent of the mean was observed for the characters like days to 50% flowering (8.40), days to maturity (6.87), and days to pod initiation (1.73).

High heritability and high genetic advance were recorded for the trait's plant height and seeds per plant.

These traits are less impacted by environmental fluctuations. Selection is simple since the above qualities are mediated by additive gene activity. These results were in accordance with Katoch *et al.* (2016) and Tambolkar *et al.* (2016). High heritability coupled with low genetic advance was recorded for the trait's days to fifty % flowering, days to maturity, primary branches per plant, secondary branches per plant, pod length, 100 seed weight, and seed yield per plant. Heterosis breeding was desirable for the improvement of these traits. These results were in accordance with Saxesena*et al.* (2014) and Sharma *et al.* (2013). Low heritability along with high genetic advance was observed in the case of pods per plant. The traits with a moderate amount of heritability with low genetic advance were observed in the case of the traits days to pod initiation, seeds per pod, swelling capacity, swelling index, protein content, and harvest index.

High heritability and moderate genetic advance were recorded for the trait biological yield per plant

High heritability with high genetic advance as % of mean was recorded for the trait's primary branches per plant, secondary branches per plant, plant height, seeds per plant, pod length, 100 seed weight, biological yield per plant, and seed yield per plant. Further improvement for the above traits can be made through selection using the above breeding lines. Traits possessing high heritability with low genetic advance as % of the mean are days to fifty % flowering and days to maturity. The traits with moderate heritability and high genetic advance as % of the mean are seeds per pod, swelling capacity, swelling index, and harvest index. Moderate heritability with low genetic advance as % of mean was observed in the traits days to pod initiation and protein content. Low heritability along with high genetic advance as % of mean was recorded by pods per plant. These results were in accordance with Dar *et al.* (2013).

Conclusion

The characters primary branches per plant, secondary branches per plant, biological yield per plant, and seed yield per plant recorded high phenotypic and genotypic coefficients of variation. These characters also possess high heritability coupled with high genetic advance as % of the mean which made the phenotypic selection based on these characters highly fruitful. Even though high heritability with high genetic advance as % of mean was recorded by the character lant height, seeds per plant, pod length and 100 seed weight, selection will be less beneficial as these traits possessed moderate and low phenotypic and genotypic coefficient of variations. Therefore, the selection criteria of the genotypes should be based not only on the genotypic and phenotypic coefficient of variation. But it is their combination with heritability and genetic advance as a percentage of the mean which is highly fruitful.

Conflict of interest

The authors declare that they have no conflict of interest.

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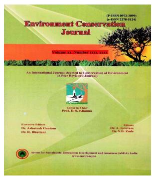

Effect of organic nutrient sources on the yield, nutrient uptake and nodulation in Cowpea (*Vigna unguiculata*) under mid-hill conditions of Western Himalayas

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ABSTRACT

Cowpea is *kharif* pulse grown mostly under rainfed conditions. It acts as a major protein source with 25 per cent content. Cowpea besides fixing atmospheric nitrogen continues to produce under harsh conditions such as low moisture and nutrient supply, which makes it a suitable candidate for dryland conditions. A field experiment was conducted during *kharif* 2019 at Research Farm, Department of Agronomy, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur to study the effect of organic nutrient sources on yield levels, nodulation, nutrient content and uptake of cowpea. Organic nutrient sources include farmyard manure, vermicompost, vermiwash, *Bijamrita*, *Jiwamrita* and *Ghanajiwamrita* can be prepared using on-farm inputs at a reasonable cost. These nutrient sources supply nutrients at a steady rate and in fewer amounts than inorganic fertilizers. The prime role of such organic sources is to sustain the soil ecosystems for longer functioning. The results of the experiment revealed that T_5 [Farm yard manure (10 t/ha) + *Ghanajiwamrita* at sowing (250 kg/ha)] was most effective and resulted in significantly higher yield level (grain yield -10.71 q/ha, straw yield - 53.14 q/ha and biological yield - 63.84 q/ha), nodulation (number of nodules - 31.7 per plant and weight of nodules - 0.47 g/plant), nutrient content (N- 3.54%, P - 0.41% and K - 1.36%) and uptake (N -141.08 kg/ha, P -19.86 kg/ha and K - 112.34 kg/ha) in cowpea.

Introduction

Post Green Revolution, India successfully achieved self-sufficiency in food grain production through increased productivity in cereal crops. The net availability of the food grains per capita per day increased from 144.1 kg year^{-1} in 1951 to 179.6 kg year^{-1} in 2019 whereas, in pulses, the net availability per capita per day decreased from 25 kg year^{-1} in 1961 to 17.5 kg year^{-1} in 2019 (Singh *et al.*, 2020). India is the largest producer as well as consumer of pulses, where production barely meets the consumption. The central reason for low pulse production is the lack of adequate inputs. These crops are generally preferred in rainfed areas resulting in poor productivity. Among pulses, Cowpea (*Vigna unguiculata*) is an important *kharif* crop that can be used either as a vegetable, grain or fodder crop. It is a major source of protein for the vegetarian populace containing about 25 per cent protein, 63.6 per cent carbohydrates, 1.9 per cent fat, 6.3 per cent fiber and vitamins such as thiamine (0.00074 per cent), riboflavin (0.00042 per cent) and niacin (0.0028 per cent) (Davis *et al.,* 2000). Due to

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its low input requirement, the appreciable survival rate in the semi-arid regions and its ability to fix atmospheric nitrogen makes it a valuable crop for resource-poor farmers and is also well-suited to intercropping with other crops.The increased production through conventional agricultural practices involved the use of high yielding varieties resulting in increased dependence on fertilizers and pesticides to tap their genetic potential but at the cost of soil degradation, environmental pollution and in the long run affecting human health. Considering the stakes, an alternative to chemical farming was inevitable and led to the emergence of a new agricultural production system. Under this system, the chemicals were replaced by farmyard manure, vermicompost, vermiwash, green manuring, etc. These help to increase the soil organic matter content, which ultimately improves the soil pH, structure, water holding capacity, cation exchange capacity, and availability of macro and micronutrients (Alabadan *et al.,* 2009). Besides organic farming, Subhash Palekars's Natural Farming is also an alternative to chemical farming wherein on-farm products are used as inputs by converting them into formulations such as *Jiwamrita, Bijamrita* and *Ghanjiwamrita*. Apart from supplying nutrients, this method helps increase the microbial population such as phosphorus solubilizing bacteria, plant growth-promoting rhizobacteria, etc.

Material and Methods

Experimental Site

The field experiment was conducted during the *kharif* season (June-November) 2019 at Research Farm, Department of Agronomy, CSK HPKV, Palampur, India. The experimental farm is situated at an elevation of 1290.8 meters above mean sea level at 76°3'E longitude and 32°6'N latitude. The soil at the experimental site was silty clay loam in texture and acidic in reaction with pH 5.38. The soil before the start of the experiment was low in available nitrogen (172 kg/ha), medium in available phosphorus (21.03 kg/ha) and potassium (248.49 kg/ha).

Treatment Details: The experiment was laid out in randomized complete block design (RCBD) with eight treatments and three replications. The treatments are T_1: *Bijamrita* (100 ml/kg of seed) + *Jiwamrita* (187.5 l/ha, 5%, 10%, and 10% respectively at 21,42 & 63 DAS), T_2: *Bijamrita* (100 ml/kg of seed) + *Ghanajiwamrita* at sowing (250 kg/ha), T_3: *Bijamrita* (100 ml/kg of seed) + *Jiwamrita* (187.5 l/ha, 5%, 10%, and 10% respectively at 21,42 & 63 DAS) + *Ghanajiwamrita* at sowing (250 kg/ha), T_4: Farm yard manure (10 t/ha), T_5: Farm yard manure (10 t/ha) + *Ghanajiwamrita* at sowing (250 kg/ha), T_6: Biofertilizer (*Rhizobium* + PSB @10g/kg of seed) + Farm yard manure (10 t/ha) + Vermiwash at 15,30 & 45 DAS (1:10), T_7: Biofertilizer (*Rhizobium* + PSB @ 10g/kg of seed) + Vermicompost (7.5 t/ha) + Vermiwash at 15,30 & 45 DAS (1:10) and T_8: Absolute control.

Statistical analysis

The data presented has been statistically analyzed by applying a Randomized Complete Block design by the method of "Analysis of Variance" as described by Gomez and Gomez (1984). The least significant difference at the 5 per cent level was worked out to determine the difference between the treatment means. The statistical analysis was done by using OPSTAT (http://14.139.232.166/opstat/onefactor.htm?flavor=One+Factor+Analysis) analysis software.

Results and Discussion

Effect on yield of the crop: The yield of the crop was significantly affected by the treatments. Significantly highest grain yield (10.71 q/ha) was obtained in T_5 (Farm Yard Manure + *Ghanajiwamrita*) while the lowest grain yield (7.67 q/ha) was recorded in T_8 (Absolute control). The results were in line with Yadav *et al.* (2017) and Sharma *et al.* (2020) where the combined application of solid organic manures *i.e.,* Farm Yard Manure + *Ghanajiwamrita* resulted in enhanced growth and yield for cowpea. The slow-release nature of FYM and *Ghanajiwamrita* made the availability of nutrients at periodic intervals thereby influencing the yield. The FYM is a major source of organic matter and storehouse of macro- and micronutrients on the other hand *Ghanajiwamrita* might have played a significant role in enhancing the biological activity of beneficial microbes (Jarvan *et al.,* 2017). The cumulative effect of these was reflected in grain yield for T_5. The omission of nutrient sources in T_8 (Absolute control) reduced the nutrient supply to the crop as compared to other treatments and resulted in the lowest yield levels. T_6 (Biofertilizers + FYM + Vermiwash) and T_7 (Biofertilizer + Vermicompost + Vermiwash) remain at par with T_4 (FYM) and T_3 (*Bijamrita* + *Jiwamrita* + *Ghanajiwamrita*) produced significantly higher grain yield over the rest of the treatments. Biofertilizer application along with organic nutrient sources such as FYM and vermicompost had a positive impact on root activity thereby enhancing available soil nutrients thus the yield in T_6 (Biofertilizers + FYM + Vermiwash) and T_7 (Biofertilizer + Vermicompost + Vermiwash) (Dekhane *et al.,* 2011; Yadav *et al.,* 2019). Organic farming input-based treatments performed at par with each other whereas among natural farming-based inputs, only the combined application of *Bijamrita* + *Jiwamrita* + *Ghanajiwamrita* in T_3 performed significantly better and was able to perform at par with organic nutrient sources such as FYM or vermicompost. Exclusion of any one of the natural farming-based inputs reduced the cumulative impact of the natural farming methodology and thus declined yield for T_1 (*Bijamrita* + *Jiwamrita*) and T_2 (*Bijamrita* + *Ghanajiwamrita* (250 kg/ha) (Table1).

Effect on pH and organic carbon

The addition of organic amendments to the soil altered the pH status of the soil but the change was not found to be significant. Although, it was observed that the treatments T_1, T_2 and T_4 showed a marked decrease in their pH values (Table 2). Natural farming formulations tend to increase the microbial population in the soil which resulted in increased mineralization, resulting in the formation of organic acids. McCauley *et al.* (2009) in their findings revealed that the addition of organic matter makes the soil biologically active resulting in the slow release of acids which in time would help reduce the soil pH in the area of their study. Similarly, there were no significant changes in the organic carbon status of the soil under different treatments. The highest change in the organic carbon status was also recorded in treatment comprising FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha).

Effect on nutrient content

The nutrient content in the grains was significantly affected by the treatments. Significantly highest nutrient content of the primary major macronutrients was recorded under T_5 (FYM @10 t/ha + *Ghanajiwamrita* @250 kg/ha) followed by T_6 (Biofertilizers (*Rhizobium* and PSB) + FYM @ 10 t/ha + Vermiwash (1:10)) and T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost @7.5 t/ha + Vermiwash (1:10)). This might be due to the application of organic manures which enhanced the nutrient availability at various growth stages of the crop due to their slow-release nature thus offsetting the leaching losses (Hameedi *et al.,* 2016; Khan *et al.*, 2017; Patil 2013). The lowest nutrient content was recorded in T_8 (Absolute Control) which was primarily due to the absence of any specific external nutrient sources (Table 3).

Effect on available nutrients: The treatment comprising of FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha) (T_5) was significantly higher in terms of available nitrogen and was also found to be at par with treatments T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)), T_4 (FYM @ 10t/ha) and T_6 (Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)). This may be due to the richness of different organic nutrient sources in nitrogen as compared to other nutrient elements (Table 3). Organic manures besides acting as nutrient sources, elevated microbial activity in the rhizosphere that improved the nitrogen fixation ability of cowpea and increased available nitrogen content in the soil (Jarvan *et al.,* 2017). *Ghanajiwamrita* acted as the microbial pool whereas *Rhizobium* inoculation-based treatments directly enhanced the crop root-*Rhizobium* symbiotic activity which resulted in a suitable micro-climate for nodule-based nitrogen fixation. Shang *et al.* (2020) in their study came to a similar conclusion that the addition of organic manures and biofertilizers from various bacteria tends to improve the soil nutrient status to varying degrees. In terms of available phosphorus and potassium, the highest was observed under treatment T_5 (FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)) followed by T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)). These treatments were also found to be statistically at par with each other. The solubilizing action of soil microbes and chelating compounds released from crop roots treated with *Rhizobium* and PSB were responsible for liberating insoluble phosphorus from cation-based complexes and thus increasing the availability of phosphorus and potassium from unavailable forms into soil solution (Dekhane *et al.,* 2011).

Table 1: Effect of different treatments on the yield in cowpea

Treatment	Grain yield (q/ha)	Straw yield (q/ha)	Biological yield (q/ha)
T_1 *Bijamrita* + *Jiwamrita*	8.64	36.86	45.50
T_2 *Bijamrita* + *Ghanajiwamrita* (250 kg/ha)	7.87	36.93	44.79
T_3 *Bijamrita* + *Jiwamrita* + *Ghanajiwamrita* (250 kg/ha)	9.17	38.44	47.62
T_4 FYM (10 t/ha)	9.40	41.75	51.15
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	10.71	53.14	63.84
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	9.91	50.76	60.67
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	9.79	43.47	53.26
T_8 Absolute control	7.67	35.71	43.39
SEm±	0.25	1.98	2.01
CD ($P \leq 0.05$)	0.77	6.00	6.09

Table 2: Effect of different treatments on pH and organic carbon status of soil

Treatment	pH	Organic Carbon (%)
T_1 *Bijamrita* + *Jiwamrita*	5.24	0.72
T_2 *Bijamrita* + *Ghanajiwamrita* (250 kg/ha)	5.27	0.73
T_3 *Bijamrita* + *Jiwamrita* + *Ghanajiwamrita* (250 kg/ha)	5.42	0.72
T_4 FYM (10 t/ha)	5.28	0.75
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	5.47	0.77
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	5.34	0.75
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	5.38	0.73
T_8 Absolute control	5.4	0.70
SEm±	0.05	0.01
CD ($P \leq 0.05$)	NS	NS

Table 3: Effect of different treatments on the content in grain and availability of nutrients in soil

Treatment	N (%)	P (%)	K (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
T_1 *Bijamrita* + *Jiwamrita*	3.37	0.35	1.17	160.6	17.7	218.5
T_2 *Bijamrita* + *Ghanajiwamrita* (250 kg/ha)	3.34	0.34	1.13	154.1	16.5	217.4
T_3 *Bijamrita* + *Jiwamrita* + *Ghanajiwamrita* (250 kg/ha)	3.41	0.365	1.23	165.5	19.3	219.4
T_4 FYM (10 t/ha)	3.42	0.37	1.25	168.0	20.9	223.1
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	3.54	0.41	1.36	169.8	21.4	231.6
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	3.50	0.39	1.33	166.2	20.1	222.7
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	3.47	0.38	1.29	168.7	21.1	227.2
T_8 Absolute control	3.26	0.33	1.08	152.3	13.4	203.5
SEm±	0.017	0.0051	0.014	1.324	0.246	1.68
CD ($P \leq 0.05$)	0.052	0.0154	0.041	4.017	0.746	5.12

***** *Jiwamrita applied @ 5,10 and 10 % at 21,42 and 63 DAS, respectively; Vermiwash applied at 15, 30 and 45 DAS***

The higher nutrient composition of vermicompost was responsible for improving nutrient availability in vermicompost- based treatments. Riba *et al.* (2018) reported that the addition of FYM in the crop in association with inorganic fertilizers improved the yield of the crop.

Effect on uptake by crop: The treatments significantly affected the uptake of nitrogen, phosphorus, and potassium by the crop plants (Table 4). Significantly highest uptake of the major macronutrients was recorded under T_5 (FYM @10 t/ha + *Ghanajiwamrita* @259 kg/ha) followed by T_6

Table 4: Effect of different treatments on the total uptake of nutrients in cowpea

Treatment	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)
T_1 *Bijamrita + Jiwamrita*	94.40	12.09	71.00
T_2 *Bijamrita + Ghanajiwamrita* (250 kg/ha)	90.55	11.57	68.33
T_3 *Bijamrita + Jiwamrita + Ghanajiwamrita* (250 kg/ha)	100.43	13.23	75.43
T_4 FYM (10 t/ha)	107.66	14.68	84.96
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	141.08	19.86	112.34
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	130.57	18.32	105.05
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	113.94	15.78	90.24
T_8 Absolute control	84.80	10.68	64.41
SEm±	1.36	0.18	1.02
CD ($P \leq 0.05$)	4.15	0.56	3.09

Table 5: Effect of different treatments on the nodulation in cowpea

Treatment	Number of nodules per plant	Dry weight of nodules per plant (g)
T_1 *Bijamrita + Jiwamrita*	20.8	0.21
T_2 *Bijamrita + Ghanajiwamrita* (250 kg/ha)	29.0	0.35
T_3 *Bijamrita + Jiwamrita + Ghanajiwamrita* (250 kg/ha)	23.1	0.33
T_4 FYM (10 t/ha)	20.7	0.21
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	31.7	0.47
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	22.0	0.25
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	28.5	0.28
T_8 Absolute control	20.1	0.20
SEm±	1.0	0.01
CD ($P \leq 0.05$)	3.03	0.04

Table 6: Effect of different treatments on the economics in cowpea

Treatment	Cost of Cultivation (₹/acre)	Gross Returns (₹/acre)	Net Returns (₹/acre)
T_1 *Bijamrita + Jiwamrita*	12941.45	33945.09	21003.64
T_2 *Bijamrita + Ghanajiwamrita* (250 kg/ha)	14408.44	31444.17	17035.73
T_3 *Bijamrita + Jiwamrita + Ghanajiwamrita* (250 kg/ha)	14964.88	35914.99	20950.11
T_4 FYM (10 t/ha)	16430.25	37191.14	20760.89
T_5 FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)	17644.31	43260.72	25616.41
T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10)	17462.2	40305.87	22843.67
T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)	25555.92	38727.09	13171.17
T_8 Absolute control	12383.39	30619.10	18235.71
SEm±	-	900.59	900.59
CD ($P \leq 0.05$)	-	2731.52	2731.52

**** Jiwamrita applied @ 5,10 and 10 % at 21,42 and 63 DAS, respectively; Vermiwash applied at 15, 30 and 45 DAS***

(Biofertilizers (*Rhizobium* and PSB) + FYM @ 10 t/ha + Vermiwash (1:10)) and T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost @7.5 t/ha + Vermiwash (1:10)). The lowest total uptake was recorded in T_8 (Absolute Control). The organic manure is able to improve the soil aeration which resulted in better root growth thereby promoting root development. This resulted in higher crop yield and hence the uptake of nutrients (Chaudhary *et al.*, 2016). Biofertilizer application especially of *Rhizobium* is reported to enhance root activity resulting in better nutrient uptake in T_6 (Biofertilizers (*Rhizobium* and PSB) + FYM @ 10 t/ha + Vermiwash (1:10)) and T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost @7.5 t/ha + Vermiwash (1:10)) (Yadav *et al.,* 2019).

Effect on nodulation: The effect of different treatments on the number of nodules per plant and the dry weight of nodules per plant were significant. The treatment T_5 (Farm Yard Manure + *Ghanajiwamrita*) recorded the highest total number of nodules (169.8) as well as dry weight per plant (21.4 g). This might be the effect of *Ghanajiwamrita* which can enhance the microbial population resulting in increased nodulation (Table 4). This treatment was found to be statistically at par with T_7 (Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10)) and T_4 (FYM (10 t/ha) (Table 5). Due to the improved number of nodules, the effect of the increased dry weight of the nodules was also observed. The organic-rich soil has the potential to host beneficial microorganisms for a longer duration in the soil due to the availability of food material which may be the reason for the increase in nodulation of the crops (Guriqbal *et al.*, 2010; Singh *et al.*, 2012). Further, *Ghanajiwamrita* has a tendency to improve the microbial population which enhances the biological activity while vermicompost enhances soil enzyme activity. Their combined application is responsible for increased nodulation (Bajracharya and Rai 2009).

Cost of Cultivation: The highest cost of cultivation (₹ 25555.92/acre) was observed for T_7 (Biofertilizer + Vermicompost + Vermiwash) whereas the lowest cost of cultivation (₹ 12383.39/acre) was observed for T_8 (Absolute Control). The highest cost of cultivation for T_8 was due to the higher cost of vermicompost application whereas the lowest cost of cultivation in absolute control was due to the lack of costs involved for nutrient application (Table 6).

Gross Returns: Gross returns were significantly affected by different treatments. The highest gross returns (₹ 43261/acre) were recorded for the treatment consisting of combined application of farm yard manure and *Ghanajiwamrita* (T_5), whereas the lowest gross returns (₹ 30619/acre) were recorded for absolute control (T_8) (Table 6). Such differences in gross returns among treatments were due to different yield levels observed in these treatments.

Net Returns: Significant effects of different treatments were observed on net returns. The highest value of net returns (₹ 25616/acre) was observed for T_5 wherein a combined application of farm yard manure and Ghanajiwamrita was done (Table 6). The lowest net returns (₹ 18235/acre) were recorded for T_8 (absolute control). Differences in gross returns and cost of cultivation lead to such significant differences in net returns among treatments.

Conclusion

From the study, it can be concluded that soil pH and organic carbon were not affected by the different organic sources whereas available nitrogen, phosphorus and potassium were significantly influenced. The treatment T_5 (FYM (10 t/ha) + *Ghanajiwamrita* (250 kg/ha)) emerged to be the best treatment in terms of yield, nutrient content, available nutrients, uptake of nutrients and nodulation. This was followed by T_6 Biofertilizers (*Rhizobium* and PSB) + FYM (10 t/ha) + Vermiwash (1:10) and T_7 Biofertilizers (*Rhizobium* and PSB) + Vermicompost (7.5 t/ha) + Vermiwash (1:10).

Conflict of interest

The authors declare that they have no conflict of interest.

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Growth analysis and yield evaluation under tillage and weed management practices in maize-wheat cropping system

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ABSTRACT

Integrated weed management strategies combine tillage systems and weed control strategies. Conservation agriculture (CA) and sustainable intensification cropping systems are potential sources of improved growth and overall productivity. This study evaluated tillage and weed management strategies effects on crop growth parameters and biological yields in maize-wheat cropping system in North Western Himalayan region. Different tillage (five) and weed management practices (three) were evaluated from 2018 to 2020 on growth indices and yield with fifteen treatments. Conservation agriculture (CA) based production system (ZT, zero tillage; crop rotation and intensification; residue management i.e. ZTR-ZTR) had higher crop dry matter accumulation (DMA), relative growth rate (RGR), crop growth rate (RGR) and biological yield of maize (28698 kg/ha) and wheat crops (18750 kg/ha). The zero tillage in maize and wheat (ZT-ZT) resulted in lowest maize (24677 kg/ha) and wheat biological yield (14009 kg/ha. Among weed management treatments, application of recommended herbicides in maize and wheat crop (H-H) resulted in higher crop DMA and biological yield of maize (27652 kg/ha) and wheat crop (19540 kg/ha). Therefore, for North Western Himalayan conditions, ZTR+H-ZTR+H (Conservation tillage combined with herbicide application in maize and wheat) is superior to other combinations for growth and yield.

Introduction

Food security for a steadily rising population and soothing poverty while sustaining agricultural production systems under the current scenario of over exhausting of natural resources, adverse effects of climatic variability, high cost of inputs and fickle prices of agricultural commodities are the primary challenges in front of most of the Asian countries. The uncertainty and insecurity of food resources and change of livelihood pattern resulting from climate change may even be a threat to national security as it may become an enigma for developing countries to have a poor resource base (Food and Agriculture Organization, 2008). Non-sustainability of agricultural production systems may be due to the soil erosion, reduction in organic matter of the soil, soil salinization and acidity whichis mainly caused by continuous deep tillage which reduce soil organic matter, degradation of soil structure, reduction in water infiltration rate, surface crusting and compaction. Therefore, significant change in agricultural production system is imperative for approaching farming productivity and sustainability of the available natural resources. Conservation agriculture (CA) a concept that appeared as a concern of sustainability of agriculture globally has cover about ~8 per cent of the world arable land (Food and Agriculture Organization, 2012). Maize-wheat cropping system is popular in many parts of

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India including the northern belt of wheat growing region. In Himachal Pradesh, the system is followed up to an elevation of 2500 m above mean sea level. Nearly 85 per cent of the total food share in the state comes from these two cereal crops (Bharti, 2013); mostly under rainfed conditions as 80 per cent of the cultivated areas of the state have scarcity of irrigation facilities resulting in lower crop productivity (Ramesh *et al.*, 2016). Therefore, boosting the viability of maize-wheat cropping sequence holds the key to the transformation of an agricultural scenario in Himachal Pradesh (Bharti, 2013). Poor socio-economic conditions, small and scattered land holdings, limited mechanization and soil and climatic constraints prevent hill farmers from taking up modern agricultural technology as practiced by their counterparts in the adjacent plains. Besides declining labour availability in agriculture, tremendous pressure on resources and fluctuations in market scenario warrants the use of available resources and technology frugal. Despite the significant achievement made in research and development, the productivity of maize-wheat cropping sequence is very low. Many production technologies have been developed, yet the farmers have failed in taking full advantage of these technologies. Farmers in the state pursue a subsistence type of agriculture. Although, conventional tillage systems may not be suitable for hilly areas due to highly prone for excessive soil erosion hazards (Ramesh *et al*., 2016). In a hilly state of India, about 70 per cent of farmers are marginal land holders and have average land holding less than 0.4 ha (Department of Economic and Statistics, 2017). Poor socio-economic conditions, small and scattered land holdings, limited mechanization and soil and climatic constraints prevent hill farmers from taking up modern agricultural technology as practiced by their counterparts in the adjacent plains. Besides declining labour availability in agriculture, tremendous pressure on resources and fluctuations in market scenario warrants the use of available resources and technology frugal. Resource conserving techniques should be the component in regional strategy for food and livelihood security, sustainability of natural resources, rural development and enhancement of profitability and improved environmental quality. Crop biomass burning is responsible for greenhouse gases emissions, reduction in soil productivity and degrading air quality (Venkatramanan *et al.*, 2021). This has become a major issue due to combine harvesting of crops and short time span between the harvesting and sowing of crops (Ravindra *et al.*, 2019). Residue burning releases pollutants, particulate matter, dust particles (Chawala and Sandhu, 2020), aerosols which retard the soil nutritious value (Jat *et al.*, 2020), human health and quality of the air (Sahu *et al.*, 2021). Other major problems like imbalance use of pesticides, nutrients deficiencies or imbalances, high energy and labour requirement and weed shift cause threat to the sustainability of cereals based cropping system (Pathak *et al.*, 2011; Ram *et al.*, 2012). Many studies have shown that continuous no-tillage for long term increases soil organic matter (Bhattacharyya *et al.*, 2009; 2015), soil health, improve soil structure (Jat *et al.*, 2018) and increases crop yield substantially than intensive tillage practices (Cannell and Hawes, 1994). Therefore, conservation strategies and modern farming techniques must be incorporated in the indigenous production systems for environmental protection, economic and social development and ecologically sustainability (Singh and Rao, 2002).Therefore, an experiment on maize-wheat cropping sequence was conducted to study the effect of tillage in relation to weed management on crop DMA, growth indices and biological yield in the North-Western Himalayas.

Material and Methods

Study area

The site of experiment was at Research Farm (32°6′ N, 76°3′ E), Department of Agronomy, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P.), India during *kharif* 2018 to *rabi* 2019-20. The experimental location has a sub-temperate mid hill zone at 1290 m above mean sea level. Experimental site has silty clay loamy soil (21% clay, 43% silt and 36% sand), according to USDA classification. The soil properties of the experimental site have been given in Table 1.The crops were irrigated whenever needed with a good drainage system.

Experimental details

The brief detail of the experimental treatments has been given in Table 2. The experiment was

Table 1: Soil properties at 0-15 cm depth before the commencement of the experiment

Particulars	Sand (%)	Silt (%)	Clay (%)	BD (g/m^3)	SOC (g/kg)	Av. N (kg/ha)	Av. P (kg/ha)	Av. K (kg/ha)
Content	21	43	36	1.18	11.0	323.0	25.8	276.4
Analytical Method employed	International pipette method (Piper, 1966)			Core Method (Singh, 1980)	Walkley and Black' rapid titration method (Piper, 1966)	Alkaline permanganate method (Subbiah and Asija, 1956)	Olsen' method (Olsen *et al.*, 1954)	Ammonium acetate extraction method (AOAC, 1970)

SOC: Soil organic carbon; Av. N: Available Nitrogen; Av. P: Available Phosphorus; Av. K: Available Potassium

Table 2: Treatments detail of the maize-wheat cropping system

a)	**Tillage and residue management (Horizontal plot) treatments**			
	Maize crop		**Wheat crop**	
	Tillage	**Residue retention**	**Tillage**	**Residue retention**
	T_1 - Conventional tillage (CT)	No	T1 - Conventional tillage (CT)	No
	T_2 - Conventional tillage (CT)	No	T2 - Zero tillage (ZT)	No
	T_3 - Zero tillage (ZT)	No	T3 - Zero tillage (ZT)	No
	T_4 - Zero tillage (ZT)	No	T4 - Zero tillage + residue (ZTR)	Yes; maize residue
	T_5 - Zero tillage + residue (ZTR)	Yes; wheat residue	T5 - Zero tillage + residue (ZTR)	Yes; maize residue
b	**Weed management (Vertical plots) treatments**			
	Weed management	**Intercropping**	**Weed management**	**Intercropping**
	W1 - Recommended herbicides (atrazine *fb* 2,4-D)	No	W1 - Recommended herbicides (isoproturon *fb* 2,4-D)	No
	W2 – IWM (Intercropping* + pendimethalin spray + hand weeding)	Yes; Soybean intercropping	W2 - IWM (Intercropping** + isoproturon spray + hand weeding)	Yes; Sarson intercropping
	W3 – Hand weeding (one hand weeding)	No	W3 – Hand weeding (one hand weeding)	No

*** intercropping of soybean in maize, ** intercropping of sarson in wheat**

conducted in strip plot design with three replications. Treatment combinations comprised five tillage and three weed control techniques. Maize crop was sown in *kharif* and wheat was *in* rabi season. Pre sowing irrigation at depth 5 cm was delivered during both *kharif* and *rabi* seasons of both the years. Except for zero tillage treatment, the plots were prepared with the help of a rotary power tiller. During seedbed preparation, crop stubble and weeds were removed to facilitate the planting operation. The left-over weeds were removed and the plots were levelled to have uniform sowing and germination thereof. The conventional tillage (CT) plots were ploughed to a fine tilth before the start of experiment. This was achieved using ploughing once, harrowing twice, and levelling. The seeds of maize variety 'Kanchan 51 hybrid' were sown in rows 60 cm apart in the first week of June and harvested in the mid to end of September every year. Sowing was done with hand plough by the kera method. Common dosage of 120 kg N, 60 kg P_2O_5, and 40 kg K_2O/ha respectively, was supplied through urea (46% N), IFFCO (12:32:16), and MOP (60% K_2O). Intercrop of soybean grown in additive series was not given any additional fertilizer dose. The net plot size was 2.7 m × 4.5 m. The crops water requirement was fulfilled according to the prevailing climatic conditions. In wheat crop, four irrigations were given in order to avoid drought stress. In both crops, all other production practices, except tillage and weed control treatments were followed as per recommendations in the package of practices. All the crops (main crops and intercrops) were harvested manually.

Soil analysis: A composite soil sample (0 to 15 cm depth) from each plot was made after the sampling from four corners of a plot with the help of tube auger and mix up it well before the commencement of the experiment i.e. before *kharif* 2018. The soil samples were air dried, processed and passed through 2 mm sieve and properly stored in polythene bags. The stored samples were later analysed for soil texture i.e. sand, silt and clay (international pipette method), soil organic carbon (rapid titration), available N (alkaline potassium permanganate), P (0.5 N Bicarbonate extraction) and K (Neutral ammonium acetate extraction).

Dry matter accumulation: The selected plant samples were taken from the sample rows of each plot from either side of net plot for recording dry matter accumulation for both the years and then pooled. At each observation, fresh plant samples (from 1 m row length) cut from the ground level were kept in the paper bags and dries at 70°C in hot air oven till constant weight is attained. The dry matter accumulation per square metre was then calculated by multiplying with the factor of 5. Total biological yield (grain + straw) from each net plot was recorded by weighing the sun-dried harvested crop.

Growth analysis: Crop growth rate (CGR) and relative growth rate (RGR) were determined using the formulas given by (Aliabadi *et al.*, 2008). However, harvest index was calculated according to the Maurya *et al.* (2021).

Statistical Analysis: The data were subjected to statistical analysis and were tested at a 5% level of significance to interpret the treatment differences. We estimated further statistical validity of the differences among treatment means by using the Fisher's Least Significant Differences (LSD) comparison method.

Results and Discussion

Weather

The average weather data for both the experimental years have been given in Figure1. The second year was relatively hotter and humid, whereas, first year received higher amount of rainfall. During 2018-19, ~20% higher rainfall (3228 mm) was received than 2019-20 (2135 mm). August month received highest rainfall 629 mm and 635 mm during 2018 and 2019, respectively. June month of the year was hotter than other months of the year. Weather conditions were felicitous during the growth and development of maize and wheat crop during both the years.

Crop DMA, CGR and RGR

Pooled data on crop DMA (g/m^2) by maize crop at 30, 60 and 90 DAS was statistically (p=0.05) influenced by tillage practices, whereas, weed management methods could not influence the DMA by crop plants (Table 3). Crop DMA increased progressively during the crop growth period. Conservation tillage in combination with residue application (ZTR-ZTR) had maximum crop DMA for all the observational periods.

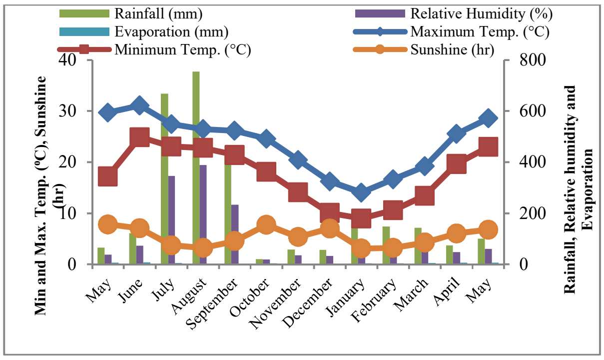

Figure 1: Pooled monthly weather data of experimental site (2018-2020)

However, CT-CT had higher crop DMA than ZT-ZT and ZT-ZTR at 30 DAS which got reduced as crop growth proceed up to harvest of the crop. Application of crop residue with zero tillage increased crop DMA during all the observational period compared to the zero tillage without residue incorporation resulted in lower DMA in all the observational periods. Data pertaining to CGR (g/m^2/day) and AGR (cm/day) had significant (p=0.05) variation for 30-60 DAS under tillage treatments, whereas weed management practices could not affect significantly. ZTR-ZTR had maximum CGR (1.96 g/m/day) which remained statistically at par with ZT-ZTR (1.80 g/m^2/ day). Minimum CGR was recorded in ZT-ZT where residue was not incorporated. Significantly higher AGR was recorded in ZTR-ZTR, CT-CT and ZT-

Table 3: Effect of tillage and weed management treatment on crop dry matter accumulation (g/plant), crop growth rate (g/m^2/day) and absolute growth rate (cm/day) of maize crop (pool data of 2 year's)

Treatment (Maize -wheat)	CDMA			CGR		AGR	
	30 DAS	60 DAS	90 DAS	30-60 DAS	60-90 DAS	30-60 DAS	60-90 DAS
Tillage							
CT-CT	8.4^{b}	43.0^{bc}	108.2^{c}	1.58^{bc}	1.52	5.54^{a}	3.73
CT-ZT	7.5^{bc}	39.6^{cd}	110.1^{c}	1.47^{c}	1.70	4.40^{bc}	4.12
ZT-ZT	6.5^{c}	36.7^{d}	103.1^{c}	1.35^{c}	1.56	4.12^{c}	4.71
ZT-ZTR	7.1^{c}	45.7^{b}	119.7^{b}	1.80^{ab}	1.86	5.25^{ab}	3.60
ZTR-ZTR	9.7^{a}	53.0^{a}	128.8^{a}	1.96^{a}	1.84	5.41^{a}	3.96
SEm±	0.3	1.5	2.2	0.08	0.09	0.29	0.28
LSD (p=0.05)	1.0	5.0	7.1	0.26	NS	0.96	NS
Weed management							
H-H	8.1	43.4	117.4	1.64	1.69	5.33	3.49
IWM-IWM	8.1	43.1	111.7	1.60	1.74	4.73	4.78
HW-HW	7.3	44.3	112.9	1.66	1.65	4.78	3.81
SEm±	0.4	1.6	3.4	0.03	0.03	0.15	0.26
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS

CT, conventional tillage; ZT, zero tillage; R, residues; H, herbicide; IWM-IWM, integrated weed management; HW, hand weeding; figures with same sign as superscript in the table in a same factor mean statistically at par with each other

Table 4: Effect of tillage and weed management treatment on crop dry matter accumulation (g/plant), crop growth rate (g/m^2/day) and absolute growth rate (cm/day) of wheat crop (pool data of 2 year's)

Treatment (Maize wheat)	CDMA					CGR				AGR			
	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	30-60 DAS	60-90 DAS	90-120 DAS	120-150 DAS	30-60 DAS	60-90 DAS	90-120 DAS	120-150 DAS
Tillage													
CT-CT	16.7^{b}	19.8^{c}	70.8^{bc}	463.4^{b}	909.0^{a}	0.106^{c}	1.698^{a}	13.087^{ab}	14.853	0.543^{ab}	0.956	1.477^{a}	0.360
CT-ZT	20.5^{a}	23.3^{b}	78.4^{ab}	484.3^{ab}	956.8^{a}	0.094^{c}	1.837^{a}	13.530^{ab}	15.748	0.593^{a}	1.033	1.254^{bc}	0.116
ZT-ZT	12.4^{c}	16.0^{d}	61.7^{cd}	419.9^{bc}	799.1^{b}	0.122^{bc}	1.523^{b}	11.939^{b}	12.641	0.445^{c}	0.935	1.106^{c}	0.377
ZT-ZTR	16.1^{b}	21.4^{bc}	52.4^{d}	379.4^{c}	748.6^{b}	0.175^{b}	1.034^{c}	10.901^{b}	12.304	0.463^{c}	1.019	1.329^{ab}	0.375
ZTR-ZTR	22.0^{a}	29.9^{a}	81.5^{a}	557.7^{a}	978.2^{a}	0.263^{a}	1.720^{a}	15.872^{a}	14.019	0.470^{bc}	0.912	1.416^{ab}	0.274
SEm±	0.7	0.9	2.9	23.5	23.2	0.016	0.085	0.843	1.144	0.023	0.030	0.052	0.076
LSD (p=0.05)	2.2	2.8	9.6	76.6	75.6	0.053	0.278	2.750	NS	0.074	NS	0.170	NS
Weed management													
H-H	18.4^{ab}	23.6^{a}	73.9^{a}	494.5	905.2^{a}	0.173	1.675^{a}	14.022	13.690	0.434^{b}	1.047^{a}	1.360	0.282
IWM-IWM	15.0^{b}	19.4^{b}	67.7^{b}	419.9	836.9^{b}	0.146	1.610^{a}	11.741	13.901	0.438^{b}	0.922^{b}	1.298	0.310
HW-HW	19.2^{a}	23.3^{a}	65.4^{b}	468.4	892.9^{a}	0.137	1.403^{b}	13.435	14.148	0.637^{a}	0.944^{b}	1.291	0.308
SEm±	0.8	0.7	1.4	15.4	11.1	0.012	0.043	0.496	0.810	0.022	0.017	0.091	0.039
LSD (p=0.05)	3.0	2.6	5.3	NS	43.4	NS	0.171	NS	NS	0.086	0.065	NS	NS

CT, conventional tillage; ZT, zero tillage; R, residues; H, herbicide; IWM-IWM, integrated weed management; HW, hand weeding; figures with same sign as superscript in the table in a same factor mean statistically at par with each other

Table 5: Effect of tillage and weed management treatment on biological yield of maize and wheat crop (kg/ha) and harvest index (%) (pool data of 2 year's)

Treatment (Maize -wheat)	Maize biological yield (kg/ha)	Maize harvest index (%)	Wheat biological yield (kg/ha)	Wheat harvest index (%)
Tillage				
CT-CT	26654[b]	28.1	16827[b]	32.9
CT-ZT	26222[bc]	27.7	16950[b]	34.1
ZT-ZT	24677c	28.2	14009[d]	33.2
ZT-ZTR	24712c	28.3	15318[c]	32.6
ZTR-ZTR	28698[a]	27.0	18750[a]	31.8
SEm±	523	0.4	229	1.4
LSD (p=0.05)	1705	NS	747	NS
Weed management				
H-H	27652	27.4[b]	19540[a]	32.2
IWM-IWM	25605	29.6[a]	10229[b]	32.7
HW-HW	25322	26.5[b]	19343[a]	33.8
SEm±	622	0.3	595	0.7
LSD (p=0.05)	NS	1.3	2336	NS

CT, conventional tillage; ZT, zero tillage; R, residues; H, herbicide; IWM-IWM, integrated weed management; HW, hand weeding; figures with same sign as superscript in the table in a same factor mean statistically at par with each other

ZTR compared to the ZT-ZT and CT-ZT. Weed management treatments could not significantly affect the CGR and AGR of maize crop at different observational periods.

This might be due to the reason that different weed management treatments could not significantly affect the crop dry matter and height which results in non significant results for CGR and AGR. In conservation agriculture, DMA, CGR and RGR were higher than in CT plots and ZT plots without residue might be due to better soil health and micro-environment created by the continuous adoption of these resources conserving practices (Ram, 2006; Memon *et al.*, 2014).Data on effect of tillage and weed management practices on wheat crop DMA at 30, 60, 90, 120 and 150 DAS have been given in Table 4. However, progressive DMA has been presented in Figure 2.The significantly (p=0.05) higher DMA was recorded in the ZTR-ZTR at all the stages of observations. ZTR-ZTR recorded statistically similar crop DMA as CT-ZT during 30, 90, 120 and 150 DAS. ZT-ZT resulted in lower crop DMA during all the observational periods. Among weed management treatments, HW-HW had significant (p=0.05) higher crop DMA during initial days which otherwise was higher in H-H during vegetative and reproductive stages. IWM-IWM recorded lowest wheat DMA during all the observational periods.Wheat crop growth was triggered under different tillage and weed management practices however such an increase was more pronounced for ZTR-ZTR compared to CT-CT. Temporal increase in crop growth rate under ZTR-ZTR was observed with maximum values (14.019 g/m^2/day) achieved at 120-150 DAS. Tillage treatments had significant (p=0.05) variation in CGR (g/m^2/day) at all stages except 120-150 DAS. ZTR-ZTR resulted in higher CGR between 30-60 DAS which was higher in CT-ZT during later stages. CT-ZT had statistical (p=0.05) similar crop growth rate as ZTR-ZTR and CT-CT between 60-90 DAS. Weed management treatments could significantly affect the CGR between 60-90 DAS. H-H had highest CGR which was statistically at par with IWM-IWM. Application of recommended herbicides recorded maximum crop growth rate in wheat crop (Khaliq *et al.*, 2013). The tillage treatments significantly affected AGR (cm/day) during 30-60 DAS and 90-120 DAS of wheat crop. CT-ZT had statistical similar AGRas CT-CT during 30-60 DAS. However, between 90-120 DAS, CT-CT had higher absolute growth rate which remaining statistically similar with ZTR-ZTR and ZT-ZTR. Among weed management treatments, HW-HW had maximum

AGR which remained statistically at par with IWM-IWM and H-H at 30-60 DAS. However, H-H had maximum AGR followed by HW-HW and IWM-IWM at 60-90 DAS. Improved growth attributes in herbicide treatments may be attributed to reduction in crop-weed competition. In conservation agriculture systems (ZTR-ZTR), the absence of weed competition led to canopy closure, which resulted in faster plant growth and accumulation of biomass led to a greater biological yield.

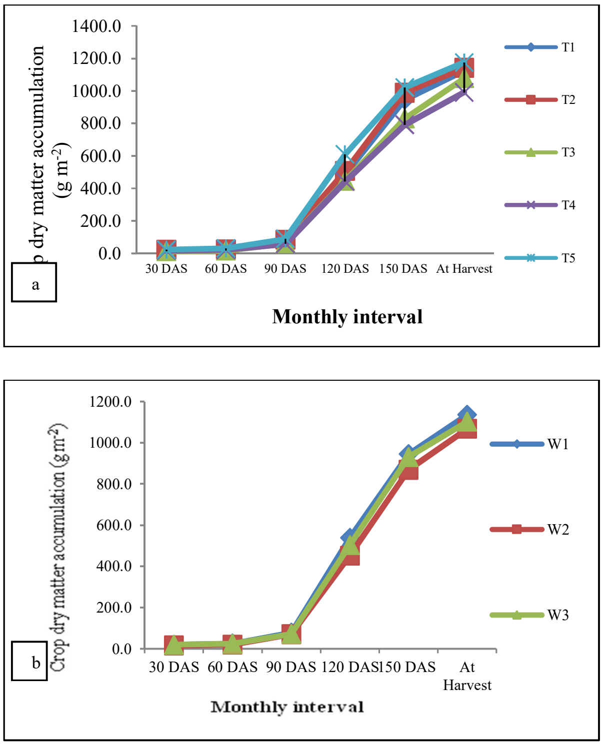

Figure 2: Progressive dry matter accumulation of wheat crop under (a) tillage and (b) weed management treatments (Pooled data for 2018-19 & 2019-20).

Biological yield and harvest index

Maize biological yield was higher in ZTR-ZTR, whereas, ZT without residue resulted in lower HI among tillage treatments. The low yield in ZT plots might be due to more weed infestation. Among weed management treatments, H-H had higher maize biological yield. In a long term field trial, Kaskarbayev *et al*., (2002) found that zero tillage (ZT) resulted in 16% yield reduction against deep CT in maize crop. Similarly, lower biological yield of maize under ZT compare to CT might be due to poor crop stand and dry matter accumulation. Singh *et al*., (2007); Singh *et al*., (2011); Wang *et al*., (2015) also reported lower biological yield under ZT. Wheat biological yield was maximum in ZTR-ZTR, whereas, CT-CT and CT-ZT remained statistically at par with each other. H-H and HW-HW resulted in higher wheat biological yield among weed management treatments. Furthermore, Sime *et al*., (2015) reported that conservation tilled plots (ZTR-ZTR) had higher yield of maize under maize-wheat cropping system than CT-CT. However, higher biological yield in herbicide applied plots may be due to the efficient weed control efficiency (Baghestani *et al*., 2008; Chhokar *et al*. 2008; Santos, 2009). Harvest index (HI) was higher in ZT-ZTR followed by ZT-ZT in maize crop. IWM-IWM resulted in significantly (p=0.05) higher HI, whereas, H-H had statistically similar HI. Pariyar *et al*. (2019) also reported that ZTR resulted in higher HI than conventional tillage. In wheat crop, tillage as well as weed management treatments could not significantly affect the HI. Ion *et al*., (2015) also found that HI of maize crop varies from 0.20-0.56 under different conditions.

Conclusion

Results from the present study showed that conservation agriculture-based maize-wheat cropping system resulted in higher crop dry matter accumulation and crop growth rate and relative growth rate under different observational periods in maize and wheat crop. ZTR-ZTR had higher biological yield in maize and wheat crop. This might be due to the reason that conservation agriculture had improved soil properties, better crop establishment and minimum lodging. Similar results were reported by Jat *et al*. (2017) and Chaudhary *et al*. (2018). Although, harvest index was higher in ZT-ZTR in maize and CT-ZT in wheat crop. Among weed management treatments, H-H resulted in higher crop DMA in maize and wheat crops. CGR was initially higher in H-H which later stages in HW-HW in wheat crop, whereas, AGR was higher in HW-HW during initial months which was in H-H as crop reach towards maturity. Although, biological yield was higher in H-H for both the cereals. Therefore, based on the results of the present study, it is suggested to follow ZTR+H-ZTR+H among all the combinations for better growth and yield from maize-wheat cropping system.

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

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Crop acreage and yield mapping of groundnut crop in erstwhile Mahabubnagar District using RS and GIS

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ABSTRACT

An investigation was carried in the erstwhile Mahabubnagar district of Telangana during *rabi* 2019-20 aiming the estimation of groundnut crop acreage and yield. The crop area was estimated using the satellite images of Landsat-8-OLI sensor from September to February covering the entire crop growth period by performing an unsupervised image classification technique with 300 classes, 300 iterations and a convergence threshold of 0.99. The groundnut yield was estimated by developing the regression equation using crop-cut yield data and NDVI values of the corresponding GPS locations. The crop area was estimated to be 57,865 ha with producer's and user's accuracy of 100 and 90% respectively, and a relative deviation of 28.6% when compared with actual ground estimates of the Department of agriculture. The crop yields were estimated with an R^2 value of 0.71 and a correlation coefficient of 0.87.

Introduction

The groundnut crop is mostly cultivated under rainfed conditions of all the districts of Telangana, mainly concentrated in Mahabubnagar, Warangal and Nalgonda districts (Agriculture at glance, 2014). Mahabubnagar district of Telangana accounts for 60.0% of the total area of the Groundnut crop. Mahabubnagar is mainly a drought prone area which suits the growing climatic conditions of groundnut. In Mahabubnagar district of Telangana, the annual average production of groundnut crop during 2013-14 was 220 thousand tons and annual average yield per hectare during the same period was 1751 kg/ha (Shruthi *et al.,* 2017). The crop statistics shows that during the year 2019-20, the crop area extended to 0.91 lakh ha in Telangana state with 0.83 lakh ha alone in Southern Telangana Zone (Rabi 2019-20, Pre-harvest price forecast of Groundnut, 2020). Groundnut production for the year 2019-20 was 2.90 lakh mt during rabi 2019-20 (Agriculture Action Plan, 2019-20). Recent developments in aerospace survey technology, digital image processing, modelling of crop production process, and geographic information systems has created promising opportunities for upgrading the agriculture statistical systems. Remote sensing data can greatly contribute to the monitoring of earth's surface features by providing timely, synoptic, cost efficient and repetitive information about the earth's surface (Justice *et al.,* 2002). Crop inventory related applications comprise of identification/ discrimination of crop covers and acreage estimation, predicting crop yield and crop growth condition assessment and cropping system analysis (Kingra *et al.,* 2016). The present study was taken up aiming the groundnut crop area and yield estimation in the groundnut belt in erstwhile Mahabubnagar district of Telangana using remote sensing and GIS techniques during the *rabi* season of 2019-20.

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Material and Methods

Study area

The present research was conducted in the erstwhile Mahabubnagar district of Telangana (Figure 1). Mahabubnagar district is one of the western districts of Telangana and lies between 15°55’ to 17°20’ Northern latitudes and 77°15’to 79°15’ Eastern longitudes. The total area of the district is 18,432 Sq. km and ranks 2nd position contributing to 6.70% of the total geographical area of the state. The average normal rainfall in the district is 604 mm and most of it is received during the south-west monsoon. The rainfall was hardly 64.0 per cent of the state average (940 mm). The year-to-year variation in the actual rainfall showed that there were more dry spells during the cropping season. The predominant soil is the chalka dubba which is about 70.0% of the total area and the water holding capacity is low (District census handbook, Mahabubnagar, 2011).

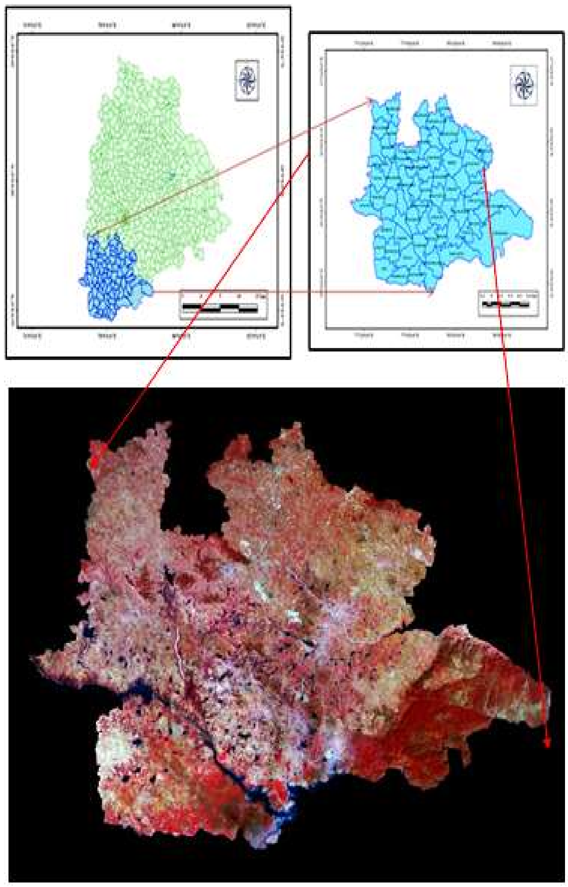

Figure 1: Geographical map of the study area

Satellite data

In this study the freely downloadable, false colour composite satellite images from Landsat-8 OLI sensor were acquired from earthexplorer.usgs.gov.in website to classify the study area. In order to study the crop from sowing to harvesting, multi-temporal, cloud free satellite images from September to February were collected for digital image processing. NDVI values were computed for the satellite data for assessing the area under vegetation. NDVI is calculated as a ratio difference between measured canopy reflectance in the red and near infrared bands respectively (Nageswara *et al.,* 2005).

Digital image processing

The satellite image processing, generation of training sites, acreage estimation and yield estimation was carried out in ERDAS 2018 imagine analysis software.

Area estimation

The satellite images were pre-processed using top of atmosphere corrections for the conversion of radiance images into reflectance images (Figure 2) which were further layer stacked band-wise and mosaic subset images were created using the vector images of the study area. These images were used for generation of NDVI images and then layer stacked into a single image. The multi-date NDVI layer stack image was used for the generation of monthly maximum NDVI composite image. NDVI thresholding is a standard technique which includes calculating of minimum and maximum NDVI values with variance to identify NDVI threshold value of cultivated areas in order to create a mask for cultivated areas and isolate these areas from the other land cover types (Abdelraouf *et al.,* 2018). NDVI thresholding was performed in spatial model maker in ERDAS imagine software with NDVI values > 0.40 and < 0.70 considering as vegetation to form the vegetation mask (Figure 3). The NDVI values < 0.40 indicates the built-up area, water bodies and other land cover types and the NDVI values > 0.70 indicates the forest area. For the vegetation mask, forest mask was applied and the crop mask was generated. The ground control points collected during the survey were superimposed on the 6 date NDVI layerstack image and drawing the area of interest for each GCP, training signatures were generated using the signature editor tool in ERDAS imagine software as presented.

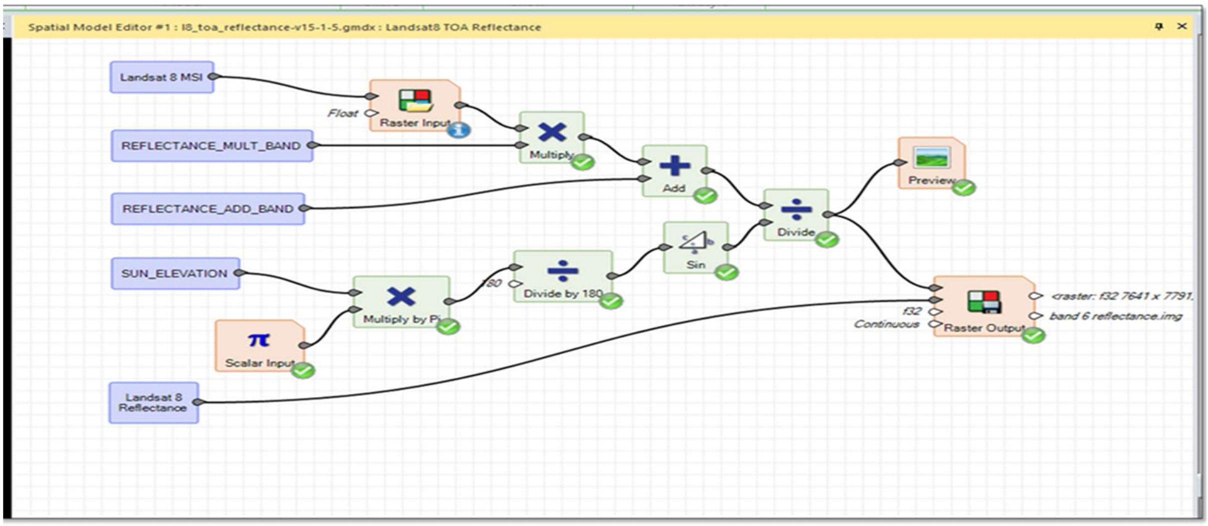

Figure 2: Spatial model used for atmospheric corrections in satellite images

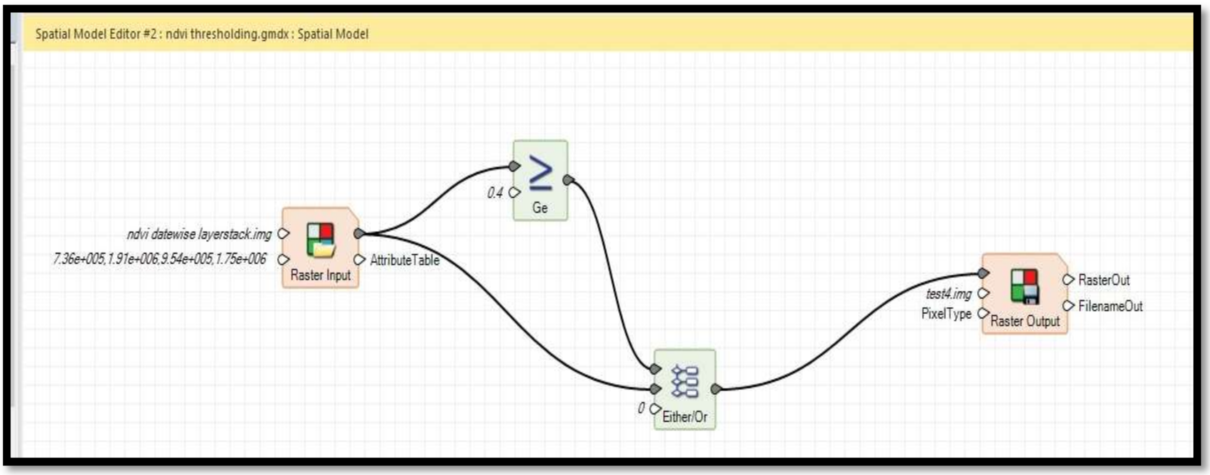

Figure 3: Spatial model used for calculating NDVI threshold

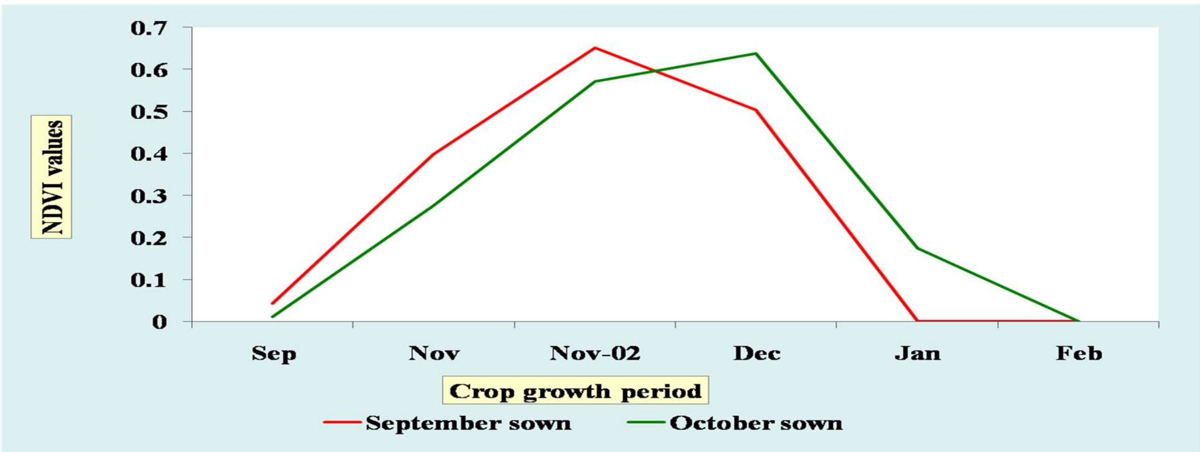

Figure 4: Typical spectral crop growth profile of groundnut crop

From the generated training signatures, by extracting the maximum, minimum, mean and standard devaition statistics, spectral growth profile curves of groundnut crop were generated (Figure 4). Unsupervised classification methods were applied in order to efficiently process a large number of unlabeled samples in remote sensing images (Zhe Ma *et al.,* 2020). Image classification was done using the crop mask and by adopting unsupervised classification technique based on K-cluster algorithm generating 300 spectral classes with 300 iterations and a convergence threshold of 0.90. From the 300 classes generated, the groundnut crop classes were segregated using the spectral signature graphs obtained from the monthly maximum NDVI composite images by overlaying the ground control points collected during the survey. Thus, only groundnut class was isolated for area estimation by manually assigning each class to groundnut by carefully studying the spectral curve from the generated spectral signatures and eliminating all other classes. The area under groundnut crop was then computed to arrive to the estimated area.

Accuracy assessment

Accuracy assessment plays a key role in remote sensing studies for assessing the results. A confusion matrix or error matrix contains information about actual and predicted classifications done by a classification system. The pixel that has been categorized from the image was compared to the same site in the field (Ayyanna *et al.,* 2018). Classification error matrix for the assessment of crop area estimates was prepared as it is one of the most common means of expressing classification accuracy, which compares the relationship between known reference data and the corresponding results of an automated classification which means the variation in total number of crop pixels and the number of correctly classified pixels. From the above information producer's accuracy and user's accuracy were calculated and the overall classification accuracy was computed by dividing the number of correctly classified pixels by total number of reference pixels.

Yield estimation

For yield estimation, ground truth was collected by conducting the crop cut experiments in 3x3 m^2 area in the selected farmers fields. For extraction of maximum NDVI values, seasonal maximum NDVI image was generated using the model maker tool by employing maximum function to the 6-date NDVI layer stack image. Then using the seasonal maximum NDVI values and the crop cut yield data of the corresponding GCP locations, a regression equation (Figure 6) was developed and the yield model thus generated was used to estimate the yields of groundnut. A simple curve was drawn between predicted and observed yields to understand how close the curve fits the data as mentioned in Figure 8. Groundnut yield map generated through remote sensing was validated using Root Mean Square Error (RMSE) and coefficient of determination (r^2) of multiple regression statistical techniques.

Results and Discussion

The area under groundnut crop was estimated by generating the crop mask implementing unsupervised classification technique for image classification and then crop area was estimated. From the above procedure, the groundnut crop coverage for the erstwhile Mahabubnagar district was computed to be 57,865 hectares. The spatial distribution map (Figure 5) of groundnut for erstwhile Mahabubnagar district has shown maximum extent of the crop in Nagarkurnool and Wanaparthy divisions (high potential zone). In this zone, the crop has been observed under cultivation both as homogenous and discrete patches at places. The groundnut crop in rest of the divisions (Narayanpet, Gadwal and Mahabubnagar) of the district was found mostly scattered and in sparse patches. Confusion matrices were used to assess the accuracy of the crop area estimation as they compare the relationship between the ground data as the reference data and the "corresponding" results of the unsupervised classification techniques (Table 1). From the results of accuracy assessment, it was observed that groundnut crop area was estimated with 100% producer's and 90.0% user's accuracy. This indicated that the scrubs omitted in the producer's category were included in the user's category resulting in a misclassification of 10.0%. The spatial distribution map of maize crop of the Mahabubnagar district of Telangana generated through unsupervised classification using Landsat-8 and Sentinel data was classified similarly with a producer's accuracy and user's accuracy of 96%

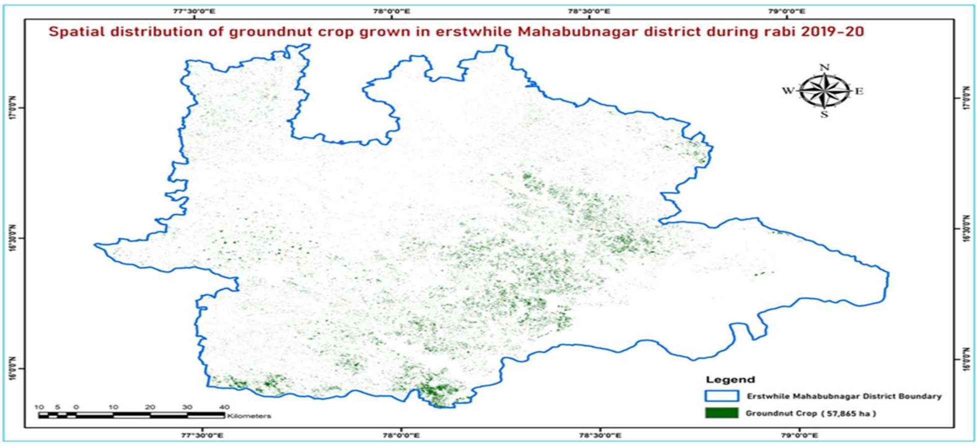

Figure 5: Spatial distribution (hectares) map of groundnut crop acreage obtained through unsupervised classification

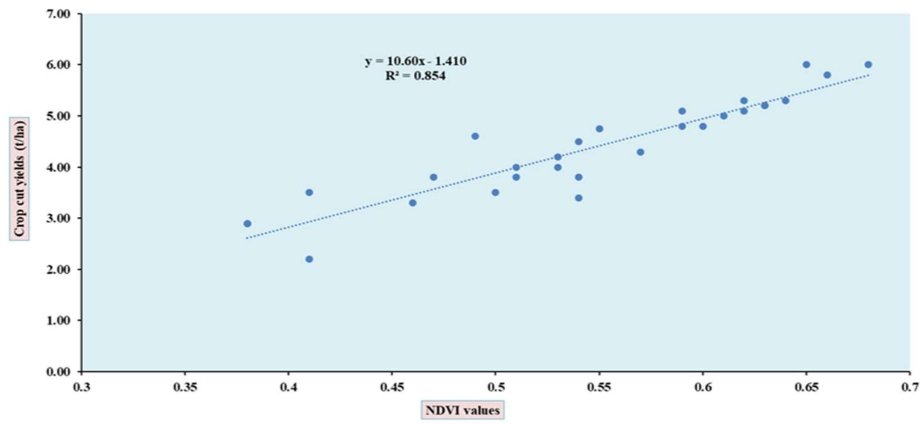

Figure 6: Yield model for estimating groundnut crop yield

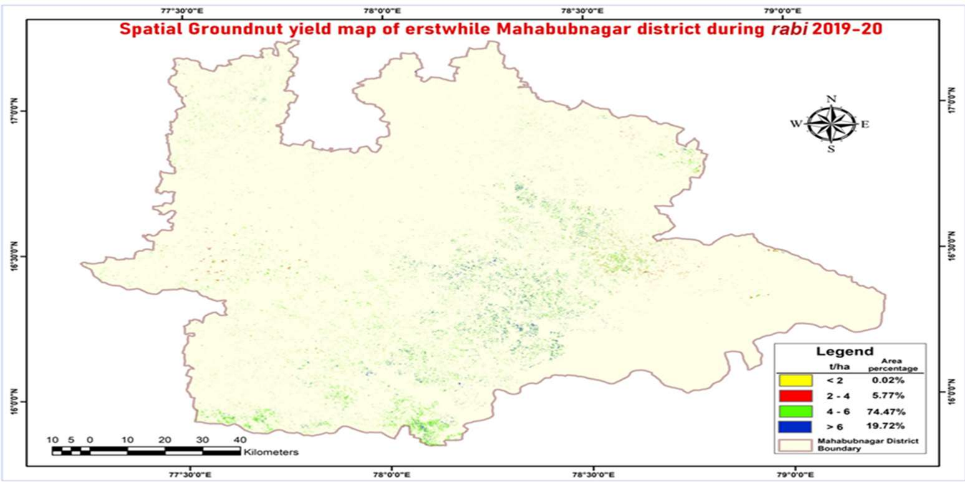

Figure 7: Spatial distribution map depicting the estimated groundnut crop yield

Table 1: Classification accuracy of groundnut crop acreage by unsupervised classification

Classified Data	Non groundnut class	Groundnut class	Row total
Non groundnut class	2	0	2
Groundnut class	0	18	18
Column Total	2	18	20
Producer's accuracy	100 per cent		
User's accuracy	90.0 per cent		

and 86% respectively (Gumma *et al.,* 2021). A similar study conducted in Andhra Pradesh for the delineation of vegetation class, non-vegetation class and water bodies also classified with vegetation class using unsupervised classification with a producer's accuracy and user's accuracy of 96% and 87% respectively (Sreelekha and Reddy, 2019).

Mixed signatures of scrubs with the crop at peak growth stage during November and December months for both September and October sown crops resulted in misclassification. Extensive rains during September and October months had resulted in excessive growth of scrubs along with the crop, thus duplicating the spectral signatures. For unsupervised classification, though the chance of error may decrease by using a lower number of information classes, the chances of pixel misclassification may actually increase in areas where multiple land cover types transit into each other, where there is a large number of instances of mixed pixels (Mukherjee and Mukherjee, 2009) or in cases where a feature may be spectrally similar to those of a different land cover type, such as the confused classes identified by Hung and Wu (2005). The remotely sensed crop area under groundnut crop was compared with ground estimates of state department of agriculture to find the deviation in remotely sensed estimates by computing the relative deviation (RD) percentage. The remote sensing estimate of groundnut acreage for the erstwhile Mahabubnagar district has been computed as 57,865 hectares with a relative deviation of 28.6% from the DOA estimates. Mixing of soil reflectance values with the crop reflectance during initial growth, non availability of cloud free data during peak growth stage and mixing of scrub with the post vegetative growth stages has led to misclassification of the crop. Also, cultivation of the crop in discrete patches owing to small and marginal fields in the study area, and the crop being short statured was misclassified as scrub in most of the regions which might have resulted in less estimation of the cultivated area in the groundnut belt. Further, it may be noted that the remote sensing area estimates were for Mahabubnagar district, while the DOA estimates were obtained from newly formed districts of re-organised erstwhile Mahabubnagar district wherein, couple of *mandals* having groundnut crop cover were added from erstwhile Rangareddy district. Hence, area estimates obtained from the Department of Agriculture were higher than the remote sensing estimates. Also, area estimates of the district could only be obtained during the study. If it were for *mandal* estimates, that accuracy could be precise.

Yield estimation

The crop yield was mainly estimated using NDVI. The NDVI values for groundnut crop ranged from 0.38 to 0.68, with a mean value of 0.55 which represent the maximum greenness value for each groundnut pixel. The groundnut yield estimation was carried out by using the ground information collected by conducting the crop cut experiments in a 3x3 m^2 area of selected farmer's fields in the erstwhile Mahabubnagar district of Telangana and developing the regression equation from the maximum NDVI values and the crop cut yields. The crop cut yields ranged from 1.67 to 6.67 t/ha with an average value of 4.71 t/ha. The yield estimated through the regression equation for the entire study area was categorized into four categories as <2.00 t/ha, 2.10-4.00 t/ha, 4.10-6.00 t/ha and >6.00 t/ha constituting 0.02, 5.77, 74.5 and 19.7% of the groundnut area respectively as mentioned in Figure 7. With the above-mentioned categories, yield map was generated in ERDAS imagine software. The average production of groundnut in the Mahbubnagar was found to be 2.19 lakh tons.

Validation of remotely sensed yield estimates

The yield prediction model estimated groundnut yield with a significant variability of 85.0% (R^2=0.85). The satellite based similar study estimates reported total soybean production as 22 lakh tons with an average productivity of 844 kg/ha when compared to ground truth at an overall accuracy of 80.7% showing the reliability of RS based crop inventory (Maurya, 2011). The regression yield model was validated using measured yield collected from the crop cut experiment plots and the predicted yields derived from the satellite image. The observed /measured yield from CCE plots ranged from 2.20-6.00 t/ha. On the other side, predicted yield for groundnut ranged from 4.06-6.81 t/ha. The predicted yield deviated from the observed /measured yield ranging from 0.07-1.92 t/ha with a mean value of 1.57 t/ha. A simple curve was drawn between predicted and observed yields to understand how close the curve fits the data which showed a strong positive

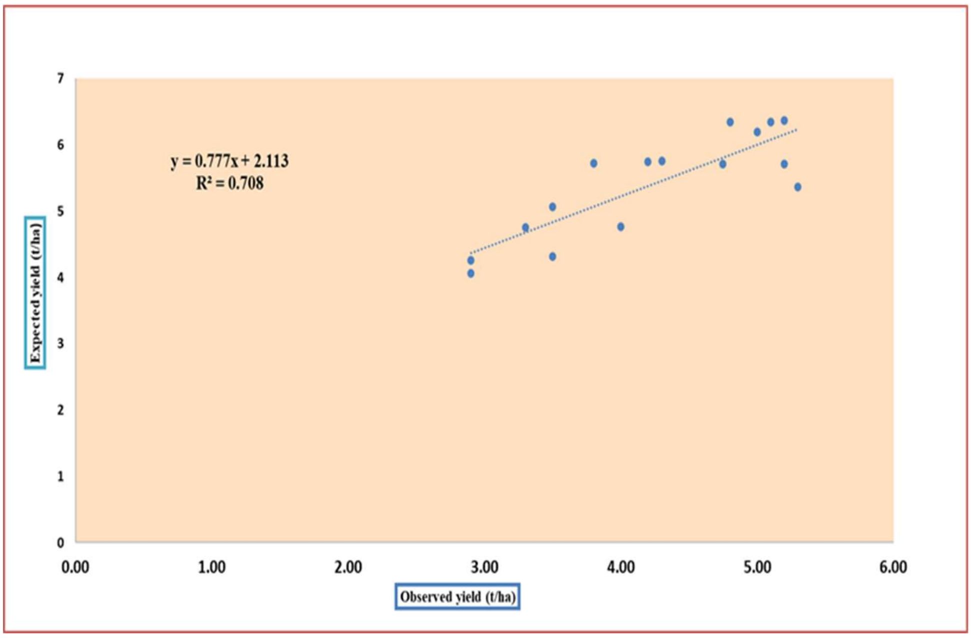

Figure 8: Scattered diagram plotted for the determination of correlation coefficient for validating the yield model

correlation of observed and predicted yields with R^2 value of 0.71 and the correlation coefficient of 0.87. The yield estimation of sugarcane crop under FASAL programme using the Landsat and Resourcesat data also arrived at a correlation coefficient value of 0.60 which may be attributed to lack of capturing ability of variation found in the sowing dates of the crop across the country (Sharma *et al.,* 2019). A significant correlation (r^2 = 0.82) between the groundnut pod yield with that of the corresponding NDVI values was also reported at 115 dryland locations at Queensland during 2004-07. The measurement of infrared reflectance from groundnut crop canopies via multispectral satellite imagery was observed to be an effective method for identifying the spatial variability in crop vigour, as well as producing high correlations with groundnut yield (r^2=0.91) and poor maturity (r^2=0.67) (Andrew *et al.,* 2007). The root mean square error (RMSE) was used to validate the performance of regression yield model (Miles and Shelvin, 2001) and measures dispersion of the observations from the true values (Longley *et al.,* 2005). The smaller the RMSE value, higher is the accuracy of the predicted values (Watson and Teelucksingh, 2002). The RMSE value for the yield estimates in the present study was found to be 1.25 which indicates that the predicted yields deviated from the observed yields by 1.25 t/ha.

Conclusion

The groundnut crop acreage estimation using multi-date NDVI images from Landsat-8 OLI sensor by adopting K-cluster algorithm technique in unsupervised image classification has realized the groundnut area of 57,865 ha during *rabi*, 2019-20 for the erstwhile Mahabubnagar district with producer's and user's accuracy of 100 and 90.0%, respectively. The remote sensing crop area estimates deferred from the estimates (81,095 ha) of the State Department of Agriculture, Government of Telangana with a relative deviation of 28.64%. The estimated crop yield was categorized into four classes viz., < 2 t ha-1, 2 to 4 t/ha, 4 to 6 t/ha and > 6 t/ha contributed from 0.02, 5.77, 74.5 and 19.7% of the study area, respectively. The crop cut yields in the study area ranged from 1.67 to 6.67 t/ha. The NDVI values for groundnut ranged from 0.38 to 0.68 representing maximum greenness for each groundnut pixel. The yield model generated based on crop cut yield data and NDVI values, predicted the crop yield with a good correlation at r^2 of 0.708 and correlation coefficient of 0.869. The predicted yields deviated from the observed yields with RMSE of 1.25 t/ha.

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

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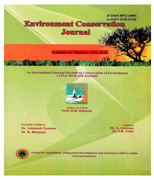

Performance of castor in heavy metal polluted soils under the treatment of various decontaminants

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ABSTRACT

In order to find out the performance of castor under decontaminant treated heavy metal polluted soils, an experiment was conducted at Students Farm, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, during *kharif* 2016 to study the performance of castor in heavy metal polluted soil under the treatments of various decontaminants (various dosages of phosphorus as well as, quick lime). The dry matter before flowering, and stalk yield at harvest of castor varied from 429 to 516, 1460 to 1758 kg/ha, respectively. Among the different decontaminants highest dry matter yield and stalk yield (516 and 1758 kg/ha at before flowering and harvest) and seed yield (1720 kg/ha) was obtained in T_5 (RDF+CaO @ 2 t/ha), which was significantly superior over all other treatments and on par with T_4 (RDF+CaO @ 1 t/ha), and per cent increase over RDF was 20.41 and 23.56, respectively for stalk, and seed yield of castor. Decontamination treatments had reduced the mean Pb, Cd, Ni and Co contents of castor to 4.51, 0.65, 0.95 and 0.63 mg/kg, and increased mean uptake to 7.62, 1.17, 1.69 and1.09 g/ha respectively, for Pb, Cd, Ni and Co in seed at harvest. The Pb, Cd, Ni and Co contents of soil after harvest of the castor crop ranged from 17.11, 0.79, 1.89 and 1.22 mg/kg in the reference control and decreased to 14.60, 0.68, 1.67 and1.02 with RDF+CaO @ 2 t/ha treatment. The reduction in Pb, Cd, Ni and Co concentration in post-harvest soil was more due to Cao at different levels when compared to high phosphorus.

Introduction

In India, due to rapid industrial development during the last few decades, disposal of industrial effluents has become serious problem, and application of those effluents to land has become a common means of disposal in the recent past (Solanki *et al*., 2019; Bhardwaj *et al.,* 2020). Besides being a useful source of plant nutrients, these effluents often contain high amounts of various organic and inorganic materials as well as heavy metals. Such industrial effluents when mixed with water used for irrigation, becomes potential threat to soil physical, chemical and biological environment, as a whole to the soil health and ecosystem functionality (Ruhela *et al.,* 2021). The unscientific disposal of untreated or undertreated effluents has resulted in huge accumulation of heavy metals in soil and finally gets entry into the human and animal food chain through the crops grown on it (Ruhela *et al.,* 2022). Restriction of crop cultivation in such toxic metal polluted soils, is the best option to check the entry of such hazardous heavy metals into the day today food chain of the animals. But looking at the ever-increasing food demand of the globe, it is not practically possible to restrain those areas from

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growing crops. Thus, the real challenge to a soil scientists lies in restoring these soils to normal levels within reasonable time and cost, as non-renewable sources like soils cannot be afforded to be left unused. The heavy metal contaminated soils can be remediated through two approaches *i.e.,* phytoremediation (Ghosh and Singh, 2005; Bhutiani *et al.,* 2019a and b) and/or chemical decontamination (Balachandra *et al*., 2021). Remediation methods available for reducing the harmful effects of these heavy metals at their contaminated sites include chemical, physical and biological techniques (Padhan *et al*., 2021). These can be grouped into two categories *i.e*., ex-situ and in-situ methods Reed *et al* (1992). The conventional ex-situ methods for remediating the polluted soils are based on excavation, detoxification and/or destruction of contaminants physically or chemically, as a result the contaminants undergo stabilisation, solidification, immobilisation, incineration or destruction (Padhan *et al*., 2021 and Balachandra *et al*., 2021). Identification of chemical amendments followed by evaluation of their cost effectiveness can be a key element in a sustainable land management strategy for reclaiming heavy metal contaminated agricultural lands. In this backdrop the experiment was taken up to study the performance of castor on application of various decontaminants in a heavy metal polluted soil.

Material and Methods

An experiment was setup to know the effect of inorganic amendments in abetting the heavy metals pollution in a polluted soil at Student Farm, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad. The basic properties of the soil were analyzed using standard protocol Marchi *et al.* (2009). The soil of the experimental site was sandy clay loam in texture, slightly acidic in reaction, normal in soluble salt content with available N (275 kg/ha), P_2O_5 (34 kg/ha) and K_2O (275 kg/ha) and DTPA extractable Pb (20.24 ppm), Cd (1.11 ppm), Ni (2.27 ppm) and Co (2.27 ppm). The experiment was laid out in randomized block design with 5 treatments and 4 replications with castor as test crop. The treatments include T_1 RDF (only), T_2: (T_1)+High phosphorus 150%, T_3: (T_1) + High phosphorus 200%, T_4: (T_1)+lime (CaO)1t/ha, T_5: (T_1)+lime (CaO) 2 t/ha. The recommended dose of fertilizers for castor was 60: 40: 30 kg N: P_2O_5: K_2O ha^{-1} as per the PJTSAU, Telangana and were applied in the form of urea, Single Super Phosphate (SSP) and Muriate of Potash (MOP) respectively. High amount of phosphorus and quick lime were applied as per the treatments and were mixed thoroughly with soil to ensure uniform distribution of the decontaminants in the soils of respective plots. The crop was sown on 20th of June 2016 and the irrigation and plant protection measures were taken up as and when required. The representative plant samples were collected from all the treatments before flowering (whole above ground biomass) and at harvest stage (stem and seeds separately) of the crop. The soil samples collected after harvesting of the crop and the plant samples collected during different growth stages were analyzed in order to find out the heavy metal concentration in each sample.

Results and Discussion

Effect of applied decontaminants on dry matter and seed yield (kg/ha) of Castor.

Dry matter is the expression of growth and development of different morphological components and it is directly related to the yield. Dry weight of plants before flowering and stalk and seed yield at harvest are given in table 1, 2 and 3 respectively.

Dry weight of plants before flowering

The dry weight of the castor plants collected before flowering ranged from 429 to 516 kg/ha with a mean of 482 kg/ha. Decontaminant treatments were found effective in bringing about significant increase in the dry weight of plants collected before flowering over the control. Also, there was significant effect of application of the two decontaminants on dry weight of castor. On an average, the dry weight of plants obtained under the decontaminant treatments i.e. high phosphorus at 150 and 200% or lime application at 1 and 2 t/ha are 470, 472, (513 and 516 kg/ha) respectively. But the dry matter yield obtained under various doses of P application were at par with each other and similar results were also obtained in case of quick lime treatments. In terms of dry weight and percentage, the dry weight of plants obtained under decontaminants contaminant treatments increased by 482 kg/ha and 14.91% over the control one. The increase in dry weight of plants under decontaminant application might be due to

Table 1: Direct effect of applied decontaminants on dry matter yield, heavy metal contents and uptake by castor before flowering (*kharif*, 2016)

Treatments	Dry matter (kg/ha)	Heavy metal contents (mg/kg)					Heavy metal uptake (g/ha)				
		Pb	Cd	Ni	Co	Total metal load	Pb	Cd	Ni	Co	Total metal uptake
T1-100% RDF	429	27.01	0.78	3.47	1.50	32.76	11.59	0.33	1.49	0.64	14.05
T2-T1+ High phosphorus 150%	470	25.01	0.70	3.36	1.47	30.54	11.75	0.33	1.58	0.69	14.35
T3-T1+ High phosphorus 200%	472	24.86	0.71	3.33	1.44	30.34	11.73	0.34	1.57	0.68	14.32
T4-T1+ lime (CaO) 1 t/ha	513	22.89	0.69	3.23	1.32	28.13	11.74	0.35	1.66	0.68	14.43
T5-T1+ lime (CaO) 2 t/ha	516	21.70	0.69	3.15	1.32	26.86	11.20	0.36	1.63	0.67	13.86
Mean of Decontaminants	493	23.62	0.70	3.27	1.39	28.97	11.61	0.34	1.61	0.68	14.24
Overall mean	482	24.18	0.71	3.30	1.41	29.60	11.60	0.34	1.59	0.68	14.21
S. Em$\pm$	13.39	0.65	0.02	0.08	0.05	0.99	0.15	0.02	0.06	0.02	0.21
CD (*P=0.05*)	40.00	1.96	0.07	0.23	0.16	2.98	NS	NS	NS	NS	NS

Table 2: Direct effect of applied decontaminants on stalk yield, heavy metal contents and uptake by castor at harvest (*kharif*, 2016)

Treatments	Stalk yield (kg/ha)	Heavy metal contents (mg/kg)					Heavy metal uptake (g/ha)				
		Pb	Cd	Ni	Co	Total metal load	Pb	Cd	Ni	Co	Total metal uptake
T_1-100% RDF	1460	12.39	3.71	2.40	1.04	19.54	18.09	5.42	3.50	1.52	28.53
T_2-T_1+ High phosphorus 150%	1605	11.68	3.45	2.34	1.00	17.66	18.75	4.51	3.58	1.51	28.34
T_3-T_1+ High phosphorus 200%	1608	11.45	3.42	2.29	0.94	17.13	18.59	4.26	3.26	1.43	27.55
T_4-T_1+ lime (CaO) 1 t/ha	1751	10.54	3.16	2.10	0.84	15.84	18.45	4.41	3.43	1.44	27.72
T_5-T_1+ lime (CaO) 2 t/ha	1758	10.29	3.15	2.08	0.82	14.64	18.09	4.24	2.00	1.41	25.74
Mean of Decontaminants	1681	10.99	3.30	2.20	0.90	16.32	18.47	4.35	3.07	1.45	33.48
Overall mean	1636	11.27	3.38	2.24	0.93	16.96	18.39	4.57	3.16	1.46	33.53
S. Em$\pm$	47	0.31	0.09	0.03	0.03	0.59	0.24	0.42	0.53	0.05	0.96
CD (*P=0.05*)	140	0.92	0.28	0.10	0.09	1.78	NS	NS	NS	NS	NS

reduced concentration and activity of toxic metals in soils there by reducing their detrimental effects on plants growth (Mathavan *et al*., 2001).

Yield at harvest

The stalk (table 2) and seed (table 3) yield collected at harvest varied from 1460 to 1758 and 1392 to 1720 with a mean of 1644 and 1587 kg/ha, respectively. Significant effect of decontaminants was seen on the stalk and seed yield over control (RDF). There was significant increase in stalk and seed yield of castor at harvest due to application of decontaminants but there was no significant difference in stalk or seed yield of castor within the doses of applied decontaminants i.e. high phosphorus (150 and 200%) and lime application (1 or 2 t/ha). Highest yield of stalk (1758 kg/ha) and seed (1720 kg/ha) was recorded when lime application was done along with RDF @ 2 t/ha. Increase in yields of castor by the application of inorganic amendments may be attributed to suppression of heavy metals toxicity, improving soil physical condition (Park *et al.*, 2011) and increase in mineral nutrition (Hamid *et al.*, 2019). Similar results were also observed by Ranjeet Kumar (2016) who reported a significant increase in yields of spinach due to application of organic and inorganic amendments in contaminated soil at New Delhi. The performance of treatments in terms of seed yield were in the order of $T_5 > T_4 > T_3 > T_2 > T_1$. Application of quick lime resulted in 23 per cent increase in seed yield of castor over control which may be due to improved workability of soil, soil aggregation, porosity and aeration which might have resulted in increased seed yield of castor. Similar results were reported by Daur and Tatar (2013). Muhammad and Khattak (2011) in wheat and Chamun *et al.* (2011) in rice also found the similar observation where increase in yield was obtained due to application of gypsum along with FYM. Muhammad *et al.* (2017) observed higher wheat yields with gypsum application in an effluent irrigated field at Multan in Pakistan, Ahmad *et al.* (2017) also reported higher wheat yields with gypsum application as a amendment in alkaline soils using irrigation water polluted with Cd and Pb for maize. Due to inherent soil acidity in area, liming resulted in increased yield. The positive effect of high phosphorus and lime in trace metal contaminated soil may be due to the reduction in bioavailability of toxic metals by complexation and adsorption. Similar results were reported earlier by Mathavan *et al.* (2001[a]) Bolan *et al.* (2003) and Rattan *et al.* (2005).

Effect of decontaminants on concentration of heavy metals in castor (before flowering)

Lead, cadmium, nickel and cobalt contents in the dry matter samples collected before flowering in the reference control (only RDF) treatment were 27.01, 0.78, 3.47 and 1.50 mg/kg, respectively. Application of decontamination treatments reduced the mean heavy metal content in the dry matter to 23.62, 0.70, 3.27 and 1.39 mg/kg, respectively. Though there was a reduction in heavy metal contents i.e. (Pb, Cd, Ni and Co) in the samples collected, but the concentrations were at par with each other were (table 1). The uptake of heavy metals (Pb, Cd, Ni and Co) in the dry matter collected before flowering were increased by the decontamination treatments except T_5 when compared to RDF (11.59, 0.33, 1.49 and 0.64 g/ha, respectively), but the increase was found to be non significant. The total heavy metal uptake varied between 13.86 and 14.35 g/ha (table 1).

Effect of decontaminants on concentration of heavy metals by castor at harvest

Heavy metal contents and uptake of stalk and seed were presented in (table 2 and 3). Heavy metal contents showed a decrease both in the seed and stalk with the application of decontaminants. Heavy metals viz., Pb, Cd, Ni and Co in the control (RDF) were 12.39, 3.71, 2.40 and 1.04 mg/kg, respectively in stalk and 5.17, 0.82, 1.17 and 0.76 mg/kg, respectively in seed which decreased to 10.99, 3.30, 2.20 and 0.90 mg/kg, respectively in stalk and 10.21, 2.46, 1.85 and 0.82 mg/kg, respectively in seed at maturity. There was significant reduction in Pb, Cd, Ni and Co contents in the stalk and seed noticed in the lime applied treatment at both the doses (1 and 2 t/ha) over control (RDF) and the reduction in Ni and Co concentration in stalk and Pb, Cd, Ni and Co in seed was also seen at high phosphorus application *i.e.,* P @ 200%. The mean total metal load ranged from 14.64 to 19.54 mg/kg in stalk and 6.74 to 7.92 mg/kg in seed.The heavy metal uptake was not significantly influenced by the decontamination treatments. The mean heavy metal uptakes recorded by different treatments were 18.39, 4.57, 3.16 and 1.46 and 7.53, 1.17, 1.68 and 1.09 g/ha in stalk, and seed, respectively.

The total heavy metal uptakes ranged from 25.74 to 28.53 and 11.02 to 11.77 g/ha in the stalk and seed, respectively. Beneficial effect of organic and inorganic amendments on heavy metal conc of plants were also observed by various scientists. For example, Park *et al.*, 2011 observed that Pb content in sunflower shoot reduced by 60-80% with amendments application in sewage polluted soils. Ranjeet Kumar (2016[a]) found the significant reduction of Pb in plant parts of spinach in amended polluted soil when compared to non amended polluted soil at New Delhi. Pandit *et al.*, 2017 also reported that the application of amendment decreased the Cd concentration to an extent of 61 percent in spinach plant compared to unamended treatment in a contaminated soil. The immobilization of Cd and Pb through adsorption, complexation and precipitation phenomena, may have resulted in reduced phytotoxicity and accumulation in plants (Geebelen *et al.*, 2002;

Table 3: Direct effect of applied decontaminants on seed yield, heavy metal contents and uptake by castor at harvest (*kharif*, 2016)

Treatments	Seed yield (kg/ha)	Heavy metal contents (mg/kg)					Heavy metal uptake (g/ha)				
		Pb	Cd	Ni	Co	Total metal load	Pb	Cd	Ni	Co	Total metal uptake
T_1-100% RDF	1392	5.17	0.82	1.17	0.76	7.92	7.20	1.14	1.63	1.06	11.02
T_2-T_1+ High phosphorus 150%	1549	4.84	0.79	1.11	0.71	7.45	7.50	1.22	1.72	1.10	11.54
T_3-T_1+ High phosphorus 200%	1560	4.76	0.77	1.08	0.70	7.31	7.43	1.20	1.68	1.09	11.40
T_4-T_1+ lime (CaO) 1 t/ha	1716	4.54	0.67	1.01	0.64	6.86	7.79	1.15	1.73	1.10	11.77
T_5-T_1+ lime (CaO) 2 t/ha	1720	4.51	0.65	0.95	0.63	6.74	7.76	1.12	1.63	1.08	11.59
Mean of Decontaminants	1636	4.66	0.72	1.04	0.67	7.09	7.62	1.17	1.69	1.09	11.58
Overall mean	1587	4.76	0.74	1.06	0.69	7.26	7.53	1.17	1.68	1.09	11.47
S. Em±	52	0.10	0.01	0.02	1.07	0.20	0.22	0.03	0.05	0.03	0.26
CD (*P=0.05*)	156	0.32	0.04	0.05	0.04	0.60	NS	NS	NS	NS	NS

Table 4: Heavy metal status (mg/kg) in post-harvest soil of castor under different decontamination treatments (DTPA)

Treatments	Available heavy metal status (mg/kg)			
	Pb	Cd	Ni	Co
T_1-100% RDF	17.11	0.79	1.89	1.22
T_2- T_1+High phosphorus 150%	16.45	0.76	1.84	1.19
T_3-T_1+ High phosphorus 200%	16.15	0.75	1.78	1.15
T_4-T_1+ lime (CaO) 1 t/ha	15.64	0.71	1.73	1.06
T_5-T_1+ lime (CaO) 2 t/ha	14.60	0.68	1.61	1.02
Mean of Decontaminants	15.71	0.73	1.74	1.11
Overall mean	15.99	0.74	1.77	1.13
S. Em±	0.51	0.02	0.05	0.04
CD(*P=0.05*)	1.53	0.06	0.16	0.12

Table 5: Heavy metal status (mg/kg) in post-harvest soil of castor under different decontamination treatments (AB-DTPA)

Treatments	Available heavy metal status (mg/kg)			
	Pb	Cd	Ni	Co
T_1-100% RDF	17.01	0.75	1.87	1.20
T_2- T_1+High phosphorus 150%	16.43	0.74	1.81	1.15
T_3-T_1+ High phosphorus 200%	16.12	0.71	1.77	1.12
T_4-T_1+ lime (CaO) 1 t/ha	15.55	0.67	1.71	1.04
T_5-T_1+ lime (CaO) 2 t/ha	14.41	0.63	1.58	1.00
Mean of Decontaminants	15.63	0.69	1.72	1.08
Overall mean	15.90	0.70	1.75	1.10
S. Em±	0.56	0.02	0.06	0.04
CD(*P=0.05*)	1.69	0.07	0.17	0.11

Seaman *et al.*, 2003). Rehman *et al.* (2015) also found that gypsum application decreased the grain and straw Cd concentration in wheat and rice crops grown on contaminated soil at Faisalabad in Pakistan. Gypsum might be better amendments for *insitu* immobilization of Cd and some other heavy metals due to its low cost and frequent availability (Illere *et al.*, 2004). Ahmad *et al.* (2017) also found the reduction of Cd and Pb concentration in wheat with the addition of gypsum at Faisalabad. The percentage reduction of all the heavy metal content due to application of decontamination treatments over the control in castor crop grown on polluted soil at harvest in seed ranged from 6.38 to 12.76 for Pb, 3.65 to 20.73 for Cd, 5.12 to 18.80 per cent for Ni, and 7.04 to 20.63 per cent for Co. The maximum percentage reduction in heavy metals over the control was in T_5 where with CaO @ 2 t/ha. The reduction in heavy metals contents in seed with the best treatment were in order as Cd>Co>Ni>Pb. According to Bolan *et al.* (2003[a]) Ca^{+2} addition through lime inhibited the translocation of Cd, Pb and other heavy metals from root to shoot as these metals were accumulated primarily on cell walls of roots with only limited amounts translocated to shoot and seed. Stabilization of metals by these amendments through adsorption, complexation and reduction reaction (Brown *et al.* 2003; O' Dell *et al.* 2007).

Heavy metal status (mg/kg) in post-harvest soil

DTPA and AB-DTPA extractable Heavy metal status

There was a significant influence of applied decontaminants on the status of DTPA extractable heavy metal status at maturity. There was a depletion of available heavy metals due to application of two different sources of decontamination treatments. The contents varied from 14.60 to 17.11, 0.68 to 0.79, 1.61 to 1.89 and 1.02 to 1.22 mg/kg, respectively for Pb, Cd, Ni and Co at different levels of applied decontaminants. The mean concentration of extractable heavy metals at maturity were 15.99, 0.74, 1.77 and 1.13 mg/kg, respectively of Pb, Cd, Ni and Co (table 4).Though the variation in contents between different levels and sources were not significant except lime @ 2 t/ha, where there was a depletion in extractable heavy metal status as extracted by AB-DTPA due to application of decontaminants at different levels. The mean AB-DTPA extractable heavy metals i.e. Pb, Cd, Ni and Co contents in the soils after the harvest of direct crop of castor were 15.90, 0.70, 1.75 and 1.10 mg/kg, respectively (table 5). The reduction in Pb, Cd, Ni and Co in post harvest soils were more due to Cao (quick lime) at different levels as compared to application of high doses phosphorus. The reduction of available heavy metal contents in post harvest soils with the application of CaO might be due to the stabilization of metals by amendments (Mathavan 2001).

Conclusion

From our study it appeared that inorganic amendment application might have decreased the available heavy metal contents in the soil and thereby providing more congenial atmosphere for castor growth due to reduction in their contents in plant. Though the application of quick lime did not contribute to mineral nutrition of the plant directly, it can be concluded that these amendments helped in increasing seed yield of castor grown on polluted soils by reducing detrimental effects of toxic metal in soil for plant growth and neutralizing the inherent soil acidity. Solubility and mobilize of heavy metals of soils was reduced due to amendment application and hence their toxicity could be reduced. It was observed that available heavy metal contents in soils decreased in amendment treated soils as compared to unamended one. A reason for decrease in plant available heavy metals in soils may be due to the stabilization of metals by amendments in order to form insoluble complexes restricting its activity in soil.

Conflict of interest

The authors declare that they have no conflict of interest.

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Role of nalas in development of landforms and land use pattern with special reference to the Stakmo village, Leh valley

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ABSTRACT

In the Leh valley, apart from the river Indus, several nalas have a great significance on development of a number of villages and settlement areas. The present work is an effort to illustrate the pivotal role of the Stakmo nala in establishment of the Stakmo village. The study also seeks to emphasis the necessity of the nala basin management to sustain the natural resources for the existence of the villages. Various problems related with Leh city and its adjacent area have been studied by several scientists. However, most of the scientists hardly show interest in depicting the comprehensive roles of the different nalas for the construction of many settlement areas and their related issues in the Leh valley. Present authors have tried to unfold the role of nalas in development of landforms and land use pattern with special reference to Stakmo village, Leh valley. Along the Leh valley there are11 important nalas which have key role to supply necessary natural resources to develop a number of important villages and settlement areas. Undoubtedly, availability of natural resources of an area greatly influenced by the process of landform development and their characteristics, because landscape characteristics play a significant role in generation of local natural resources. Hence, in order to illustrate the significant role of the Stakmo nala in development of landforms and land use pattern in Stakmo village and its adjacent area, the authors analysed vivid geomorphic features of the Stakmo nala basin based on quantitative approach including morphometric techniques and field observation. Apart from morphometric analysis, field based data regarding nala sediment size, rock types, micro features, varied land use pattern of the Stakmo village including perception study of the local people have been tried to discuss in the work.

Introduction

Leh valley is situated in high altitude cold desert area under periglacial environment. Most of the part of Ladakh area is under barren land due to lack of water resource. Hence, existence of lives in this hard climatic region is very difficult. That is why development of settlement area in Ladakh area is mainly restricted in valleys where snow melt nalas are present. The current paper has tried to explain the role of the Stakmo nala in development of landforms and land use characteristics.Though, various problems regarding landforms and land use of Leh city and its adjoining areas of Ladakh area have been studied by several scientists.The rain shadow Ladakh area has been several times highlighted in many studies for its cloud burst induced flash flood vulnerability previously in different years specially in Leh valley (Ziegler *et al.,* 2016; Gupta *et al.,* 2012; Arya; 2011; Juyal, 2010).A number of scientists (Barrett,2014) have focused their studies on climate change and associated glacial retreat problems in the area. Searle (2011) had benchmark contribution in the study of geology in Ladakh and Karakoram ranges. Lal *et al.* (2018) has emphasised the tectonic influences on the

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landform development in Ladakh region. Many scientists (Sant *et al.,* 2011) have also emphasised on geomorphological classification of Leh valley emphasising on periglacial landforms. Koul *et al.* (2016) published report on glacier status over the past 50 years (1962-2013) on remotely-sensed volumetric changes of glaciers in Drass glacier basin, Ladakh Mountain, North-West Himalaya. Gupta *et al.* (2017) elaborated vividly the characteristics of the soils of Ladakh region. Mukhopadhyay (1980) had elaborated geomorphology of peri glacierised area of upper Tista basin.Sangode *et al.* (2017) have analysed the sedimentary and geomorphic signatures of 2010 cloudburst triggered flash flood in Leh valley. Mujtaba *et al.* (2017) identified the geomorphic imprints of the paleolakes, alluvial fans and other landforms around Leh. Many renowned scientists (Patel *et al.,* 2008 ; Raghuvanshi *et al.,* 2019) have contributed their valuable research works on agricultural land use patterns and associated problems in Leh and its adjoining areas. Dame *et al.* (2019) nicely elaborated the scenario of rapid urbanisation of the Leh town as well as the conversion of the barren land and agricultural land into built up area.

However, the earlier studies have hardly illustrated the role of nalas in development of different geomorphic features and land use pattern in different villages and settlement areas of the Leh valley. Hence, the present research has focused on quantitative analysis of the Stakmo nala basin emphasising the landform and land use relationship in a quantitative manner. The investigators have also vividly explained the characteristics of the landforms and their impacts on land use pattern in the Stakmo valley to study the local resource vulnerability.

Geographical location of the Stakmo village:

The Stakmo village is mainly located in the Leh valley.The longitudinally trending NW-SE Leh Valley extends from Karoo in SE (33°54′48″N, 77°44′14″E) to Pathhar Sahib in NW (34°11′31″N, 77°22′19″E). The Leh valley bordered by snow covered mountainous Ladakh and Zanskar hill ranges. Ladakh range lies between the Indus and Shyok river valleys, stretching to 230 miles (370 km). The entire eastern flank within Leh valley is marked by 11 major transverse streams arising from the Ladakh batholithic range and opening into the main Indus valley. Among the 11 nalas,Stakmo (figure 2)is one of the important nalas which has a great influence on the development of the Stakmo village (figure 1).The figure 3shows the upper course of the Stakmo nala flowing from the Ladakh range. The Stakmo village is situated over dry fan (fan apex,34° 7' N & 77° 42' 1" E) in the Leh valley. Along the fan surface, the Stakmo village (77° 42' 21.56" E, 34° 01' 36.90" N), has been developed which is under Leh block.

Figure 1: Location of the Stakmo Village and Leh City. (Source: Toposheet, S.O.I)

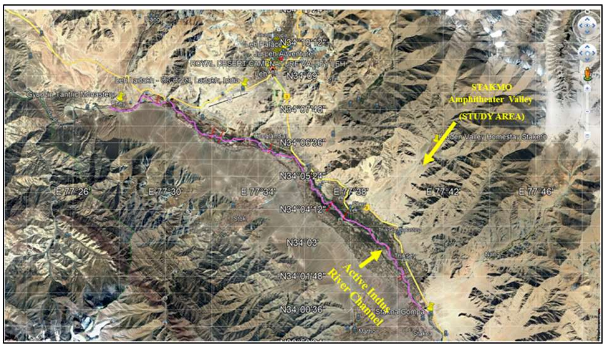

Figure 2: Location of Active Indus Channel and Stakmo Nala (study area) in Leh Valley, (source: Google Satellite Image, 2021)

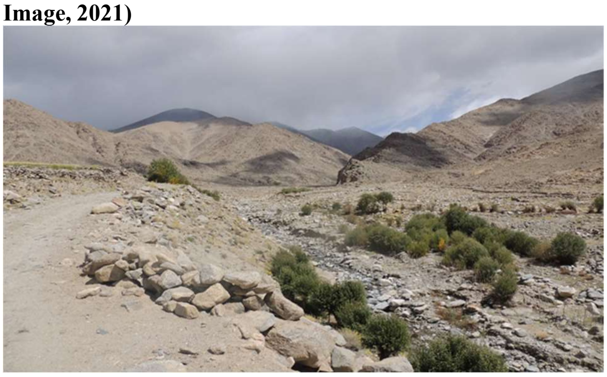

Figure 3: Location of the Stakmo nala (Source: Field survey, 2014)

Material and Methods

Stage-I: The paper is prepared on several secondary data and primary data including field observation. The secondary data includes toposheet (SOI), satellite images (google earth,2020),valuable literatures and the primary data consists of local people perception and field recorded data with photographic evidences. Based on several previous literature review specific research gap regarding *significance of nala* has been identified .

Stage-II: Field verification was done to gather different types of primary data of the Stakmo nala basin (landform study, nala morphology analysis, nala bed sediment study, observation of land use practices), photographic evidences including the people perception about the Stakmo village as well as the Stakmo nala .

Stage-III: Analysis of the secondary data was so import to delineate the Stakmo nala basin as well as fan area and establish the relationship among the different physical setup (physiography, ground water availability, climate, geology) .Morphometric interpretation of the basin has been done based on SRTM DEM. Visual interpretation of the satellite image (google earth,2021) was also very relevant for better understanding about the land use pattern of the basin.

Stage-IV: Depending on the analysis of the collected primary and secondary data role of the Stakmo nala in development of landform and land use has been evaluated and few effective measures have been concluded towards the sustainability of the natural resources of the Stakmo basin.

Objective: The main objective of the present paper is to illustrate the influence of the Stakmo nala on the development of landforms and landuse pattern in the Stakmo basin, Leh block.

Results and Discussion

Role of Physical setup for scarcity in ground water resources

The Leh city and its adjacent area are located in Leh valley which lies in the rain shadow zone because the Great Himalaya and the Karakoram range are located towards south and north respectively of the valley and the Tibatan plateau is situated towards east of the valley. As a result of the orographic barriers the area receives very little amount of rainfall. Annual average high and low temperature at Leh are 12.8 and -1.3 degree celcius respectively. The annual average rainfall at Leh is 105.5 mm (IMD, 2018). In the rain shadow region, due to lack of moisture a desert environment has been developed in Ladakh area. As a result of acute deficiency of soil moisture existences of trees and other vegetation are not found except the narrow valley portion. Extended parts of the area are under barren land. Apart from the climatic condition, geological setup of the area is also not favourable for the storage of ground water resources. The district is underlain by consolidated formation in maximum part. Ground water in these formations occur in fissures and fractures developed due to repeated tectonic activity. Large scale ground water development is not possible in consolidated formations but limited development of ground water resources can be taken up. As a result, ground water development in the study area is on moderate scale restricted only to the valley portion. At present, the development of ground water resources is very insufficient. According to the report by the Central Ground Water Board CGWB (2014), Leh district is about 81840 sq. km. and the total valley area is approx. 81 sq. km. The number of wells constructed by CGWB is only 25. In 1st phase(1973-2001) it was 16 and in 2nd phase(2005-2008) 9 wells were constructed. According to the Census report (2011) of India, total population of the Leh district is about 147104. The data is enough to establish the acute inadequacy in storage and supply of ground water resource in compare to total population resource in the are under study.

Broad drainage features including nalas: The Leh valley is mainly drained by the river Indus. Two main rivers flowing in this area Nobra and Shyok rivers. Nubra is a perennial river and is originated from Siachen Glacier and flows in North west to South east direction. Shyok river is also a perennial river and it originates from South Rimo Glacier and Central Rimo Glacier. Beside the main river Indus, in Leh valley there are so many nalas flowing from the north-eastern Ladakh Batholith Ridge. The Nalas are locally termed as Lungpa. Those nalas are mainly fed with glaciers and snowmelt water. The important nalas are such; Taru Nala, Phyang Nala, Khardung nala, Sabu Nala, Stakmo nala, Nang nala etc. Those nalas have great influence on the development of landforms and land use pattern in Leh valley. Along the nalas, series of fans have been developed and depending on the fan topography as well as water resources of the nalas a number of settlement areas and villages have been developed (table 1& plate 1). All of the alluvial fans in the Leh valley area represent potentially important aquifers. On the other hand, during cloudburst triggered flash flood these nalas cause excessive water and sediment discharge as well as debris flow from the Ladakh Batholith Ridge into Leh valley causing severe damage of lives and properties. A study by Sangode *et al.* (2017) on 2010 flash flood event, reveals that the small stream drainage area produced extra stress on the pre-existing narrow channels, forcing the rapid lateral erosion and mass transfer. The field photograph also proves prominent Stakmo channel incision caused during 2010 flash flood (figure 6D).

Table 1: Important settlement areas developed along the nalas in Leh valley

Name of the important nalas	Settlement situated along the nala
Taru	Taru village
Phyang	Phyang village
Khardung	Leh city
Sabu	Sabu village
Stakmo	Stakmo village
Thikse	Thikse Monastery and adjacent settlement
Nang	Nang Village
(Based on Sangode *et al.,* 2017 & Google earth Image,2020)	

Effective character of the nalas in development of settlement areas:

Under the adverse environmental conditions nalas and springs have enormous roles to enrich the soil moisture and natural vegetation resources along the different valleys. The settlement pattern of people in this district are mainly concentrated in the river and nala valleys. The ground water development in these areas is of utmost importance. All the major irrigation and drinking water supplies depend on natural springs, rivers and nalas. Sangode *et al.* (2017) have clearly identified eleven important nalas in Leh valley, originating from the Ladakh Batholith Ridge Crest.

Broad geomorphological characteristics of the Leh valley:

Geomorphology of the Leh valley has a great influence on the development of ground and surface water availability in some parts of Leh valley. The present authors think that there are three important geomorphic zones where development of settlement area including land use patterns have been possible; (1) Indus floodplain (2) Amphitheatre valley with nalas (3) dry alluvial fans.In broad valley, snow fed surface and ground water is an important natural resource to sustain the lives. The broad glaciated valleys are mainly filled with glacio-fluvial alluvium, moraines, weathered materials including stone lag deposits, sand dunes etc. The unconsolidated formations like alluvium, scree and talus deposits present along the river valleys which plays a vital role in terms of occurrence and movement of ground water. The moraine formations consist of boulders and clastics in a matrix of gravel, sand, silt and clay which from the aquifer. Depth in water level in moraine formations is very deep which varies from 60 m bgl to 75 m bgl (CGWB ,2014). The Stakmo village is located along the Stakmo nala valley. The upper part bears prominent characteristics of amphitheatre valley and the lower part the Stakmo nala has developed an elongated dry fan over which the Stakmo village has been developed.

Morphometric analysis of the Stakmo nala basin:

The Stakmo nala basin is extended from 34°0'north to 34°12' north and 77° 30' east to 77° 47'east. The river is mainly snowfed originated from the Ladakh range. It is 4th order nala (figure 5). The nala has developed an elongated dry fan which has been demarked in the basin map. A relative relief map of the entire basin has been prepared based on SRTM DEM. The apex of the fan (fan apex ,34° 7' N & 77° 42' 1" E) is shown in the map. Above the apex of the fan prominent amphitheatre valley is located. Excessive terminal moraines are observed over the floor of the amphitheatre valley. The moraines are heavily weathered under periglacial environment. Heaps of weathered moraines are the source of sediment of the Stakmo fan. According to the villagers during 2010 cloudburst induced flash flood debris flow from the upper course of the basin or from amphitheatre valley caused devastation in downstream settlement area over the Stakmo fan and the campus of the Druk a Lotus school.

Plate 1: Important settlement areas including Leh city and several villages developed along different nalas (Source: Google earth image, 2021)

On the other hand, the average slope map shows maximum slope 41°-69°in the upper part of the basin where mass wasting process is very active. Over the fan surface the average slope is 0 – 12 ° where the Stakmo village is located. The Stakmo nala is flowing over this fan area with its characteristic channel form. In the upper part of the basin maximum intensity of dissection (0.113-0.156) is observed (figure 4). Mainly glacial erosional process is responsible for this higher intensity of dissection index. The absolute relief map of the Stakmo basin clearly indicates fan surface with gradual decline of the elevation. The elevation of the upper fan is 3780 – 4222m. On the other hand ,lower fan is located at the elevation of 3290-3780m (figure 4).

Major periglacial landforms in Stakmo Nala basin:

The landforms of the Stakmo basin indicate clear evidences of periglacial features.Several geomorphic processes are working over the entire landscape that are characterised with glacial, periglacial, glacio-fluvial, aeolian, lacustrine and tectonic processes.The landforms prominently observed by the authors during the field survey are;amphitheater valleys (Glacial erosional), moraines (Glacial depositional), sand dunes and sand sheet (aeolian depositional), alluvialfans (fluvial depositional), glacial outwash (glacio-fluvial), palaeo lake sediments (lacustrine process), flood plain(fluvial depositional) etc. Beside these, stone lag deposits, tor, dells are the observable periglacial features. The researchers have observed the important features during field visit.

Glaciated amphitheatre valley: Many scientists (Sant *et al.* 2011) have identified amphitheatre valleys characterised with triangular funnel shaped deglaciated valley bounded by steep rocky slopes on three sides within Ladakh hill range comprising glacial out wash and lag deposits all along the valley floor. At the upper part of the Stakmo river a prominent amphitheatre valley has been surveyed (figure 6B) by the authors. The Stakmo amphitheatre valley is characterised with steep rocky slopes on three sides with lag deposits all along the valley floor.

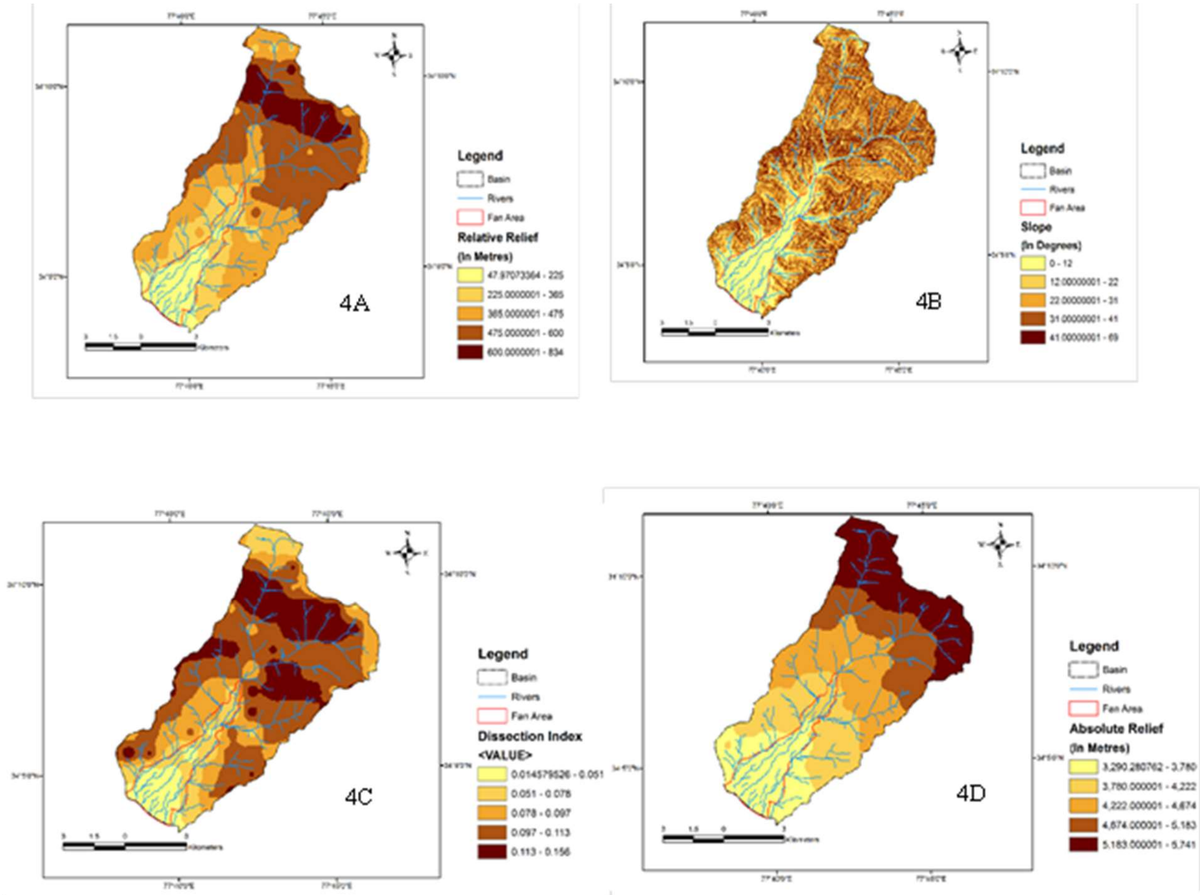

Figure 4: Morphometric Analysis of Stakmo Nala Basin (4A:Relative Relief, 4B: Slope, 4C: Dissection Index, 4D: Absolute Relief (Source: SRTM, DEM)

According to the Stakmo villagers the debris flow originated from the upper part of the amphitheatre valley during flash flood 2010. Towards the lower part of the Stakmo valley a dry alluvial fan has been developed . Heavily weathered rocks and debris of the amphitheatre valley are the major source of sediments for development of the Stakmo alluvial fan in downstream.

Dry periglacial fan: Periglacial dry fans are formed during deglaciation and their development is dependent upon the temporary abundance of glacial debris. In the present study the authors have given a special emphasis on dry fans which are noticeable periglacial feature of the area. The authors have observed that these dry periglacial fans (figure 6c) have great impacts on the development of city and other settlement areas along the Leh Valley. The present investigators think that the series of dry alluvial fans observed in the north-east part of the Leh valley are developed depending on the pre existing foothill topography and important nalas (Taru , Phyang , Khardung , Sabu, Stakmo , Nang nala etc) which are fed by snow, glaciers and sometimes rain water as well as heterogeneous sediments which are the important factors in sculpturing the surface of the fan topography.

Sand sheet: Existence of sand sheets Kumar *et al.* (2016) in the Stakmo basin has been observed by the authors. The authors have identified a palaeo lake covered with sand sheet on the way from Shey to Stakmo village.

Role of the Stakmo nala in development of the Stakmo village: The Stakmo valley (elevation about 3800 m.) is situated at a distance of 26 km from Leh city. It is believed that the name of the village is derived from the village's shape which resembles as a Tiger or 'Stak' in Ladakhi language.

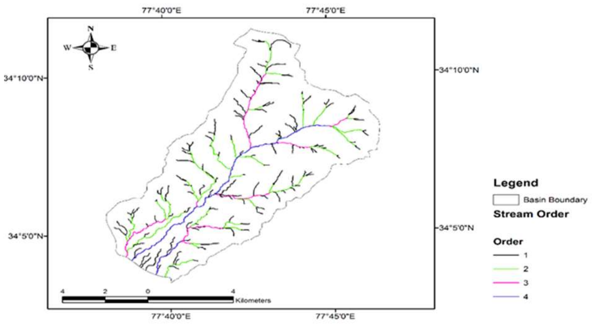

Figure 5: Stream Ordering of Stakmo Nala Basin (Source: SRTM, DEM)

The present investigators have studied the dry fan over which the village of Stakmo is located though according to the studies of several scientists (Sant *et al.* 2011) the Stakmo valley(upper part) is actually falls under amphitheatre valleys characterised with different types of moraines. The Stakmo valley is situated about 7 km. far towards the north- east direction of the Shey Fort as well as the famous Druk Padma Karpo school. Depending on the field verification the authors have identified varied micro landforms and land use features over the fan (figure 6 &7) specially along the Stakmo nala. A prominent trenching of more than 6 metres height was observed along the Stakmo nallah(upper course), which is very significant evidences regarding the supply of sediments from source area . The dry fans are trenched because of the relatively rapid depletion of the erodible glacial debris and the subsequent reduction of sediment loads from the drainage basin Eckis (1928).The fan is comparatively steeper and the upper part is covered with the soils shallow to very shallow in depth, generally yellowish brown, coarse textured loamy sand with common occurrence of gravels and pebbles. Depending on the water resource and shallow soil resource of the Stakmo fan, a large portion of the area is utilized for cultivation of barley and also different type of vegetables and simultaneously used for orchard .The Stakmo nala provides the water to the whole Stakmo village. More than fifty families live in the village. There is a large stretch of barren land which is being converted into agricultural and built-up area. Though different literatures (Patel *et al.,* 2008; Dame *et al.,* 2019)also have described the rapid conversion of barren land into built-up area in the entire Leh valley. The present researchers have evidently observed the geomorphic signature of the 2010 flash flood along the Stakmo nala (figure 6C&D) in its upper reaches where the cloud burst resulted into significant vertical and lateral cutting increasing the channel width along with a sharp axial incision of Stakmo bed. The feeder nala (Stakmo nala) has actively eroded its bank in the form of toe erosion. The feeder channel is filled with granitic gravels in heterogeneric size (figure 6C) that studied by the authors during field survey. The flash flood events have brought lots of fresh gravels and boulders in the channel of Stakmo. Prominent breaks in longitudinal profile of the nalla has been observed from the field survey .According to the local source, at the upper reach of the dry fan ,mainly extensive part of the left bank was totally washed away in form of debris flow by the devastating 2010 flash flood. Distinct altitudinal variation in between the both sides fan surface of the river clearly indicates that (figure 3). Plantation of Willow trees are seen in different parts of the village. There is an extended part of barren land covered with lag stone and deep weathered (figure 6F) rocks mainly in the upper part of the fan area. Organised irrigation network of earthen channels (called 'yuras') are constructed to properly distribute (figure 7A) of Stakmo river water into agricultural fields.Stakmo river water is the only source of irrigation water. Prominent agro-based livelihood of the villagers has been observed during field survey in September, 2014 by the authors (figure 7C&D). Apart from the agriculture they depend on animal husbandry. Along the elongated dry fan (figure 8) different types land utilisation patterns are observed. In order to understand the capacity of the Stakmo nala at its maximum flow analysis of sediment size in terms of bed load was done in random sampling approach. About fifteen samples of bed loads along the nala about 969m. reach from the fan apex were studied. In this reach , maximum intermediate axis of bedload was found 2.1m.(granite) and minimum was recorded 0.3 m(granite).About 86.66 percent of bed loads are granite and 6.66 percent of bed load are basalt and dolerite respectively .About 53.33 percent of surveyed samples were elongated in shape. Apart from it, platy (13.33%) , rectangular (13.33%) and

Figure 6: Different landform features in Stakmo nala basin; (A) dells or small dry valley (B) Stakmo amphitheatre valley (C) Stakmo channel characterised with heterogeneous size of sediments over the upper part of Stakmo fan (D) Prominent channel incision due to cloudburst,2010. (E)Moraines. (F) Deep weathered upper part of the basin. (Source: Field observation, 2014)

Figure 7: Important land use pattern along Stakmo nala;(A) Man-made irrigation channel and barren land used for plantation (B) Way to Stakmo Gompa. (C) House type and fencing with boulders as land divide in Stakmo village.(D) Agricultural field observed in Stakmo village.(E) Location of the Stakmo primary school and village road in Stakmo valley. (F) Human induced check dam to store Stakmo nala water for drinking and irrigation purpose. (Source: field survey,2014)

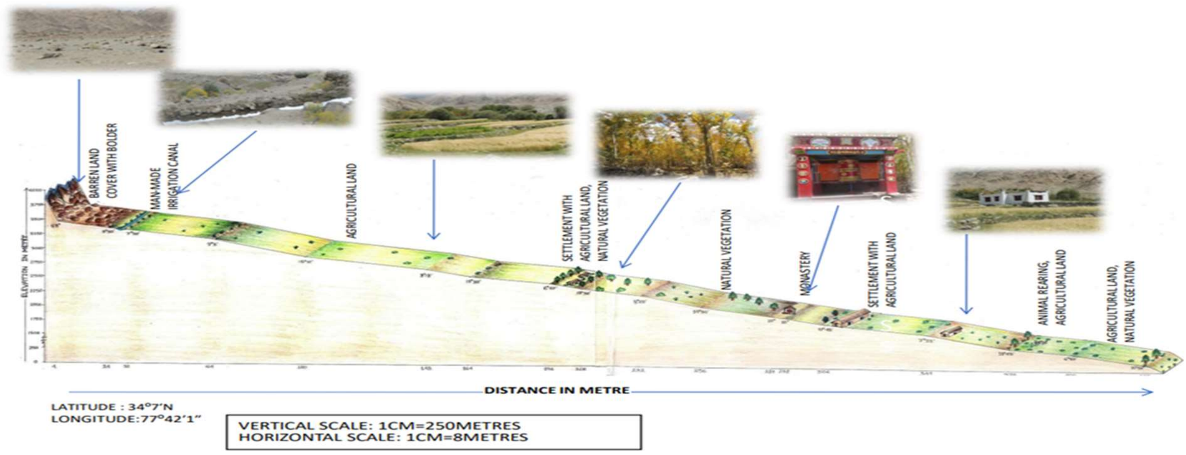

Figure 8: Diagrammatic illustration of the Stakmo dry fan showing land cover land use types (source: field survey, 2014)

circular (13.33%) shaped samples were also observed respectively (table:3). Based on field data analysis and observation of nala bed sediment sizes it can be understood that the main source of nala bed sediments are glaciated moraines and those heavy loads are mainly transported by mass wasting process specially by flash flood events.Though, according to the views of the local people, as a result of glacial melting and lesser trend of snowfall, the amount of water flow in the nala is gradually decreasing compare to earlier condition.

Major findings:

Based on the above study some important findings have been observed. Those are;(1)Naturally, in the high altitude cold desert environment there is an acute scarcity of surface as well as ground water resource. Hence, extensive area is under barren land. (2) Presence of the Stakmo nala is the only reason to develop the village and its different land use pattern. The snow melt water of the nala is the main source of drinking and irrigation. (3) The result of the morphometric techniques of the Stakmo basin clearly suggest the fan topography which is influenced by mainly glacio-fluvial processes. (4) Apart from the river Indus, there are many nalas (Taru, Phyang, Khardung, Sabu, Stakmo, Nang etc) that play significant role to develop elongated and slopy dry fans over which many settlement areas have been developed depending on its water and sediment resources.(5) Based on secondary sources and field observation the authors have identified dry Stakmo fan and its vivid geomorphic features. The upper portion of the fan clearly indicates a prominent amphitheatre valley and the downstream area an elongated dry fan has been developed, which is topographically and hydrologically favourable for the development of Stakmo village. (6) Considering the wide ranging role of the Stakmo nala on the physical and cultural environment of the village scientific nala basin management can be introduced to minimise the intensity of hazards like soil erosion and cloudburst induced flash flood etc.

Conclusion

It is true that the Stakmo nala is not so significant like river Indus in the entire Leh valley but undoubtedly, the nala has pivotal role for providing sustainable resources like; favourable fan topography, surface and ground water resources, riverine alluvium, natural vegetation etc. Depending on those natural resources the entire Stakmo village and the livelihood of the villagers have been established. Water of the Stakmo nala is the key issue behind the socio-economic activities of the villagers like; agriculture, plantation, animal husbandry etc. Like Stakmo nala other nalas have also significant role in establishment of many other villages and settlement area in the entire Leh valley. In near future there is a possibility in decline of the amount of the nala water due to deglaciation or the demand of water by the local people may exceed the level of availability. In this possible adverse situation the existence of the village may be under threat. So, the local people, planners and researchers have great challenge to develop the alternative sources of water resource availability in the Stakmo and other villages of the Leh valley for the sustainability. In this regard proper management of the nala basin including snow resource management can be an effective planning for the sustainability of the nalas.

Conflict of interest

The authors declare that they have no conflict of interest.

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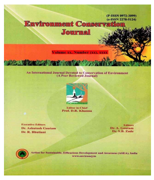

Effect of zinc and silicon nanoparticles on yield, quality and economics of lowland paddy

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ABSTRACT

An investigation to know the effect of nano zinc and silicon on quality and yield of rice was conducted at AHRS, Bavikere, KSNUAHS, Shivamogga. The experiment consisted 12 treatments replicated thrice and was laid in completely randomized block design. The different treatment combinations of seed treatment of nano zinc and silicon, foliar spray of zinc and silicon nanoparticles were compared with conventional sources and the control. Application of both zinc and silicon (T6) in nano form as foliar @ 40 ppm each at 40 DAT registered significantly higher no. of productive tillers (18.72), protein content (11.41 %), starch content (75.70 %) grain yield (6034 kg/ha), straw yield (6693 kg/ha) with higher economic net returns (98631 Rs ha^{-1}), foliar spray of zinc nano particles alone @ 40 ppm at 40 DAT showed next best results.

Introduction

Rice, grown in global area of 162.06 m ha, serves as a staple food crop of most populated continent Asia and alone in India it occupies 43.39 m ha area with 104.32 million tonnes of production and 2404 kg/ha of productivity (Anon., 2020). About two billion people are benefitted with secure livelihood through paddy cultivation. Exponentially growing population in the country is barring boundaries and the demand to fulfill the hungry stomach is a nutcracking job. To ensure national food security and food sufficiency, by the end of this decade there is an urge for increasing productivity by 4.03 t/ha in order to meet the growing demand of milled rice (130 m tonnes) (Anon., 2021). Nutrient management is the most effective agronomic key factor for enhancing productivity in rice cultivation as it has quick response. The importance of macro nutrients (nitrogen, phosphorus and potassium) is well-known by the farmers and supplementing these by chemical fertilizers is now a mandatory practice. But, micro and beneficial nutrients are also deciding factors for good harvest in rice. Any deficiency during critical stages-tillering, panicle initiation and grain filling results in drastic yield loss (Boonchuay *et al.*, 2013). Zinc, a micronutrient is inevitably important to humans, animals and plants as well. It is directly involved in reducing oxidative stress which safeguards plant cell wall and stabilize chromosomal fraction. Being directly involved in auxins and nitrogen metabolism it has a vital role to play in enhancing enzymatic activities. Silicon, Despite the fact that it doesn’t fix in an essential element list but tops at beneficial elements especially for rice. Wholesome effect of silicon is evidently seen in cereals particularly in rice and is tagged as siliciferous cereal. Silicon is less mobile in plants

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and its constant supply is essential for sustainable production (Pati *et al.,* 2016). Silicon has ample of benefits like protection from abiotic and biotic stress, reduce toxicity of elements like Mn, Fe, Cd and Al. it also enhances the availability of nutrients to the plant (N, P, K, Ca, Mg, S, and Zn) (Wattanapayapkul *et al.*, 2011). Today major constrain in rice cultivation that has to be addressed right away is low nutrient use efficiency caused due to runoff, leaching (less retention in soil) resulting in low absorption by the plants. Best way to tackle this problem is by adopting innovative technologies that are effective. in increasing nutrient use efficiency. Nanotechnology which is an engineering technique of manipulating of materials at smallest scale of 1 to 100 nanometers size. Its small size reduces the quantity of application when compared to conventional fertilizers and are also environmentally friendly. Application of nanotechnology in agriculture was seldom and now it is holding its ground in agricultural as nano fertilizers or some time quoted as smart fertilizers. What makes them smart is the increase in release rate of elements and reduce the time of uptake that would coincide to the critical stages of the crop and fulfill its nutritional demands. In Indian nanotechnology has an ability to bring huge transformation in fertilizer manufacturing, usage pattern and reducing losses.

Material and Methods

This investigation was conducted in the field of Zonal Agricultural and Horticultural Research Station (AHRS), Bavikere, UAHS, Shivamogga (Figure 1) located at 75°51` E longitude and 13°42` N latitude at an altitude of 695 meters above the mean sea level. The 12 treatments were laid in randomized block design. The variety used was Sahyadrimegha, and the crop was taken up in *Kharif.* The treatment details are seeds were treated with zinc nanoparticles (100 ppm) (T_1), seeds treated with silicon nanoparticles (200 ppm) (T_2), combination of T_1 and T_2 (T_3), foliar spray of zinc nanoparticles (40 ppm) at 40 DAT (T_4), foliar spray of silicon nanoparticles (40 ppm) at 40 DAT (T_5), combination of T_4 and T_5 (T_6), EDTA $ZnSO_4$ (0.5%) foliar spray at 40 DAT (T_7), potassium silicate (0.5%) foliar spray at 40 DAT (T_8), EDTA $ZnSO_4$ and Potassium silicate foliar spray @ 0.5% at 40 DAT (T_9), zinc sulphate soil application @ 25 kg/ha (T_{10}), rice hull ash soil application @ 2 t/ha (T_{11}) and control (T_{12}). All the treatments were replicated thrice. The seeds were subjected to priming with nano zinc and silicon for twelve hours as per the treatments. The sowing of these treated seeds was taken up in different nursery beds. Two to three seedling per hill were transplanted at 20*10 spacing. The nano zinc and silicon solution of different concentrations were prepared with the help of sonicator and was sprayed to the crop at prescribed intervals. Along with FYM @ 10 t/ha, recommended dose of fertilizers (100:50:50 kg N: P_2O_5: K_2O) were applied in common to all the treatments. The timely observations on growth, yield attributes and yield were recorded at different growth stages of the crop (30, 60, 90 DAT and at harvest). leaves (YFELs) were used to examine leaf morphology and structure using scanning electron microscope (SEM) following processing as described by Li *et al.* (2017).

Figure 1: General view of experimental plot

Results and Discussion

Yield attributes

Increased no. of productive tillers hill^{-1} (18.72), filled grains (100.5 hill^{-1}), panicle length (27.6 cm), grain yield hill^{-1} (13.63) and test weight were obtained with foliar spray of zinc and silicon nanoparticles each @ 40ppm @ 40 DAT. This was statistically best when compared to remaining treatments with regard to productive tillers, panicle length and grain yield per hill except foliar spray of zinc nanoparticles (40 ppm) at 40 DAT (17.26, 26.5 and 12.7, respectively) which recorded on par results. Whereas, in the case of number of filled grains hill^{-1}, zinc treatments (T_4, T_1, and T_7) were found on par and significantly excelled over soil application of $ZnSO_4$ (25 kg/ha). The 1000 grains weighed (test weight) across treatments indicate significant differences among treatments with highest being registered with zinc and silicon nanoparticles (40 ppm) foliar spray @ 40 DAT (24.82 g). This was significantly superior over treatments; control (22.30 g) and rice hull ash application @ 2 t/ha (22.77 g) and on par with rest of the treatments. Foliar spray of zinc either in the form of EDTA $ZnSO_4$ (0.5%) or nanoparticles, seed treatment with nano zinc @ 100 ppm were found to be significantly superior over plots which received $ZnSO_4$ as soil application @ 25 kg/ha with respect to panicle baring tillers, length of the panicle, no. of filled grains panicle^{-1} and yield hill^{-1}. The 1000 grain weight did not vary among zinc treatments irrespective of source and method.The silicon treatment viz., seed treatment in nano form @ 200 ppm, foliar application @ 40 ppm @ 40 DAT and potassium silicate (0.5 %) foliar spray @ 40 DAT were found to be statistically significant over soil application of rice hull ash @ 2 t/ha in recording productive tiller, panicle length and filled grains panicle^{-1} over rice hull ash which registered the least values for these parameters (10.65, 19.2 cm and 73, respectively). All silicon treatments (T_2, T_5, T_8 and T_{11}) are on par with respect to grain yield per hill and test weight but in order of merit numerically. The plots which received both zinc and silicon (T_6, T_9 and T_3) showed statistical differences in yield parameters except test weight. Foliar spray of zinc and silicon nanoparticles both @ 40 ppm @ 40 DAT resulted in significantly higher number of productive tillers (18.72) and panicle length (27.6 cm) over EDTA $ZnSO_4$ (0.5 %) and potassium silicate (0.5%) foliar spray (16.18 and 22.4 cm) and seed treatments with nano from (14.72 and 22.1 cm). With respect to filled grains and grain yield hill^{-1}, nano form is on par with conventional source of silicon and significantly superior over seed treatment. Treatment T_6, T_9 and T_3 is in the order of merit and is presented in the Table 1. In paddy, panicle size is affected by adverse environmental conditions. Any biotic or abiotic stress like drought, high temperature and diseases can reduce panicle length, hence application of silicon helps the plant to overcome the stress there by increase panicle size. Silicon is reported to improve panicle fertility and number of filled grains; and panicle length (Wang *et al.*, 2015) and are less prone to blast (Wattanapayapkul *et al.*, 2011). Further, nano zinc is also known to increase panicle length (Anzer-Alam and Kumar, 2015), number of spikelets per panicle and test weight and grain weight (Ghasemi *et al.*, 2014). Application of nano zinc at panicle initiation meet out the nutritional requirement and helps in grain filling, silicon on the other hand reduce stress level. Therefore, the synergetic effect of both zinc and silicon is reflected in grain yield of rice, since, most of the dry matter accumulation in the grain gets increased up to 15 to 20 days after flowering. Hence, applying silicon and zinc in nano form at panicle initiation will help to overcome stress, meet out nutritional demand thereby increased filled grains and test weight. Test weight (1000 grains) is an index of grain yield. The grain weighed across treatments indicates significant variations among treatments with highest being registered with foliar spray of both zinc (40 ppm) and silicon (40 ppm) nanoparticles at 40 DAT (24.82 g) (Table 1). Zinc deficiency can lead to reduction in grain number and grain weight and the response of plant also related to uptake of Zn from soil. Ghasemi *et al.* (2014) reported highest grain weight with nano zinc oxide application @ 20 and 40 ppm at panicle initiation and full heading stage.Further, lesser chaffy seeds were recorded in treatment with foliar spray of both zinc (40 ppm) and silicon (40 ppm) nanoparticles at 40 DAT (T_6) i.e., 67.11 per cent less than control, 12.5 per cent less than T_3 and 6.58 per cent less than T_9 is another reason for higher grain yield. As a result of less carbohydrate accumulation in the plant, insufficient carbohydrates translocation to sink (lower harvest index) leading to more chaffiness in treatments viz., control, rice hull ash application and conventional sources (Table 1). The results are in line with findings of Lavinsky *et al.* (2016) who reported higher filled grains per hill with the application of nano silicon at reproductive stage. As per grain yield is concerned, it is directly influenced by the yield attributes the best treatment T_6 recorded highest grain yield, straw yield and harvest index.

Grain yield

The treatments receiving both zinc and silicon nanoparticles foliar spray (T_6) each at 40 ppm each

Table 1: Effect of application methods of nano zinc and silicon on yield parameters of paddy

Treatments	No. of productive tillers hill^{-1}	Panicle length (cm)	No. of filled grains Panicle^{-1}	Grain Yield hill^{-1} (g)	Chaffiness (%)	Test weight (g)
T_1: ST with nano zinc @ 100 ppm	13.14	22.0	92.9	10.87	06.0	22.87
T_2: ST with nano silicon @ 200 ppm	13.77	21.9	90.0	09.38	07.9	22.77
T_3: ST with zinc (100 ppm) and silicon (200ppm) nanoparticles	14.72	22.1	93.0	10.68	06.9	23.53
T_4: FA of zinc (40 ppm) nanoparticles at 40 DAT	17.26	26.5	95.4	12.87	05.1	23.91
T_5: FA of silicon (40 ppm) nanoparticles at 40 DAT	12.82	22.5	89.1	08.76	07.1	22.98
T_6: FA of zinc and silicon nano particles both @ 40 ppm at 40 DAT	18.72	27.6	100.5	13.63	05.0	24.82
T_7: EDTA $ZnSO_4$ foliar spray @ 0.5% at 40 DAT	12.88	21.7	90.0	09.49	06.0	23.71
T_8: Potassium silicate foliar spray @ 0.5% at 40 DAT	13.48	21.0	80.3	08.10	08.1	22.71
T_9: EDTA $ZnSO_4$ and Potassium silicate foliar spray @ 0.5 % at 40 DAT	16.18	22.4	95.9	12.00	06.0	23.84
T_{10}: Zinc sulphate soil application @ 25 kg/ha	12.06	19.2	68.4	08.45	13.7	22.49
T_{11}: Rice hull ash soil application @ 2 t/ha	10.65	18.6	73.0	07.85	12.4	22.77
T_{12}: Control	9.89	18.2	68.2	07.18	15.2	22.30
S.Em+/-	0.73	0.62	2.48	00.59	0.77	0.76
C.D. at 5%	2.15	1.83	7.27	01.72	2.26	2.22

@ 40 DAT recorded highest grain yield of 6034 kg/ha (Table 2.) which was significantly superior over all other treatments excluding the treatments with zinc nanoparticles (40 ppm) foliar spray at 40 DAT which recorded 5720 kg/ha of grain yield. Foliar application of both zinc and silicon in nano form was found to be superior over corresponding seed treatment (5212 kg/ha). In treatments with zinc alone; zinc and silicon nanoparticles foliar spray both @ 40 ppm @ 40 DAT registered highest grain yield of 5720 kg/ha than nano zinc (100 ppm) seed treatment (4815 kg/ha) and EDTA zinc sulphate (0.5 %) foliar (4624 kg/ha) and $ZnSO_4$ (25 kg/ha) as soil application (4732 kg/ha) including control. With regard to silicon, foliar application of nano form (4391 kg/ha) and spray of potassium silicate at 40 DAT (4300 kg/ha) recorded lower yield when compare to seed treatments with nano silicon (5308 kg/ha). Among nano particle treatments, foliar application of nano zinc was found superior than nano zinc seed treatment and EDTA form and the results were in line with Rana *et al.*, 2014. Similarly, in silicon applications, seed treatment with nano form significantly excelled over other foliar application of nano form and conventional potassium silicate and rice hull ash soil application at 2 t/ha. The treatment receiving both zinc and silicon showed that foliar applications > conventional form > seed treatments are in the order. Least grain yield was registered in control plot (4274 kg/ha). Grain yield is the result of genotype, climate, soil and also the agronomic management practices. Grain is the final economical part of the plant which is of greater importance. Similarly, Kheyri *et al.* (2019) have reported no significant difference between combined and sole application of zinc and silicon nano particles for foliar application. The yield improvement in these two treatments was to an extent of 41 and 33.83, 20 and 11.8, and 12 and 4.72 per cent over control (T_{12}), seed treatments with both nano silicon and silicon (T_3) and combined application of zinc and silicon in conventional form (T_9), respectively. The increase in yields due to application of both silicon and zinc in nano form as foliar @ 40 DAT is attributed to higher silicon and zinc uptake i.e., 0.98 and 20.8 and 0.86 and 20.9

Table 2: Effect of zinc and silicon nanoparticles on straw yield, grain yield and harvest index of paddy

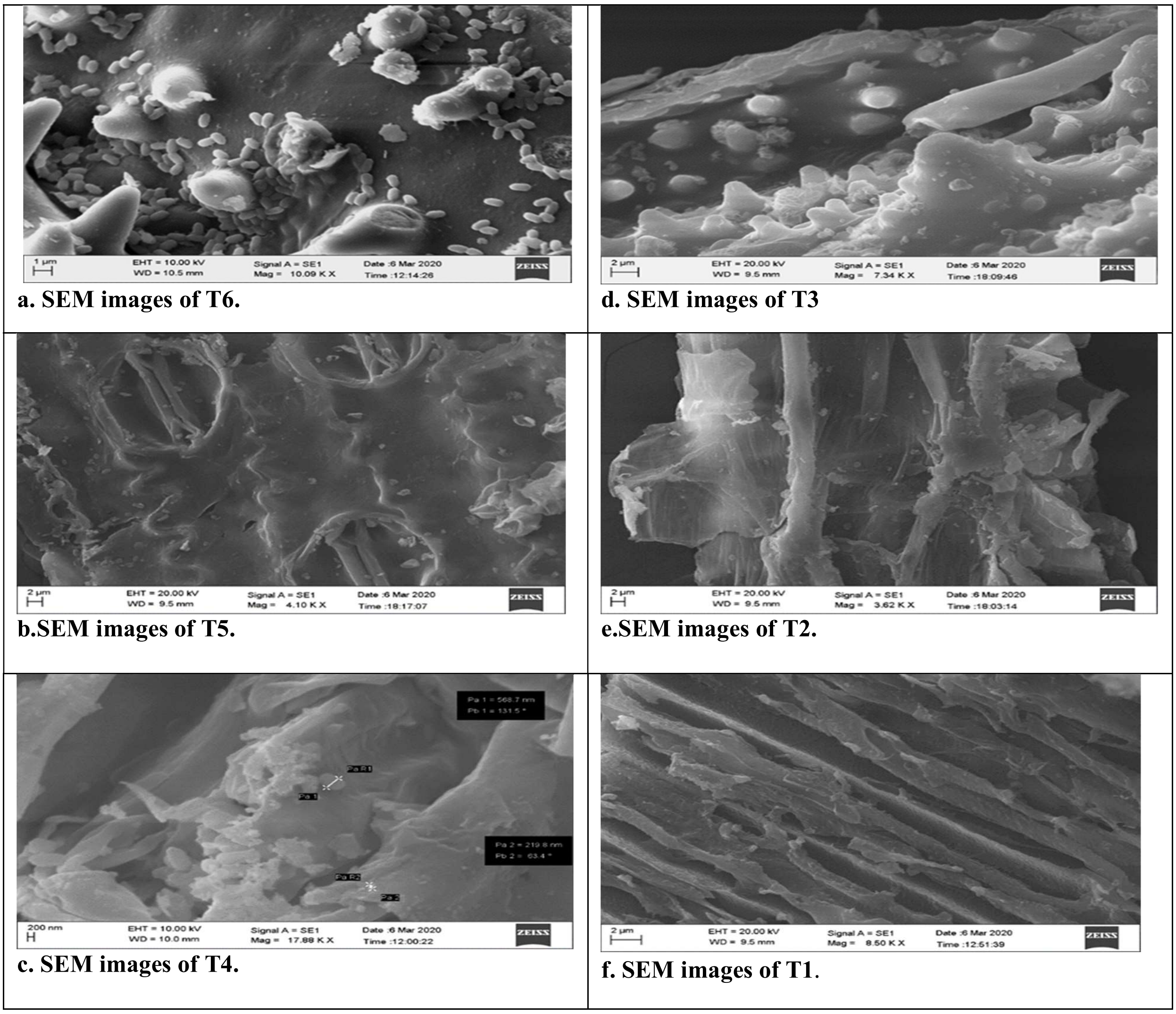
Treatments	Grain yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)
T1: ST with nano zinc @ 100 ppm	4815	5329	47.5
T2: ST with nano silicon @ 200 ppm	5308	5947	47.2
T3: ST with zinc (100 ppm) and silicon (200ppm) nanoparticles	5212	5761	47.5
T4: FA of zinc (40 ppm) nanoparticles at 40 DAT	5720	6131	48.3
T5: FA of silicon (40 ppm) nanoparticles at 40 DAT	4391	4991	47.0
T6: FA of zinc and silicon nanoparticles both @ 40 ppm at 40 DAT	6034	6693	48.4
T7: EDTA ZnSO4 foliar spray @ 0.5% at 40 DAT	4624	5152	47.3
T8: Potassium silicate foliar spray @ 0.5% at 40 DAT	4300	4971	46.4
T9: EDTA ZnSO4 and Potassium silicate foliar spray @ 0.5 % at 40 DAT	5541	6084	47.7
T10: Zinc sulphate soil application @ 25 kg/ha	4732	5734	46.8
T11: Rice hull ash soil application @ 2 t/ha	4278	4823	46.2
T12: Control	4274	4550	45.2
S.Em+/-	148.79	137.1	1.57
C.D. at 5%	436.4	461	4.61

Table 3: Effect of zinc and silicon nanoparticles on protein and starch content of rice

Treatments	Proteincontent (%)	Starch content (%)
T_1: ST with nano zinc @ 100 ppm	11.27	72.00
T_2: ST with nano silicon @ 200 ppm	10.87	74.97
T_3: ST with zinc (100 ppm) and silicon (200ppm) nanoparticles	11.33	72.81
T_4: FA of zinc (40 ppm) nanoparticles at 40 DAT	11.34	74.80
T_5: FA of silicon (40 ppm) nanoparticles at 40 DAT	10.42	73.11
T_6: FA of zinc and silicon nanoparticles both @ 40 ppm at 40 DAT	11.41	75.70
T_7: EDTA $ZnSO_4$ foliar spray @ 0.5% at 40 DAT	10.69	71.56
T_8: Potassium silicate foliar spray @ 0.5% at 40 DAT	10.40	74.82
T_9: EDTA $ZnSO_4$ and Potassium silicate foliar spray @ 0.5 % at 40 DAT	10.96	74.23
T_{10}: Zinc sulphate soil application @ 25 kg/ha	10.53	73.38
T_{11}: Rice hull ash soil application @ 2 t/ha	10.45	73.78
T_{12}: Control	10.34	70.17
S.Em+/-	0.49	3.64
C.D. at 5%	NS	NS

kg/ha zinc and silicon by straw and grain, respectively and improvement in yield components. The SEM analysis of the leaf sample (Fig. 1) collected after the foliar application showed the presence of nano particles which justifies the fast entry of the nutrients into the site of action. This hampers the accumulation of the zinc and silicon in the plant system. It was seen that the more nano particles were accumulated near and around the chloroplast. The nano particles took their entry through the epidermis as well as stomata. However, the leaf samples of the treatment receiving seed priming (Fig. 2) did not exhibit any nano particles this was due to the fact that nano particles absorbed during priming were completely utilized by the plant system.

Higher silicon uptake was noticed in seed treatment than foliar application and conventional source. Similarly, Lavinsky *et al.* (2016) reported 45 per cent higher grain yield due to application of silicon at panicle initiation stage. It is in line as opined by Kheyri *et al.* (2019) that the higher yields due to foliar application of nano zinc and silicon can be reasoned out for zinc activity which involved in enzyme activation and thereby increases dry matter production. Which will be stored in the sink.



a. SEM images of T6.
d. SEM images of T3
b.SEM images of T5.
e.SEM images of T2.
c. SEM images of T4.
f. SEM images of T1.

Figure 2: SEM images of leaf samples of nano zinc and silicon treated treatments. (a. SEM images of treatment T_6, b-T_5, c-T_4, d-T_3, e-T_2, f-T_1 respectively).

Straw yield

The same trend as of yield attributes and grain yield was seen in straw yield and also harvest index. Highest straw yield (6693 kg/ha) was recorded in treatment T_6 (Table 2.), foliar application of zinc nanoparticles at 100 ppm (6131 kg/ha) was the next best treatment and followed by foliar application of EDTA zinc sulphate and potassium silicate each @ 0.5 per cent. Control treatment recorded the least with 4550 kg/ha. Harvest index was also recorded higher (48.4 %) in the same treatment and was closely followed by T_4 (48.3 %) and foliar application of both EDTA zinc and potassium @ 0.5 per cent (47.7 %).

Quality parameters

Protein content

Statistically no significant differences were observed among the treatments. However, numerically superior protein content (11.41 %) was recorded in treatment with foliar spray of zinc and silicon nanoparticles both at 40 ppm at 40 DAT, closely followed by the treatments receiving nano zinc as foliar @ 40 ppm at 40 DAT, treatment receiving both nano zinc (100 ppm) and silicon (200 ppm) seed treatment (11.33 %) and treatment receiving seed treatment with nano zinc (100 ppm) (11.27 %) as presented in Table 3.

Table 4: Effect of zinc and silicon nanoparticles on economics of rice

Treatments	Gross returns (Rs/ ha)	COC (Rs/ha[1])	Net returns (Rs/ha)	B:C
T_1: ST with nano zinc @ 100 ppm	94663	33427	61236	1.8
T_2: ST with nano silicon @ 200 ppm	97465	35750	61715	1.7
T_3: ST with zinc (100 ppm) and silicon (200ppm) nanoparticles	102458	35217	67241	1.9
T_4: FA of zinc (40 ppm) nanoparticles at 40 DAT	112155	35347	76808	2.2
T_5: FA of silicon (40 ppm) nanoparticles at 40 DAT	92517	35276	57241	1.6
T_6: FA of zinc and silicon nanoparticles both @ 40 ppm at 40 DAT	138294	39663	98631	2.5
T_7: EDTA $ZnSO_4$ foliar spray @ 0.5% at 40 DAT	89969	34290	55679	1.6
T_8: Potassium silicate foliar spray @ 0.5% at 40 DAT	80883	32427	48456	1.5
T_9: EDTA $ZnSO_4$ and Potassium silicate foliar spray @ 0.5 % at 40 DAT	108840	35757	73083	2.0
T_{10}: Zinc sulphate soil application @ 25 kg/ha	82589	33010	49579	1.5
T_{11}: Rice hull ash soil application @ 2 t/ha	74133	31460	42673	1.4
T_{12}: Control	65008	29960	35048	1.2

Foliar application was superior over seed treatment and soil application. Treatments $T_6 > T_4 > T_3 > T_1 > T_9$ are in the order of merit. Protein content increased by 10.33 per cent in the plot treated with foliar spray of both zinc (40 ppm) and silicon (40 ppm) nanoparticles at 40 DAT (T_6) and 9.66 per cent with foliar application of zinc in nano form which was on par. Higher protein content is mainly attributed to role played by Zn enzyme activation which are directly involved in carbohydrate metabolism, protein synthesis, auxin regulation and pollen formation. Protein content is highly correlated with a value r = 0.82 to zinc and silicon uptake. Similar results recorded by Rehman *et al.* (2012). Further, Sharifi *et al.* (2016) have also recorded higher wet gluten content, sedimentation value and protein content in wheat due to zinc application. Silicon mitigates the adverse drought condition and increases amylose content which in later stages leads to protein formation. Improvement in crude protein and total carbohydrate of radish roots was reported by Mahmoud *et al.* (2019).

Starch content

The treatment with foliar spray of zinc and silicon nanoparticles both @ 40 ppm at 40 DAT recorded numerically higher starch content (75.7 %) closely followed by silicon nanoparticles (200 ppm) seed treatment (74.97 %), and treatment receiving potassium silicate foliar spray (0.5 %) at 40 DAT (74.82 %). Least starch content was noticed in control treatment (70.17 %). But no statistical significance was found among the treatments (Table 3.). Application of zinc and silicon nanoparticles as foliar spray or seed treatment did not have significant effect on starch content. Improvement in starch content was to an extent of 7.88 and 6.84 per cent in T_6 and T_2 when compared to control, respectively. It is due to the deficiency of other essential elements at the early stages.

Economics

Increased net returns, gross returns and B:C ratio were obtained in the treatment receiving foliar spray of zinc and silicon nanoparticles both @ 40 ppm at 40 DAT (Rs. 1,38,294, Rs. 98,631 and 2.5, respectively) as indicated in Table 4., followed by the treatment receiving foliar spray of zinc (40 ppm) nanoparticles at 40 DAT (Rs.1,12,155, Rs. 76,808 and 2.2, respectively) and the treatment receiving foliar application of both EDTA $ZnSO_4$ and potassium silicate each @ 0.5 per cent (Rs.1,08,840, Rs. 73,083 and 2.0, respectively). Whereas, least was recorded in the control (Rs. 65,008, Rs. 35,048 and 1:2, respectively). Zinc and silicon in nano forms are highly efficient and are required in less quantity when applied as foliar due to reduced losses. Higher benefit cost ratio is due to the higher yield and higher market prize for economic and biological yield.

Conclusion

It is evident from the present investigation that the foliar application of both zinc (40 ppm) and silicon (40 ppm) nanoparticles at 40 DAT on enhanced no. of productive tillers, no. of panicles, filled grains, total grain yield, straw yield and net returns. Further nutrients supplied in nano form increases the efficacy by enhancing their availability directly at the site of action hence is the best know way to increase nutrient use efficiency and render higher returns to the farmers.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Influence of gamma radiation on growth, flowers and morphological changes in Gladiolus

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ABSTRACT

An experiment was conducted at Sam Higginbottom University of Agriculture Technology And Sciences, Allahabad in the year 2017-2018 to check the effect of different doses of Gamma radiation (0kR, 1.5kR, 3kR, 4.5kR, and 6kR) on corms of three different gladiolus cultivars namely Praha, Tiger flame, and Snow Princess, it was established that till 3 kR most of the characters were stimulated but started to sink from 4.5kR. However, 6kR treated corms produced leathery and narrower leaves with shorter plant height (57.41 cm), less leaf number (6.75), less sprouts/corm (1.57), and least spikes/plant (1.14) with an abnormal spike. While 1.5 kR treatment proved the most beneficial for various growth parameters including Plant height (87.21cm), the number of leaves (10.76), early sprouting (14.40 days), Early flowering (72.37 days), Longest flower spike (75.41 cm) and more spikes per plant (2.90). Among the Cultivars Tiger flame and Praha were found to respond well to lower doses of gamma radiation and consider being more suitable for gamma treatment. Moreover, discolor basal floret was found in Praha in 1.5kR treated corms, and Chimera was found on Tiger Flame variety at 3kR.

Introduction

The Indian Floriculture sector is undergoing a rapid transformation. Floriculture adds up to a major component of the total agricultural production in India and it plays an important role in increasing the GDP. And Cut flower industry dominates the Indian Floriculture sector. Among the cut flowers bulbous flowers are considered one of the most important categories and have become an integral part of the floriculture wealth. Gladiolus which is considered the "Queen of Bulbous" crop has become one the most popular commercial cut flower crop in India. Gladiolus offers a range of colors, shapes, and sizes that are deemed ideal as cut flowers, good for bed and herbaceous borders beside grown as a pot plant. However these traits are considered rare among flowering plants and gladiolus can be cultivated in almost all countries of the world where summer and spring conditions are favorable including both plain and hills areas, (Cantor and Tolety, 2011). Meanwhile, the spikes of Gladiolus are considered alluring and elegant with subtle florets of various shades that follow the sequential opening of the floret. This permits the flower to grow for an extended period and delivers a good vase life of cut spikes (Singh, 2006). Due to this reason, there is a growing demand for Gladiolus flowers in both Domestic and International Market. As the demand for gladiolus flower is increasing gradually genetic improvement of the crop has become a prerequisite and gladioli have mostly evolved through conventional breeding and a few through mutation breeding. As, Gladiolus has many characteristics of economic significance like flower traits which include novelty, doubleness, petaloid, vase life,

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biotic and abiotic resistance, etc. they are considered ideal for the application of mutation induction techniques and they can be easily monitored after the mutagenic treatment. Besides Gladiolus are heterozygous and are raised through vegetative propagation. The main advantage of mutation induction in vegetatively propagated crops is the ability to alter one or a few characters of otherwise outstanding cultivars without changing the remaining and often unique part of the genotype. So, this makes Gladiolus a potential test material for inducing physical mutagenesis that further creates variability for further improvement (Datta, 2012). The mutation is credited as one of the best approaches for the development of new varieties through genetic manipulation. In different ornamental plants, mutation techniques like ionizing radiations, chemicals, and other mutagens, have produced and released a large number of new promising varieties. Approximately 70% of the world's mutant varieties have been induced through gamma-rays (Sisodia and Singh, 2014). Gamma radiation is high-frequency rays consisting of high-energy protons that penetrate the cell and cause ionization leading to disruption of the normal processes of the cell. Hence, the present research was conducted and the experiment aimed to find out desirable variations caused by different gamma doses on morphological characters and flowering of gladiolus varieties.

Material and Methods

The Gladiolus cultivars Praha (V_1), Tiger Flame (V_2), and Snow princess (V_3) were irradiated using ^{60}CO source with gamma chamber-900 model (Baba Atomic Research Centre, Mumbai) at National Botanical Research Institute, Lucknow. The five different gamma doses with irradiation durations i.e. 0 kR (T_1) , 1.5 kR (T_2) for 11seconds, 3 kR (T_3) for 22 seconds, 4.5 kR (T_4) for 33 seconds, 6 kR (T_5) for 44 seconds respectively. The irradiated corms were planted in the beds with a spacing of 35 cm x 35 cm. The trial was positioned out in a Factorial Randomized Block design and was replicated 3 times. Various morphological and flowering characters along with Morphological and physiological changes were observed and recorded at regular intervals which were then statistically analyzed.

Study area

The experiment was conducted in the Horticulture experimental field, the Sam Higginbottom University of Agriculture Technology And Sciences, Allahabad. Allahabad is positioned at 25° 45' North latitude, 81°85'East longitude, and at an altitude of 98m (322ft) above mean sea level (MSL). The area of Allahabad district comes under the sub-tropical belt in southeastern Uttar Pradesh, which experiences extremely hot summer and fairly cold winter. In cold winter, the temperature sometimes is as low as 4°C in the months of December-January and very hot summer with the temperature reaching up to 48°C in the month of May and June (Canty and Associates LLC, 2013).

Results and Discussion

Changes in vegetative characters of Gladiolus due to different doses of Gamma radiation

From Table 1 (a), it can be seen that the minimum days required for sprouting (14.40 days), was observed in corms treated with 1.5kR which was significant par with corms treated with 3kR (14.66 days) while the maximum days (17.67 days) was observed in irradiation dose 6kR. Cultivar Tiger flame, took minimum days (14.37 days) to sprout while maximum days were from cultivar Snow Princess (16.84 days) and followed by Praha (16.38 days). The maximum sprouting/corm (3.39) was recorded in corms irradiated with a dose of 1.5kR followed by 3kR which was significantly par with control. While minimum sprouts/corm (1.57) was recorded in irradiation dose 6kR and was significantly par with 4.5kR. Cultivar Praha recorded maximum sprouting/ corm (2.65), followed by a cultivar Tiger flame (2.53) and there was no statistical difference between them. While the minimum sprouting/ corm (1.74) was observed in cultivar Snow princess. The best interaction effect of cultivars and gamma doses as seen in Table 1 (b) was in the cultivar Tiger flame in 1.5kR dose and it took the least number of days to sprout the corms (12.55 days) and this data was significantly par 3kR irradiation of the same cultivar. Interaction effect of maximum sprout/ corm (4.04) was recorded in the cultivar Praha with irradiation dose at 1.5kR which was significantly par with cultivar Tiger flame (3.74) of the same dose. At lower doses of gamma radiation stimulate early sprouting due to activation of

Table 1(a): Effect of different doses of Gamma radiation on Vegetative growth of Gladiolus

Treatments	Days taken for sprouting	Number of sprouts/ corm	Plant height (cm)	Leaf / plant
Cultivar				
V_1: Praha	16.38	2.65	77.44	8.47
V_2: Tiger flame	14.37	2.53	78.68	10.05
V_3:Snow princes	16.84	1.74	67.11	7.54
S. Em. (±)	**0.140**	**0.063**	**0.509**	**0.138**
C.D. at 5%	**0.409**	**0.184**	**1.483**	**0.402**
Dose				
T_1: 0 kR	15.60	2.44	85.13	9.77
T_2 :1.5 kR	14.40	3.39	87.21	10.76
T_3: 3 kR	14.66	2.53	82.74	8.81
T_4: 4.5kR	16.99	1.69	60.54	7.29
T_5: 6kR	17.67	1.57	57.41	6.75
S. Em. (±)	**0.528**	**0.082**	**0.657**	**0.178**
C.D. at 5%	**0.181**	**0.238**	**1.914**	**0.519**

Table 1(b): Interaction effect of different doses of Gamma radiation and different Gladiolus Cultivar on Vegetative Growth

Treatments	Days taken for sprouting	Number of sprouts / corm	Plant height (cm)	Leaves / plant (cm)
$V_1 \times T_1$	15.92	2.67	87.76	8.87
$V_1 \times T_2$	14.66	4.04	91.11	10.44
$V_1 \times T_3$	15.73	3.22	86.65	8.68
$V_1 \times T_4$	17.10	1.59	63.39	7.11
$V_1 \times T_5$	18.44	1.73	58.29	7.10
$V_2 \times T_1$	13.97	2.55	92.40	11.77
$V_2 \times T_2$	12.55	3.74	94.08	12.78
$V_2 \times T_3$	12.84	2.53	88.95	10.44
$V_2 \times T_4$	15.98	2.20	60.70	8.11
$V_2 \times T_5$	16.43	1.90	57.19	7.16
$V_3 \times T_1$	16.88	2.11	72.25	8.66
$V_3 \times T_2$	15.97	2.39	76.45	9.08
$V_3 \times T_3$	15.30	1.84	75.63	7.73
$V_3 \times T_4$	17.86	1.29	57.46	6.64
V_3 T_5	18.10	1.08	53.43	5.97
S. Em. (±)	**0.314**	**0.142**	**1.139**	**0.309**
C.D. at 5%	**0.914**	**0.412**	**3.316**	**0.402**

synthesis of RNA or enzyme or protein that are directly involved in auxin formation for germination and increased activity of gibberellins which break dormancy and thereby increase sprouting of corm. (Dilta *et al.*, 2003; Patil *et al.*, 2017; Sahariya *et al.*, 2017 reported that higher gamma doses adversely affect sprouting and sprout/ corm. This is because application of Gamma radiation higher doses generate detrimental effects on auxins and other growth substances affecting the chromosomes of the plant including the tissue (Srivastava *et al.*, 2007).

Gamma irradiation had a significant effect on plant height as seen from Table 1 (a). The growth data shows that 1.5kR irradiated corm produced a plant of height (87.21cm) which was the highest of all the treatments while 6kR irradiated corm produced the shortest plant height (57.41cm). Among the cultivar, the tallest plant was recorded from Tiger flame (78.68 cm) which was significantly par with Praha (77.44 cm) while Snow princess produced the shortest plant among the 3 cultivars with an average of (67.11cm) when irradiated with gamma radiation. The best interaction was recorded from Tiger flame irradiated with 1.5kR (94.08 cm) plant height and it was on par with control treatment of cultivar Tiger flame and 1.5kR treated corms of Praha while the shortest plants were from Snow princess treated with 6kR (53.43cm) and was in par with Snow princess treated with 4.5kR (57.46 cm) as seen in table 1 (b). Thus it can be noticed that a higher gamma dose retarded the growth and vice versa. The stimulating effect at a lower dose may be due to stimulation of cell division, and positive alteration of metabolic activities which affect the synthesis of nucleic acid, while higher doses of mutagen cause chromosomal and physiological impairment as reported by (Tiwari *et al.*, 2010; Kumari *et al.*, 2015) in gladiolus.

The number of leaves at 90 days was significantly affected under different irradiation treatments. As shown in Table 1 (a), corms irradiated with a 1.5kR dose of gamma rays produced the most number of leaves (10.76). It was observed that the number of leaf/plant decreased with an increase in irradiation dose and at 6kR, the minimum number of leaf/plant was recorded (6.75). Among the cultivar, Tiger flame produced the maximum leaves (10.05) and the least by Snow Princess (7.54) leaves. The best interaction between Cultivars and Gamma doses was found in Tiger flame treated (1.5kR dose) with 12.78 leaves and the minimum leaves in Snow princess (6kR) with 5.97 as seen in Table 1 (b). The present findings are in line with the findings of (Patel *et al.*, 2010; Sisodia, 2015a; Yadav *et al.*, 2016) in

Gladiolus. where higher gamma dose reduces the number of leaves. This is due to the fact that higher gamma doses causes inhibitory effect and retard cell division by arresting mitotic cells causing adverse effects on auxins synthesis thus significantly reducing the number of leaves.

Changes in flowering characters of Gladiolus due to different doses Gamma radiation

Floral characters of Gladiolus cultivars Praha, Tiger Flame, and Snow princess were significantly influenced by different gamma rays irradiation. From Table 2 (a) it can be seen that corms treated with 1.5kR gamma rays dose induced Spike initiation faster than the rest of the treatment with (72.37 days), faster color break stage with (90.07 days), and took only (93.35 days) for basal floret to open. It was then followed by corms treated with 3kR with (73.79 days) for spike initiation, (92 days) for color break Stage, and (95.47 days) for floret to open. However spike initiation, color break stage, and opening of basal floret were delayed when doses reach 4.5kR and 6kR took the maximum days for spike initiation (81.21 days), color break stage (100.37 days), and floret to open (104.37 days). From Table 2 (a), it is revealed that cv. Tiger flame flower earlier than Praha and Snow princess took the longest time. Meanwhile, from Table 2 (b) it can be seen that the best interaction between Cultivar and Gamma doses was found in the Tiger flame cultivar at 1.5kR (69.67 days) for Spike initiation, (86.57 days) for color break stage and (89.53 days) for basal floret opening. The result coincides with an experiment conducted by (Karki *et al.*, 2010) in Gladiolus where application of lower doses of Gamma radiation produced the earliest spike. This may be because Gamma doses activate the growth regulators and block the growth inhibitors, this helps to increase the root and shoot length, thus helping to absorb more nutrients and performed more photosynthesis, which ultimately resulted in early spike emergence. And due to this reason, there is an early color break stage and early opening of floret (Cantor *et al.*, 2002). While a delay in spike emergence with higher doses may be due to disturbance in biochemical pathways which are altered during the radiation process and these are directly or indirectly linked with the flowering physiology. The treatment of 1.5kR (13.33) was significantly par with 3kR (13.07) and shown to increase the number of florets per spike, as compared to control, while the least number was recorded from 6kR (9.54 floret/spike) as seen from Table 2 (a). The present data are in agreement with several findings that recorded the positive effect of gamma irradiation at lower doses and detrimental on higher doses and exhibited a negative response on the number of florets. A study conducted by (Kuldeep, 2017) showed reduced in the number of floret per spike at the higher dose and an increase in floret/ spike at lower doses of gamma rays in 10 varieties of Gladiolus. These results are in discord with the observation of (Singh *et al.*, 2017) who recorded the maximum number of florets per spike in untreated plants in Tuberose. Meanwhile, the cultivars Tiger flame bear the maximum florets per spike (12.28) followed by Praha (11.71) and Snow princess bear the least floret (11.06). As seen from Table 2 (b), interaction of different Cultivars and Gamma doses was found significant on florets/spike. The best interaction was found in the Tiger flame cultivar at 1.5kR (14.88) florets/spike and was par with Tiger Flame at 3kR (13.97) florets/spike. While the least florets/spike was recorded from Snow princess treated with 6kR (8.64). The reduction of floret number at higher doses may be due to destruction of auxin, irregular synthesis of auxin, failure of assimilation, inhibition of mitotic and chromosomal changes, or due to damage linked with secondary physiological damage (Kole and Meher, 2005).

The size of basal floret was also influenced significantly by irradiation. As seen from Table 2(a), the maximum size (10.53 cm) diameter was recorded with 1.5kR treated plants and a smaller basal floret size (7.97 cm) was recorded at 6kR gamma rays treatment. The beneficial effect was seen till 3kR and floret size got drastically reduced as doses increased showing a detrimental effect. Among the cultivars, Praha bears the biggest size basal floret (9.69 cm) which was statistically par with Tiger flame (9.59cm). Snow princesses produced the smallest size floret (8.72cm). Meanwhile, the interaction between Cultivars and Gamma doses was also significant where the most effective was found in cultivar Tiger flame treated with 1.5kR producing a mean basal floret of (11.10cm) diameter and the smallest basal floret was recorded from Snow princess treated with 6kR (7.83) as seen in Table 2 (b).

Table 2 (a): Effect of different doses of Gamma radiation on flowering characters of Gladiolus

Treatments	Spike emergence (DAP)	Color break stage (DAP)	Basal floret opening (DAP)	Floret/ spike	Diameter of first floret (cm)	Spike length (cm)	Rachis length (cm)	Number of Spikes/ plant	Vase life (days)
Cultivar									
V_1:Praha	77.21	96.13	99.82	11.71	9.69	64.03	31.18	2.33	11.45
V_2: Tiger flame	74.08	92.28	95.85	12.28	9.59	65.90	32.61	2.12	11.87
V_3: Snow princes	78.16	97.20	101.25	10.86	8.72	55.23	25.84	1.53	9.87
S. Em. (±)	**0.097**	**0.302**	**0.195**	**0.155**	**0.136**	**0.179**	**0.200**	**0.063**	**0.062**
C.D. at 5%	**0.284**	**0.881**	**0.567**	**0.452**	**0.397**	**0.522**	**0.583**	**0.182**	**0.181**
Dose									
T_1: 0 kR	76.29	95.62	98.51	11.7	9.91	68.44	32.10	2.17	11.74
T_2: (1.5 kR)	72.37	90.07	93.35	13.33	10.53	75.41	37.47	2.90	13.28
T_3:(3 kR)	73.79	92.00	95.47	13.07	9.55	74.113	37.10	2.40	12.34
T_4:(4.5kR)	78.77	97.96	102.84	10.44	8.71	47.322	22.22	1.33	9.30
T_5: (6kR)	81.21	100.37	104.68	9.54	7.97	43.65	20.49	1.14	8.64
S. Em. (±)	**0.126**	**0.391**	**0.251**	**0.201**	**0.176**	**0.674**	**0.259**	**0.081**	**0.080**
C.D at 5%	**0.336**	**1.137**	**0.732**	**0.584**	**0.512**	**0.232**	**0.375**	**0.235**	**0.233**

Table 2(b): Interaction effect of different doses of Gamma radiation and Gladiolus Cultivar on flowering characters

Treatments	Spike emergence (DAP)	Color break stage (DAP)	Basal floret opening (DAP)	Number of Floret/ spike	Diameter of first floret (cm)	Spike length (cm)	Rachis length (cm)	Number of Spikes/ plant	Vase life (Days)
V_1 x T_1	77.22	98.11	99.44	11.66	10.91	70.88	33.52	2.40	11.55
V_1 x T_2	72.97	91.43	94.45	12.53	11.04	75.51	37.58	3.44	13.98
V_1 x T_3	74.67	93.67	96.67	12.99	9.62	76.89	38.71	2.89	12.76
V_1 x T_4	79.09	96.11	103.24	11.11	8.81	50.22	23.49	1.57	9.75
V_1 x T_5	82.11	101.34	105.32	10.23	8.09	46.66	22.59	1.33	9.19
V_2 x T_1	74.23	90.97	94.47	12.43	10.08	73.73	34.5	2.33	13.00
V_2 x T_2	69.67	86.57	89.53	14.88	11.10	82.52	42.06	3.06	14.63
V_2 x T_3	71.13	88.11	91.87	13.97	9.94	79.14	40.73	2.67	13.33
V_2 x T_4	76.78	97.33	100.96	10.33	8.82	48.31	24.02	1.44	9.52
V_2 x T_5	78.63	98.47	102.42	9.78	7.99	45.76	21.75	1.11	8.87
V_3 x T_1	77.43	97.77	101.64	11.00	8.73	60.69	28.3	1.78	10.69
V_3 x T_2	74.47	92.23	96.09	12.55	9.44	68.21	32.77	2.22	11.24
V_3 x T_3	75.61	94.22	97.89	12.23	9.10	66.31	31.85	1.65	10.92
V_3 x T_4	80.44	100.47	104.31	9.88	8.52	43.43	19.15	1.00	8.64
V_3 x T_5	82.89	101.33	106.31	8.64	7.83	38.52	17.14	1.00	7.87
S. Em. (±)	**0.218**	**0.676**	**0.435**	**0.347**	**0.305**	**0.401**	**0.44**	**0.140**	**0.139**
C.D. at 5%	**0.635**	**1.969**	**1.268**	**1.012**	**0.887**	**1.168**	**1.03**	**0.408**	**0.404**

This was supported by (Dobanda, 2004) in Gladiolus; (Mahure *et al*., 2010) in Chrysanthemum cultivar 'Red Gold' and Chrysanthemum variety 'Maghi'; (Kapadiya *et al*., 2014) who reported that higher gamma doses decreases the size of the floret while lower doses enhance. The decrease in flower size could be credited to the poor growth of the plant as a result of physiological, morphological, or cytological interruption caused by gamma rays on the irradiated plants. As compared to control corms, longer spikes length and Rachis length were observed in corms treated with 1.5kR and 3kR while the corms treated with 4.5kR and 6kR shows detrimental effect where both the spike and rachis length got considerably shorter compared to control. The length of the spikes was measured (75.41 cm) in 1.5 Kr and (43.65 cm) in 6kR treated corms. In the case of Rachis length, the data shows that the corm treated with 1.5kR and 3kR were statistically par measuring (37.47 cm) and (37.10 cm) respectively while the shortest measured (20.49 cm) which was 6kR treated corms. Meanwhile the cultivar Tiger Flame produced the longest Spikes (65.90 cm) and Rachis length (32.61cm) and Snow princess recorded the lowest among the 3 cultivars measuring (55.23 cm) for Spikes and (25.84 cm) for Rachis as shown in Table 2(a).The Cultivars and Gamma doses too showed a significant effect, the best interaction for Spike length (82.52 cm) and Rachis length (42.06 cm) was recorded in cultivar Tiger flame treated with 1.5kR. While the shortest Spike length (38.52 cm) and Rachis length (17.14 cm) was recorded from Snow princess treated with 6kR as shown in Table 2 (b). A similar finding was observed by (Sisodia, 2015b) in 8 Gladiolus varieties and (Singh and Kumar, 2013) in 10 varieties of Gladiolus.

The increase in Spike length and Rachis length may be due to rapid activation of necessary growth regulators which in turn increase the plant growth and photosynthetic process

Similarly, the number of spikes per plant was significantly higher in corms that were treated with lower doses of gamma radiations and significantly reduced at higher doses. The number of spikes /plant in 1.5kR treated corms was statistically higher compared to all the treatments recording (2.90 spikes/plant) while the least number was recorded from 6kR treated corms with (1.14 spikes/plant). Besides, both 4.5kR and 6kR doses were found to have a detrimental effect on the corms as they produced fewer spikes beside some plant remains blind. Among the cultivar, spike production was highest in Praha (2.33 spikes/plant) followed by Tiger flame (2.21 spikes/plant) while Snow Princess produced the least spike at (1.53 spikes/plant) as shown in Table 2 (a).In all the cultivars some plants produced blind shoot at higher gamma doses and did not flower at all. The best interaction was recorded from cultivar Praha treated with 1.5kR (3.44 Spikes/Plant) which was statistically par with Tiger flame irradiated with 1.5kR (3.06 spikes/plant) and a mean of 1 spike/plant was recorded from Snow princess treated with 4.5kR and 6kR which was considered the least and it was par with both Praha (1.33 spikes/plant) and Tiger flame (1.11 spikes/plant) treated with 6kR as sown in Table 2(b).. The observation also coincides with finding made by (Patil and Dhaduk, 2009; Karki and Srivastva, 2010; Patil, 2014) who reported spike yield increases in lower doses and decreases in lowers doses. Irradiations increase the photosynthetic and enzyme activities that help to trigger gibberellins and auxins action causing a higher rate of sprouting percentage which produced many spikes per plant. However, at higher irradiation, there are changes in plant metabolic activities that trigger a negative response of plant hormones which led to no or less flower production

From Table 2 (a) it can be seen that, Gamma radiation treatments at lower doses increases the vase life of gladiolus. Corms irradiated with 1.5kR produced higher vase life of (13.28 days) followed by 3kR with (12.34 days). Higher doses reduced the vase life of cut spikes and minimum vase life was recorded from 6kR irradiated with (8.64 days). Cultivar Tiger flame and Praha had recorded maximum vase life of (11.87 days) and (11.45 days), respectively, in comparison to Snow princess (8.97 days). As seen in Table 2(b), Cultivars and Gamma doses were statistically significant and the best interaction was from variety Praha treated with 1.5kR with a vase life of (13.98 days) which is statistically par with the same dose in Tiger flame with (13.33 days). A similar observation was made by (Kumar *et al.*, 2003; Misra *et al.*, 2006) in Tuberose. An increase in Vase life may be due to a rise in sugar content when irradiated which might have boosted some metabolic processes in the plant subsequently resulting in increasing vase life and longevity of flower in gladiolus.

Morphological changes due to gamma irradiation

Several qualitative changes were observed in the gladiolus plants and flowers in all the three Gladiolus cultivars. In cultivar Praha, plant abnormal spike growth was observed at 4 .5 kR and 6kR dose of gamma irradiation (Figure 1 & 2) and the nodal distance was noticeably decreased, the floret was not grown in sequential order and two flower buds emerged from the nodes in a spike (Figure 3).

Figure 1: Abnormal spike growth in cv. Praha at 4.5

Figure 2: Abnormal spike growths in cv. Praha at 6 kR Irradiation

Whereas in cultivar Tiger flame twining of spike was observed at 4.5kR of gamma irradiation (Figure 4). Emergences of two flower buds from the same nodes in a spike at 4.5kR gamma dose (As shown in Figure 5) and an abnormal emergence of new spike growth from the main spike were observed (Figure 6). And in cultivar snow princess the node length was considerably decreased from 4.5kR and 6kR (Figure 7). In the Praha cultivar, a discolored basal floret was seen at 1.5kR (Figure 8). Flower color mutation was detected in Tiger flame irradiated with 3kR as sectorial chimeric forms, changed in color was only in one or two floret in a spike (Figure 9) which may be due to physiological disturbances caused by reshuffling of histogen layers (Banerji and Datta, 2003). While flower shape variation was observed in the Snow Princess cultivar at 4.5kR, (Figure 10). However, these changes in flower color might be due to the effect of radiation treatments along with the temperature and light that impacted the flower pigmentation. Several studies reported the ionizing radiation effects on the shape, form, and color of the floral organs (Khalaf, 2008) on *Amaranthus caudatus* ; (Singh *et al*,. 2009) in Marigold; (Kumari *et al.*, 2013b and Tarek *et al.*, 2014) in *Chrysanthemum morifolium*; (El-Mokadem and Hoda, 2014) in *Catharanthus roseus*; (Sathyanarayana *et al*., 2019) in Gladiolus. Changes in flower form may be due to chromosomal deletion or change of the factor governing the normal form or structure, as well as to the effect of gamma-rays on the ontogeny of flower organ tissue through the selective destruction of one or more cell layers in the apical floral meristem (Abdel-Maksoud, 1992). However, some of the irradiation responses may be due to point mutations or chromosomal aberrations, and in most cases, the abnormality in plants reverts to normal growth during a recovery period suggesting the basic cause to be a non-genetic physiological disturbance.

Figure 3: Disorder in florets arrangement in cv. Praha at 4.5 kR

Figure 4: Twining of spike in cv. Tiger Flame at 4.5 kR Irradiation


Figure 5: Appearance of double buds from same node in cv. Tiger Flame at 4.5 kR Irradiation


Figure 6: Development of abnormal emergence of new spike from main spike in cv. Tiger Flame at 4.5 kR.


Figure 7: Shortened nodal length in cv. Snow Princess at 6 kR Irradiation


Figure 8: Discolored basal florets in cv. Praha at 1.5 kR Irradiation


Figure 9: Sectorial chimeric in cv. Tiger Flame at 3kR Irradiation


Figure 10: Flower variations in cv. Snow princess at 4.5 kR Irradiations.

Conclusion

From the experimental findings, it can be concluded, that the low doses of gamma rays i.e., 1.5kR to 3kR produced superior vegetative and floral growth compared to control as well as the higher doses of irradiation treatments. The low dose of irradiations ranging from 1.5 to 3 kR was found to be desirable treatments for inducing phenotypical changes. Whereas, at 4.5kR and 6kR irradiation treatment showed a detrimental effect on the overall performance of the plant. The best interaction among the cultivars and Gamma doses was found in V_2 x T_2 (Tiger flame X 1.5kR) with the highest No. of sprout (3.74), Plant height (94.08cm), No. of leaves (12.78), Early spike emergence (69.67 days), Floret per spike (14.88), Spike length (82.52cm) and Vase life (14.63 days). Color changes at the basal floret were observed in Praha from Red to light orange at 1.5kR and chimera was found at 3kR in the Tiger Flame cultivar. And among the cultivars, it was observed that Tiger Flame and Praha responded well to various gamma-ray doses for inducing vegetative and floral characters and may be used for the improvement of varieties.

Conflict of interest

The authors declare that they have no conflict of interest.

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Natural extracts from *Marchantia polymorpha* against plant pathogens growth inhibition

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ABSTRACT

The health of plants, animals, and humans is seriously threatened by the production of toxins by bacteria and fungi. The aim of the current work is to find a dependable and eco-friendly microorganism biocontrol technique to alleviate this concern. In this work, the antibacterial abilities of *Marchantia polymorpha* (liverwort) extracts were examined. These extracts were collected from several altitudinal ranges in the Kumaon region in Uttarakhand province, India, in the western Himalayas. Using microbroth dilution methods, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the crude extracts were determined. The results demonstrated that the *Marchantia polymorpha* extracts exhibited potent antifungal activity against *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Colletotrichum acutatum*, as well as antibacterial activity against *Pseudomonas syringae*, *Xanthomonas campestris*, *Staphylococcus aureus*, and *Bacillus subtilis*. According to the study results, *Marchantia polymorpha* extracts may have applications as natural antimicrobials in a number of sectors, including medications, agriculture, and preserving food. The research we performed demonstrates the potential of liverwort extracts as a promising biocontrol agent against bacterial and fungal diseases and as an inducer of plant disease resistance, providing a safer and more environmentally friendly alternative to synthetic chemicals that is beneficial to both human health and the environment.

Introduction

It is commonly known that plant medicines can be used to treat a variety of ailments. In a similar manner, plant-derived pesticides were widely used before the discovery of synthetic pesticides. Tesfahun *et al.* (2000), revealed that farmers in the Welo region use a combination of physical, cultural, and chemical methods to control pests and diseases in their crops. Natural pesticides including *Phytolacca dodecandra*, *Euphorbia tirucalli*, *Croton macrostachys*, and *Aloe spp.* to keep crops free from pest and infections. Chemical pesticides are utilized in the majority of plant disease treatments. These temporary strategies may provide short-term protection, but they ultimately make farming operations susceptible to harmful chemicals and increase their potential for environmental contamination.Therefore it is suggested that bryophytes extracts be further researched as potential bioagents for plant development and disease resistance. Bryophytes, which include hornworts, liverworts, and mosses, are the second biggest macro-group of terrestrial plants and have considerable biotechnological applications in pharmaceuticals, agriculture, and

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healthcare (Nikolajeva *et al.,* 2012). Bryophytes, a group of non-vascular plants, encompass approximately 25,000 to 28,000 species (Chavhan, 2017). These plants have developed mechanisms to control their growth in the face of challenging environmental conditions, including both abiotic stressors such as temperature and ultraviolet radiation, as well as biotic stressors like irregular water supply, predation, and infectious attacks, which persist as an evolutionary force that tests their adaptability and defensive strategies (Commisso *et al.,* 2021). Sunsequently, it was reported that numerous bryophytes, including *Bazzania*, *Conocephalum*, *Diplophyllum*, *Dumortiera*, *Marchantia*, *Metzgeria*, *Lunularia*, *Pellia*, *Plagiochila*, *Porella*, *Radula*, and *Riccardia*, exhibited antimicrobial properties (Vollar *et al.,* 2018).

Marchantia polymorpha, a liverwort, has been established as a model plant for studying morphological and physiological responses to various environmental factors for over two centuries, and has recently emerged as a valuable model plant for investigating plant-microorganism interactions (Poveda, 2020). Additionally, it has been used in traditional medicine and pharmaceuticals in China, North America, and India to treat various ailments, such as diuretic activity, hepatitis, open wounds and burns, fractures, snake bites, convulsions, uropathy, pneumonia, and neurasthenia (Ludwiczuk and Asakawa, 2019; Tran *et al.,* 2020). Phytochemical research has revealed that *M. polymorpha* contains polyphenols, long-chain polyunsaturated fatty acids, and terpenoids, among which bis-bibenzyls have exhibited significant antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Cai *et al.,* 2022). However, therapeutic studies of bryophytes have been limited, with less than 10% of species studied to date (Rao, 2021).

Due to their microbicidal properties, liverworts, including *M. polymorpha*, can be used to control plant diseases, and the Himalayan region boasts the greatest diversity of bryophytes. Although *M. polymorpha* is one of the most extensively studied liverwort species, aspects of its bioactivity against plant microbes remain poorly understood. Therefore in this study, we investigated the antifungal and antibacterial efficacy of *M. polymorpha* extracts obtained using different solvents on plant pathogenic bacteria and fungi. Our primary objective was to identify and gather information on the traditional uses of *M. polymorpha* for managing plant infections. Our aim was to evaluate the potential of *M. polymorpha* extracts as a source of novel antimicrobial compounds against significant plant bacteria and fungi, offering a sustainable and environmentally friendly approach to disease control.

Material and Methods

Marchantia polymorpha, a type of liverwort, was collected from various substrates including soil, rocks, walls, and the trunks and leaves of vascular plants in the Kumaon region of the Western Himalaya (213-2100 m), Uttarakhand, India. The collection was conducted from two different altitudinal ranges, namely Artola (29º23.711'N 79º28.000'E, Alt. 6790 ft.) Uttarakhand, India. The selection of the sampling area was based on the fact that liverworts usually grow in humid locations where they form mats and cushions over soil and rocks (Ludwiczuk and Asakawa, 2019). The collected *M. polymorpha* samples were stored in sterilized polythene bags and transported to the Laboratory of Environmental Science, ITM University Gwalior, India.

Sample Preparation

For analysis, *M. polymorpha* were rinsed with distilled water to remove soil and plant residues. Further cleaning involved multiple rinses (2-3 times) with distilled water. Finally, the liverworts were dried on blotting paper in the shade at room temperature. This ensures that the liverworts are free from extraneous materials and provides accurate and reliable data for further investigation.

Extraction of *Marchantia polymorpha* for Antimicrobial Activity

The liverworts were first dried in room temperature and then subjected to an electric grinder to obtain a fine powder. The powder was then extracted using a hot Soxhlet extraction method with 80 percent solvent, including petroleum ether, chloroform, acetone, ethanol, methanol, and water. By using multiple solvents for the extraction, a diverse range of bioactive compounds from the *M. polymorpha* liverworts can be extracted and studied which will be conducted in further research. To optimize the extraction process, 10g of *M. polymorpha* powder was combined with 100mL of solvent. After the extraction, the samples were filtered using muslin and obtained crude extracts were then concentrated using a rotary evaporator (Biogen) to produce extracts at various concentrations ranging from 1000, 500, 250 and 125 µg/ mL.

Preparation of Extract solution

To prepare the extract solution for testing, the mother extract was first prepared by dissolving the extract in a separate solvent at a concentration of 1000 µg/mL. This was achieved by mixing the extract and solvent in a 1:2 ratio, and thoroughly stirring until the extract was completely dissolved.

Once the mother extract was prepared, three additional doses were made by diluting the solution with the same solvent. These doses were 125 µg/ mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL respectively. These dilutions were tested for their antimicrobial activity against phytogenic fungal and bacterial strains.

Phytopathogenic Bacterial strain and Fungal strain

The bacterial Strain *Pseudomonas syringae* (MTCC No- 1604), *Xanthomonas campestris* (ITCC BU0001), *Staphylococcus aureus* (MTCC No- 737), *Bacillus subtilis* (MTCC 441) and fungal strain *Macrophomina phaseolina* (ITCC 7209), *Fusarium oxysporum* (ITCC 4998), *Rhizoctonia solani* (MTCC 2356), *Colletotrichum acutatum* (ITCC 4214), were obtained from reputable sources such as Institute of Microbial Technology (IMTECH), Chandigarh India and The Indian Council of Agricultural Research (ICAR), New Delhi, India for the present study. These strains have a pathogenic relationship with many plants, making them ideal candidates for testing the antimicrobial activity of the extract. Each bacterial isolate was grown in tryptic soy agar (TSA) (Merck, Germany) for 24 h at 37°C, and stored in Luria-Bertani (LB) broth containing 25% glycerol at -70°C (Changa and Fang, 2007; Gu *et al.,* 2011). The fungal strains were maintained on Potato dextrose agar (PDA) (Himedia M096) and Sabouraud Dextrose Agar (SDA) (Himedia M063) at 27 ± 2 °C.

Antibacterial and Antifungal Activity of *Marchantia polymorpha*

Bioassay for fungus:

In order to determine the antifungal activity of the organic extracts of *M. polymorpha*, 48-hour-old phytopathogenic fungal culture discs were placed on a agar plate. This plate was impregnated with varying concentrations of *M. ploymorpha* extract (ranging from 1000, 500, 250 and 125 µg/ mL) for treatment. In order to evaluate the efficacy of the liverwort extract in inhibiting the growth of fungal pathogens, the agar plates were incubated at a specific temperature of 27 ± 2 °C and monitored closely for 24, 48, and 72 hours, then compared the colony diameter of the poisoned plate (with plant extract or positive control) to the non-poisoned plates (solvent) to estimate the percentage of mycelial growth inhibition. Nystatin was used as positive control. Through this meticulous observation, we were able to determine the potency of the *M. polymorpha* extract in inhibiting the growth of fungal pathogens.

The inhibitory effect was worked out by using following formula:

$$\textbf{Percent inhibition} = \frac{C-T}{C} \times 100$$

“The equation compares the control and treatment data to determine the percent blockage of a specific process or activity.”

“C” represents the colony diameter of the non-poisoned plate (control).

“T” represents the colony diameter of the poisoned plate (with plant extract or positive control).

Bioassay for Bacteria

The method used is the Disc diffusion method, which involves placing four discs, two of which are treated with plant extract (T) and two control discs (C), on solid agar plates. These plates were then incubated with 1 mL of bacterial culture. After 24 hours, the inhibition zone was measured in mm, to determine the effectiveness of the plant extracts on the bacterial strains tested. To ensure accurate results, two positive control antibiotics - Tetracycline and Streptomycin were included.

Determination of MIC (Minimum Inhibitory Concentration)

The quantities of the extracts' inhibitory and bactericidal/fungicidal properties were measured using a micro broth dilution assay. Diluents included freshly made potato dextrose broth for fungi and nutrition broth for bacteria. Freshly revived cultures of the test microorganisms were multiplied by 100 in the broth (100 µl of microbes in 10 mL broth) to assure accuracy.

Using an optical density measurement at 620 nm using a UV-visible spectrophotometer, the CFU was calculated and was found to be 1X 10^6 CFU/mL for bacteria and 1X 10^9 CFU/mL for fungi (Genesys). In a two-fold dilution series, plant extract at progressively lower concentrations (1000 to 125 µg/mL) were introduced to test tubes containing live microbe cultures. All tubes containing bacterial and fungal species underwent a 24-hour and 72-hour incubation period at 37 °C and 28 °C, respectively. Using a UV visible spectrophotometer, the visible turbidity and optical density of cultures were assessed at 620 nm.

MIC was determined as the lowest concentration that significantly inhibited the growth of test organisms and MBC was defined as the lowest concentration that had no effect on microbial growth. These findings are significant as they provide critical information on the effectiveness of organic extracts in inhibiting bacterial and fungal growth.

Results and Discussion

In this study, the antibacterial and antifungal potential of five different organic extracts of *M. polymorpha* was evaluated against eight common microorganisms, including four bacteria (*Pseudomonas syringae*, *Xanthomonas campestris*, *Staphylococcus aureus*, and *Bacillus subtilis*) and four fungi (*Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Colletotrichum acutatum)*. The results showed that the methanol extract of *M. polymorpha* exhibited the highest antibacterial and antifungal activity among the tested extracts. The observed concentration-dependent (1000 to 125 µg/mL) growth inhibition further supports the antimicrobial potential of the plant extract. Additionally, the extract's MIC and MBC values (Table 1) are important metrics for assessing the strength of antimicrobial agents were measured in relation to the microorganisms. The MBC/MFC values were greater than the MIC values for most of the extracts, indicating that the methanol extract demonstrated the highest antibacterial activity against *X. campestris* with a zone of inhibition (ZI) of 14.4 ± 0.20 mm, MIC of 1.25 µg/mL, and MBC of 1.75 µg/mL at the maximum used concentration of 1000 µg/mL (Tables 1 & 3). The zone of inhibition for each bacterial strain was measured, and the results were compared to the positive control antibiotics, tetracycline and streptomycin. The findings indicated that the methanol extract had a significantly higher zone of inhibition against all four bacterial strains, with values of ZI= 12.40 ± 0.35 mm (*P. syringae*), ZI=14.4 ± 0.20 mm (*X. campestris*), ZI= 55.06 ± 0.5 (*S. aureus*) and ZI= 13.4 ± 0.21 mm (*B. subtilis*) (Table 2-5). However, the zone of inhibition for the methanol extract was not significantly higher than that of the positive control antibiotics. These results suggest that the methanol extract may have potential as a natural antibacterial agent against a broad range of bacterial strains. Similarly, the methanol extract demonstrated the highest antifungal activity against *M. phaseolina* with a ZI of 65.65 ± 0.11 mm, MIC of 2.50 µg mL^{-1}, and MBC of 3.00 µg mL^{-1} at the maximum used concentration of 1000 µg mL^{-1} (Table 1 & 6). Moreover, the zone of inhibition of the methanol extract against *M. phaseolina* (ZI= 65.65 ± 0.11 mm), *F. oxysporum* (ZI=47.45 ± 0.46) and *R. solani* (ZI= 55.06 ± 0.5) and the zone of inhibition of the chloroform extract against C. acutatum (ZI= 34.12 ± 0.77) were significantly higher than that of the positive control, nystatin (39.36 ± 0.14, 26.41 ± 0.37, 19.52 ± 0.24 and 28.46 ± 0.45 respectively) (Tables 6-9). The results indicate that *M. polymorpha's* methanol and chloroform extracts possess greater antifungal potency than the commonly used antifungal agent, nystatin. However, acetone extract had no effect on fungal populations. Since fungal infections pose a significant threat to plants, causing stress and serious diseases, these extracts may prevent infection and minimize the risk of fungal contamination from soil, seeds, crop debris, weeds, and nearby crops. Therefore, the study's findings suggest a strong link between the plant's traditional use in plant disease management and its antibacterial effects in vitro.

While all of the extracts showed some degree of activity against each of the tested fungi and bacteria, the methanolic extract was determined to be the most effective. Both gram-positive and gram-negative bacteria were killed by petroleum ether extracts of Barbula and Timmiella species, as

Table 1:Minimum Inhibitory Concentrations (MIC), Minimum Fungicidal Concentrations (MFC), and Minimum Bactericidal Concentrations (MBC) of different extract of *Marchantia polymorpha* against different plant pathogens (µg/ml)

Pathogen	Petroleum ether		Methanol		Chloroform		Ethanol		Acetone		STANDARDS*
Bacteria	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC / MFC**	**MIC**	**MBC/ MFC**	**MIC (MBC/MFC)**
P. s.	1.50	2.25	2.00	5.00	1.50	1.75	1.75	2.50	-	-	0.50 (0.80)
X. c.	1.75	2.50	5.00	6.00	0.75	2.50	2.50	3.00	-	-	1.00 (1.25)
S. a.	2.00	4.50	1.25	1.75	0.75	2.00	0.25	0.75	0.50	1.50	0.50 (0.60)
B. s.	0.75	1.50	1.00	2.00	1.25	2.50	1.25	1.75	1.50	2.25	0.50 (0.60)
Fungi											
M. p.	-	-	2.50	4.50	-	-	1.50	2.50	-	-	0.50 (0.70)
F. o.	3.0	4.0	2.50	2.75	0.25	0.75	1.75	2.50	-	-	0.25 (0.25)
R. s.	1.75	2.50	0.50	1.50	1.25	1.75	2.00	4.50	-	-	0.65 (0.80)
C. a.	0.75	1.50	0.50	1.25	1.50	2.25	0.75	1.50	-	-	0.65 (0.75)

P.s.=*Pseudomonas syringae,* X.c.= *Xanthomonas campestris*, S.a.=*Staphylococcus aureus,* B.s.=*Bacillus subtilis,* M.p.=*Macrophomina phaseolina,* F.o.= *Fusarium oxysporum*, R.s.= *Rhizoctonia solani,* C.a.=. *Colletotrichum acutatum*

***Nystatin, Tetracycline and Streptomycin are used as standards for fungi and bacteria respectively.**

Table 2:Antibacterial activity (expressed as zone of inhibition in mm) of *Pseudomonas syringae* with different extract of *Marchantia polymorpha*

Nature of extract	**Concentration (µg ml^{-1})***Values are represented as mean ± SD.			
	1000	**500**	**250**	**125**
Petroleum ether	6.00 ± 0.15	2.50 ± 0.15	1.25 ± 0.10	0.0
Methanol	12.40 ± 0.35	10.70 ± 0.15	9.40 ± 0.21	7.70 ± 0.12
Chloroform	8.20 ± 0.10	6.20 ± 0.15	5.40 ± 0.20	4.40 ± 0.26
Ethanol	11.40 ± 0.17	10.50 ± 0.10	9.50 ± 0.10	8.30 ± 0.26
Acetone	0.0	0.0	0.0	0.0
Tetracycline	22.90 ± 0.36	22.50 ± 0.15	22.10 ± 0.52	21.10 ± 0.47

Table 3:Antibacterial activity (expressed as zone of inhibition in mm) of *Xanthomonas campestris* with different extract of *Marchantia polymorpha*

Nature of extract	**Concentration (µg ml^{-1})***Values are represented as mean ± SD.			
	1000	**500**	**250**	**125**
Petroleum ether	6.20 ± 0.10	5.20 ± 0.15	3.40 ± 0.20	1.40 ± 0.26
Methanol	14.4 ± 0.20	12.4 ± 0.21	10.4 ± 0.20	8.4 ± 0.12
Chloroform	8.4 ± 0.21	6.6 ± 0.26	5.5 ± 0.15	4.6 ± 0.21
Ethanol	11.5 ± 0.31	10.5 ± 0.12	8.6 ± 0.10	6.7 ± 0.10
Acetone	0.0	0.0	0.0	0.0
Tetracycline	20.1 ± 0.40	20.2 ± 0.46	20.2 ± 0.32	20.0 ± 0.21

Table 4:Antibacterial activity (expressed as zone of inhibition in mm) of *Staphylococcus aureus* with different extract of *Marchantia polymorpha.*

Nature of extract	**Concentration (µg ml^{-1})***Values are represented as mean ± SD			
	1000	**500**	**250**	**125**
Petroleum ether	8.25 ± 0.21	6.25 ± 0.26	5.50 ± 0.15	3.15 ± 0.21
Methanol	13.4 ± 0.21	11.5 ± 0.26	9.5 ± 0.15	7.6 ± 0.21
Chloroform	10.6 ± 0.15	8.6 ± 0.15	6.4 ± 0.10	4.0 ± 0.12
Ethanol	11.5 ± 0.26	10.4 ± 0.10	8.4 ± 0.15	6.6 ± 0.30
Acetone	0.0	0.0	0.0	0.0
Streptomycin	25.6 ± 0.75	24.6 ± 0.17	25.6 ± 0.25	26.0 ± 0.20

discovered by Gupta and Singh (1971). The chemical composition of different plant species varies, and this can be affected by factors such as where the plants were grown and when they were harvested (Burt 2004). The findings of this research are consistent with prior studies that have revealed antibacterial efficacy of bryophyte extracts against pathogenic bacteria and fungi.

Mewari and Kumar (2011) examined the antifungal activity with methanolic extracts of *Marchantia polymorpha*, *Dryopteris filix-mas*, and *Ephedra foliate*. *Marchantia polymorpha* methanolic extract had the most powerful antifungal activity against all fungal infections of the three plant species that were tested. In another study, Mewari *et al.* (2008) evaluated the crude methanol and flavanoid extracts of *M. polymorpha* and discovered that the methanolic extract was the most efficient, demonstrating the best antibacterial activity against three bacterial strains (*E. coli*, *P. mirabilis*, and *S. aureus)*, and four fungal strains (*A. flavus, A. niger, C. albicans, and T. mentagrophytes*). According to the findings of Tadesse *et al.* (2003), the ethyl acetate extract of *Marchantia polymorpha* had the highest antifungal activity against *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Rhizoctonia solani* and inhibiting the growth of the fungi at low concentrations. Several bryophyte extracts were found to have strong antifungal activity against the plant pathogenic fungi examined by Tadesse *et al.* (2003), with some extracts exhibiting equivalent or greater activity than the commercial fungicide carbendazim. The authors also discovered terpenoids and phenolic chemicals, both of which have antibacterial capabilities, in the most active extracts.

A review of the literature on several research demonstrating *Marchantia polymorpha's* antifungal activity and its application in plant protection is provided by Dey & De (2011). According to the authors, *Marchantia polymorpha* extract can be utilized to create antifungal pharmaceuticals that can treat human fungal infections. Additionally, the use of *Marchantia polymorpha* extract as a biocontrol agent in agriculture has the potential to lower the usage of chemical fungicides, which have negative effects on both the environment as well as human health. *M. polymorpha* was shown to be active against *S. aureus*, *S. pyogens*, and the majority of the Gram-negative bacteria when Kamory *et al.* (1995) isolated Marchantin from *M. polymorpha* and investigated its antimicrobial activity against five Gram-positive and five Gram-negative bacteria. In another study Bodade *et al.* (2008) conducted invitro screening for antibacterial activity using extracts from six bryophytes species. They discovered that *Polytrichum commune* and *Sphagnum* spp. had the strongest effects on fungus and both Gram-positive and Gram-negative bacteria. Additionally, the authors noted differences in the antibacterial efficacy of the various extracts from each species, suggesting the potential existence of several bioactive substances. In contrast to conventional anti-snail therapies, bryophyte extract is just as effective, according to the research of Frahm (2004). Extracts from bryophytes are non-lethal alternatives to poisons for controlling snails and slugs. Similarly, Chen *et al.* (2021) studied the induced defense response of two bryophyte species, *Hypnum plumaeforme* and *Thuidium tamariscinum*, to snail herbivory. The results demonstrated that both species produced secondary metabolites that reduced herbivory and favorably benefited plant growth and reproduction.

Currently, it seems that there is a lack of scientific evidence supporting the use of bryophyte extracts in crop protection for their ability to prevent disease in vivo. However, several studies have revealed that higher plant extracts have direct protective effects. In contrast to untreated plants, Abo-Zaid, Matar, and Abdelkhalek *et al*. (2020) showed that *Streptomyces cellulosae* isolated Actino 48 significantly decreased disease symptoms and TMV accumulation levels in tomato tissues. Additionally, compared to untreated plants, tomato plants exhibited greater growth. The study by Otero-Blanca *et al.* (2021) advances knowledge of the complex interactions between fungi and bryophytes and sheds light on the morphological and molecular alterations that take place in *P. patens* after *C. gloeosporioides* infection. In order to manage fungal diseases in bryophytes and other plants, approaches to management can be developed using findings from the research. Gimenez-Ibanez *et al.* (2019) conducted studies to find out more about the immunological mechanisms underlying interactions between M. polymorpha and the plant pathogenic bacterium Pseudomonas syringae. According to the authors, when P. syringae was present on the liverwort, it triggered an immunological response, which included effector activity within the liverwort cells. The form and degree of this response varied among various strains of *Pseudomonas syringae*, demonstrating a unique interaction between the bacterium and the plant.

Results of the study indicate that bryophytes might be a useful source of antibacterial chemicals. However, more investigation is required to pinpoint the precise chemical components responsible for the observed bioactivity and to examine the possible applications of bryophytes in areas including agriculture, medicine, and environmental remediation. The study emphasizes the necessity of researching bryophyte's bioprospecting potential as a means of uncovering novel sources of antibacterial compounds.

Table 5:Antibacterial activity (expressed as zone of inhibition in mm) of *Bacillus subtilis* with different extract of *Marchantia polymorpha*

Nature of extract	**Concentration (µg/ml)***Values are represented as mean ± SD.			
	1000	**500**	**250**	**125**
Petroleum ether	6.4 ± 0.21	4.5 ± 0.25	3.4 ± 0.15	1.3 ± 0.15
Methanol	13.4 ± 0.10	11.5 ± 0.25	10.5 ± 0.10	9.1 ± 0.10
Chloroform	10.4 ± 0.20	8.4 ± 0.20	6.3 ± 0.42	5.5 ± 0.31
Ethanol	11.3 ± 0.21	10.1 ± 0.15	8.2 ± 0.21	6.2 ± 0.15
Acetone	9.4 ± 0.21	7.5 ± 0.25	6.4 ± 0.15	4.3 ± 0.15
Tetracycline	19.9 ± 0.36	19.6 ± 0.38	19.9 ± 0.21	19.4 ± 0.50

Table 6: Percent inhibition in the growth of *Macrophominaphaseolina*with different extract of *Marchantia polymorpha*

Nature of extract	**Concentration (µg ml-1)** *Values are represented as mean ± SD.											
	Time (hrs.)											
	1000			**500**			**250**			**125**		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	65.65 ± .11	59.72 ± .38	47.65 ± 0.6	28.64 ± .16	27.16 ± .71	25.10 ± .36	21.60 ± .35	20.50 ± .67	18.07 ±0.34	8.81 ± .53	8.85 ±0.27	8.45 ±0.48
Chloroform	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol	45.33 ±0.54	38.36 ±0.6	26.08 ±0.55	39.99 ±0.83	31.82 ±0.4	20.81 ±0.42	21.27 ±0.72	16.95 ±0.54	12.29 ±0.42	8.67 ±0.74	8.89 ±0.64	8.02 ±0.30
Acetone	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	46.52 ±0.30	39.36 ±0.14	38.40 ±0.48	31.48 ±0.48	28.56 ±0.35	25.49 ±0.42	15.48 ±0.40	14.41 ±0.41	13.61 ±0.32	14.48 ±0.40	12.48 ±0.46	11.68 ±0.37

Table 7:Percent inhibition in the growth of *Fusarium oxysporum* with different extract *of Marchantia polymorpha*

Nature of extract	**Concentration (µg/ml) *Values are represented as mean ± SD.**											
	Time (hrs.)											
	1000			**500**			**250**			**125**		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	45.65 ±0.36	43.60 ±0.34	40.65 ±0.37	34.50 ±0.48	33.50 ±0.47	30.60 ±0.41	29.42 ±0.50	28.43 ±0.38	24.46 ±0.48	27.20 ±0.06	25.59 ±0.50	24.45 ±0.48
Methanol	47.45 ±0.46	46.55 ±0.48	45.61 ±0.37	46.58 ±0.50	45.30 ±0.49	43.52 ±0.44	43.57 ±0.49	42.53 ±0.49	40.74 ±0.33	41.37 ±0.51	39.54 ±0.46	38.55 ±0.36
Chloroform	33.44 ±0.49	32.35 ±0.33	30.55 ±0.49	30.42 ±0.50	28.44 ±0.49	25.43 ±0.50	25.65 ±0.26	23.34 ±0.44	20.48 ±0.34	22.44 ±0.41	19.45 ±0.48	15.44 ±0.26
Ethanol	38.45 ±0.43	36.38 ±0.40	32.53 ±0.45	33.49 ±0.43	32.60 ±0.31	30.46 ±0.42	28.42 ±0.38	26.40 ±0.41	25.65 ±0.39	18.36 ±0.51	16.59 ±0.36	14.48 ±0.40
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	26.41 ±0.37	25.66 ±0.37	21.55 ±0.46	24.62 ±0.42	23.38 ±0.42	21.59 ±0.15	22.55 ±0.41	20.69 ±0.19	18.38 ±0.41	19.61 ±0.32	16.38 ±0.42	13.34 ±0.35

Table 8: Percent inhibition in the growth of *Rhizoctonia solani* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (μg/ml) *Values are represented as mean ± SD.											
	Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	27.31 ±0.35	25.90 ±0.67	24.0 ±0.17	26.83 ±0.27	25.14 ±0.26	22.0 ±0.57	23.21 ±0.33	20.07 ±0.39	18.0 ±0.12	19.22 ±0.25	13.56 ±0.46	12.0 ±0.07
Methanol	55.06 ±0.50	52.89 ±0.85	50.03 ±0.62	45.69 ±0.57	44.70 ±0.46	43.91 ±0.51	40.0 ±0.45	38.0 ±0.35	35.0 ±0.25	30.0 ±0.26	28.0 ±0.15	26.0 ±0.60
Chloroform	14.55 ±0.50	12.97 ±0.48	10.0 ±0.48	12.50 ±0.43	13.23 ±0.50	10.0 ±0.48	11.43 ±0.32	10.0 ±0.48	10.0 ±0.48	8.74 ±0.58	6.0 ±0.48	0.0
Ethanol	49.85 ±0.48	45.01 ±0.84	44.94 ±0.27	40.20 ±0.50	38.12 ±0.62	32.44 ±0.50	29.13 ±0.45	24.52 ±0.24	23.01 ±0.65	20.35 ±0.46	16.21 ±0.03	15.42 ±0.54
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	19.52 ±0.24	14.58 ±0.49	12.57 ±0.48	16.37 ±.42	17.44 ±0.41	14.50 ±0.25	15.48 ±0.31	13.61 ±0.32	11.63 ±0.33	14.36 ±0.43	13.64 ±0.35	12.33 ±0.45

Table 9: Percent inhibition in the growth of *Colletotrichum acutatum* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (μg/ml) *Values are represented as mean ± SD.											
	Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	26.23 ±0.20	24.05 ±0.39	22.52 ±0.41	23.16 ±0.16	20.49 ±0.17	17.68 ±0.54	20.36 ±0.22	16.94 ±0.54	16.30 ±0.39	15.25 ±0.05	12.01 ±0.63	11.34 ±0.36
Methanol	25.13 ±0.20	23.24 ±0.39	22.45 ±0.43	23.29 ±0.42	22.66 ±0.54	20.27 ±0.12	20.49 ±0.34	18.45 ±0.38	15.42 ±0.38	18.58 ±0.28	15.64 ±0.36	12.53 ±0.44
Chloroform	34.12 ±0.77	30.49 ±0.42	28.73 ±0.32	26.76 ±0.38	25.66 ±0.30	23.76 ±0.15	25.31 ±0.35	23.53 ±0.19	20.33 ±0.16	13.29 ±0.32	10.52 ±0.37	9.30 ±0.38
Ethanol	23.80 ±0.48	25.56 ±0.37	22.51 ±0.49	26.93 ±0.31	23.41 ±0.39	21.70 ±0.58	24.41 ±0.38	19.94 ±0.25	18.52 ±0.47	15.24 ±0.12	12.87 ±0.27	11.77 ±0.54
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	28.46 ±0.45	25.59 ±0.51	23.47 ±0.38	22.41 ±0.37	20.46 ±0.28	24.34 ±0.44	21.49 ±0.46	17.40 ±0.41	19.46 ±0.40	20.49 ±0.42	16.37 ±0.42	14.57 ±0.30

Conclusion

In conclusion, our research lends credence to the use of bryophytes for conventional plant disease management and to their in vitro antibacterial activities. The observed differences in antibacterial and antifungal potential between different bryophyte extracts can be attributed to variations in their chemical content. Our results are consistent with earlier investigations that have demonstrated the efficacy of bryophyte extracts against pathogenic bacteria and fungi. These findings suggest that bryophytes may be a promising new source of natural antibacterial agents, with potential applications in both the pharmaceutical and agricultural industries. Further research is needed to identify the specific chemical constituentsresponsible for the observed antibacterial activity and explore their potential use in antimicrobial applications or treatment formulations derived from natural sources. Overall, our study contributes to the development of innovative and effective strategies for combating bacterial infections and highlights the importance of harnessing the therapeutic potential of natural products.

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Conflict of interest

The authors declare that they have no conflict of interest.

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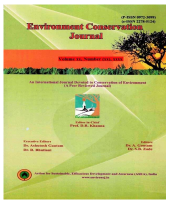

Antibacterial screening of *Terminalia chebula* Retz. against certain bacterial strains

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ABSTRACT

The aim of the study was to determine the antibacterial effectiveness of four different crude drug extract of *Terminalia chebula* (Badi Harad) extracted in methanol, ethanol acetone and aqueous solvent against certain food borne microorganisms i.e. *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio cholerae, Bacillus cereus,* and *Bacillus subtilis.* Methanolic extract had the strongest antibacterial effect, followed by acetone, ethanol, and aqueous extract. Maximum antibacterial activity obtained at 100% crude drug extract in methanolic was recorded against *E. coli* (25.6 mm) whereas in acetonic extract highest inhibition at 100% conc. was visualized against *S. typhi* and *V. cholerae* (22 mm).All four solvent crude extract crudes showed varient response in term of inhibition zone against all test organisms. Phytochemical analysis by standard methodology as well as by HPLC of the extracted crude samples showed presence of various phytoconstituents.

Introduction

Medicinal agents have been produced by natural resources since antiquity, and an incredible number of modern drugs have been discovered from these sources, especially those of plant origin, many of which were based on their use in traditional medicine. (Cowan, 1999). Viral infections and other infectious disorders have showed considerable potential for phytomedicines made from plants. About 25% of all medications are thought to be obtained either directly or indirectly from higher plants (Wickramsinghe, 2006). Eighty percent of the world's population, or about four billion people, use herbal remedies (Mukherjee, 2002; Bodeker *et al.*, 2005; Wickramsinghe, 2006). Therapeutic properties of the plants are based on their metabolites which are stored in different parts of plant with their properties.The primary cause of death in humans is infectious disease. It is estimated that infectious diseases account for about 50% of fatalities (Iwu *et al.,* 1999). Antibiotics were first discovered in the 20th century provided a new line of treatment for controlling the infectious disease.Credit goes to Sir Alexander Fleming (1929), who discovered and introduced the first antibiotic to the world i.e. Penicillin. The treatment of infectious disease with antibiotics has been successfully done, but the scientists are forced to discover/launch the new antibiotics because of the failure or less effectiveness against the disease which were very effective earlier. Due to indiscriminate use of these antibiotics, various pathogenic microorganisms have gained resistance to these antibiotics. The issue of antimicrobial agent (antibiotic) resistance has gained importance on a global scale. This has generated a new generation of pathogens i.e. multi-drug resistant pathogens. Infectious illness management and treatment have been hampered by these multi-drug resistant pathogens. Due to the recurring occurrence of drug resistance in human diseases against currently prescribed antibiotics, a quest for novel antimicrobial chemicals from other sources, including plants, has become imperative. Since, the plants posses a large number of chemical components within them to protect themselves against a large number of pathogens. In addition, a

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variety of human ailments have been treated using plants from the dawn of humanity. There is a wealth of knowledge on the utilization of plants as antibacterial agents to combat human diseases. Large number of workers are carrying out researches on different aspects of plant based molecules. But most important aspect in this area is the study of antimicrobial activity of raw drug plant material extract or grinded powdered crude drug extract against human pathogenic organisms which provides a basic platform for further investigations and different aspects including enhancement of treatment of range of human diseases described in ancient text. Therefore this aspect becomes more significant. Large number of the workers are actively engaged in this area and voluminous reports are regularly coming but some of the important contribution are being quoted here. Several workers reported antimicrobial activity of different medicinal plants (Dwivedi *et al*., 2012; Chopra *et al.,* 2013; Adebayo *et al.,* 2014; Asimuddin *et al*., 2017; Lee and Hwang, 2021; Nam and Hwang, 2021).Due to its excellent healing properties, *Terminalia chebula* Retz., also known as the "King of Medicine" in Tibet, consistently ranks at the top of the "Ayurvedic Materia Medica" list. (Bag *etal.,* 2009). In Asia's tropical and subtropical regions, which include China and Tibet, the plant is widely dispersed. *T. chebula* contains a variety of phytoconstituents, including as tannins, flavonoids, sterols, amino acids, fructose, resins, etc. It is a top-ranked plant for treating gout, gouty arthritis, bleeding piles, sore throats, and asthma in the Ayurvedic Materia Medica (Aneja and Joshi, 2009). The rejuvenating properties of haritaki include laxative, astringent, anthelmintic, nervine, expectorant, tonic, carminative, and stimulant of appetite. The herb is used to make "Triphala," a laxative intended to treat persistent constipation. Triphala is the most adaptable herbal remedy and is recommended for candida infection and cardiotonic effects (Kaur *et al.,* 2005). It is recommended for those who have leprosy (which includes skin issues), anaemia, narcosis, piles, chronic intermittent fever, heart disease, diarrhoea, anorexia, cough, excessive mucus secretion, and a number of other complaints and symptoms. The goal of the current study was to study the antibacterial properties of *T. chebula* fruit against gram positive and gram negative bacterial strains.

Material and Methods

Collection of plant material and soxhlet extraction procedure:The fruits of *Terminalia chebula* were collected locally. The fruits were cleaned with running tap water, then sterilised distilled water, and thendried.. After proper drying fruits of *T. chebula* (Badi harad) were grinded into coarse powder. The coarse powder was extracted in soxhlet extractor for 24 hrs using different extractants methanol, ethanol, acetone, and water The solution was then filtered through filter paper (Whatman No.1.). The resulting extract solution was referred to as stock solution (100 percent concentrated drug solution). The 100% concentrated solution was subsequently diluted with distilled water to produce 75%, 50%, and 25% concentrations.

Test organisms: Pure cultures of *Vibrio cholerae* (MTCC 3904), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 6728), *Salmonella typhi* (MTCC 3216), and *Staphylococcus aureus* (MTCC 7443) were obtained from the Microbial Type Culture Collection of the Institute of Microbial Technology (IMTECH) Chandigarh and *Escherichia coli* was obtained from from SGPGI Lucknow.

Antibacterial activity testing: To evaluate the effectiveness of plant extracts against bacterial strains, the agar well diffusion method was employed (Bell and Grundy, 1968). To 100 ml of nutritional agar medium, 2 ml of bacterial suspension was added. Nutrient agar medium was substituted with Luria bertani media for *V. cholerae.* The flask was gently swirled to ensure that the test organisms were distributed evenly. After that, sterile petri plates were filled with the inoculated culture media and allowed to solidify. A sterile cork borer with a 6mm diameter was used to create 5 wells in the set of each petriplate, 4 of which were located on the periphery and 1 in the centre. Four peripheral wells received 0.1ml (100µl) solutions of plant extract at 100%, 75%, 50%, and 25% of the various concentrations. A 0.1 ml solution of the control was poured into the centre wells. Methanol, ethanol, acetone, and sterilised distilled water were employed as controls for methanolic, ethanolic, acetonic, and aqueous extract. Each Sample was assayed in triplicate and value was observed and recorded.

Phytochemical Analysis: The plant extracts were underwent qualitative screening to determine the different plant constituents as per standard procedures (Kokate *et al.,* 2007; Khandelwal, 2004).

Detection of alkaloids: One drop of Mayer‟s reagent was added to the extracts. The formation of precipitation with color change was observed.

Detection of Flavonoids: Add a few drops of concentrated H_2SO_4 producing deep yellow coloured solution indicated the presence of flavones and flavanols.

Detection of Steroids: Libermann-Burchard Test:- The extract was heated and then cooled after being treated with a few drops of acetic anhydride. Sulphuric acid was added from the side of the test tube, and a brown ring developed at the junction of two layers.

Detection of Saponin: Because they resemble soap, they are referred to as saponins. Separately, 1 ml of the extract was diluted to 20 ml with distilled water and stirred for 15 minutes in a graduated cylinder. A centimeter-thick layer of foam signify saponins.

Detection of Tanins: Ferric Chloride Test:- A 5% ferric chloride solution in water or ethanol was used to treat the extract. When many drops of solution are added to an extract, a blue-black or blue-green colour results, signifying the presence of tannins.

Detection of phytochemicals by HPLC

HPLC analysis of *T. chebula* (ethanolic and acetonic extracts) were carried out in order to detect the presence of phytoconstituents.

Results and Discussion

Result of antibacterial potency/efficacy of different extracts of *Terminaliachebula* based on four different solvent extraction i.e. methanol, ethanol, acetone and water against certain Gram-positive bacterial strains *B.subtilis*, *B.cereus, S.aureus* and Gram- negative bacterial strains *E.coli, S.typhi, V.cholerae* have been presented in table-1 and 2, Photoplate 1. Results of phytochemical screening of most prominent plant extracts of *T. chebula* have been depicted in table-3. Results of HPLC analysis have been shown in table-4. A positive correlation between drug concentration and drug potency has been found against all the test organisms in all four extracts. With the dilution of a 100% concentrated extract, the drug's effectiveness in terms of the inhibitory zone decreased.

Table 1: Antibacterial activity of methanolic and ethanolic extract of *T. chebula*

Extract Type	Test organism	Effective Zone of Inhibition ±SD (mm)			
		Extract concentration			
		100%	75%	50%	25%
Methanol	*Salmonella typhi*	23.0±0.00	20.6±0.57	16.3±0.57	14.0±0.00
	Escherichia coli	25.6±0.57	23.3±0.57	21.0±0.00	19.3±0.57
	Vibrio cholerae	23.0±1	21.3±0.57	18.0±0.00	14.0±0.00
	Staphylococcus aureus	24.0±1.00	22.3±0.57	20.0±1.00	17.6±0.57
	Bacillus cereus	18.6±0.57	16.6±0.57	15.3±0.57	13.6±0.57
	Bacillus subtilis	21.3±0.57	19.3±0.57	16.6±0.57	14.3±0.57
Ethanol	*Salmonella typhi*	19.0±1	16.0±0.00	14.0±1.00	11.6±0.57
	Escherichia coli	20.0±1	19.0±0.00	17.0±0.00	14.0±1.00
	Vibrio cholerae	21.6±0.57	19.0±0.00	16.0±1.00	14.0±0.00
	Staphylococcus aureus	20.6±0.57	18.0±1.00	16.0±0.00	13.0±1.00
	Bacillus cereus	20.3±0.57	17.3±0.57	16.3±0.57	13.6±0.57
	Bacillus subtilis	20.6±0.57	18.3±0.57	16.6±0.57	14.3±0.57
control		-	-	-	-

***Effective inhibition zone= Average value of inhibition zone of three replicates-well size(-)= No activity**

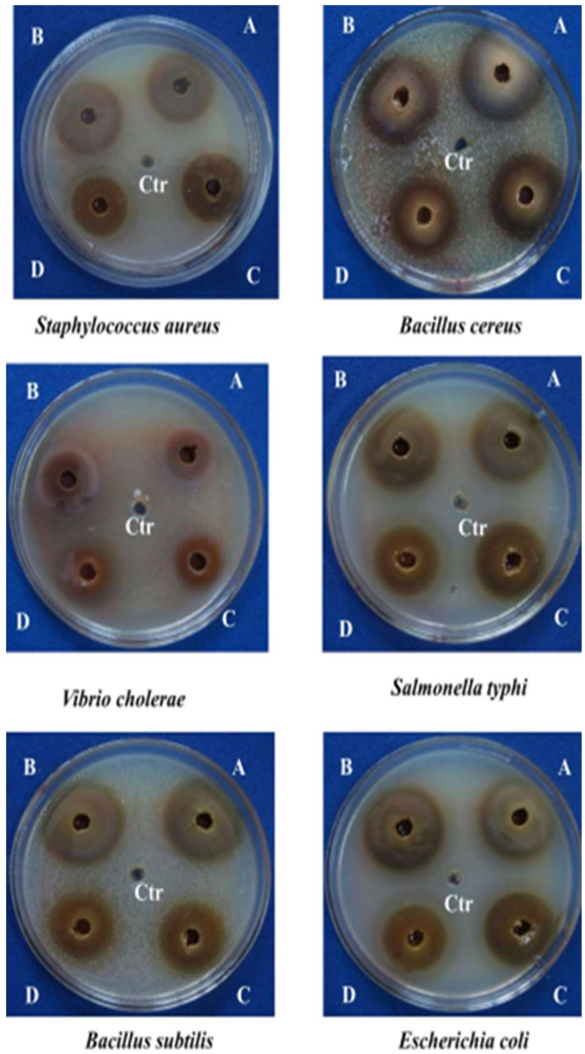
Methanolic extract of *T. chebula* was found more effective followed by acetone, ethanol and aqueous extracts. Maximum inhibitory activity in methanolic extract at 100% conc. was recorded against against *E. coli* followed by *S. aureus, S. typhi, V.cholerae, B. subtilis* and *B. cereus* whereas ethanolic extract revealed maximum activity against *V. cholerae* followed by *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* and *S. typhi*.

Table 2: Antibacterial activity of acetonic and aqueous extract of *T. chebula*

***Effective inhibition zone= Average value of inhibition zone of three replicates-well size(-)= No activity**
SD= Standard deviation±of three replicates

Extract Type	Test organism	Effective Zone of Inhibition ±SD (mm)			
		Extract concentration			
		100%	75%	50%	25%
Acetone	*Salmonella typhi*	22.0±1.0	20.0±0.00	17.0±1.00	15.0±1.0
	Escherichia coli	21.0±1.0	19.6±0.57	16.0±0.00	13.0±1.0
	Vibrio cholerae	22.0±0.0	19.0±1.00	16.6±0.57	14.0±0.0
	Staphylococcus aureus	21.0±1.0	19.0±1.00	16.6±0.57	14.0±1.0
	Bacillus cereus	17.0±0.0	16.0±1.00	14.0±0.00	11.0±1.0
	Bacillus subtilis	20.0±0.0	16.6±0.57	15.3±0.57	11.0±1.0
Aqueous	*Salmonella typhi*	12.0±1.0	10.3±0.57	9.0±0.00	5.0±1.0
	Escherichia coli	12.0±1.0	9.6±0.57	8.6±0.57	3.6±1.15
	Vibrio cholerae	10.3±1.1	8.6±0.57	7.6±0.57	3.0±0.0
	Staphylococcus aureus	12.6±0.5	10.3±0.57	7.0±1.73	4.0±1.0
	Bacillus cereus	16.0±0.5	14.3±0.57	11.6±0.57	5.6±0.57
	Bacillus subtilis	14.6±0.5	13.3±0.57	11.6±0.57	7.6±0.57
Control		-	-	-	-

Theacetonic crude extract of plant showed highest inhibitory activity against *S. typhi* and *V. cholerae* followed by *E. coli*, *S. aureus, B. subtilis* and *B. cereus* at 100% concentration. The aqueous plant extract at 100% conc. displayed maximum inhibitory activity against *B. cereus* followed by *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli* and *V. cholerae.* The findings of earlier researchers support our findings. Sah *et al*. (2012) screened the antimicrobial potential of *T. chebula* fruit extracts, extracted in petroleum ether and methanol against *B. subtilis, S. aureus, E. coli, Klebsiella* spp. and *S. paratyphi* and found that methanolic extract was better than petroleum ether extracts. Similar to this, Jayalakshmi *et al.* (2011) assessed the antibacterial potential of *T. chebula* fruit extracts in petroleum ether, chloroform, ethyl acetate, and methanol against *E. coli, K. pneumonia, B. subtilis, B. cereus, S. typhi, E. aerogenes,* and *S. aureus*.In their research, petroleum ether, ethyl acetate, and methanolic extracts all shown the highest levels of inhibitory activity. Results of present study are also supported by findings of Kumar *et al*. (2013). The methanolic extract of *T. chebula* fruit was more effective than that of acetone, ethanol, water (cold and hot) extracts against certain bacterial species i.e. *B. amyloliquefaciens*, *S. epidermidis*, *E. coli*, *Salmonella enteric* ser *typhi* and *A. fumigates* which might be due to fact that more organic compounds were leached in methanol solvent as evidenced by Kumar *et al.*(2013).



Photoplate 1: Antibacterial activity of methanol extract of *T. chebula*against *S. aureus, B.cereus, V. cholera, S.typhi, B. subtilis* and *E. coli*.
Abb: A=100%, B=75%, C= 50%, D=25%

Results of preliminary phytochemical analysis has been depicted in table-3. Methanol extract of *T.chebula* were found to be rich in flavonoids, saponins and tannins but alkaloids, steroids and glycosides showed absence while in acetonic extract alkaloids, flavonoids, tannins, steroids and glycosides were analysed and detected but saponins showed absence. Gram-positive and Gram-negative bacteria are effectively inhibited by methanolic extract, which may be attributed to the individual or combined effects of these phytochemicals. It is generally known that certain alkaloids and tannins have antibacterial properties (Sing and Bhat, 2003), probably due the mode of antimicrobial action of methanolic extract which is related with inactivation of microbial enzymes along with transport of proteins. In the present study, moderate activity in aqueous extracts has been observed against both Gram-negative and Gram-positive bacteria as also reported by Kumar *et al*. (2009).

Table 3: Phytochemical components present in different crude extracts of grinded test drug material of *T. chebula*

Extract type	Alkaloids	Flavonoids	Saponins	Tannins	Steroids
T. chebula (Methanol)	–	+	+	+	–
T. chebula (Acetone)	+	+	–	+	+

Table 4: HPLC analysis of *T. chebula*

Extract type	Quantification on percent dry weight basis of different compounds					
	Chlorogenic acid	Caffeic acid	Rutin	Myricitin	Quercetin	Kaempferol
T. chebula (Ethanol)	0.225977	0.155168	0.070655	0.124353	ND	ND
T. chebula (Acetone)	0.161361	0.202259	ND	ND	0.00601	ND

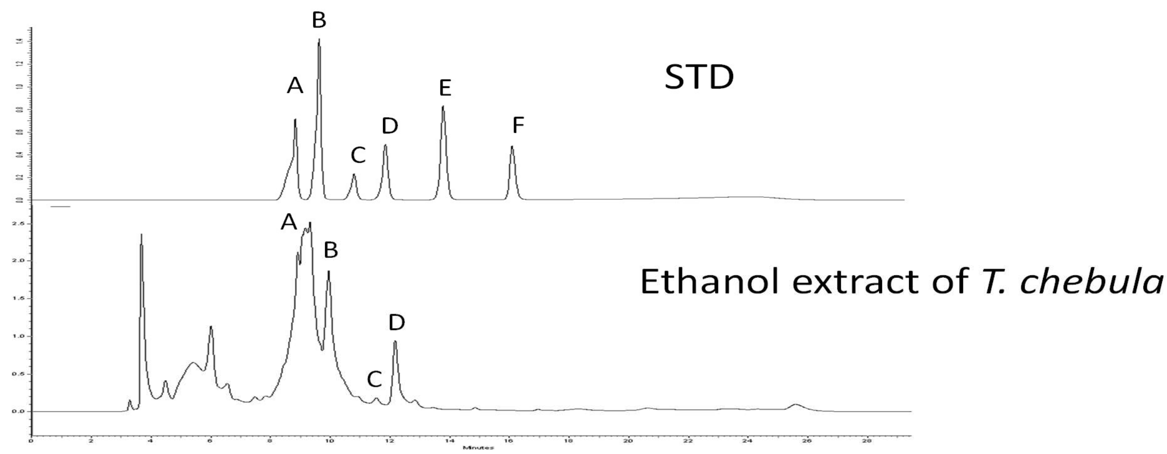


Figure 1: HPLC analysis of ethanol extract of *T. chebula*

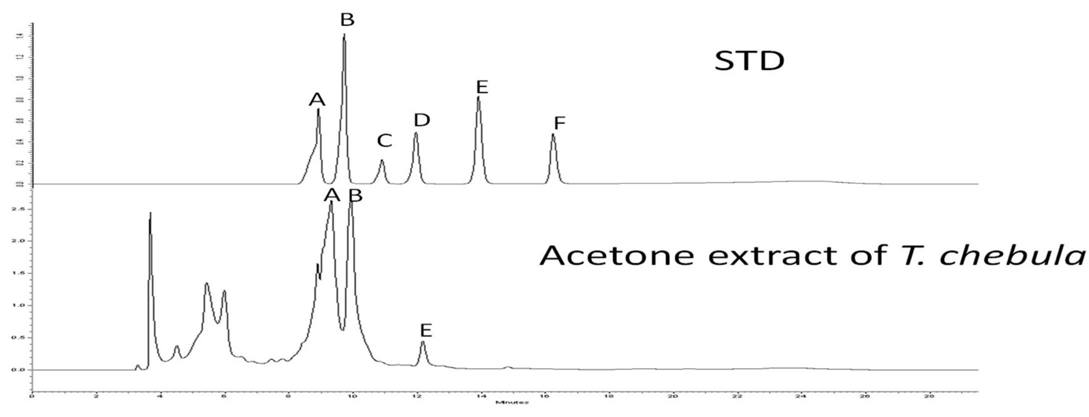


Figure 2: HPLC analysis of acetone extract of *T. chebula*
Description of peaks found:
A-Chlorogenic acid, B-Caffeic acid, C-Rutin, D-Myricitin, E-Quercetin, F-Kaempferol, STD: Standard

This may be due to the fact that variation in the extracted chemical component as well as very lesser quantity of the extracted component whichcould not effect the growth of test organisms. Lowered inhibitory effect of aqueous extract of *T. chebula* fruit is supported with the findings made by Ahmad *et al.* (1998). They evaluated three different extracts, extracted in alcohol, hexane and water and recorded lowest antibacterial activity in aqueous extract among all of them.

T. chebula ethanolic extract's HPLC examination revealed the presence of chlorogenic acid, caffeic acid, rutin, myricitin but quercitin and kaempferol were not detected (table-4) (Figure 1,). Similarly, the results of HPLC analysis of *T. chebula* acetonic extract (table-4; Figure-2) showed presence of three compounds i.e. chlorogenic acid, caffeic acid and quercitin. While rutin, myricitin and kaempferol were not found in acetone extract of *T. chebula.* These compounds have been found associated with antimicrobial activity. Observation made by HPLC analysis of *T. chebula* revealed that chlorogenic acid played a significant role in inhibition of different bacterial strains. Chlorogenic acid obtained from *Helichrysum* (Asteraceae) exhibited significant antimicrobial activity against *B. subtilis* and *B. cereus* (Albayrak *et al.,* 2010), whereas chlorogenic acid derived from carrot extracts showed higher antimicrobial activity against *L. monocytogenes* (Babic *et al.,* 1994). Infact chlorogenic acid derived from different variety of plants exhibits high antimicrobial activity against *E. coli* strains (Babic *et al.,* 1994; Albayrak *et al.,* 2010; Xia *et al.,* 2011a & b). Other constituents such as rutin, myricitin, quercitin have also found in *T. chebula*. The literature that is currently available demonstrated that these compounds play a substantial role in antibacterial action. Myricetin reported to have inhibitory activity against both Gram-positive and Gram-negative pathogenic bacteria (Tsai *et al.,* 2008; Freeman *et al.,* 2010). Askun *et al.* (2009 a & b) and Santas *et al.* (2010) reported antibacterial activity of quercitin against *B. subtilis* and *B. cereus* and *E. coli*.

Conclusion

With a broad range of pharmacological and therapeutic actions, *T. chebula* is one of the most popular plant world wide. This versatile plant's use as a medicine makes it a special source of several kinds of chemicals with distinct chemical structures. Our research indicated that the methanolic, acetonic *T. chebula* extracts have demonstrated positive antibacterial activity against all of the investigated bacterial pathogens, which accounts for its use in traditional systems of medicine. Consequently, *T. chebula* can be used as a source of natural antimicrobials as a complement to traditional medications.

Conflict of interest

The authors declare that they have no conflict of interest.

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Standardization of roots of *Taraxacum officinale*

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ABSTRACT

Taraxacum officinale **(Dandelion) belongs to the Asteraceae family; an edible herb commonly found in subtropical and temperate regions worldwide. Traditionally dandelion is used in diarrhea, gout, jaundice, diabetes, pneumonia, urinary problems and to purify the blood. The phytochemical study of dandelion discovered the occurrence of β- sitosterol, stigmasterol, taraxsterol, lactucopicrin, lactucin, cichorin, taraxacoside, taraxacerin, campesterol, homotaraxasterol, etc. Anti-inflammatory, immunostimulating, antimicrobial, antioxidant and antidiabetic activities of dandelion were studied. This plant has great therapeutic value and in order to overcome the problem of adulteration, the present study was aimed to standardize and preserve the quality parameters of the plant. Roots of** ***Taraxacum officinale*** **were subjected to measurements including macroscopy, microscopy, foreign organic content, ash value, extractive value, phytochemical screening, fluorescence analysis and chromatographic analysis. HPTLC analysis confirmed the presence of various phytocomponents. The macroscopic, microscopic and physico-chemical criteria presented here can help to identify the drug and to prepare the monograph.**

Introduction

Medicinal plants have historically been used to treat various ailments (Barnes *et al.*, 2007). Medicinal plants are found all over the world, but tropical countries have the greatest abundance (Duke and Martinez, 1994). Now a day's in medical practice there is a growing awareness and acceptance of the role of herbal drugs. Over eighty percentages of the global residents in underdeveloped nations depend on herbal treatments to lead a healthy life (Bodeker, 2005). The increasing demand of medicinal plants has resulted in a range of adulteration of herbal products, leading to consumer disappointment and, in some cases, serious consequences (Tewari, 1991). The main disadvantage of herbal medicines is lack of standardization techniques. Because of lack of standardization there is a chance of adulteration of plant material. To overcome the problem of adulteration plant must be properly standardized (Bauer, 1998).

The plant *Taraxacum officinale* belongs to the Compositae or Asteraceae family. It is an edible herb commonly known as dandelion. The plant is commonly found in open meadows and grassland up to 3500 m of the temperate and subtropical constituency of Asian and European, countries (Stewart-Wade *et al.*, 2002). It is the most recognizable weed in the world. Plant leaves are adopted as a salad; traditionally used as a laxative, stomachic, and hepatic stimulant (Dearing *et al.*, 2001). It has been used to treat diarrhea, gout, liver and spleen problems, diabetes, pneumonia, urinary problems, blood purifier, and diuretic (Schütz *et al.*, 2006). Liver protecting impact of root extract of dandelion investigated (Mahesh *et al.*, 2010). Mosquito repellant action of the milky latex of *Taraxacum officinale* was also reported (Stuart, 1979). Biologically this plant has been assessed for antiviral activity (Rehman *et al.*, 2016), anti-inflammatory (Koh *et al.*, 2010), immunostimulatory (Yoon, 2008), antidiabetic (Akhtar *et al.*, 1985), antimicrobial and antioxidant (Ghaima *et al.*, 2013) activities. Various parts of

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dandelion are used to prepare foodstuff in addition to being employed in therapeutic applications. The underground parts of plant after roasting are used as a coffee substitute. Moreover, plant extracts are used as flavoring agent for a variety of foods, drinks, soft drinks, and frozen dairy items (Hfaiedh *et al.*, 2016).

Phytochemically major active constituents present in the root of *Taraxacum officinale* are β- sitosterol, stigmasterol, and taraxsterol. Taraxinic acid or lactucopicrin, lactucin, cichorin, taraxacoside, taraxacerine, taraxasterol , campesterol, homotaraxasterol, luteolin-7-glucoside, caffeic acid, ferulic acid, quercetin-7-glucoside, α-amyrin, β-amyrin, lupeol, taraxol, taraxaserol, arnidiol, faradiol, neoxanthin, flavoxanthin, chrysanthe-maxanthin, β-D-glucopyransoides, lutein-5-6-epoxide, taraxacoside, asparagine, apigenin-7-glucoside, chlorogenic acid are isolated from different parts of *Taraxacum officinale* (Singh *et al.*, 2008).

This plant has great therapeutic value; hence the current study was done to standardize the root of selected plant to establish the quality parameters so that plant can be differentiate from other similar plant species. Roots of *Taraxacum officinale* were subjected to macroscopy, microscopy, foreign organic content, ash value, extractive value, phytochemical screening, fluorescence analysis, and chromatographic analysis.

Material and Methods

Taraxacum officinale root was procured from the Doddabetta area of Ootacamund, Tamilnadu, India and was identified by Dr. S. Rajan, Survey of Medicinal Plants and Collecting Unit, Arts College, Ootacamund. Proper examination of the untreated root sample of *Taraxacum officinale* was performed according to WHO guidelines (1998) under diffused sunlight and an artificial source to observe the color of selected root sample, other organoleptic characters like size, odor, shape and taste were also determined. To evaluate the microscopic features 10 to 12 mm thick sections were prepared using a rotary microtome. Toluidine blue used to stain sections. After staining, sections were examined under a microscope (Sanderson, 2020). The alcohol extract of coarse powder of *Taraxacum officinale* root was prepared. Hot extraction methods and cold extraction method were used for extraction of phytoconstituents; and qualitatively tested by phytochemical screening using the standard procedure (Sati and Kumar, 2015)(Archana *et al.*, 2012). Various physico-chemical parameters such as total ash value, acid-insoluble ash value, and water-soluble ash value, loss on drying, foreign organic matter and fluorescence analysis were carried out according to WHO (1998) guidelines. For chromatographic analysis, 1 g powdered drug was extracted with ethanol to prepare the sample, followed by filtration through Whatmann filter paper. Precoated HPTLC silica gel G254 plates (Merck) were used in the study. A solvent solution of chloroform: methanol (8:2) was used as the mobile phase. Sample was applied to the HPTLC plate as an 8 mm bend with the help of CAMAG Linomat IV applicator. CAMAG Twin Trough Chamber was used to develop the plate. A densitometer was used to scan a plate of *Taraxacum officinale* alcoholic extract at 254 nm (Sherma, 2010).

Results and Discussion

A systematic approach is required in pharmacognostic study to confirm and determine the identity, purity and quality of a raw drug. This comprehensive and rigorous pharmacognostic study will provide useful information for future research. It is important to note that macroscopic plant evaluation is subjective; hence substitutes or adulterants can be very similar to the original material (Rehman *et al.*, 2016). Therefore, the macroscopic findings must be validated and authenticated. Morphological evaluation of *Taraxacum officinale* root was performed based on organoleptic parameters such as color, smell, shape, size and taste. It was found that the length of the root was about 30 cm and the thickness was about 15-25 cm. The outer surface of the fresh root was yellowish brown while the inner surface was white and fleshy. Thin and fibrous rootlets were present. Upon drying, the occurrence of longitudinal wrinkles was observed. The taste was bitter. The fracture was short (Fig. 1).

The microscopic evaluation is essential to identify the crude drugs. It is one of the crucial pharmacognostic parameter in preparation of modern monographs (Koh *et al.,* 2010).T.S. and

L.S. of the root sample is studied. Microscopic study showed the occurrence of periderm, secondary phloem, secondary xylem, vessels and xylem parenchyma and seive tube member. The periderm consists four to five layers of cells. The cortex region is wide and homogenous. The cortical cells are parenchymatous in nature. Secondary phloem is cylindrical in shape having sieve element and parenchymatous cells. Secondary xylem has several vessels and fibres. The vessels are variable in diameters. The xylem fibers are thick and lignified (Fig. 2). Analysis of physicochemical parameters of a crude drug is very important as it helps in identification and in setting of proper standards (World Health, 1998).

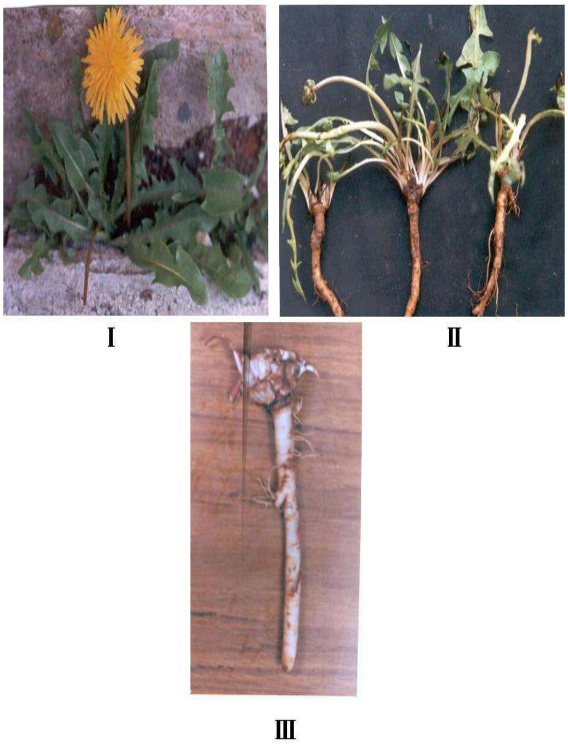

Figure 1: *Taraxacum officinale* (I: Flower, II: Aerial parts and Root, III: Root).

Ash values, extractive values, loss on drying, and fluorescence reaction with various chemical reagents were investigated and the results are present in Table 1. Any organism or part other than plant material are known as foreign organic material; was determined according to WHO guideline, found to be 0.189%. The presence of moisture in the drug sample can lead to microbial contamination due to enzymatic hydrolysis.The loss of water content on drying was determined, The ash value is useful for identifying excess sand and depleted low-grade drugs. and was found to be 7.863%. This test is based on gravimetric analysis.

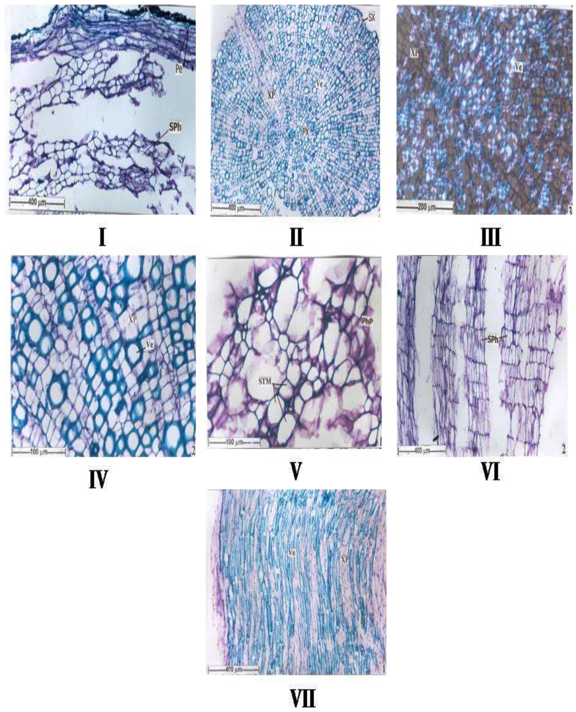

Figure 2: Microscopic Characters of root of Taraxacum officinale (I to VII). (Pe:Periderm, SPh: Secondary Phloem, SX: Secondary Xylem, XP: Xylem Parenchyma, Ve: Vessel, PX: Primary xylem, PhP: Phloem Parenchyma, STM: Sieve Tube Members).

Table 1: Results of physiochemical parameters

Parameters	Percentage
Foreign organic matter	0.189 %
Loss on Drying	7.863 %
Total ash	5.546 %
Sulphated ash	3.571 %
Acid insoluble ash	1.496 %
Water soluble ash	1.581 %
Alcohol soluble extractive value (Hot Extraction Method)	12.57 %
Alcohol soluble extractive value (Cold Extraction Method)	8.943 %

If the value of the ash is not within the limit, then this directly affects the purity of the drug, and indirectly affects the quality of the drug. Inorganic radicals like phosphates, carbonates and silicates of sodium, calcium and magnesium etc. are present in ash. When a powdered drug is ignited at a temperature 400°C, ash is obtained, which is known as total ash. It was determined and was 5.546%. When the residue obtained after boiling the total ash with dilute hydrochloric acid and then ignited at 400°C, it is known as acid-insoluble ash; gives an idea about the concentration of silica, which is mainly present as sand. Hydrochloric acid is used because silica is soluble in hydrochloric acid. The acid-insoluble ash was found to be 1.496%. When powder drugs are first burned to ash, then treated with sulfuric acid and burned to in turn get the ash known as sulphated ash, and it was found to be 3.571%. The weight difference between the total ash and the residue obtained after treating the total ash with water is known as water soluble ash; was determined and found to be 1.581%. The extractive value helps to evaluate solubility of drug constituents that cannot be calculated in any other way. It gives an idea about the solubility pattern of phytoconstituents based on the polarity of solvent. The extractive value can be determined by hot extraction methods and cold extraction methods. Depending on the choice of solvent, different solvents can be used to determine the extraction value. The alcohol soluble extract value of the root of *Taraxacum officinale* was determined by the hot extraction method and the cold extraction method and was found to be 12.57% and 8.943%, respectively. Fluorescence analysis of the drug was observed under daylight and UV light using different solvents such as hydrochloric acid, sulfuric acid, ammonia solution, aqueous sodium hydroxide. The fluorescence behavior tabulated in Table 2.

Table 2: Observation under fluorescence analysis

Treatment	Visible	Short UV light 254 nm	Long UV 366nm
Powder as such	Light- brown	Dark- brown	Dark- brown
Powder +distilled water	Light- brown	Dark- brown	Dark- brown
Powder +5% aqueous NAOH	Yellowish- brown	Dark -brown	Dark- brown
Powder + ammonia solution	Light- green	Light- green	Black
Powder +conc. H_2SO_4	Light -brown	Light- brown	Black
Powder + 50 % HCL	Light- brown	Dark- brown	Dark- brown

Preliminary phytochemical screening was carried out to determine the presence of chemical constituent's category. The results of phytochemical screening are shown in Table 3. Preliminary phytochemical screening of root of *Taraxacum officinale* gave the colors with modifications according to colors of extract indicating the presence of the various active phytoconstituents metabolites as follows: carbohydrate (Molish's violet ring at the junction of two liquids, Fehling's yellow to red, Bendict's greenish yellow to red), protein (Biuret violet, Xanthoprotein yellow precipitates), amino acids (Ninhydrin purple colour) glycosides (Legal pinkish red), steroids and sterol (Liebermann-Burchard purple / violet , Salkowski red color ring at junction of two layer) tannins (lead acetate white precipitates, Ferric chloride bluish black) and saponin (Foam test persistent of white foam at upper layer). Root extract of *Taraxacum officinale* revealed the presence of cardiac glycosides, terpenoids (antiviral activity), phenols (Immuno modulatory and antihyperglycemic) (Kenny *et al.*, 2015) , tannins (wound healing activity) (Ajaz *et al.*, 2019), flavonoids (antioxidant and anti-inflammatory activity) (Hagymási *et al.*, 2000), steroids and sterols (Antihyperglycemic and Anti inflammatory activity) (Petlevski *et al.*, 2003)(Jones and Persaud, 1998) carbohydrates, proteins and amino acids with absence of alkaloids and fixed oil. Also similar finding reported in the other species of the same family (Jaramillo-Jaramillo *et al.*, 2016). HPTLC is one of the most demanding technical methods for the quantitative and qualitative analysis of medicinal plants (Marston, 2007). According to HPTLC results ethanolic extract of selected sample under 254 nm UV light, showed the presence of seven spots suggesting presence of various compounds in the extract. The Rf value of various phytocomponents present in alcoholic extracts was found to be 0.07, 0.10, 0.24, 0.34, 0.55, 0.62 and 0.83. HPTLC analysis showed the presence of seven components. It could not be found the name of component there is further need to isolate each component.

Table 3: Result of Phytochemical Screening

Tests	Ethanol extract
Alkaloids	
a) Dragondorff's Test	-
b) Wagner's Test	-
c) Mayer's Test	-
d) Hager's Test	-
Carbohydrates	
a) Molisch Test	+
b) Fehling's Test	+
c) Benedict's Test	+
Proteins	
a) Biuret Test	+
b) Xanthoprotein Test	+
Amino Acid	
a) Ninhydrin Test	+
Glycoside	
a) Legal Test	-
b) BaljetTest	-
Steroids and Sterols	
a) Libermann Burchard Test	+
b) Salkowsky Test	+
Flavonoids	
a) Extract + Tin + HCI	+
Tannins and Phenol	+
Triterpenoids	+
Saponin Test	
a) Foam Test	+
Fixed oils	
a)spot Test	-

+ Represent positive result while – represent negative result

Conclusion

For thousands of years, medicinal plant used as a home remedy to treat a variety of diseases in both developed and developing countries. In recent years, consciousness about the utilization of medicinal plants increased, and pharmaceutical companies are trying to formulate dosage forms based on herbs due to less side effects of medicinal plants compared to synthetic drugs. The overexploitation of crude drugs is increasing due to increasing demand, and instead of genuine drug, lower quality plant materials or substitute or adulterated drugs are being used. Therefore it is necessary to ascertain the quality parameters of herbal drugs. The current study emphasized the standardization of the root portion of *Taraxacum officinale*. The root part was chosen because it has been found in the literature that the root of *Taraxacum officinale* has been reported for various pharmacological activities. In order to overcome the problem of root adulteration with other similar species of genus *Taraxacum*, a standard for identifying the drug needs to be established. Despite the modern method of analysis, identification and standardization by pharmacognostical studies is still more consistent, precise and economical means. The macroscopic, microscopic and physico-chemical criteria presented here could be helpful to enhancement information about the identification, authentication and standardization. Terpenoids, phenols, tannins, flavonoids, steroids, carbohydrate, protein, and amino acid confirmed in selected drug sample. In the HPTLC analysis, the presence of various phytocomponents was confirmed. The information established can also be helpful to arrange monograph of plant.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Diversity, composition and conservation status of avian fauna in the forest and the wetland sites of Hastinapur wildlife sanctuary, India

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ABSTRACT

Almost, in all flora and fauna, the species richness is affected by environmental changes; hence it is important to survey regularly to understand the current composition and diversity of different wildlife species in an ecosystem. The present study surveyed to assess the species distribution, conservation status, abundance and diversity of birds using the point count method, at two different habitats, a wetland (Bhikund Jheel; BJ) and a forest (Arjun Van; AV) in Hastinapur wildlife sanctuary, India. We surveyed early in the morning (before and after sunrise) and evening (before sunset) for 20 min at every point from January 2019 to April 2019. A total of 96 bird species were recorded, which belong to the 40 families and 15 orders. Out of 96 species, we recorded 67 resident bird species and 29 migratory bird species. The number of bird species specific to the type of habitat was higher in Bhikund Jheel than those in Arjun Van. The species richness and abundance were higher in the wetland (81; 5605) than in the forest habitat (45; 1716); however, evenness (J') and diversity [Shannon-Weiner (H') and Simpson diversity (D)] indices were higher in the forest habitat (J': 0.827, H': 3.420, D: 0.960) than in the wetland habitat (J': 0.669, H': 2.940, D: 0.827). Overall, we found that in the wetland habitats had more species richness than in the forests.

Introduction

The three sides of water and the range of mountains on another side around the Indian subcontinent make it different from any other subcontinent. Variations in geographical and climatic conditions are responsible for different types of ecosystems which lead the establishment of distinguished flora and fauna as mega biodiversity in the subcontinent. Birds are worldwide distributed because they have varied anatomical and ecological adaptations (O'Connor *et al.,* 2011). Indian avian diversity is very varied and contains approximately 13% (1200-1300) species of birds out of the 9600 according to the new classification (Ali *et al*., 1987; Grimmett *et al.,* 1999; Javed and Kaul, 2000). Out of total of 75 families of birds, 48 families are found in the Indian subcontinent. Avian diversity acts as an important ecological indicator to assess the quality of habitats since their habitat is roughly divided into forests, scrubs and wetlands and also the mixed type of habitat for many species to sustain their requirement (Blair, 1999). Avifauna is not similar in the each side of Himalayas as the survey showed that 4/5 parts of the bird species of the Indian subcontinent were found in the eastern Himalayan region which was twice as than the western Himalayas (Price *et al.,* 2003) and this region is the most diverse in the world (Stattersfield *et al.,* 1998). In Asia, Indian Himalayas are particularly important as a large number of threatened avian species are found in this part of the Oriental region (Acharya and Vijayan, 2010). Being a natural predator of insects and rodents, bird diversity helps

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mankind by acting as a bio-pest controlling agent, via reducing the use of chemical pesticides. Therefore, birds are not only important for preserving ecological balance but also useful for economic importance (Simeone *et al.,* 2002). Based on different habitats, anatomical differences in body colours, beak shape, and feet structures are found when we compare water birds and land inhabiting birds. Avian population and diversity at a particular time and area is a bio-indicator and helpful model for reviewing a range of environmental problems (Urfi *et al.,* 2005). In the Indian subcontinent, approximately 310 were wetland bird species out of more than 1200 species of bird and some of these were migrants (Kumar, 2005). Being ecologically important with high nutritional value and productivity, wetlands support good diversity of birds (Paracuellos, 2006; Gibbs, 1993). The presence of numerous water bodies provides food and shelter to birds, therefore Hastinapur wildlife sanctuary is one of the important habitats for native as well as migratory birds. Many studies have shown that the abundance of various bird species is decreasing in human-inhabited parts of the world, and it is of great concern because of urbanization and increasing population (Emlen, 1974; Donaldson *et al.,* 2007). Anthropogenic activities and expanding global urbanization, subsequently, have reduced avifaunal diversity. To understand the urban biodiversity bird surveys are required (McDonnell *et al.,* 2009; Marzluff *et al.,* 2008) and such studies are also helpful for human well-being (Fuller *et al.,* 2007) as well are an important indicator of change in environmental conditions (Dearborn and Kark, 2010). Hence, the regular evaluation of avifauna in a particular area helps to understand the abundance of birds and the variety of other organisms (Turner, 2003). According to Gregory *et al.,* 2003, birds are conspicuous elements and biological indicators of diversity for monitoring the health of an ecosystem. A well-designed bird survey can directly or indirectly provide a clear view about inhabiting organisms, their biological associations, and can also serve as a biological monitoring agent to understand the how organisms adapt with changing environmental conditions. The aim of this study is to investigate the diversity, abundance and distribution of bird species, in two different habitats of the Hastinapur wildlife sanctuary.

Material and Methods

Study area

Hastinapur wildlife sanctuary covers a 2073 km^2 area (between 28^{0}46'– 29^{0}35'N and 77^{0}30'– 78^{0}30'E) of the upper Gangetic plain in the northwest region of Uttar Pradesh and represents the Gangetic grassland biome in biogeographic classification (Rodgers & Panwar, 1998). According to previous studies 83% area of the sanctuary wasused for cultivation and presence of the township resulted in noticeable anthropogenic disturbance (Khan, 2010; Agarwal, 2009; Khan *et al.,* 2003). According to Khan *et al.,* 2003; the vegetated area (17%) of Hastinapur wildlife sanctuary comprised of tall wet grasslands (35.3%), short wet grasslands (23.5%), dry scrub grasslands (29.4%) and plantations (11.8%). We selected two major distinguishable habitats of the Hastinapur wildlife sanctuary.

a): Forest (Arjun Van): Arjun Van is located beside the middle Ganga canal (29^{0}15'N and 77^{0}99'E) and is densely vegetated with shrubs and trees. Major cultivators of this area include sugarcane, wheat in winters, and rice in summers.

b): Wetland (Bhikund Jheel): Characterized with low vegetation, Bhikund Jheel is located near river Ganga (29^{0}17'N and 78^{0}03'E) and have similar agricultural cultivars as of Arjun Van.

Timing of the survey

The study was conducted in January 2019 to April 2019 at Hastinapur Wildlife Sanctuary, Uttar Pradesh. The periodic observation was done twice daily: morning hours (before and after sunrise) and evening hours (before sunset). Each point is chosen randomly and separate from the others with at least 250m in both the habitats of the sanctuary. In terrestrial habitats, bird species were recorded for the time duration of 20 minutes within a close circle of a 30m radius while for wetland habitats open radius circular plots were laid for the equal time.With the help of binoculars (Olympus: 8-16X40 Zoom DPS I, UV protective) eye observations were made to record the avian biodiversity. A digital camera (Sony cyber-shot (DSC-HX 100V; 16.2 megapixels with 30x optical zoom; full HD movie)) was used for photography. The identification of birds was done using a field guide "A pictorial guide to the birds of the Indian subcontinent" (Grimmett and Inskipp, 2018; Ali *et al.*, 1987; Kumar, 2005).

Data analysis

Data were arranged to obtain the following parameters:

1) The relative abundance of bird species per habitat was determined using the:

Relative abundance = n/N

Where N is the total number of birds of all species and n represents the total number of birds of a particular species.

2) Diversity of bird species: The richness of species is the number of different species present in an area. Species richness was estimated for each habitatby:

a. Shannon-Weiner index (H'): Based on previous studies, Shannon-Weiner formula (H'=-[∑Pi*LN(Pi)]) was used to calculate species diversity based on species abundance (Hutcheson, 1970).

Where H' is the Diversity Index, LN (Pi) is the natural logarithm of this proportion and Pi is the proportion of each species in the sample.

b. Evenness: to compare the similarity of the population size of each bird species, Evenness Index (J') was calculated according to Kiros *et al.,* (2018); using the ratio of observed diversity to maximum diversity (J' = H'/Hmax). Where H' is the Shannon Wiener Diversity index and Hmax is the natural log of the total number of species.

c. Simpson Index (D): It measures the probability of any two individuals drawn from a noticeably large community belonging to different species (Simpson, 1949). It was calculated by the following formula: D = 1- ∑ n(n-1)/N(N-1).

Where N is the total number of birds of all species and n is the total number of birds of a particular species.

Results and Discussion

Composition

A varied structure of the bird community was recorded at Hastinapur wildlife sanctuary. Table 1 shows the checklist of all bird species recorded in two different areas (AV: Arjun Van and BJ: Bhikund Jheel) during the period January 2019 to April 2019. Out of 96 recorded bird species of 40 distinguished families and 15 orders, 29 and 67 bird species were migratory and residential species respectively. Passeriformes order was represented by 11 families including 26 species of which five were migratory and 21 were residents (Figure 1). Six families including 15 species, eight migratory, and seven residents were from Order Charadriiformes. Bird species of the only single family were recorded from seven orders, namely: Podicipediformes, Anseriformes, Galliformes, Columbiformes, Psittaciformes, Cuculiformes, and Strigiformes (Figure 1). Anatidae family with 12 species recorded as the highest number of species of a particular family. According to the IUCN Red List of Threatened Species (Table 1) among recorded avian species 87 species were Least Concern; five and four species were Near Threatened and Vulnerable categories, respectively. During the investigation, we recorded 56 water bird species, 26 migratory species, and 30 resident's species. Throughout the study, 81 bird species were recorded from the BJ area and 45 from the AV area. 30 species were common as they were present in both AV and BJ areas (Table 1).

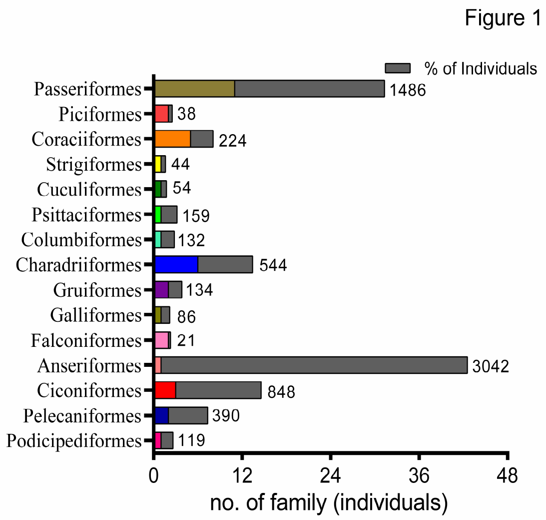

Figure 1: Colour bar diagram represents the number of families in a particular order and black bar represents the percentage of recorded individuals in Hastinapur Wildlife sanctuary, India. The total numbers of individuals are mentioned for each order

Table 1: Composition and status of avifauna recorded from the two habitats of Hastinapur Wildlife Sanctuary

Order	Family	Common name	Scientific name	Status	IUCN status	BJ	AV
PODICIPEDIFORMES	Podicipedidae	Little Grebe	*Tachybaptus ruficollis*	R	LC	+	-
PELECANIFORMES	Phalacrocoracidae	Little Cormorant	*Phalacrocorax niger*	R	LC	+	-
"	"	Indian Shag	*Phalacrocorax fuscicollis*	M	LC	+	-
"	"	Great Cormorant	*Phalacrocorax carbo*	M	LC	+	-
"	Anhingidae	Darter	*Anhinga melanogaster*	R	NT	+	-
CICONIFORMES	Ardeidae	Little Egret	*Egretta garzetta*	R	LC	+	+
"	"	Median Egret	*Mesophoyx intermedia*	R	LC	+	-
"	"	Large Egret	*Casmerodius albus*	R	LC	+	-
"	"	Cattle Egret	*Bubulcus ibis*	R	LC	+	-
"	"	Grey Heron	*Ardea cinerea*	R	LC	+	-
"	"	Purple Heron	*Ardea purpurea*	R	LC	+	-
"	"	Indian Pond-Heron	*Ardeola grayii*	R	LC	+	+
"	"	Black-crowned Night-Heron	*Nycticorax nycticorax*	R	LC	+	-
"	Ciconiidae	Painted Stork	*Mycteria leucocephala*	R	NT	+	-
"	"	Asian Openbill-Stork	*Anastomus oscitans*	R	LC	+	-
"	"	Black Stork	*Ciconia nigra*	M	LC	+	-
"	"	White-necked Stork	*Ciconia episcopus*	R	VU	+	-
"	Threskiornithidae	Oriental White Ibis	*Threskiornis melanocephalus*	R	NT	+	-
"	"	Black Ibis	*Pseudibis papillosa*	M	LC	+	-
"	"	Eurasian Spoonbill	*Platalea leucorodia*	R	LC	+	-
ANSERIFORMES	Anatidae	Lesser Whistling-Duck	*Dendrocygna javanica*	M	LC	+	-
"	"	Greylag Goose	*Anser anser*	M	LC	+	-
"	"	Bar-headed Goose	*Anser indicus*	M	LC	+	-
"	"	BrahminyShelduck	*Tadorna ferruginea*	M	LC	+	-
"	"	Cotton Teal	*Nettapus coromandelianus*	M	LC	+	-
"	"	Gadwall	*Anas strepera*	M	LC	+	-
"	"	Eurasian Wigeon	*Anas penelope*	M	LC	+	-
"	"	Northern Shoveller	*Anas clypeata*	M	LC	+	-
"	"	Northern Pintail	*Anas acuta*	M	LC	+	-
"	"	Common Teal	*Anas crecca*	M	LC	+	-
"	"	Common Pochard	*Aythya ferina*	M	VU	+	-

"	"	Red-crested Pochard	*Rhodonessa rufina*	M	LC	+	-
FALCONIFORMES	Accipitridae	Black Kite	*Milvus migrans*	R	LC	-	+
"	"	Shikra	*Accipiter badius*	R	LC	+	+
"	Falconidae	Laggar	*Falco jugger*	R	NT	-	+
GALLIFORMES	Phasianidae	Indian Peafowl	*Pavo cristatus*	R	LC	+	+
GRUIFORMES	Gruidae	Sarus Crane	*Grus antigone*	R	VU	+	-
"	Rallidae	White-breasted Waterhen	*Amaurornis phoenicurus*	R	LC	+	-
"	"	Common Moorhen	*Gallinula chloropus*	R	LC	+	-
"	"	Common Coot	*Fulica atra*	R	LC	+	-
CHARADRIIFORMES	Jacanidae	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	R	LC	+	-
"	"	Bronze-winged Jacana	*Metopidius indicus*	R	LC	+	-
"	Charadriidae	Little Ringed Plover	*Charadrius dubius*	R	LC	+	-
"	"	River Lapwing	*Vanellus duvaucelii*	R	NT	+	-
"	"	Red-wattled Lapwing	*Vanellus indicus*	R	LC	+	+
"	"	White-tailed Lapwing	*Vanellus leucurus*	M	LC	+	-
"	Scolopacidae	Spotted Redshank	*Tringa erythropus*	M	LC	+	-
"	"	Common Redshank	*Tringa totanus*	M	LC	+	-
"	"	Common Greenshank	*Tringa nebularia*	M	LC	+	-
"	"	Green Sandpiper	*Tringa ochropus*	M	LC	+	-
"	"	Common Sandpiper	*Actitis hypoleucos*	M	LC	+	-
"	Recurvirostridae	Black-winged Stilt	*Himantopus himantopus*	M	LC	+	-
"	Glareolidae	Small Pratincole	*Glareo lalactea*	R	LC	+	+
"	Laridae	Pallas's Gull	*Larus ichthyaetus*	M	LC	+	-
"	"	River Tern	*Sterna aurantia*	R	VU	+	-
COLUMBIFORMES	Columbidae	Blue Rock Pigeon	*Columba livia*	R	LC	+	+
"	"	Eurasian Collared-Dove	*Streptopelia decaocto*	R	LC	+	+
"	"	Yellow-legged Green-Pigeon	*Treron phoenicoptera*	R	LC	-	+
PSITTACIFORMES	Psittacidae	Rose-ringed Parakeet	*Psittacula krameri*	R	LC	+	+
CUCULIFORMES	Cuculidae	Asian Koel	*Eudynamys scolopacea*	R	LC	-	+
"	"	Greater Coucal	*Centropus sinensis*	R	LC	-	+
STRIGIFORMES	Strigidae	Spotted Owlet	*Athene brama*	R	LC	+	+
CORACIIFORMES	Alcedinidae	White-breasted Kingfisher	*Halcyon smyrnensis*	R	LC	+	+
"	"	Lesser Pied Kingfisher	*Ceryle rudis*	R	LC	+	-
"	Meropidae	Small Bee-eater	*Merops orientalis*	R	LC	-	+
"	Coraciidae	Indian Roller	*Coracias benghalensis*	R	LC	+	+

"	Upupidae	Common Hoopoe	*Upupa epops*	R	LC	+	+
"	Bucerotidae	Indian Grey Hornbill	*Ocyceros birostris*	R	LC	-	+
PICIFORMES	Capitonidae	Brown-headed Barbet	*Megalaima zeylanica*	R	LC	-	+
"	Picidae	Yellow-fronted Pied Woodpecker	*Dendrocopos mahrattensis*	R	LC	-	+
PASSERIFORMES	Alaudidae	Ashy-crowned Sparrow Lark	*Eremopterix grisea*	R	LC	+	+
"	Hirundinidae	Common Swallow	*Hirundo rustica*	M	LC	+	-
"	Motacillidae	White Wagtail	*Motacilla alba*	M	LC	+	+
"	"	Large Pied Wagtail	*Motacilla maderaspatensis*	R	LC	+	+
"	"	Citrine Wagtail	*Motacilla citreola*	M	LC	+	-
"	"	Paddyfield Pipit	*Anthus rufulus*	R	LC	+	+
"	Campephagidae	Small Minivet	*Pericrocotus cinnamomeus*	R	LC	-	+
"	Pycnonotidae	Red-vented Bulbul	*Pycnonotus cafer*	R	LC	+	+
"	Muscicapidae	Bluethroat	*Luscinias vecica*	M	LC	-	+
"	"	Indian Robin	*Saxicoloides fulicata*	R	LC	+	+
"	"	Pied Bushchat	*Saxicola caprata*	R	LC	+	+
"	"	Common Stonechat	*Saxicola torquata*	M	LC	+	-
"	"	Indian Chat	*Cercomela fusca*	R	LC	+	+
"	"	Common Babbler	*Turdoides caudatus*	R	LC	+	+
"	"	Large Grey Babbler	*Turdoides malcolmi*	R	LC	-	+
"	"	Jungle babbler	*Turdoides striatus*	R	LC	+	+
"	Nectariniidae	Purple Sunbird	*Nectarinia asiatica*	R	LC	-	+
"	Passeridae	House Sparrow	*Passer domesticus*	R	LC	+	+
"	"	Baya Weaver	*Ploceus philippinus*	R	LC	-	+
"	Sturnidae	Asian Pied Starling	*Sturnus contra*	R	LC	+	+
"	"	Common Myna	*Acridotheres tristis*	R	LC	+	+
"	"	Bank Myna	*Acridotheres ginginianus*	R	LC	+	+
"	Dicruridae	Black Drongo	*Dicrurus macrocercus*	R	LC	+	+
"	Corvidae	RufousTreepie	*Dendrocitta vagabunda*	R	LC	-	+
"	"	House Crow	*Corvus splendens*	R	LC	+	+
"	"	Jungle Crow	*Corvus macrorhynchos*	R	LC	+	+

AV- Arjun Van (forest habitat), BJ- Bhikund Jheel (wetland habitat); Status: M- Migratory, R- Resident; IUCN Status: NT- Near Threatened, VU- Vulnerable, LC- Least Concern; Sign (+) shows the presence of species, Sign (–) shows the absence of species; Bold common name = Waterbird.

Diversity indices for the total number of birds in two different habitats

Table 2 showed the diversity index, BJ had a greater number of birds (5605) and species richness (r = 81) than AV (1716, r = 45). Incontrast, evenness (J'), Shannon-Weiner diversity (H'), and Simpson's index of Diversity (D) were higher in AV (J' = 0.90), (H'= 3.42), and (D = 0.96) than in BJ (J' = 0.67), (H' = 2.94), and (D = 0.83).

Table 2: Diversity indices of birds at Hastinapur Wildlife Sanctuary

Diversity index	Arjun van	Bhikundjheel
Overall abundance	1716	5605
Species richness	45	81
Evenness (J')	0.898	0.669
Shannon-Weiner (H')	3.420	2.940
Simpson diversity (D)	0.960	0.827

Relative abundance of bird species

Supplementary Information Table 1 (SI Table 1) represented the bird species numbers for both habitats. In the BJ, species were recorded in descending order as follows: Bar-headed Goose, Brahminy Shelduck, Cattle Egret, and Gadwall and their numbers were the highest as follows: 2258, 278, 214, and 206 individuals, respectively. Their relative abundance was 0.403, 0.050, 0.038, and 0.037, respectively. In the AV, the species were recorded in descending order as follows: Rose-ringed Parakeet, Jungle Babbler, Common Myna, Asian Pied Starling, and Small Bee-eater (124, 118, 115, 97, and 92 individuals recorded, and their relative abundancewas 0.072, 0.069, 0.067, 0.057 and 0.054, respectively. A timely bird survey is important for the health of an ecosystem. Richness of species indicates variability for the survival of various types of species while evenness of species indicates that a particular species survives in that ecosystem or environment. We found that wetland has more richness of the species of birds while the Arjun Van area (forest) has less species richness since the water of river Ganga throughout the year provides different types of habitats and opportunity to support this sanctuary to develop and maintain different types of ecosystems. Since India is an agriculture-dependent country, the use of Indo-Gangetic plains for food production has increased deforestation, extreme exploitation of natural resources, and pollution (Khan and Abbasi, 2015; Khan *et al.,* 2013). This might be the reason for fewer species richness in the Arjun Van (forest). Also, bird species (migratory and resident) abundance and numbers were high in BJ; because of habitat suitability, which supports free running water and abundant food supply (insects, grasses, and aquatic fauna) as well as nesting and resting sites. On the other hand, the forest habitat has more evenness and diversity as compared to the wetland since the numbers of individuals per species were highly skewed in the wetland (BJ) (Table 2). The survey in the months of late winters or early summers showed migratory birds, especially Bar-headed Goose (2258 individuals, SI Table 1) which migrates from central Asia to India to overwinter (Hawkes *et al.,* 2011). However, the range of individuals was more even in forest habitats although less in number than in wetland areas. Almost 30 species were common between forest and wetland habitats which might be due to the amount and type of food resources available or the presence of water in the wetland area. A recent survey of the Hastinapur Wildlife sanctuary has shown that there is little to no protection of the habitats and encroachment has increased in the recent decade (Khan and Abbasi, 2015). Also, in the analysis of the social impact of conservation, it was found that the locals were mostly ignorant about the conservation and biodiversity status with around 25% of people admitting to having no idea about the trend in biodiversity in this sanctuary since the last decade (Khan and Abbasi, 2015). As evident from previous studies and conservation mechanisms that the action to sustain biodiversity and the progress of the local community in a viable way requires sharing of knowledge with the community and accurate use of natural resources since locals have a better understanding of the area and the bio-geographical and socio-economic aspects of their instant environment will be useful for designing a local policy for management and conservation of environment and its resources.

Conclusion

The present study showed the variation in the distribution of bird species between two different habitats of Hastinapur Wildlife sanctuary. The composition, diversity, evenness, and richness were dependent on the area and the number of individuals present as AV showed more evenness and diversity and BJ showed more numbers and richness. We also observed that BJ has more daily and seasonal human agricultural activities which disturb the birds. The anthropogenic activities and rapid urbanization (e.g. national highways passing near wetland areas) can be a potential reason for reduced avifaunal diversity by directly or indirectly causing habitat loss, noise pollution, and water pollution. More regular studies of these kinds will increase awareness and add to the conservation of biodiversity.

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

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Green fabrication of zinc oxide nanoparticles by *Anagallis arvensis* ethanolic extract and their antibacterial properties

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ABSTRACT

Green approach of zinc oxide nanoparticle fabrication is a reliable reaction that has compatibility with many biological properties. In the present study the approach of zinc oxide nanoparticle has been synthesized by *A. arvensis* aerial part using ethanol extract. The morphological, compositional and structural properties have been investigated by SEM, XRD, and FTIR studies. XRD technique demonstrated the crystallite size of 17nm with the help of Debye-Scherrer's equation which was obtained in nanorange. SEM technique demonstrated their microscopic agglomerated crystal image of green synthesizes metal in zinc oxide nanoparticle. FTIR technique represents the different types of biomolecules i.e. phenol, alkynes etc. that are responsible for good nanoparticle fabrication. These biomolecules work as encapsulation and stabilization agents for nanoparticle fabrication. These all properties of nanoparticle fabrication have been responsible for the antimicrobial activity.

Introduction

Nanotechnology is an advanced field at the present period, works with chemistry, physics, biological science, molecular and different other fields interactually. In this technology, nanomaterials play a versatile role in cosmetic, electrical, pharmaceutical, power generation, environment and textile industries. Nanomaterials moved through nanotechnology, constitute 1-100nm scale range nanoparticles. Different types of metal oxide and metal nanoparticles are already exist eg- copper oxide, copper, zinc oxide, titanium oxide, iron oxide, iron etc (Marassi *et al.*, 2018; Rastogi *et al.*, 2017). This metal based nanoparticle is formed from different kinds of modes such as – chemical, physical and biological. In chemical and physical modes, more energy is required and causes various side effects (Awwad *et al.*, 2020; Kharissova *et al.*, 2019). To over come this situation, the biological and green approach of nanoparticle synthesis is the best method by using of plant extract. This is budget-friendly, safe, and less dangerous has been fabricated by new scientists. Among already exist nanoparticles; zinc oxide is the safest, multipurpose, more ecofriendly and USDA (US administration of drug and food) approved. It is applied in many applications. For instance- photodetectors, chemical sensors, antimicrobial, wound healing and gas sensors (Chang *et al.,* 2012; Dadi *et al.,* 2019; Li *et al.*, 2018). Several types of plant part like fruit, stem, flower, leaf, peel, seed etc applied in zinc oxide nanoparticle synthesis. For instance, many scientists have demonstrated ZnO NPs from leaf extract of *Echinacea angustifolia* (Iqbal *et al.,* 2021), *Camellia sinensis* (Senthilkumar & Sivakumar,2014), *Eucalyptus globulus Labill* (Barzinjy *et al.*, 2020); pod extract of *Papaver somniferum*; root extract of *Rubus fairholmianus* (Rajendran *et al.,* 2021). At a recent proposal, we fabricated a green approach of zincoxide nanoparticle by *A. arvensis* aerial parts and used it for antimicrobial effect. *A.arvensis* is used by tribals people for medicinal purposes

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contain therapeutic based phytochemical substances such as triterpenoids, glucosides, flavonoids and so on substances work as encapsulating and stabilizing mediator for the fabrication of zinc oxide nanoparticle (Kawashty *et al.,* 1998) but there is lack lot of work of *A.arvensis* plant based zinc oxide nanoparticle synthesis and their properties.

Material and Methods

Aerial extract preparation

A.arvensis aerial parts were washed with water many times to clean mud, soil particle and dirt content. After that aerial part was dried. The dried part was a grind with mortar and an electric grinder. Soxhlet apparatus is used for the extraction process. 80-100g sample used in soxhlet and ethanol solvent was run in soxhlet according to the polarity. Then, the extract was filtered with filter paper 1 and the solvent was saturated with the rota evaporator, preserved at 4^0C for ahead processing (Redfern *et al.* ,2014).

Fabrication process

The precursor zinc acetate was mixed with 400mg extract in distilled water. Then, ethanolamine was added as an encapsulation mediator. Ethanolamine and precursor compound was maintained at 1 molar concentration. After that solution was vibrated for half an hour. Now, NaOH solution was added drop by drop until the endpoint of the solution is not measured, centrifuged the solution and dried the sample (Li *et al*. 2004; Perveen *et al*., 2020).

Identification

For the identification of metal oxide nanoparticle fabrication, many systematic instruments were used. For instance- XRD depicted the size of crystallite with the help of identified phases and dimensions. Debye-Scherrer's equation was applied in the estimation of D value (where, D = crystallite size) of zincoxide preparation. FTIR spectra were applied for the functional group identification which is responsible for encapsulation in metal oxide nanoparticle preparation. These functional groups are sometimes conjugated between bioadsorbant and nanomaterial. Different groups were found in the spectra of FTIR, ranged in between (4000-500cm^{-1}), and analyzed with KBr pellet (Torres-Rivero *et al*., 2021). SEM microscopic instrument was used in the morphology of nanomaterial respectively.

Antimicrobial effect

The microbicidal effect was conducted by agar well diffusion, which evaluated the inhibitory action of pathogens. ZnO nanomaterial (50mg) was tested against *Streptococcus pyogenes* MTCC 442 and *Klebsiella pneumonia*MTCC 4030. Muller Hinton plates were applied for bacterial strains. Then 45µl sample was added toan appropriate well of plates. After all complete procedures, plates were incubated 310K for 24h (Archana & Abraham, 2011).

Results and Discussion

Determination of functional biomolecules

Various types of biomolecules illustrated peaks in FTIR spectrum of extract and biosynthesized ZnO NPs shown in figure 1. Figure 1 demonstrated a comparison spectrum of extract (a) and bio reduced ZnO NPs(b). Many peaks were identified as the spectrum of FTIR at 2335, 1560, 1424 and 640cm^{-1}. 640cm^{-1} peak represents ZnO NPs which is found inrange 800-400cm^{-1} (Degefa *et al.,* 2021). The peak at 1424 corresponds to C=C–C stretching. Different intensities at 1560 and 2335cm^{-1} originatedue to the polyphenol aromatic ring of C=C stretching and internal alkynes (Rahman *et al*., 2022).

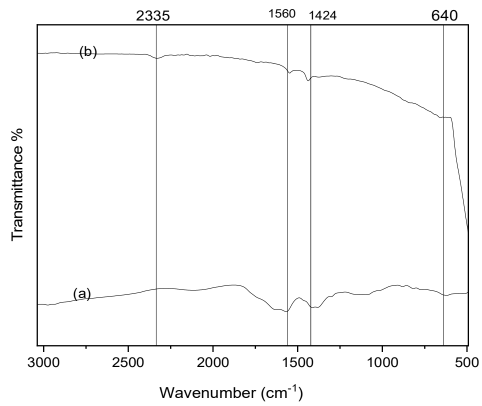

Figure 1: Functional group identification spectra of FTIR

Determination of crystallite size

Different intensity peaks of XRD spectra demonstrated the wide range of phases which indicates the fabricated nanomaterial was found in nano dimension range (figure 2). XRD value of plant mediated zincoxide nanoparticles 112 planes sequentially. The large identified peak 36.36 present on 101miller indices showed a crystallite size of about 19nm and the average crystallite size of all identified peaks was found to be 17nm. Previously studies have been found similar at these studies demonstrated by Degefa *et al.,* 2021; Senthilkumar & Sivakumar, 2014.

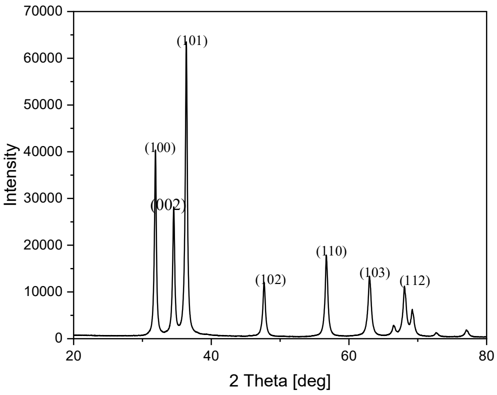

Figure 2: Identified phase and dimension of crystallite of zinc oxide nanoparticle *A. arvensis*aerial part ethanol extract

Determination of morphology

The morphology of nanomaterial was determined by FESEM microscopic instrument. Figure 3 demonstrated the agglomerated form of small nanomaterial which becomes aligned and forms crystal shape structure (RSC). These agglomerated shape of nanomaterial showed spherical structure of zincoxide nanoparticle which was similar from previous studies. These agglomerations indicate the presence of encapsulating agent (Muhammad *et al.,* 2019).

Antimicrobial property

This study was conducted against two pathogens *Streptococcus pyogenes* MTCC 442 and *Klebsiella pneumoniae* MTCC 4030 respectively. The outcomes of the study were found 14mm and 16mm towards ZnO NPs, 18mm zone of inhibition toward antibiotic clindamycin against these pathogens. Thus, ZnO NPs showed the satisfied results of the antimicrobial towards MTCCpathogens, enter in the cell wall, generated demonstrated the different angle 31.87, 34.53, 36.36, 47.67, 56.73, 63.00, 68.09, 69.20 correspond to the reflection of 100, 002, 101, 102, 110, 103, theROS, and causes the cell destroy (Jiag *et al.,* 2020).

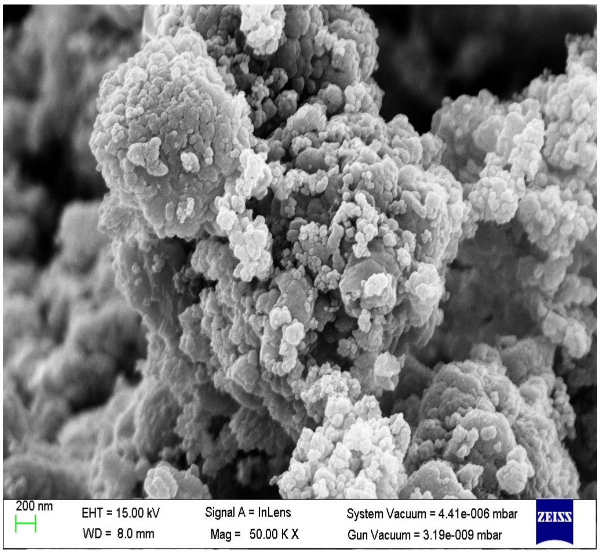

Figure 3: Microscopic photograph of SEM of zinc oxide nanoparticle *A. arvensis* aerial part ethanol extract

Conclusion

We have designed successfully appropriate ZnO NP fabrication from *Anagallis arvensis* aerial part by green synthesis. All characterizations have an important role in the designed study of the therapeutic field. FTIR studies showed organic functional group act as an encapsulation of ZnO NP fabrication. Morphological studies demonstrated by SEM showed nano particle fabrication. These all properties of prepared ZnO NPs impact on the antimicrobial study. The antimicrobial study indicates zinc oxide nanoparticle as a drug indicator. Thus it has been applied for pharmaceutical and medicinal applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Efficiency assessment of 3.2MLD MBR based sewage treatment plant of IFFCO township Aonla, Bareilly, Uttar Pradesh, India

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ABSTRACT

Increasing urbanization and industrialization is continuously putting a pressure on the ground and fresh water resource in form of quality and quantity. Therefore water recycling through wastewater treatment is the need of the present hour. Therefore in the present study the efficiency of the 3.2MLD sewage treatment plant (STP) based on membrane bioreactor technology (MBR) located in Indian Farmers Fertilizer Cooperative Limited (IFFCO) township Aonla, Bareilly, Uttar Pradesh, India was studied. The plant was recently commissioned on 10th of June 2022. The plant shows highest efficiency for turbidity (98.6%) followed by total suspended solids (TSS) (95.7%), chemical oxygen demand (COD) (89.0%), iron (86.7%), and biochemical oxygen demand (BOD) (85.0%). The efficiency for the rest of the parameters is below 50%. The MBR based STP is working efficiently as the values of parameters in treated water is within the discharge standards of central pollution control board (CPCB) listed in The Environment (Protection) Rule, 1986. One of the major problems of MBR based STP reported in literature is membrane fouling which is also rectified in the current treatment plant by using sodium hypochlorite for membrane cleaning.

Introduction

Water is a natural resource of vital importance and act as elixir of life. It makes around 80% part of the protoplasm and is essential for all the cell metabolic activities. Water is used in almost all the activities of human routine life but we all underestimate the importance of water and thus despite this much of importance this natural resource is categorized as mismanaged resource. Continuously we are heading towards water shortage due to more extraction of groundwater than recharge (Bhutiani *et al.,* 2019; Kumar *et al.,* 2010). More than 80% of the supplied to a residency returned as wastewater which is called as domestic wastewater or sewage (Bhutiani and Ahamad, 2018; Ruhela *et al.,* 2020). Sewage is mixed water containing the water from kitchens, bathrooms, floor washing and surface runoff during rainy seasons (Kumar *et al.,* 2010). Globally two million tonns of wastewater is generated and about 80% of this wastewater is discharged without any treatment (UNEP, 2010; WWAP, 2017). As per the central pollution control Board (CPCB) report of 2021, in class I cities of India, the percentage of sewage treatment in 1978 was 39.3% (treatment of 2755MLD out of 7006MLD generation), in 1988 was 21.7% (treatment of 2633MLD out of 12145MLD

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generation), in 1999 was 24% (treatment of 4037MLD out of 16662MLD generation), and in 2008 was 30.8% (treatment of 11787MLD out of 38254MLD generation). Presently in each state of India, sewage generation capacity is more in comparison to sewage treatment. This data shows that public as well as government interests have been increased in recent decades towards the water recycling (Qayoom *et al.,* 2021).

The direct dispose of untreated wastewater makes the ground as well as surface water bodies contaminated (Yaqub *et al.,* 2020; Osmani *et al.,* 2021). The consumption of contaminated water, unhealthy sanitation and hygiene practices alone contribute about 7% in disease and 19% in child mortality rate (Cairncross *et al.,* 2019: Bhutiani *et al.,* 2021). Due to the upgraded soaps, detergents, toothpaste, shampoos and increased lavish life style, the nature of sewage also changes (Von Sperling, 1996; Forrez *et al.,* 2011). Now days, besides organic matter it also contains various chemicals, heavy metals and pathogens (Ali *et al.,* 2022). Therefore the treatment of sewage becomes an urgent need of the present time. Establishment of new STP's requiring large land areas which are also an issue in developing countries like India. Therefore efficiency enhancement is the sustainable solution to the above discussed problem so that more water is treated effectively in short time period (Nelson *et al.,* 2017).

Due to complex nature of wastewater originated conventional wastewater treatment technologies shows low efficiency (Sahar *et al.,* 2011). Therefore, trend of advanced treatment technologies application is going on these days. These technologies include membrane bioreactor (MBR) (Tadkaew *et al.,* 2011), Nano filtration (NF) and reverse osmosis (RO) (Nghiem *et al.,* 2004), and UV oxidation (Lekkerkerker-Teunissen *et al.,* 2012).

About MBR based STP

MBR technology is an advanced version of classical activated sludge (CAS) process. Membrane Bioreactor Technology (MBR) is a combination of two phenomenon's that is the Membrane Filtration (Micro and Ultra Filtration) process and the Biological Process. Therefore MBR based Sewage Treatment Plant (STP) means STP's where the numbers of membranes are used for the removal of smaller impurity particles from the sewage water mainly (greater than 0.1 micro-meter size) and these membranes are submerged in aerated biological reactors where the biological process will occur. In this biological process the air is added or blown into the sewage water so that the bacterial concentration will increases very rapidly in the sewage so that they easily degrade or digest/decompose the organic matter present in the sewage water and also breaks down the organic matter into smaller parts and this process also similar to the activated sludge process (Figure 1).

So in this MBR section of the STP plant these two processes works together such that firstly the Biological process converts organic matter into smaller parts and after that the Membranes easily removes or filtered out these impurities from the sewage water so that we got highly treated effluent from this MBR section.

MBR technology possesses several advantages such as smaller physical footprint and higher efficiency in comparison to CAS (Tadkaew *et al.,* 2011; Nguyen *et al.,* 2013). Some Advantages of MBR based STP plants are-

1- It requires less space in comparison to ASP plants.
2- In these plants the operation should be performed easily.
3- We got the High-quality treated effluent from this type of STP.
4- It produces less volume of sludge.

MBR based STP plants can be used for Municipal wastewater treatment, Industrial wastewater treatment and many other places having less space so these plants are very effective. But there is one major problem of this MBR based STP plant is that the problem of membrane fouling in the membranes of the MBR chamber and due to this major problem, the efficiency of the plant decreases to some extent. Membrane fouling means the membranes or filter medias used for the removal of different impurities from the wastewater are to be blocked or clogged due to the cake formation of these impurities and it affect the efficiency of the plant but this MBR system is better than activated sludge plants and also, we easily solve the problem of membrane fouling by the help of coagulation,

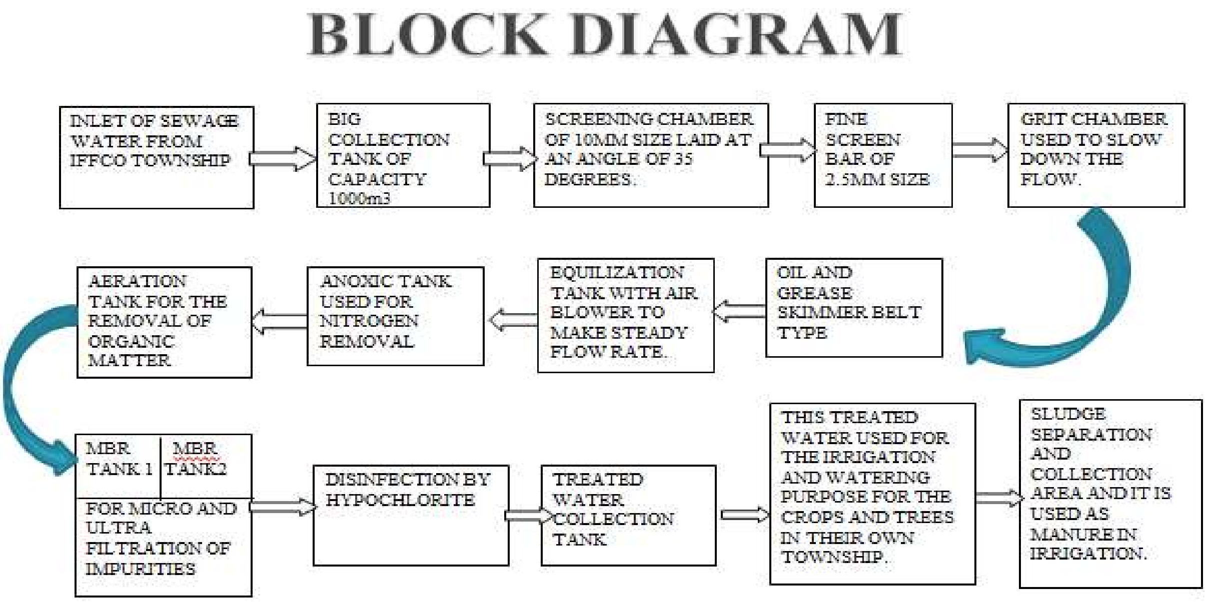

Figure 1: Showing block diagram of MBR-STP

adsorption process and aerobic granulation technique. Therefore by the help of these methods, we should improve the efficiency and also increases the life of the membranes used in the MBR section of this STP plant. Literature review suggests that anoxic MBR based STP show maximum efficiency (Sima *et al.,* 2011; Xiang *et al.,* 2014; Miura *et al.,* 2015; Schaeffer *et al.,* 2018; Shen *et al.,* 2019).

Material and Methods

Study area

MBR based STP plant of 3.2 MLD is situated in IFFCO Township near IFFCO Urea Plant in Aonla, Bareilly, UP, India at the 28.208303 latitudes and 79.245088 longitudes. This plant starts working from 10th of June 2022 and having a capacity of 3240 m3/day. Inside the IFFCO Township this STP plant is located at the very back side where the sewage water of the whole Township collected in proper way. The nearest location around this plant is Chakarpur Ramnagar.

Sample Collection

Water samples were collected from inlet and outlet point in the morning hour. The sampling was performed for five months (from August 2022 to December 2022). Samples were collected in the prewashed plastic containers of 2 litres throughout the study period. The parameters studied during the study period were turbidity, total dissolved solids (TDS), total suspended solids (TSS), pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total hardness, calcium, magnesium, iron, sulphate, alkalinity, chloride, sodium, and salinity. All the parameters were analyzed following the standard methods (APHA 2017; Trivedy and Goel, 1986 and Khanna and Bhutiani, 2008).

Results and Discussion

The average results of different physicochemical parameters are presented in table 1 and figure 2. Turbidity is due to the presence of solids present in suspended form in the water. Turbid water interferes in pathogen removal process (WHO, 2004). The turbidity in raw sewage ranged from 30-75NTU with an average value of 62.2NTU±19.0 and in treated water 0.5-1.4NTU with an average value of 0.8NTU±0.3. Therefore the efficiency of the plant for turbidity reduction was 98.6%. Our results are in full agreement with Nguyen *et al.* (2013) which observed the turbidity below 0.2NTU in all the samples in laboratory scale MBR based STP. Total Suspended Solids (TSS)is a measure of the floating particulate content of the wastewater

Table 1: Showing the minimum, maximum and average values of different physicochemical parameters of treated and untreated sewage and the efficiency of STP (n=5)

Parameters	Inlet point (Untreated water)				Outlet point (Treated water)				Efficiency
	Average	SD	Minimum	Maximum	Average	SD	Minimum	Maximum	
Turbidity (NTU)	62.2	19.0	30.0	75.2	0.8	0.3	0.5	1.4	98.6
TDS (mg/l)	361.8	21.4	350.0	400.0	289.4	39.7	245.0	326.0	20.0
TSS (mg/l)	71.8	25.6	28.0	92.0	3.1	0.3	2.6	3.5	95.7
PH	7.2	0.3	7.0	7.6	8.3	0.1	8.1	8.4	-14.3
BOD (mg/l)	33.6	13.6	18.0	48.0	5.1	2.8	1.5	8.8	85.0
COD (mg/l)	94.0	19.6	70.6	124.0	10.4	6.9	4.0	18.9	89.0
T. Hardness (mg/l)	94.9	12.3	80.0	110.0	52.7	9.6	38.0	62.4	44.67
Calcium (mg/l)	59.9	21.2	30.0	80.7	39.7	9.4	25.0	48.0	33.8
Magnesium (mg/l)	15.8	12.2	4.0	35.0	9.4	7.4	2.0	19.0	40.5
Iron (mg/l)	3.0	0.3	2.5	3.3	0.4	0.3	0.2	0.8	86.7
Sulphate (mg/l)	61.5	30.4	30.0	105.0	32.8	17.3	20.0	60.0	46.7
Alkalinity (mg/l)	310.4	32.1	280.0	360.0	252.0	52.2	200.0	320.0	18.8
Chloride (mg/l)	55.0	22.6	40.0	95.0	44.8	20.1	32.0	80.0	18.5
Sodium (mg/l)	132.4	14.8	110.0	150.0	70.4	19.6	49.0	100.0	46.8
Salinity (mg/l)	408.0	52.6	330.0	470.0	304.2	48.5	250.0	380.0	25.4

having the diameter greater than 2 micrometre and is an indicator of the clarity of the wastewater (Johal *et al.,* 2014). During the study period, the range of TSS was observed from 28-92mg/l with an average value of 71.8mg/l±25.6 in inlet and in outlet from 2.6-3.5mg/l with an average value of 3.1mg/l±0.3. Therefore the efficiency of the plant for TSS reduction was 95.7%. The reduction in suspended solids may be due to sedimentation of these particles. Besides this, biological degradation in aerobic zone is also another method observed (Wang *et al.,* 2010). Xiang*et al.* (2014) also observed the similar results (more than 96%) in MBR based STP. Total Dissolved Solids (TDS)value of the wastewater is mainly due to the ions/salts added during the use of water or dissolved from the rock dissolution (Salunke *et al.,* 2014). During the study period, the range of TDS was observed from 350-400mg/l with an average value of 361.8mg/l±21.4 in inlet and in outlet from 245-326mg/l with an average value of 289.4mg/l±39.7. Therefore the efficiency of the plant for TDS reduction was 20.0%. TDS removal may be due to oxidation and biological degradation of dissolved solids (Singh and Varshney, 2013; Bhutiani *et al.,* 2016; Ruhela *et al.,* 2020).

pH is the indicator of salinity or acidity of the water and wastewater. Efficiency of the treatment plant depends of the pH value because it determine the transformation of many pollutants and is an essential measure to maintain the nutritive balance for the proper development of aquatic biota (Bolawa and Gbenle, 2012; Ruhela *et al.,* 2020; Osmani *et al.,* 2021), and it's optimum range essential for bacterial activity (Sincero and Sincero, 1996). During the study period, the range of pH was observed from 7.0-7.6 with an average value of 7.2±0.3 in inlet and in outlet from 8.1-8.4 with an average value of 8.3±0.1. Therefore the efficiency of the plant for pH gain was 14.3%. Less removal percentage in pH was observed due to chlorine dosing to remove the color, odour and pathogens in treated water (Bhutiani and Ahamad, 2018; Showkat and Najar, 2019).

Microbes degrade organic matter present in the wastewater and for this purpose they demanded the oxygen. Thus the amount of oxygen demanded by the microbial population present in fixed quantity of wastewater to degrade the organic matter is known as Biochemical Oxygen Demand (BOD). Therefore the value of BOD is the indicator of amount of organic matter of wastewater (Hur and Kong, 2008). During the study period, the range of BOD was observed from 18-48mg/l with an average value of 33.6mg/l±13.6 in inlet and in outlet from 1.5-8.8mg/l with an average value of 5.1mg/l±2.8. Therefore the efficiency of the plant for BOD reduction was 85.0%. BOD removal is indicative of the efficiency of biological treatment processes and is the most widely used parameter to

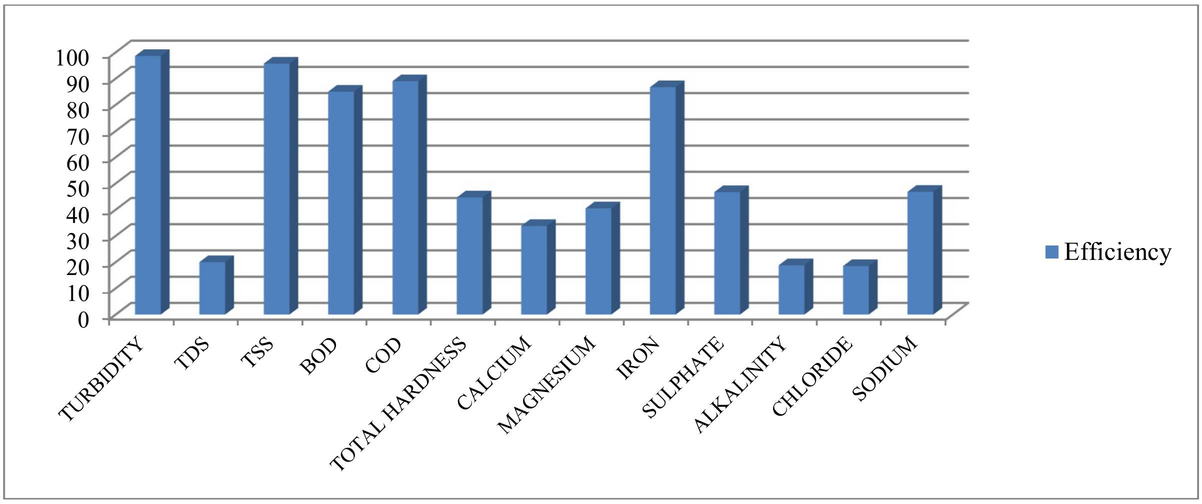

Figure 2: Showing the efficiency of STP for different physicochemical parameters

measure wastewater quality. The results of this study are concurrent to that Bolong *et al.* (2022) and Ali *et al.* (2021) who observed BOD reduction upto 92%.Chemical Oxygen Demand (COD) is the oxygen demand raised during chemical breakdown of organic and inorganic matter (Bhutiani *et al.,* 2017; Kumar *et al.,* 2018). Higher COD values of wastewater results into drastic oxygen depletion in the receiving water bodies. During the study period, the range of COD was observed from 70.6-124.0mg/l with an average value of 94.0mg/l±19.6 in inlet and in outlet from 4.0-18.9mg/l with an average value of 10.4mg/l±6.9. Therefore the efficiency of the plant for COD reduction was 89.0%. The decrease may be linked to the aeration and digestion processes, which have also been confirmed by Xiang *et al.* (2014), Jafarzadeh *et al.* (2014), Johal *et al.* (2014) and Ali *et al.* (2021) who obtained 84.5%, 94%, 98%, and 91.5% respectively reduction in their studies. COD test is complex in nature but less time consuming. In our study COD reduction was achieved much higher than those of Fluidized aerobic bioreactor (FAB) based STP (29.00%) and less than sequential batch reactor (SBR) based STP (22.32%) conducted by Qayoom *et al.* (2021). Total Hardness in water is due to the presence of carbonate, bicarbonate, chloride, and sulphate of calcium and magnesium The main sources of Ca and Mg in wastewater are calcite, dolomite, magnesite, anhydrite, gypsum feldspar, pyroxene and amphiboles present in catchment. The range of Total Hardness was observed from 80.0-110.0mg/l with an average value of 94.9mg/l±12.3 in inlet and in outlet from 38.0-62.4mg/l with an average value of 52.7mg/l±9.6. Therefore the efficiency of the plant for Total Hardness reduction was 44.67%. Decrease in concentration could be attributed to the grit separation, sedimentation process and active uptake of calcium and magnesium by microorganisms during treatment (Showkat and Najar, 2019). During the study period, the range of calcium was observed from 30.0-80.7mg/l with an average value of 59.9mg/l±21.2 in inlet and in outlet from 25.0-48.0mg/l with an average value of 39.7mg/l±9.4. Therefore the efficiency of the plant for calcium reduction was 33.8%. During the study period, the range of magnesium was observed from 4.0-35.0mg/l with an average value of 15.8mg/l±12.2 in inlet and in outlet from 2.0-19.0mg/l with an average value of 9.4mg/l±7.4. Therefore the efficiency of the plant for magnesium reduction was 40.5%. Similar reduction in hardness, calcium and magnesium ion (from 39.9% to 53.97%) was observed by Bhutiani *et al.* (2017) and Ruhela *et al.* (2020). Iron in raw effluent ranged from 2.5 to 3.3mg/l with an average value of 3.0mg/l±0.3 and from 0.2-0.8mg/l with an average value of 0.4mg/l±0.3 in treated water. Therefore the efficiency of the plant for iron reduction was 86.7%. In our study iron reduction was achieved much higher than those of Fluidized aerobic bioreactor (FAB) based STP (29.22%) and less than sequential batch reactor (SBR) based STP (52.28%)

conducted by Qayoom *et al.* (2021). Similarly, value of sulphate ranged from 30.0-105.0mg/l with an average value of 61.5mg/l±30.4 in inlet and from 20.0-60.0mg/l with an average value of 32.8mg/l±17.3 in outlet. Therefore the efficiency of the plant for sulphate reduction was 46.7%. In our study sulphate reduction was achieved much higher than those of Fluidized aerobic bioreactor (FAB) based STP (25.39%) and sequential batch reactor (SBR) based STP (8.79%) conducted by Qayoom *et al.* (2021).Alkalinity in water is due to presence of carbonate, bicarbonate, phosphates, and nitrates along with hydroxyl radical in their free state (Bhutiani *et al.,* 2016). In the raw sewage, alkalinity ranged from 280.0-360.0mg/l with an average value of 310.4mg/l±32.1 and from 200.0-320.0mg/l in treated effluent with an average value of 252.0mg/l±52.2. Therefore the efficiency of the plant for alkalinity reduction was 18.8%. Less reduction in alkalinity is due to the use of base to adjust the pH value during the treatment processes. Higher concentration of chloride in water is the indicator of the degree of contamination by a large quantity of sewage inputs, detergents and soaps (Von Sperling, 1996). It shows that the contamination at the site is higher. During the study period, the range of chloride was observed from 40.0-95.0mg/l with an average value of 55.0mg/l±22.6 in inlet and in outlet from 32.0-80.0mg/l with an average value of 44.8mg/l±20.1. Therefore the efficiency of the plant for chloride reduction was 18.5%. Less reduction in chloride value is pointing towards the use of high chlorine dosing (dosing of poly aluminum chloride) to remove the odour, color and pathogens which is also indicating towards the slow functioning or high organic loading in influent (Rao and Shruthi, 2002). In raw effluent, the range of sodium was observed from 110.0-150.0mg/l with an average value of 132.4mg/l±14.8 in inlet and in outlet from 49.0-100.0mg/l with an average value of 70.4mg/l±19.6. Therefore the efficiency of the plant for sodium reduction was 46.8%. Salinity is the amount of salts dissolved in a unit volume of water. Salinity can take three forms, classified by their causes: primary salinity (also called natural salinity); secondary salinity (also called dryland salinity), and tertiary salinity (also called irrigation salinity). During the study period, the range of salinity was observed from 330.0-470.0mg/l with an average value of 408.0mg/l±52.6 in inlet and in outlet from 250.0-380.0mg/l with an average value of 304.2mg/l±48.5. Therefore the efficiency of the plant for salinity reduction was 25.4%.

Conclusion

The performance of the MBR based STP located in IFFCO township area of Bareilly was conducted for a period of 5 months (August 2022 to December 2022). The overall efficiency is in the order Turbidity>TSS>COD>Iron>BOD>Sodium>Sulphate>Total Hardness> Magnesium>Calcium> Salinity >TDS>Alkalinity>Chloride. In case of chloride less removal efficiency was observed showing the use of chlorine in the treatment process for the purpose of disinfection. The treated waste can be re-used for irrigation purpose. In order to improve the efficiencies of the STPs, the treatment systems must be properly operated and maintained, sources of raw sewage need to be identified, and existing facilities should be upgraded accordingly. In terms of proper operation and maintenance, trained and experienced workers are required in a defined period of time to assess treatment performance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Floristic inventory of S. R. T. campus of HNB Garhwal University (Badshahi Thaul, Tehri Garhwal, Uttarakhand): A semi-natural environment

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ABSTRACT

The paper provides a floristic account of the S. R. T. Campus (HNB Garhwal University, Tehri Garhwal, Uttarakhand) which enumerates 193 taxa, distributed under 158 genera and 70 families of Angiosperms, Gymnosperms and Pteridophytes. Life form analysis revealed the presence of 65.28% herbs, 17.62 % shrubs 12.44 % trees, and. climber 4.66%. Asteraceae, Poaceae, Fabaceae, Lamiaceae, and Rosaceae revealed as the dominant families (in term of number of species) which together contributed 36.46% in the total species count. *Desmodium* with 4 species revealed as the dominant genus followed by *Carex*, *Solanum*, and *Indigofera* (3 species each). Of these, 17.13% species bears white flower, 15.47% yellow, 11.61% Pink, 9.94% green, blue 7.185.52 purple, 4.42% red while rest of the species (28.74%) bears flowers of other than colour class.

Introduction

The biodiversity of the Indian Himalayan Region (IHR) is being threatened by many natural as well as anthropogenic factors. The biodiversity in this region experienced some noticeable impacts, such as changes in species abundance and range, shifts in habitat and change in phenology (Gaur, 1999). Anthropogenic disturbances such as agricultural expansion, over grazing, habitat degradation, deforestation, forest fire and infrastructure development, may give a way to encroachment of invasive alien species, loss of pollinators, disease and pest out breaks (Pokhriyal *et al.,* 2012; Rawat *et al.,* 2016a, b, 2020; Kaushal *et al.,* 2021). The majority of rural households of the country depend on locally available natural resources to meet their daily needs especially fuelwood and fodder (Bhatt & Badoni, 1990).Floristic records, particularly from the Uttarakhand Himalaya and adjacent parts were attempted by a number of workers (Uniyal *et al.* 2007; Pusalkar and Srivastava, 2018). From the Garhwal Himalayan region of Uttarakhand, the floristic accounts are available in various forms e.g. District Flora, Block Flora, Valley Flora, Forest Flora, and Flora of protected areas. Knowing the taxonomic and ecological implications plant diversity even in smaller semi-natural environment, several workers conducted floristic surveys in the different University Campuses of the country viz. Katoch *et al.* (2012; Jammu University Campus, Jammu & Kashmir), Ambrish *et al.* (2022; Dr. Y.S.P. University of Horticulture and Forestry Campus, Himachal Pradesh), Barik *et al.* (2023; North Orissa University Campus, Odisha), Kumari *et al.* (2023; Indira Gandhi National Open University (IGNOU) Campus, New Delhi), Saranya *et al.* (2023; Nehru Arts and Science College, Tamil Nadu), etc. S. R. T. campus of HNB Garhwal University (Badshahi Thaul, Tehri Garhwal, Uttarakhand) is has a good vegetation cover including both natural and planted floristic elements. Dangwal *et al.* (2011) surveyed the weeds of the campus and documented a total of 72 weed species belonging to 56 genera and 27 families. However, the complete floristic inventory

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of the campus is not attempted by the earlier workers. Therefore, the present study aimed to provide a checklist of higher plants (Angiosperms, Gymnosperms and Pteridophytes) of the campus.

Material and Methods

Study area

The Swami Ram Teerth Campus (SRT Campus), one of the three campuses of the Hemvati Nandan Bahuguna Garhwal University (HNB Garhwal University), is located in the in the lap of Himalayan range in the Garhwal region of Uttarakhand. Administratively, it is situated at the Badshahi Thaul, block Chamba, district Tehri Garhwal, Uttarakhand at an altitude of ca. 1700 m asl. It is about 10 km from the new Tehri city (district Headquarters), 65 from the Rishikesh city while 90 km the Srinagar Garhwal (Main campus of HNB Garhwal University). Topographically the Badshahi Thaul area is undulating hilly. It is representing lower montane to montane climatic condition. The weather remains pleasant throughout the year. The forests of the area are dominated by *Pinus roxburghii* on drier and exposed slopes while board leaved species form mixed forests of shaded and moist slopes (especially along the streams).The study on the flora of the S. R. T. Campus (HNB Garhwal University, Uttarakhand) was carried out during 2020–2022.The plant specimens were collected in regular interval in different seasons from the vegetation cover area of the areas and its adjacent regions.Voucher specimens of all the plant species were collected and processed following conventional method (Jain & Rao, 1977). All the specimens were deposited in the Herbarium of Deptt. of Botany (Govt. P. G. College, New Tehri) after incorporating all the relevant field information for future records. An elaborated inventory of alien plants occur in these protected areas was prepared based on the fresh collections.

Figure 1. Site of the study, S. R. T. campus of HNB Garhwal University (Badshahi Thaul, Tehri Garhwal, Uttarakhand) (Source: Google Map)

Results and Discussion

The present floristic investigation in S. R. T. Campus (HNB Garhwal University, Tehri Garhwal, Uttarakhand) have resulted in the documentation of 193 plant species, distributed in 158 genera belonging to 70 families of Angiosperms, Gymnosperms and Pteridophytes (Table 1).

Appendix 1: List of floristic elements of S. R. T. Campus (HNB Garhwal University, Badshahi Thaul, Tehri Garhwal, Uttarakhand)

Name of family/ species	Habit	Period of Fl. & Fr.	Colour of flower
Dicotyledons			
Ranunculaceae			
Anemone rivularis Buch.-Ham. ex DC.	H	April-Aug.	White-purple
Clematis grata Wall.	ClS	July-Sept.	Creamy
Clematis montana Buch.-Ham. ex DC.	ClS	April-May	White
Delphinium denudatum Wall. ex Hook. f. & Th.	H	April-May	Blue
Ranunculus diffusus DC.	H	May-July	Yellow
Menispermaceae			
Stephania elegans Hook. f. & Th.	ClH	Aug.-Sept.	Purple
Stephania glabra (Roxb.) Miers.	ClS	July-Aug.	Greenish-yellow
Berberidaceae			
Berberis asiatica Roxb. ex DC.	S	Mar.-May	Yellow
Berberis lycium Royle	S	Mar.-May	Yellow
Brassicaceae			
Cardamine impatiens L.	H	Mar.-April	White
Violaceae			
Viola pilosa Blume	H	April-June	Pale-blue
Viola serpens Wall.	H	April-July	Liliac
Polygalaceae			
Polygala chinensis L	H	Aug.-Sept.	Yellow
Caryophyllaceae			
Stellaria media (L.) Villars	H	Feb.-April	White
Stellaria semivestita Edgew. & Hook. f.	H	July-Aug.	White
Vaccaria pyramidata Medikus	H	April-May	Pink
Malvaceae			
Sida cordata (Burm. F.) Borss.	H	Sept.-Nov.	Pale-yellow
Urena lobata L.	S	July-Nov.	Pink
Tiliaceae			
Grewia optiva J.R. Drummond ex Burret	PlT	April-May	Yellow
Triumfetta pilosa Roth.	S	Sept.-Oct.	Yellow
Linaceae			
Reinwardtia indica Dumortier	H	Mar.-April	Yellow
Geraniaceae			
Geranium nepalense Sweet	H	May-Sept.	Purple
Geranium ocellatum Cambess.	H	Mar.-June	Pink
Oxalidaceae			
Oxalis acetosella L.	H	July-Sept.	Pink
Oxalis corniculata L.	H	July-Sept.	Pale-yellow
Balsaminaceae			
Impatiens balsamina L.	H	Aug.-Sept.	Pink
Rutaceae			
Boenninghausenia albiflora (Hook.) Reichb. ex Meisn.	H	July-Sept.	White
Murraya koenigii (L.) Sprengel	S	May-July	White
Zanthoxylum armatum DC.	S	April-May	Pale-yellow
Meliaceae			
Cedrela serrata Royle	T	May-June	Green-yellow
Celastraceae			
Euonymus pendulus Wall. ex Roxb.	T	May-June	Green
Vitaceae			
Parthenocissus semicordata (Wall.) Planch.	ClS	April-May	Yellowish-green
Aceraceae			
Acer oblongum Wall. Ex DC.	T	Mar.-April	White-green
Anacardiaceae			
Rhus cotinus L.	S	April-June	Purple
Rhus parviflora Roxb.	S	May-June	Yellowish-green
Coriariaceae			
Coriaria nepalensis Wall.	S	Mar.-April	Green
Papilionaceae			
Astragalus leucocephalus Graham ex Benth.	H	May-Aug.	Pale-yellow
Astragalus trichocarpus Grah.	H	May-Aug.	Pink
Desmodium concinnum DC.	S	Sept.-Feb.	Purple

Desmodium laxiflorum DC.	H	Sept.-Feb.	Pale-yellow
Desmodium microphyllum (Thunb.) DC.	S	July-Sept.	Purplish-brown
Desmodium motorium (Houtt.) Merr.	S	July-Sept.	Yellow
Erythrina suberosa Roxb.	T	Mar.-April	Red
Indigofera atropurpurea Buch.-Ham. ex Hornem	S	Mar.-May	Purple
Indigofera dosua Buch.-Ham. ex D.Don	S	May-June	Pinkish-purple
Indigofera heterantha Wall. ex Brandis	S	May-June	Pink-purple
Smithia ciliata Royle	H	July-Sept.	Blue-White
Trifolium repens L.	H	April-July	White-pink
Caesalpiniaceae			
Bauhinia racemosa L.	T	Sept.-Feb.	Pale-yellow
Cassia gluca Lamk.	PT	April-Aug.	Yellow
Mimosaceae			
Albizia chinensis (Osbeck) Merrill	T	May-June	Yellowish-white
Rosaceae			
Agrimonia pilosa Ledebour	H	July-Sept.	Yellow
Cotoneaster affinis Lindley	S	April-June	White
Potentilla fragarioides L.	H	July-Aug.	Yellow
Prunus cerasoides D.Don	T	April-May	Pink
Pyrus pashia Buch.-Ham. ex D.Don	T	Mar.-April	White
Rubus ellipticus Smith	S	Mar.-April	White
Rubus niveus Thunb.	S	Feb.-May	Pinkish-red
Saxifragaceae			
Bergenia ciliata (Royle) Raizada	H	Mar.-April	White-pink
Crassulaceae			
Sedum multicaule Wall. ex Lindley	H	July-Aug.	White-yellow
Melastomaceae			
Osbeckia stellata Wall. ex D.Don	S	Aug.-Sept.	Pink-purple
Lythraceae			
Woodfordia fruticosa (L.) Kurz	S	Feb.-May	Red
Punicaceae			
Punica granatum L.	T	April-May	Orange-red
Begoniaceae			
Begonia picta Smith	H	Aug.-Sept.	Pink
Apiaceae			
Centella asiatica (L.) Urban	H	May-June	Reddish-brown
Cornaceae			
Benthamidia capitata (Wall. ex Roxb.) Hara	T	April-Sept.	Yellowish-green
Cornus macrophylla Wallich	T	April-Oct.	Cream-white
Rubiaceae			
Argostemma verticillatum Wallich	H	July-Aug.	White
Galium aparine L.	H	July-Aug.	White
Galium asperifolium Wallich ex Roxb.	H	July-Aug.	Reddish-pink
Randia tetrasperma (Roxb.) Brand.	S	Mar.-April	Cream-yellow
Rubia cordifolia L.	ClH	July-Oct.	Red
Valerianaceae			
Valeriana hardwickii Wall.	H	July-Sept.	White-Pink
Asteraceae			
Ageratum conyzoides L.	H	Sept.	Pale-blue
Ainsliaea aptera DC.	H	Sept.	Pink-white
Anaphalis busua (Buch.-Ham. ex D.Don) DC.	H	Sept.-Oct.	White
Artemisia capillaris Thunb.	H	Sept.-Oct.	Greenish-yellow
Bidens bipinnata L.	H	July-Sept.	Yellow
Conyza japonica (Thunb.) Lessing ex DC.	H	April-Oct.	Pale-yellow
Conyza stricta Willd.	H	July-Aug.	Yellow
Echinops niveus Wallich ex Royle	H	Aug.-Sept.	Whitish-blue
Erigeron alpinus L.	H	June-Sept.	Purple-white
Eupatorium glandulosum H.B.K.	H	July-Sept.	Pale-white
Gerbera gossypina (Royle) G. Beauv.	H	May-Nov.	Pale-pink
Inula cappa (Buch.-Ham. ex D.Don) DC.	S	July-Oct.	Yellow
Inula cuspidata (DC.) C.B. Clarke	S	July-Oct.	Yellow
Saussurea heteromalla (D.Don) Hand-Mazz.	H	April-Aug.	Purple-red
Senecio alatus Wallich ex DC.	H	Aug.-Oct.	Yellow
Tagetes erecta L.	H	Aug.-Sept.	Red-yellow
Tagetes minuta L.	H	Aug.-Sept.	Pale-yellow

Taraxacum officinale Weber	H	Mar.-Nov.	Yellow
Xanthium strumarium L.	H	April-Nov.	White
Ericaceae			
Lyonia ovalifolia (Wallich) Drude	T	April-May	White
Rhododendron arboreum Smith	T	Mar.-April	Red
Primulaceae			
Anagallis arvensis L.	H	April-May	Bluish-purple
Primula floribunda Wallich	H	Dec.-April	Yellow
Oleaceae			
Jasminum humile L.	S	May-June	Yellow
Nyctanthes arbor-tristis L.	T	Aug.-Oct.	Orange-white
Gentianaceae			
Gentiana argentea (D.Don) C.B. Clarke	ClH	April-May	Blue
Swertia alata (Royle ex D.Don) C.B. Clarke	H	Sept.-Oct.	Green-yellow
Boraginaceae			
Cynoglossum glochidiatum Wallich ex Benth.	H	June-Sept.	Blue
Cynoglossum nervosum Benth. ex Cl.	H	June-Sept.	Blue
Convolvulaceae			
Ipomoea dichroa (Romer & Schult.) Choisy	H	Aug.-Sept.	Pale-blue
Ipomoea eriocarpa R. Br.	H	Aug.-Sept.	Pink
Solanaceae			
Datura stramonium L.	S	July-Sept.	White
Nicandra physalodes (L.) Gaertner	H	July-Sept.	Pale-blue
Solanum nigrum L.	H	Aug.-Sept.	Blue
Solanum surattense Burm. f.	S	May-Oct.	White
Solanum viarum Dunal	S	July-Sept.	White
Scrophulariaceae			
Bacopa procumbens (Miller) Greenman	H	Aug.-Sept.	Pale-yellow
Lindernia nummularifolia (D.Don) Wettst.	H	Sept.-Oct.	Red-purple
Verbascum thapsus L.	H	May-Sept.	Yellow
Veronica persica Poiret	H	Sept.-Oct.	Blue
Gesneriaceae			
Chirita bifolia D.Don	H	July-Sept.	Purple-blue
Acanthaceae			
Barleria cristata L.	S	July-Nov.	Purplish
Dicliptera roxburghiana Nees	H	May-Dec.	Pink
Lepidagathis incurva Buch.-Ham. ex D.Don	S	Mar.-May	White
Pteracanthus angustifrons (Clarke) Brem.	S	Sept.-Dec.	Pale-purple
Rostellularia japonica Thunb.	H	July-Oct.	Pinkish-blue
Verbenaceae			
Caryopteris grata (Wallich) Benth.	S	Mar.-April	White
Lamiaceae			
Ajuga lobata D.Don.	H	Mar.-Oct.	Blue
Ajuga parviflora Benth.	H	Mar.-Oct.	Blue
Leucas lanata Benth.	H	July-Oct.	White
Micromeria biflora (Buch.-Ham. ex D.Don) Benth.	H	April-Oct.	Pink
Nepeta graciliflora Benth.	H	Oct.-Nov.	Pink
Ocimum basilicum L.	H	Sept.-Oct.	Pink-purple
Origanum vulgare L.	H	Aug.-Oct.	Pink
Plectranthus striatus Benth.	H	Aug.-Oct.	White
Pogostemon benghalense (Burm. f.) Kuntze	S	Jan-Feb.	White-pink
Salvia mukerijeea Bennett & Raizada	H	July-Oct.	Yellow
Salvia nubicola Wallich ex Sweet	H	Aug.-Oct.	Yellow
Nyctaginaceae			
Boerhavia diffusa L.	H	April-May	Pink
Amaranthaceae			
Achyranthes aspera L.	H	Sept.-Oct.	Pink
Achyranthes betendifolia L.	H	Sept.-Oct.	Pink
Aerva scandens Wallich	ST	Aug.-Sept.	White
Alternanthera pungens Humb.	H	Mar.-April	White
Polygonaceae			
Polygonum capitatum Buch.-Ham.	H	July-Aug.	Pink
Polygonum nepalensis (Meissn.) H. Gross	H	July-Oct.	Purple-pink
Rumex hastatus D.Don	H	Oct.-Nov.	Greenish-purple

Rumex nepalensis Sprengel	H	Oct.-Nov.	Greenish-red
Euphorbiaceae			
Mallotus philippensis (Lam.) Muell.-Arg.	T	Oct.-Nov.	Greenish-yellow
Urticaceae			
Urtica parviflora Roxb.	H	Aug.-Sept.	Green
Juglandaceae			
Engelhardtia spicata Leschenault ex Blume	T	Mar.-April	Green
Juglans regia L.	T	Mar.-May	Green
Myricaceae			
Myrica esculenta Buch.-Ham. ex D.Don	T	May-Dec.	Green
Fagaceae			
Quercus leucotrichophora A. Camus	T	April-May	Pale-green
Monocotyledons			
Zinziberaceae (Scitamineae)			
Hedychium acuminatum Roscoe	H	Aug.	Yellow
Hedychium ellipticum Buch.-Ham. ex Smith	H	Aug.	Crimson
Roscoea alpina Royle	H	June-July	Dark-purple
Roscoea procera Wallich	H	Aug.-Sept.	Lilac
Haemodoraceae			
Ophiopogon intermedius D.Don	H	July-Aug.	White
Dioscoreaceae			
Dioscorea belophylla (Prain) Voigt ex Hain.	TH	Aug.-Sept.	Pale-green
Dioscorea bulbifera L.	TH	July-Aug.	Pale-green
Liliaceae			
Asparagus curillus Buch.-Ham.	SS	Feb.-Oct.	White
Asparagus gracilis Royle	S	June-Aug.	White
Commelinaceae			
Cyanotis cristata (L.) D.Don	H	July-Sept.	Blue
Araceae			
Arisaema flavum (Forsk.) Schott.	H	June-July	Green-yellow
Colocasia esculenta (L.) Schott.	H	Aug.-Sept.	Pale-green
Remusatia vivipara (Roxb.) Schott.	H	May-July	Yellow-green
Cyperaceae			
Carex condensata Nees ex Wight	H	Sept.-Oct.	Brown
Carex filicina Nees ex Wight	H	Sept.-Oct.	Reddish-brown
Carex setigera D.Don	H	July-Sept.	Pale-brown
Cyperus cuspidatus H.B.K.	H	Sept.-Oct.	Reddish-yellow
Cyperus niveus Retz.	H	May-June	White
Poaceae (Gramineae)			
Alloteropsis cimicina (L.) Stapf.	H	Aug.-Sept.	White-green
Apluda mutica L.	H	Aug.-Sept.	Green
Arundinella spathiflora Trinius	H	Aug.-Sept.	Green
Avena fatua L.	H	June	Yellowish-green
Brachiaria ramosa (L.) Stapf.	H	Aug.-Sept.	Green
Capillipedium assimile (Steudel) A. Camus	H	Sept.-Oct.	Green
Chrysopogon distans W. Watson	H	Aug.-Sept.	Purple
Cynodon dactylon (L.) Persoon	H	May-Sept.	Green
Digitaria biformis Willd	H	Aug.-Sept.	Silvery-white
Eragrostis poaeoides P. Beauv.	H	Aug.-Sept.	Pale-green
Microstegium nudum (Trinius) A. Camus	H	Sept.-Oct.	Green
Neyraudia arundinacea (L.) Henrard	H	Sept.-Oct.	Pale-purple
Oplismenus compositus (L) P. Beauv.	H	Aug.-Sept.	Green
Saccharum bengalensis Retz.	H	Sept.-Oct.	Pale-purple
Setaria gluca (L.) P. Beauv.	H	Sept.-Oct.	Yellow
Sporobolus indicus (L.) R. Br.	H	Sept.-Oct.	Green
Tripogon filiformis Nees ex Steud	H	Aug.-Sept.	Purplish-green
Gymnosperms			
Pinaceae			
Cedrus deodara (Royle ex D.Don) G.Don	PlT	Aug.-Nov.	-
Pinus roxburghii Sargent	T	Jan-June	-
Ferns			
Adiantaceae			
Adiantum incisum Forrsk	Tf	-	-
Adiantum venustum D.Don	Tf	-	-

Aspidiaceae			
Tectaria coadunata (Wall. ex Hook. et. Grev.) C.Chr.	Tf	-	-
Athyriaceae			
Athyrium pectinatum (Wall. ex Mett.) Moore	Tf/Ep	-	-
Athyrium schimperi Moug. ex Fee	Tf/Ep	-	-
Cheilanthaceae			
Cheilanthes farinosa (Forsk) Kaulf	Tf	-	-
Dryopteridaceae			
Dryopteris caroli-hopei Fr.-Jenkins	Tf	-	-
Polystichum squarrosum (D.Don) Fee	Tf	-	-
Polypodiaceae			
Microsorium membranaceuum (D.Don) Ching	Ep/Tf	-	-
Polypodium microrhizoma Clarke	Tf	-	-

Abbreviations used:H= Herb S= Shrub T= Tree PlT= Planted tree ClS= Climbric shrub ClH= Climbric herb TH= Twining herb Tf= Terristrial fern Ep= Epiphitic fern.

Floristic details given in Appendix 1 include species and family, binomial, growth form, flowering and fruiting times and colour of flower. Of these, 10 species in 8 genera and 6 families belong to Pteridophytes (Ferns) and 181 species of angiosperms distributed over 148 genera and belonging to 63 families. Among them, the dicotyledonous flora represents 146 species from 120 genera belonging to 55 families and the monocotyledonous flora with 35 species from 28 genera belonging to 8 families. The Gymnosperms are represented only by 2 species.

Table 1: Statistical synopsis of floristic inventory of S. R. T. Campus, HNB Garhwal University, Tehri Garhwal

Plant group	Family	Genera	Species
Dicotyledons	55	120	146
Monocotyledons	8	28	35
Pteridophytes (Ferns)	6	8	10
Gymnosperms	1	2	2
Total	**70**	**158**	**193**

The family Asteraceae has the maximum number of species (19 species), followed by Poaceae (17 species), Fabaceae (12 species), Lamiaceae (11 species), Rosaceae (7 species), Acanthaceae, Cyperaceae Ranunculaceae, Rubiaceae and Solanaceae (5 species each) (Table 2). Some of the dominant genera in the area are *Desmodium* (4 species), *Carex*, *Solanum* and *Indigofera* (3 species each). The enumerated plants were classified into their respective growth forms viz., trees (natural and planted), shrubs (including under shrubs, woody parasites), herbs (vines, epiphytic herbs and ferns)and climbers (woody and non-woody) (Table 2). Asper the type of growth form,126 species

Table 2: Ten dominant families of the S. R. T. Campus

Family	Genera	Species
Asteraceae	16	19
Poaceae	17	17
Fabaceae	6	12
Lamiaceae	10	11
Rosaceae	6	7
Ranunculaceae	4	5
Rubiaceae	4	5
Solanaceae	3	5
Acanthaceae	5	5
Cyperaceae	2	5
Total	**73**	**91**

(65.28%) were herbs, 34 species (17.62%) were shrubs, 24 species (12.44 %) were trees and 9 species (4.66%) were climbers (Figure 2). Of these, 17.13% species bears white flower, 15.47% yellow, 11.61% Pink, 9.94% green, blue 7.185.52 purple, 4.42% red while rest of the species (28.74%) bears flowers of other than colour class (creamy, crimson, liliac, purplish, etc.).

A comprehensive and updated checklist on the floristic diversity of a area (including semi-natural environment) serve as a ready reference for researchers, policy makers and for better management and conservation of its indigenous species (Rawat *et al.,* 2016a). Thorough collection, identification and documentation of plants from any smaller eco-region is an essential step that evaluates the total biodiversity wealth of the district, state and country (Rawat *et al.,* 2016b). The S. R. T. Campus (HNB Garhwal University, Tehri Garhwal, Uttarakhand) is rich in natural floristic plant diversity however the current study and a previous

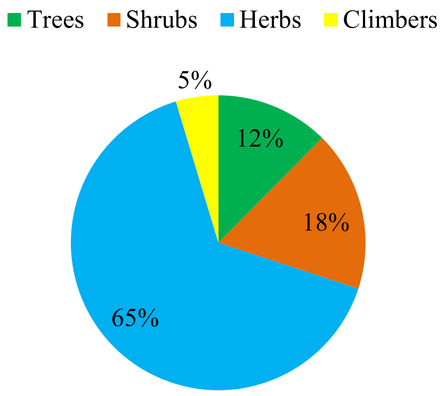

Figure 2: Habit-wise distribution of taxa in S. R. T. Campus

study (Dangwal *et al.,* 2011) revealed that the several invasive species have established themselves in within the campus and adjacent areas. Encroachment of several invasive alien species into uphill of Himalaya need matter to be concern, as some of the invasive species are able to form large carpet of them on the land surface and change the natural habitats which may eradicate several habitat specific native species from the area (McGeoch *et al.,* 2010; Rawat *et al.,* 2016b). The garden worker may follow any of the four main suggested strategies (Syrett *et al.,* 2000) to control or eradicate invasive species (manual, mechanical, chemical and biological) in the campus area.

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

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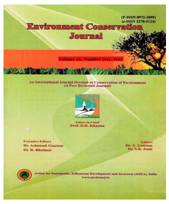

Biological control of *Fusarium*-wilt *and* quality improvement of *Sesamum indicum* cv. ST-1 using fluorescent *Pseudomonas*

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ABSTRACT

Seven plant growth-promoting bacterial strains (LES1-LES7) were isolated from rhizosphere of *Lycoperscion esculentum* Mill. (Tomato) and further screened based on colony morphology, carbon source utilization and biochemically characterized as fluorescent *Pseudomonads*. Among the isolates prominent strain identified as *Pseudomonas aeruginosa* LES4 produced maximum siderophores *in vitro* besides indole acetic acid, hydrocyanic acid, solubilized insoluble inorganic phosphate and secreted β-1, 3-glucanase urease and chitin solubilizing enzymes chitinase. It also exhibited a strong antagonism against *Fusarium oxysporum* f.sp. *sesami* when co-cultured on nutrient agar medium and inhibiting the growth of the pathogen by 69% after 5 days incubation at 28 ± 1°C. Sesame (*Sesamum indicum* L. cv. ST-1). When surface sterilized seeds bacterized with *P. aeruginosa* LES4 showed enhancement in seedling sprouting early vegetative growth, and increased seed yield components viz. biomass accumulation, and all other yield and quality improving components. Strain LES4 significantly reduced the wilt disease of sesame in *F. oxysporum* f.sp. *sesami*-infested soil. Moreover, *Tn*5 induced streptomycin resistant trans-conjugants of spontaneous tetracycline-resistant LES4 (designated LES4$^{tetra+strep+}$) used to exhibit efficient rhizosphere colonization of sesame. Such properties of fluorescent *P. aeruginosa* LES4 prove it as a beneficial and potential microbial agent against wilt causing sesame.

Introduction

Sesame (*Sesamum indicum* L.) is one of the most ancient oil seed crop cultivated in all over the world. Wherever, sesame is grown, it is likely to attack by a number of serious phyto-pathogenic fungi (El-Shazly *et al.* 1999; Jyothi *et al.* 2011). Fungal pathogens cause wilt by invading and gradually blocking the conducting tissues. *Fusarium oxysporum* is a notorious phyto-fungal pathogen may be soil or seeds/seedling borne which completely block water conducting xylem vessels and photosynthate supply leads to wilting of plants (Bateman *et al*. 1996). Sesame is susceptible and liable to attack by various phytopathogens on different developmental stages and resulted in serious damage or great crop loss. *F. oxysporum* f.sp. *sesami* (Zaprometoff) Castellani is one of the serious pathogen of sesame wilt and leads to reduced sesame production. Armstrong and Armstrong (1950), reported for the first time from America. Use of broad-spectrum biocides, soil fumigation and cultivation resistant cultivars are the common methods to control this pathogen. However, use of fungicides and soil fumigation are not environmentally (Kumar *et al.* 2011). Recently, Gricher *et al.* (2001a, b) observed negative effects of these phytochemicals on sesame and accumulation harmful chemicals have been reported in seeds, oil and oil cake of Sesame

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(Shinde *et al.* 2021) leads to reduction in sesame export and consumptions. Due to which, sometime export consignments in international market are cancelled and leads to loss of revenues (Duhoon *et al*. 2004). Thus the use of fungicide, synthetic fertilizers and pesticide not only distract soil fertility, ecology, microbiome but leads pollutes water resources and consequently drastically affect human health directly and indirectly (Ayala and Rao 2002; Kumar *et al*., 2009).

Biocontrol of plant pathogens is one of the ecological safe methods being used in recent years. Among the bacterial antagonists, pseudomonads have shown a promising potential biocontrol rhizobacteria against soil as well as seed borne phytopathogens with plant growth-promoting (PGP) activities under controlled and field conditions (Kloepper *et al.* 1989; Gupta *et al.* 2002, Kumar *et al.* 2005). Pseudomonads are able to colonize plant roots and dominant rhizospheric microbiome over the native soil-microflora as compared with other inoculated strains (Kumar *et al.*, 2009; Lim *et al.* 2002). The disease management involves antagonism through iron deficiency due to chelation by siderophores, which may lead to elimination of fungal pathogens in the rhizosphere). The present study was designed to evaluate the efficiency of seed bacterization with fluorescent *P. aeruginosa* LES4 on seed germination, root colonization, plant biomass accumulation, crop yield, oil contents and its requisite population in rhizosphere to suppress the sesame wilt caused by *F. oxysporum* f.sp. *sesami*.

Material and Methods

Bacterial inoculum

The fluorescent Pseudomonad strains were isolated from the rhizosphere ofvigorously growingplant of*Lycopersicon esculantum* L. (Tomato) growing in waste land seems to be deficient in nutrients. The strains were isolated by serial dilution method on nutrient agar medium (NAM), and the petri-plates were incubated at 30 ± 2°C for 48-78 h. The fluorescent water soluble pigment producing bacterial colonies were selected and further streaked onto a NAM addition of 100 µg ml^{-1} tetracycline and streptomycin to evaluate the antibiotic-resistant strain. All the isolates were cultured and maintained on NAM slants at 4°C for further studies. Morphological, physiological and biochemical characterization of isolates was carried out with Microbial Type Culture Collection (MTCC) standard strain as described by Holt *et al*. (1994). *F. oxysporum* f. sp. *sesami* was isolated from seeds, roots and infected plant parts of sesame on potato-dextrose agar f(PDA) and identified following Barnett and Hunter (1972) and maintained on PDA slants for further uses.

Direct and indirect plant growth promoting (PGP) properties of all the promising strains were determined by using log phase cultures (24 h old). Siderophores production was estimated on Chrom Azurol agar medium (CAS) qualitatively following the method of Schwyn and Neilands (1987). The strains were separately inoculated on CAS agar medium, and the Petri-plates were incubating at 30 ± 2°C for 78 h. Hydrocyanic acid (HCN) production was evaluated (modified) as described by Miller and Higgins (1970), and indole acetic acid (IAA) production was determined correspondingly to the method of Gupta *et al.* (1999).Phosphate solubilization abilities of strains were appropriately determined by inoculating a loopful culture of isolates on Pikovskya's agar plates (Pikovskaya's 1948).

Chitinase activity

The chitin minimal medium (CMM) was used test for production of extra cellular chitinase activities. Bacterial cultures were infused on CMM plates and incubated at 28 ± 1^0C up to 5-6 days. Formation clear halo zone around the bacterial spots indicated secretion of chitinase enzyme (Dunne *et al*. 1997). Similarly, ß-1-3 glucanase enzymatic activity determined by supplementing the laminarin (0.2%) in growth medium as a sole source of carbon and observed by appearance of turbid growth.

Antagonistic activities

Antagonistic activities of bacterial strains were evaluated against wilt causing pathogen *F. oxysporum*f.sp. *sesami*on NAM using a dual culture method of the as described by Skidmore and Dickinson (1976). Five days old mycelial agar blocks of 5 mm diameter were placed on 90 mm diameter NAM plate, and the log phase culture were infused 2 centimeters apart from the fungal pathogen and incubated at 30 ± 2°C along with control (Without bacterial strain). Antagonistic activities were evaluated by calculate the growth inhibition of fungal colonies by bacterial strains, and represented in percent by following formula:

Growth inhibition = Control -Treatment/Control X100

The experiment was performed in three replicates. The mycelia of *F. oxysporum*f.sp. *sesami* were periodically recovered from zone of inhibition for deformities and microscopic examination as described by Gupta *et al.* (2001).

Preparation of fungal inoculum

Potato Dextrose Broth (PDB) was used to prepared the pathogen inoculum by culturing the fungus for 15 days at 27± 1°C. Actively growing pathogen colony was gather on sterilized filter paper to remove the extra water, and the inoculum was prepared by pulverized 100 gram of fungal mycelia in 1000 mlDW by using 10 mlinoculum to provide 1 g for each *flower*-pot for pathogeninfested soil.

Seeds treatment and pot trial assay

Sesamum indicum L. cv. ST-1 certified seeds were obtained from Center for Transgenic Plant Development, Department of Biotechnology, Jamia Hamdard Deemed to be University, Hamdard Nagar, New Delhi, India. Surface sterilized seeds were bacterized by the modified method given by Weller and Cook (1983). Surfaced sterilization was done with the help of 90% alcohol followed by 0.1% (w/v) $HgCl_2$ for 2-3 minutes and then 7-8 time washed with sterilized distilled water (SDW) and dried under aseptic conditions in Laminar Air Flow (LAF). The actively growing log phase culture of LES4 was centrifuged at 8000 revolutions per minutes for 12min at 4°C by ultra-centrifuge. After centrifuge LES4 pellets were washed with SDW and obtained a cell density of 1×10^8 CFU ml^{-1} in SDW. Above mixture used for seed coating with the help of 1% carboxymethylcellulose (CMC) slurry and air-dried overnight under LAF. The sesame seeds coated with CMC slurry (without *P. aeruginosa* LES4) treated as the control.

Bacterized and control seeds (without bacterization only coated with CMC) were sown separately in earthen pots of 15 cm diameter containing about 1 kg sterilized (steam) soil with composition of 0.097% total organic carbon, 14.4 % silt, 12.3% clay, 75.2% sand, pH 6.9 and 38% WHC and arranged all treatments as: Treatment 1: *F. oxysporum* f.sp. *sesami*inoculated soilplus non-bacterized seeds; Treatment 2: *P. aeruginosa* LES4 coated seeds plus soil; Treatment 3: *F. oxysporum* f.sp. *sesami* plus *P. aeruginosa* LES4 coated seeds; Treatment 4: *F. oxysporum* f.sp. *sesami* plus standard MTCC1934 strain Treatment 5: Seeds shown without bacterized seeds and *F. oxysporum* f.sp. *sesami* treated ascontrol**.** All the growth and development parameters including plant shoot fresh and dry weight, number of capsules per plants, number of seeds per capsules, seeds weight, oil contents and seedling infected with *Fusarium*-wilt were observed up to 90 DAS. All the treatments were arranged in completely random design (CBD) in triplicate number.

Root colonization

Antibiotic resistant marker strain was used to study rhizosphere colonization of *S. indicum*. Tetracycline and streptomycin resistant strain of Pseudomonads inoculated on Tryptic Soy Agar medium, supplemented with 100 mg l^{-1} of tetracycline designated as *P. aeruginosa* LES4^{tetra+}. The very same strain was acquainted resistance for another antibiotic streptomycin and designated *P. aeruginosa* LES4$^{tetra+strept+}$ with the help of *Tn*5 delivery suicidal vector in donor *Escherichia coli* strain WA803 (pGS9) (Selvaraj and Iyer, 1983; Kumar *et al.* (2003). Quantitative examination of rhizospheric colonization and changes in microbiome of sesame was studied. Seedlings emerged with bacterized seeds (with *P. aeruginosa* LES4$^{tetra+strept+}$)was sampled after 30 to 90 DAS and population dynamics was analyzed. Freshly collected sesame roots pieces of one centimeter about 1 g weight were vortexes in measured volume of SDW to liberate the root-adhering microbes into water. Optimum amount of the dilution was plated on Tryptic Soy Agar medium containing both antibiotic i.e. tetracycline and streptomycin (100 mg l^{-1}) to study the colonization and rhizospheric population dynamics. Further, analysis of bacterial population was done after 24 h incubation at 30 ± 2°C, and colony forming units per gram of sesame root segment were enumerated.

Pathogen infestation and *Fusarium*-wilt

Assessment of diseases incidence of *Fusarium* infestation and wilt was done in five, one-centimeter-long root pieces of sesame, surface-sterilized with the help of 3% NaClO for 2-3 min, and incubated on to potato dextrose agar plates supplemented with antibiotics penicillin (100,000 units litre^{-1}) and streptomycin (0.2 gram litre^{-1}) at 30°C for 5-6 days, the occurrence of *F. oxysporum* f.sp. *sesami* was observed in root samples (Siddiqui *et al*. 2000). To identify *Fusarium* wilt, sesame plants showing wilt symptoms were leniently uprooted, individually kept in sterilized poly-bags for further study in research lab. *Fusarium* wilt symptoms on root were observed by cut and peel back a section of the epidermis and cortical tissues just above the soil, observing with a 10x field lens and conidia under microscope. The isolate was collected, identified and maintained on PDA plate and corroboration of isolate was done as following the Koch,s postulation and microscopic analysis in laboratory.

Estimation of oil content

Seed oil content was extracted and estimated with the help of n-hexane in research laboratory using cold percolation technique by rapid gravimetric determination of oil content by modified methods (Kartha and Sethi, 1957) and represented in percentage.

Results and Discussion

Isolation of rhizobacteria

A large number of rhizospheric fluorescent pseudomonads were isolated and further screened for direct and indirect plant growth promoting (PGP) activities. Among the isolates seven isolates were found potent for PGP activities. Among the isolated strains, LES4 was found as most encouraging strain, in view of fact that it solubilized inorganic phosphate, produced plant growth hormone and phytostimulating hydrogen cyanide, along with inhibition of *F. oxysporum* f.sp. *sesami* in dual culture. In biochemical screening LES4 was producing urease, oxidase, and catalase. However, found negative for mannose utilization and starch solubilization forming smooth-round colonies and bacterial cells were found motile, Gram-negative, rod, and not forming spores on NAM plates after 24 h of incubation at 28 ± 1 ºC. The strain was positive for catalase, oxidase, urease and indole production and was negative for starch hydrolysis and mannose utilization. Based on above morphological and biochemical characteristics when compared with standard strain MTCC strain 1934, found very similar to *P. aeruginosa*. Hence, LES4 was identified as *P. aeruginosa*.

Siderophore are low molecular weight proteins which chelates iron in rhizosphere. LES4 produced sidrophore as indicated by the formation of orange zone when inoculated on Chrome Azurol agar plates. The log phase culture filtrate of *P. aeruginosa* LES4 produced hydroxamate-type siderophores as evident by occurrence of major peak at 400 nm. Hydrogen cyanide production indicated by turning the color of yellow filter paper to reddish brown in 3 days of incubation. IAA production evident by the production of pink color with and without addition of tryptophan in both cell as well as cell free culture filtrates. *P. aeruginosa* LES4 develop a clear zone around the bacterial colony on the Pikovskaya's agar plates showed production of phosphatase and solubilization abilities of phosphate after 48 h of incubation. Formation of clear halo around the bacterial colony on chitin supplemented medium indicated that strain LES4 is positive for chitinase activities. Chitinase activity started after 24 h and successively increased and attained maximum at 120 h of incubation. The activity was declined after further incubation. On the other hand, ß-1-3 glucanase activity started after 20 h and observed highest after 36 h of inoculation.

***In vitro* antagonistic activity**

*P. aeruginosa*LES4 firmly inhibited the mycelial growth and vigorously restrict colony expansion of *F. oxysporum* f.sp. *sesami* on dual culture plates 28 ± 1ºC and reached maximum after 5 days of inoculation which leads to 69% growth reduction, finally diffidence of *F. oxysporum* f.sp. *sesami* as compared to the control.

Root colonization

Direct correlation has been observed between reduction of diseases incidence and in-*vitro* antagonism activities. *P. aeruginosa* LES4$^{tetra+strept+}$ treated as makers strain vigorously colonized rhizosphere of sesame. Higher root colonization was observed by *P. aeruginosa* LES4$^{tetra+ strept+}$ in *F. oxysporum* f.sp. *sesami* infested soil as compared to *P. aeruginosa* LES4$^{tetra+ strept+}$ alone (6.9 x 10^4 and 5.8 × 10^4 CFU ml^{-1}, respectively) after 90 DAS. The colony-forming unit (CFU) increased with incubation from 30 up to 90 DAS (Table 1).

Pot experiments

The seeds bacterized with LES4 resulted in significant ($P<0.01$) increase in seedling biomass as compared to that of non-bacterized seeds. Growth

Table 1: Population dynamics of *Pseudomonas* LES4^{tetra+} in rhizosphere of sesame (Values are means of three replicate)

Treatments	CFU g^{-1} root segments of sesame		
	30 DAS	60 DAS	90 DAS
Pseudomonas LES-4	1.5 x 10^3	4.5 x 10^4	5.8 x 10^4
Pseudomonas+ *F. oxysporum* f.sp. *sesami*	4.3 x 10^3	6.7 x 10^4	6.9 x 10^4
MTCC1934	2.5 x 10^3	6.3 x 10^4	6.7 x 10^4

CFU- Colony-forming unit

parameters viz., number of capsules per plants, seed weight and oil contents were significantly influenced by *P. aeruginosa* LES4. Higher numbers of capsules per plants (58) were observed with treatment III (*P. aeruginosa* LES4 + *F. oxysporum* f.sp. *sesami*) which is 48.95% higher in comparison to control. Lesser numbers of capsules (22) were obtained in *F. oxysporum* f.sp. *sesami* invaded soil. An increased seed weight by 79.80% and oil contents by 90.87% has been recorded seedlings emerged with bacterized seeds. In contrast a decline in plant growth, biomass yield and oil content was recorded in *F. oxysporum* f.sp. *sesami* invaded soil. All plants were infected by *F. oxysporum* f.sp. *sesami* grown in infested soil but about 95 % reduction in wilt incidence was recorded with use of LES4 bacterized seeds in *F. oxysporum* f.sp. *sesami* infested pots. Standard strain MTCC-1934 also reduced the disease incidence about 70% (Table 2). Pathogen was re-isolated from infected plants and wilt-sick pots. Even though plants are infected at an early stage, they seem able to "keep fighting" with *Fusarium* wilt until flowering and capsule formation. Symptoms of disease appear 2 to 3 weeks DAS. The preliminary visible indication was the wilting of leaves and loosing normal morphology followed by chlorosis and turning bright yellow before complete wilting but retained on plants (Figure 1 A-D). When the bark of wilted plants peeled off, browning or blacking coloration, blocking of xylem vessels from root system to stem (Fig. 2A & B) and presence of conidia in internal tissues (Fig. 1C) were observed.

P. aeruginosa LES4 produced siderophore, chitinase, β-1,3-glucanase besides hydrogen cyanide, and phytohormone *in-vitro*. Recently, Arora *et al*. (2007) reported that fluorescent pseudomonad from *Solanum tuberosum* rhizosphere exhibited the same plant growth promoting and unsympathetic activities against *Phytophthora capsici* and *Rhizoctonia solani*.

Similarly, *P. aeruginosa* LES4 inhibited the mycelial growth and induced deformities in *F. oxysporum* f.sp. *sesamiin vitro*. *Pseudomonads* exhibits antagonism against phytopathogens via the production of antifungal compounds includes antibiotics, siderophore and HCN. *P. aeruginosa* LES4 reduced the *Fusarium*-wilt incidences in pot experiment and also enhanced the plant growth and yield components of sesame. Furthermore, strain LES4 proved as a competent rhizosphere colonizer, resulting enhance seed germination and seedling emergence (84%) even in presence of *F. oxysporum* f.sp. *sesami*.

Approximately, 56% of fluorescent pseudomonads isolates from tomato rhizosphere inhibited the growth of *F. oxysporum* f.sp. *sesamiin vitro* but most promising effect was caused by LES4. siderophores, chitinase, β-1,3-glucanase, HCN and antibiotics etc produced by *P. aeruginosa* LES4 exhibited reduction in growth of phytopathogen. This is proven by facts: (1) inhibition of fungal growth because of diffusible substances (2) in dual culture have all necessary nutrients, hence competition for nutrients can be excluded, (3) chitinase secretion was observed during *in vitro* study, which may be involve in degradation of fungal cell wall, (4) implication of antifungal compound produced by LES4 in the reduction of pathogen growth was established by using supernatant of PGPR to restrict *in vitro* germination of conidia and mycelial growth of phytopathogen.

This study has shown that 69% growth inhibition of *F. oxysporum* f.sp. *sesami* might be cumulative action of all cytosolic antibiosis amalgam of various biochemical such as Hydrogen cyanide, iron chelators, lytic and chitin solubilizing enzymes. Production of antifungal metabolites by fluorescent pseudomonads and its antagonistic actions on fungal pathogens (Bano and Musarrat 2003; Gupta *et al*. 2001). Similarly, LES4 restrict the conidia and mycelial growth of *F. oxysporum*

Fig. 1. Pot trail assay: (A) Soil inoculated with *F. oxysporum*f.sp. *sesami* (B) Soil inoculated with *P. aeruginosa* MTCC100+ *F. oxysporum*f.sp. *sesami*(C) Soil inoculated with *P. aeruginosa* LES4 + *F. oxysporum*f.sp. *sesami*(D) Soil inoculated with *P.aeruginosa* LES4 + *F. oxysporum*f.sp. *sesami*

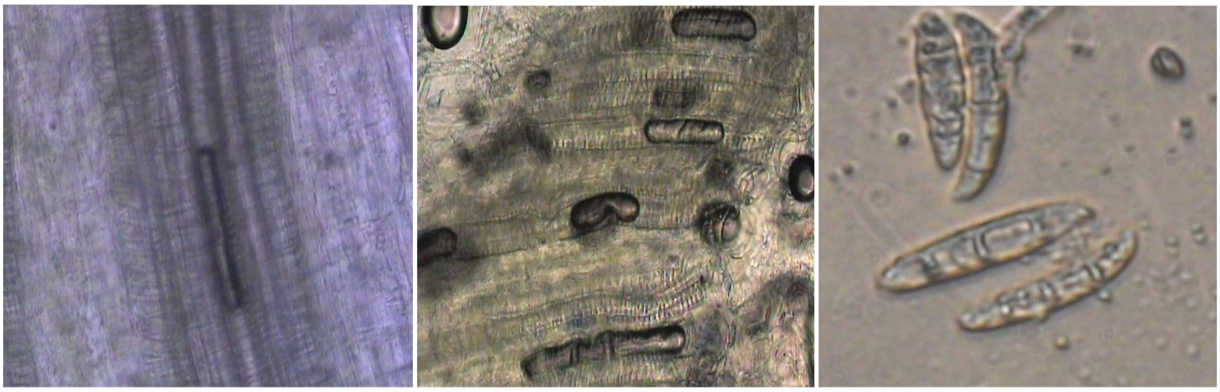

Fig. 2 (A & B) Vessels blocking found in wilted Sesame plant stem inoculated with *F. oxysporum*f.sp. *sesami*
(C) *Fusarium* conidia found in the internal tissues of wilted plant parts.

f.sp. *sesami*. Likewise, chitinase, pyrazine, HCN and low molecular weight proteins including iron chelators secreted by pseudomonads are known to restrict the growth of phytopathogens (Lim and Kim 1995; Gupta *et al*. 2006). Loss of structural integrity and solubilization of chitin cell wall by lytic enzyme chitinase is well known (Fridlender *et al*. 1993; Gupta *et al*. 2006). Upadhyay and Jayaswal (1992) reported reduction of conidia germination and mycelial degradation and hyphal lysis of fungal pathogens by *Pseudomonas cepacia* due to production antagonist compounds. Similarly, LES4 also inhibited conidia production and germination and mycelial deformities.

Seed coating with rhizospheric competent fluorescent pseudomonads is well known along with PGP activities. Suppression of plant pathogenic fungi under controlled and field conditions is highly essential for commercial and economic viabilities (Kumar and Dube, 1992). Increase in growth and crop yield because of PGP activities of fluorescent pseudomonads is well documented by several researchers (Kumar *et al*., 2009). Similarly, Hofte *et al*. (1991) reported that *P. aeruginosa* $7NSK_2$ secreted antifungal compounds, aggressively colonize rhizosphere of different vegetables and cereals and enhance growth and yield. But *P. aeruginosa* LES4 showed positive PGP activities (Kumar *et al*., 2009) and significantly influenced all the vegetative as well as yields components of sesame.

First and foremost, for biocontrol agent and plant growth promoting rhizobacteria it is highly essential that particular strain must survive and colonized the rhizosphere of that particular crop. Many workers documented that inadequate colonization leads to decrease biocontrol activities (Kloepper *et al*. 1988; Maheshwari *et al.* 2012).

LES4 exhibited successive survival and colonizing properties in sesame rhizosphere along with enhance seed germination and biological control against the *F. oxysporum* f.*sp. sesami.Pseudomonas* sp. dominating microbiome over other rhizospheric communities due to its fast growing nature and

Table 2: Effect of seed bacterization with *P. aeruginosa* LES4 on seed germination, diseases reduction, growth and yield component of *Sesamum indicum* L. cv. ST-1 after 90 DAS

Test organism	Germination (%)	Root			Shoot			Capsule /Plant^{-1}	1000 seeds weight (g)	Oil Content (%)	Wilt disease (%)
		Length (cm)	Fresh weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)				
F. oxysporum f.sp. *sesami*	55.00	4.4	11.0	6.2	49.1	73.4	28.5	22.2	2.00	45.0	100
P. aeruginosa LES4	90.00	8.4	25.4	12.5	65.6	101.7	50.8	49.8	2.78	49.2	-
P. aeruginosa LES4+ *F. oxysporum* f.sp. *sesami*	93.00	9.3	26.6	13.5	69.0	108.7	57.5	57.6	3.02	50.4	5
MTCC1934 + *F. oxysporum* f.sp. *sesami*	84.00	7.6	22.8	12.3	62.4	98.0	40.0	34.0	2.29	47.6	20
Control	78.00	5.2	13.7	7.2	54.2	86.0	32.1	28.2	2.14	45.8	2
CD@ 1%	8.94	3.05	1.62	2.36	0.39	1.78	0.95	1.00	0.86	0.75	
± SEM	1.88	0.64	0.34	0.49	1.88	8.43	4.51	4.73	0.40	3.56	

Values are mean of 5 randomly selected plants from each treatment

antibiosis with the help of various organic biochemical (Howell and Stipanovic 1980; Bakker *et al.* 1986). Our results suggest that seed bacterization with *P. aeruginosa* LES4 can protect sesame from diseases caused by a high concentration of the *F. oxysporum* f.*sp. sesami* infested soil and likewise potentially biocontrol agent to reduce the wilt disease in economic and ecofriendly manners. Hence, we might assume that the LES4 is potent biocontrol control agent against the *F. oxysporum* f.*sp. sesami* has exhibited its antagonisms and enhanced plant growth and yield components after bacterization, production of phytohormone (IAA), solubilization of inorganic phosphate, urease, and rhizospheric competent to restrict the fungal growth and growth enhancements. *F. oxysporum* f.*sp. sesami* by its extracellular enzyme chitinase, β-1,3-glucanase and siderophores production responsible for mycelial deformities, hyphal perforation, cellular lysis and conidia development due to complicity of antifungal compounds perhaps could not be avoided.

Conclusion

The present investigation conclusively revealed that application of rhizospheric competent strain *P. aeruginosa* LES4 have biological control potential for *Fusarium*-wilt and excert positive effect on vegetative growth, productivity and increase oil contents significantly higher in all the treatments in

comparison to control in ecofriendly manners probably due to aggressive root colonization, enhancement of nutrient uptakes, production of phytohormone and lytic enzymes to control the fungal pathogen.

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Conflict of interest

The authors declare that they have no conflict of interest.

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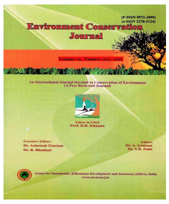

Biodegradation of quinalphos by gram negative bacteria *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A

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ABSTRACT

Five bacterial strains for biodegradation study were initially isolated from quinalphos exposed soil from cotton field of Indian Agricultural Research Institute, India by Serial dilution and enrichment technique. Amongst them, two strains *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A were exposed to different concentrations (50-250 ppm, parts per million) of quinalphos to evaluate their tolerance and the optimum concentration of quinalphos supported growth. Growth kinetics of the isolates was studied by means of optical density of the culture media (150 rpm, rotation per minute) at 30°C for 15 days. The optimal concentration of quinalphos for the growth of *Pantoea agglomerans* was 100 ppm, while for *Acinetobacter sp.* dcm5A was 200 ppm. Bacterial strains *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A degraded quinalphos 60% and 79.7% respectively in 7 days incubation and 100% degradation by both strains was observed after 13 days of incubation.

Introduction

Quinalphos (O,O-Diethyl O-quinoxalin-2-yl phosphorothioate), a potent organophosphorus insecticide was introduced into the market by Bayer AG under the trade name Bayrusil. Owing to its high insecticidal properties, this chemical is widely used to suppress and control the pest attack in a variety of crops such as rice, cotton and groundnut (Armes *et al.,* 1992). It is an acetylcholinesterase inhibitor and acts through direct contact and stomach action. The toxic effects of quinalphos in animals have been reported (Bokonjie *et al.,* 1987; Srivastava, 1989). In soil, rapid degradation of quinalphos into quinoxalin-2-ol has been observed under aerobic conditions with a DT_{50} of about three weeks. Further breakdown of quinalphos into CO_2 and polar metabolites was also reported (Schimdt, 1972). Quinalphos degraded in soil under anaerobic conditions in a red sandy loam and sandy clay soil with a half-life of 13 days and in a black clay soil with half-life of 15 days have been reported. The metabolite 2-hydroxyquinoxaline was identified and quantified and could be detected in soil after complete dissipation of parent compound after 20 days (Babu *et al.,* 1998). The hydrophilic nature of quinalphos is weakly sorbed by soil particles. Hence, there is a threat that it can be seen in wastewater arising from quinalphos manufacturing units or it may enter into the rainwater and groundwater when sprayed on crops because of leaching. The leaching upto 0.025 $\pm$ 0.0013 μg quinalphos g-1 d. wt. soil was observed in 60 days (Mayanglambam *et al.,* 2005).

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The leaching of insecticide quinalphos was observed up to a depth of 9 inch in soil (Awasthi *et al.,* 1984). The translocation of quinalphos in soil was also reported (Karoly *et al.,* 1988; Bhavani, 2020). In soil thin layer and column chromatography, quinalphos shows lower mobility. Due to leaching of this insecticide quinalphos, groundwater may be contaminated and it also affect the diversity of non-target soil organisms.

Decomposition of toxic organics by soil microorganisms is the most important and safe and natural technology and a good alternative to chemical and physical methods such as incineration and solvent extraction (Comeau *et al.,* 1993). Bacteria which has the capacity of degrading several pesticides, were isolated from soil. They include diazinon-degrading *Flavobacterium sp.* (Sethunathan and Yoshida, 1973), chlorpyriphos-degrading *Flavobacterium sp.* (Mallick *et al.,* 1999), monocrotophos-degrading *Arthrobacter atrocyaneus* MCM B-425 and *Bacillus megaterium* MCM B-423 (Bhadbhade *et al.,* 2002), metametron-degrading *Rhodococcus sp.* (Parekh *et al.,* 1994), ethoprophos-degrading *Pseudomononas putida* (Karpouzas and Walker, 2000), monocrotophos-degrading *Pseudomononas aeruginosa* F10B and *Clavibacter michiganense* subsp. *inidiosum* SBL 11 (Subhas and Singh, 2003; Kaur *et al.,* 2021; Garg *et al.,* 2022). The role of microbes in biodegradation is well known in all over the world. However, a scanty information is available on biodegradation of quinalphos by bacteria in soil. Therefore, this study has been conducted to evaluate the capability of the bacterial strains isolated from soil exposed to quinalphos by enrichment and adaptation culture technique in degradation of quinalphos.

Material and Methods

Isolation, screening and growth conditions of the microbial isolates

Five bacterial strains for biodegradation study were isolated from quinalphos exposed soil (25% EC@4L/ha, sprayed at regular intervals) from cotton field of IARI, India by Serial dilution and enrichment technique. Enrichment was done by adding 5.0 g of soil sample in 100 ml basal medium BM and then incubating at 37°C for 15 days. The basal medium consists of Glucose (5.0 g), K_2HPO_4 (0.2 g), KH_2PO_4 (0.2 g), $(NH_4)_2SO4$ (1.0 g), NaCl (0.1 g), $MgSO_4.7H_2O$ (0.2 g), KCl (0.2 g), $FeCl_3$ (0.003 g), $CaCl_2$ (0.02 g). The medium was prepared by dissolving all the chemicals in 998 ml double distilled water followed by maintaining the pH at 7.2 using 1N NaOH / 1N HCL. The medium was autoclaved at 121°C temperature and 1.5 kg /cm^2 for 15 minutes. After autoclaving, 2.0 ml of sterile Trace Element Solution was added to the medium *(6)*. The composition of the Trace Solution was as follows: H_3BO_3 (2.80 g), $MnCl_2.4H_2O$ (1.86 g), $CuSO_4.5H_2O$ (0.20 g), Na_2MoO_4 (0.75 g), $CoSO_4.7H_2O$ (0.20 g), $ZnSO_4.7H_2O$ (0.25 g), 8 M HCl (2.0 ml) in 998 ml double distilled water. After 15 days of incubation, different dilutions of medium were prepared in sterilized distilled water, and then 100 µL of this solution was spread evenly using a sterilized glass spreader on Nutrient agar plates which composed of Peptone (10 g), Yeast Extract (5 g), NaCl (5 g), Agar (16 g) in 1000 ml double distilled water at 6.5 pH. The plates were then incubated at 30°C for 24 hrs. Each bacteria colony was treated as a different strain and pure homogenous strains were acquired by repeated streaking for more than 20 times.

The dependence of the bacterial strains on quinalphos (technical grade obtained from Montari Industries Limited, Nehru Place, New Delhi, India) was determined by variably modifying the composition of Basal Medium BM (the medium with both carbon and phosphorous sources) with a known concentration of quinalphos into Modified Basal Medium One MBM1 (the inorganic phosphorus sources, K_2HPO_4 and KH_2PO_4 in BM were replaced with quinalphos) and Modified Basal Medium Two MBM2(both the inorganic phosphorus and carbon sources, K_2HPO_4, KH_2PO_4 and glucose in BM were replaced with quinalphos). The isolates were initially screened for their efficiency in degradation of quinalphos by growing them in Basal medium BM with quinalphos (100 ppm) as additional source of carbon and phosphorus. The cultures were kept on the shaker with 150 rpm (revolutions per minute) speed at 30°C.

Soil characteristics

Soil was analyzed for physico-chemical parameters using standard methods. Soil pH was calculated by using saturated soil paste (1:2.5 :: soil : water). Organic carbon was calculated by standard method (Walkley and Black, 1934).

Identification of the bacterial strains

Two best growing bacterial strains were selected out of the initially screened five isolates. DNA isolation was done using DNeasy Tissue Kit (50, Qiagen) followed by 16S rDNA sequence analysis.

. Optimization of quinalphos concentration

Optimum concentration of the pesticide at which there is the maximum growth of two selected strains of the bacteria was studied by taking 25 ml each of MBM1 and MBM2 broth mediums supplemented with various concentrations of quinalphos ranging from 50 ppm-250 ppm in 100 ml flasks. These broths were inoculated with two cultures separately (1.0 ml each of inoculum) and incubated under constant shake condition (150 rpm) in a shaker incubator at 30ºC for 15days. Bacterial growth was measured at regular intervals at 576 nm using a spectrophotometer.

Growth pattern of the isolates

To check the growth pattern of bacterial strains, the isolates (1.0 ml each of inoculum) were inoculated in 50 ml each of broth BM, broth MBM1 and broth MBM2 supplemented with optimum concentration of quinalphos (100 ppm for *Pantoea agglomerans* and 200 ppm for *Acinetobacter sp.* dcm5A*)* in 100 ml conical flasks. The flasks were then incubated on rotary shaker (150 rpm) at 30ºC. At regular intervals, 1.0 ml of the sample was taken out in an Eppendorf tube for growth curve study. The experiment was conducted in triplicate and growth was measured at 576 nm spectrophotometrically.

Extraction and estimation of residual quinalphos by Gas Liquid Chromatography (GLC)

3 ml aliquots of the inoculated culture mediums were withdrawn on alternate days for 15 days from the flasks where bacterial strains were grown in MBM2 broth and incubated (150 rpm at 30ºC). Quinalphos was extracted by partitioning 3 ml of the culture medium containing the pesticide with equal amount of ethyl acetate. This process was repeated for three more times to ensure the complete transfer of Quinalphos from the medium to ethyl acetate. The extracts were evaporated to near dryness (Univapo vacuum evaporator) and dissolved in 100 µL of ethyl acetate. The extracts were then analyzed by GLC.

The extracted samples were redissolved in 5 ml Ethyl acetate, and analyzed on Shimadzu chromatograph model GC-17 AAF, V3, 230 V LV model equipped with electron capture detector (ECD). SGE column; 30 x 0.25 mm i.d., I D-DPI 0.25 UM (Made in Australia). The flow rate of carrier gas used (Nitrogen) was 20 ml min^{-1}, the temperature of Injector, Column and Detector was 300ºC, 200ºC and 250ºC, respectively. One microlitre of sample was injected into the GLC with a 10 µL Hamilton micro syringe. The retention time for Quinalphos was 12.1 minutes. Quinalphos as low as 0.01 g L^{-1} could be detected accurately. The recoveries of quinalphos were routine more than 90%.

Results and Discussion

Results of present study of preliminary screening of bacteria, which were isolated from soil samples for biodegradation studies are given **(Table 1).** Among the five isolates (T1, T2, T3, T4 and T5),T1 and T2 showed maximum growth in broth supplemented with quinalphos as additional source of carbon and phosphorus. Hence, the two strains of bacteria were selected for further studies.

Table 1: Growth of Five Bacterial Strains[a] in Terms of Optical Density (λ 576nm) in Basal Medium with Quinalphos as Additional Source of Carbon and Phosphorus

Days	T1	T2	T3	T4	T5
0	0.201	0.219	0.183	0.187	0.182
3	0.399	0.310	0.294	0.301	0.212
5	0.602	0.699	0.425	0.330	0.250
7	0.814	0.900	0.614	0.411	0.479
9	0.985	1.086	0.745	0.617	0.682
11	1.193	1.302	0.998	0.823	0.820
13	1.269	1.375	0.914	0.831	0.801
15	1.397	1.414	0.904	0.800	0.711

[a]T1, T2, T3, T4 and T5 are the bacterial strains which were initially isolated for biodegradation study, and two best growing strains T1 and T2 were identified as *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A respectively.

Both the bacterial strains isolated have the capacity that they can survive in the soil either due to tolerance or due to their ability to degrade it, as the soil used in the present investigation was quinalphos exposed (25%EC@4L/ha, sprayedat regular intervals). The role of those organisms capable of responding to pesticides as a substrate and thereby derives energy and utilizable nutrients for their metabolism is significant in bioremediation study. Availability, low microbial toxicity and high nutritive value of the pesticide seem to be the properties that enhance the biodegradation of pesticides in the soil.

Soil characteristics

Soil used in the current investigations was sandy loam (71% sand, 21% silt and 8% clay) with pH 7.02, organic matter 0.92% and 9.8% moisture.

Identification of bacterial strains

Based on the DNA sequencing results, the bacterial strains T1 and T2 were identified as *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A respectively.

Optimum concentration of quinalphos for bacterial growth

Optimum concentration with quinalphos as a sole carbon and phosphorus sources for the growth of *Pantoea agglomerans* was 100 ppm while for *Acinetobacter sp.* dcm5Awas 200 ppm **(Figure 1).** Rests of the experiments with these two bacterial strains were performed at its optimum concentration of quinalphos.

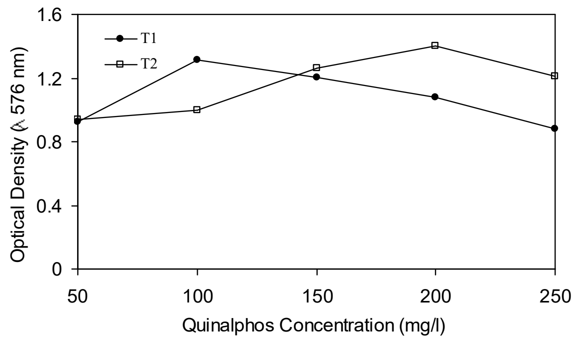

Figure 1: Optimum concentration of quinalphos utilized by *Pantoea agglomerans* T1 and *Acinetobacter sp.* dcm5A T2 as sole carbon and phosphorus sources

Growth pattern of the isolates

Growth kinetics of the isolates was studied by means of optical density of the culture media as well as the bacterial biomass, in 15 days culture. The growth behaviour in terms of optical density as obtained from the present study is given in Figure 2.a for *Pantoea agglomerans* and in Figure 2.b for *Acinetobacter sp.* dcm5A.It was apparent from the figures that when both the bacterial strains were grown in BM broth with normal carbon and phosphorus sources, followed almost linear growth pattern, in which growth increased regularly with days of incubation till 15 days. This was due to the presence of optimum conditions with sufficient and easily available carbon and phosphorus sources in the medium. Bacterial strains while grown in MBM1 broth and MBM2 broth, sigmoid growth pattern was observed in which lag phase followed by log phase and then a period of stability. This is because bacteria initially take more time to adapt themselves in the medium. Once adapted, they produce the enzymes that degrade quinalphos and utilized it as a sole carbon and phosphorus source. No further increase in growth rate was observed after 7 and 9 days for *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A, respectively which may be due to depletion of food sources. This shows that quinalphos concentration might be acting as a limiting factor.

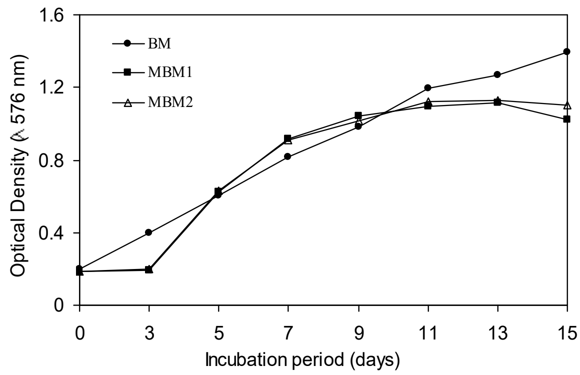

Figure 2a: Growth pattern of *Pantoea agglomerans* in terms of optical density in Basal Medium BM, Modified Basal Medium One MBM1 and Modified Basal Medium Two MBM2

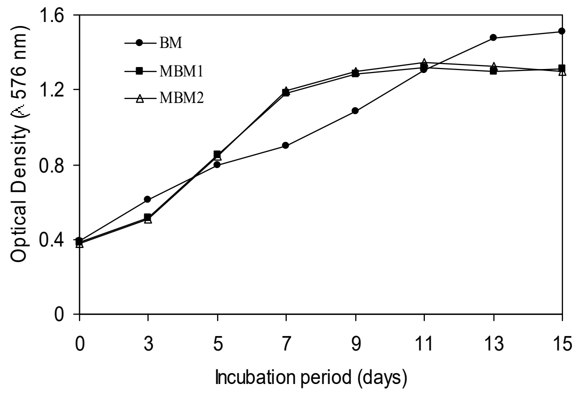

Figure 2b: Growth pattern of *Acinetobacter sp.* dcm5Ain terms of optical density in Basal Medium BM, Modified Basal Medium One MBM1 and Modified Basal Medium Two MBM2

Biodegradation of quinalphos in the medium

GLC analysis confirmed that both the bacterial strains degraded quinalphos and derived energy and utilizable nutrients for their metabolism in the medium supplemented with quinalphos as sole carbon and phosphorus sources. Quinalphos residues by bacterial strains *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A are shown in Table 2. On zero day just after inoculation of bacteria into the growth medium, 96.4 ± 0.11 µg/ml and 187 ± 0.52 µg/ml of quinalphos residues were detected for *Pantoea agglomerans* and *Acinetobacter sp.* dcm5A, respectively in the medium.

Table 2: Quinalphos residues (µg ml^{-1}) after the inoculation of *Pantoea agglomerans* and *Acinetobacter sp.* dcm*5A* in the Medium with Quinalphos as Sole Carbon and Phosphorus Source

Days	T1[a]	T2[b]
0	96.4 ± 0.11	187.0 ± 0.52
3	90.6 ± 0.02	162.4 ± 0.18
5	55.0 ± 0.26	99.5 ± 0.02
7	38.6 ± 0.04	38.0 ± 0.56
9	13.3 ± 0.03	22.7 ± 0.02
11	0.07 ± 0.01	11.4 ± 0.06
13	N D[c]	0.04 ± 0.01
15	N D	N D

[a]**T1 - *Pantoea agglomerans***
[b]**T2 - *Acinetobacter sp. dcm5A***
[c]**N D means that concentrations were below the detection limit**

In case of bacterial strain *Pantoea agglomerans* only 6.02 % of quinalphos was observed to be degraded till 3rd day. Thereafter quinalphos was degraded at a faster rate almost 60% on 7th day which further degraded to 99% by 11th day, but rate of degradation was proceed slower in comparison to degradation rate from 3-5th day. Most significant rate of degradation was observed between 3-5th day which was found to be 60.7%. These may be probably due to nutrient depletion during the log phase of degradation from 3-5th day. 100% degradation of quinalphos by this strain was observed after 11 days of incubation.Similarly, bacterial strain *Acinetobacter sp.* dcm5A degraded quinalphos upto 13% by 3rd day followed by sharp increase in growth rate resulting 80% degradation of quinalphos on 7th day. Most significant degradation rate of quinalphos was observed between 3-5th day that was 61.2% which showed that *Acinetobacter sp.* dcm5A is more efficient in utilizing quinalphos at higher concentration as their nutritive materials for their metabolism in comparison to *Pantoea agglomerans* (Figure 3). After this, degradation rate became slower and 100% degradation of quinalphos by this bacterial was observed after 13 days of incubation.

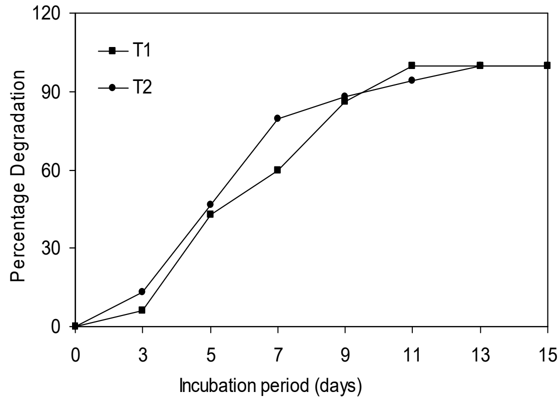

Figure 3: Percentage degradation of quinalphos by *Pantoea agglomerans* T1 and *Acinetobacter sp.*

dcm5A T2 in the medium supplemented with pesticideas a sole carbon and phosphorus sources

Degradation or detoxification of organophosphorus pesticides takes place by the action of microbes. It is generally through the hydrolysis of P-O alkyl and P-O aryl bonds. This reaction is termed as the important step in the detoxification and bacterial metabolism of organophosphorus compounds. The hydrolyses enzyme responsible for catalyzing this reaction is called as an esterase or phosphotriesterase (Brown, 1980; Kumar *et al.,* 1996) or Phosphatases (Rosenberg and Alexander, 1979). As a result of hydrolysis of quinalphos, there is a formation of 2-hydroxyquinoxaline as a primary metabolite like other organophosphorus insecticides, (Babu *et al.,* 1998). However, during the present study, no experiment was done for identification of the degradation products due to the nonavailability of authentic compounds of the metabolism. Similar report on the formation of two unidentified metabolites in culture media that were inoculated with *Chlorella vulgaris, Scenedesmus bijugatus, Synechococcus elongates, Phormedium tenue* and *Nostoc linckia,* isolated from a black cotton soil has been observed (Megharaj *et al.,* 1987). GLC analysis thus indicates that both the bacterial strains were capable of degrading quinalphos and derives nutritive materials from it when they were grown in the medium without any additional carbon and phosphorus sources. The parent compound (Rt 12.1 min) was degraded into two unidentified metabolites at Rt 5.6 and 14.4 min by 7th day onwards. Figure 4(A) & 4(B).

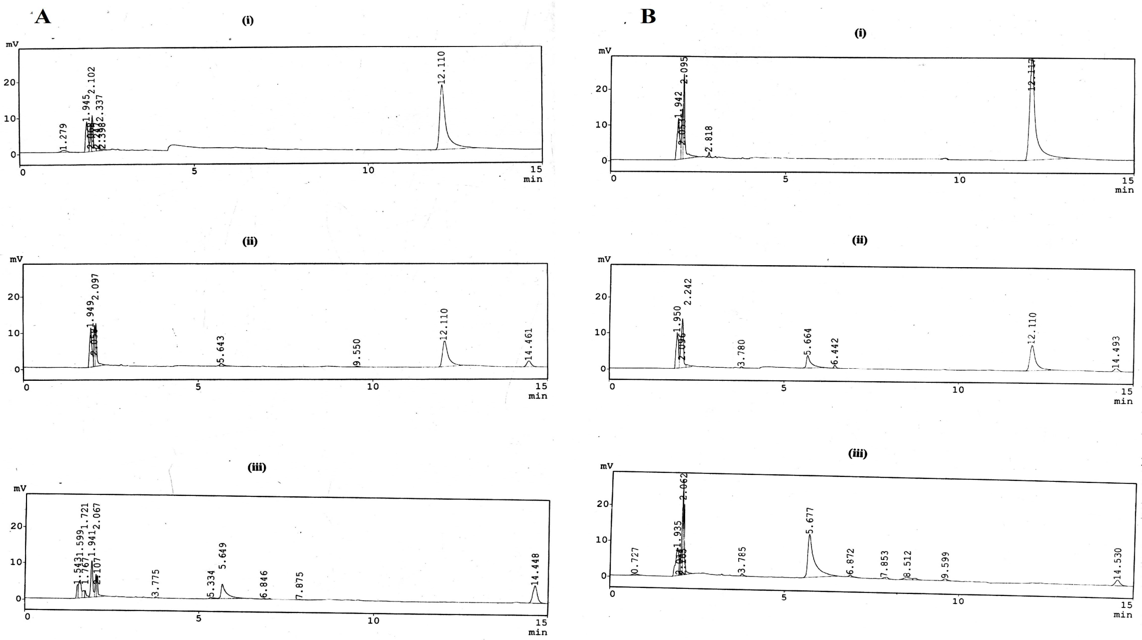

Figure 4: (A) Chromatogram of quinalphos (Rt 12.1 min) degradation by *Pantoea agglomerans* on: (i) Zero day (ii) 7th day (iii) 13th day. (B) Chromatogram of quinalphos (Rt 12.1 min) degradation by *Acinetobacter sp.* dcm5A on: (i) Zero day (ii) 7th day (iii) 15th day

Bacterial degradation of quinalphos has also reported earlier (Rangaswamy and Venkateswarlu, 1992). Both *A. lipoferum* and *Bacillus sp.*, were isolated from quinalphos treated soil, degraded quinalphos 32% and 44% respectively by the end of 7 days of incubation. Nearly 56% and 76% were degraded by both bacterial strains *A. lipoferum* and *Bacillus sp.*, respectively on 14 days. There was a notable decrease in the concentration of both monocrotophos and quinalphos during the incubation period even in un inoculated controls. From the uninoculated medium, about 40% of added monocrotophos was loss as compared to quinlphos as 16% loss of quinalphos from the corresponding uninoculated samples during the period of 11 days (Rangaswamy and Venkateswarlu, 1992). The results of this study clearly suggested that quinalphos is highly susceptible to microbial metabolism, thus supporting and conforming to the result of earlier reports (Megharaj *et al.,* 1987;Rangaswamy and Venkateswarlu, 1992).

Conclusion

The pattern and behavior of quinalphos in environment and the impacts of its residues on the population dynamics of soil microbes need further investigation in different fields having different crops. The results of current study provide important information about the microbial degradation of quinalphos. The study indicates that soil bacteria are good degraders of quinalphos and therefore could be a reason for its low persistence in the soil. Furthermore, progress in selecting suitable strains of bacteria, which can detoxify or convert the complex pesticide molecules into simple non-toxic residual molecules and use them as a source of nutrition, should lead to the development of field technology for site specific bioremediation for the treatment of soil or ground water which is contaminated by quinalphos.

Conflict of interest

The authors declare that they have no conflict of interest.

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Assessment of seasonal variations in the fine particulate matter of indoor air in sub urban area of Jammu District (J&K), India

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ABSTRACT

Indoor aerosol $PM_{2.5}$ is more harmful due to its penetration deep into lungs most ofpeoplespendingmore than 90% of their time indoor. The present study is the first timeinvestigation to evaluate the indoor aerosols ($PM_{2.5}$) in the households located in residential, commercial and industrial sub urban areas Jammu District (J&K) during different seasons of the two year study period (2017-2019). The indoor $PM_{2.5}$ was observed to exhibit deceasing trend i.e. more in summer> winter >rainy season. In non-wood fuel burning households exhibited annual average indoor $PM_{2.5}$ values below the values prescribed by CPCB and wood fuel burning households exhibited values above the values prescribed by CPCB. Moreover the indoor aerosols ($PM_{2.5}$) was observed be to almost four times higher in wood fuel burning households as compared with that of non-wood fuel burning households.

Introduction

Environmental and health conditions prevailing in the country are determined by air quality of a country (Singh, 2016; Ruhela *et al.*, 2022a; Ahamad *et al.*, 2022). Major proportion of the world's population resides in sub-urban and rural areas and consuming fuels like kerosene oil, wood and cow dung cakes to fulfil their energy demands (Ampitan and Oleyerind, 2015). Due to lack of complete premixing of the fuel and air during burning inside the cooking and heating stoves solid fuels are difficult to burn therefore liquid and gaseous fuels are preferred in which complete premixing of the fuel and air during burning take place easily (Smith, 2000; Mac Kinnon *et al.,* 2019; Ruhela *et al.*, 2022b). A lot of work and financial budget has been spent to control the major outdoor air pollutants but indoor pollution has received attention only recently. Indoor air pollution has been considered as being among the top five environmental risks to the public health by the U. S. Environment Protection Agency (EPA) as people spend long period indoors at home and at workplace (Morowska, 1999). One of the major environmental issues that need to be addressed in megacities was reported to be cooking fume pollution (Lin *et al.*, 2014) which not only causes indoor air pollution, but significantly contributes fine particulate matter ($PM_{2.5}$) in the ambient atmosphere (Zheng *et al.*, 2002; Robinson *et al.*, 2006; Abdullahi *et al.*, 2013).Diameter between 0.002µ to 100 µ of any solid or liquid droplet is called particulate matter (PM) which can be PM_{10} with an aerodynamic diameter ≤10µm or $PM_{2.5}$ with an aerodynamic diameter ≤ 2.5µm or ultrafine particles with an aerodynamic diameter of ≤0.1µm). $PM_{2.5}$ and ultrafine particles are considered most harmful because of their tendency to penetrate deeply into the lungs. Cooking operations emit $PM_{2.5}$ which poses great danger to several major organ systems like emissions industrial, power, mobile, residential, agricultural sources (Abbey *et al.*, 1995, Romieu *et al.*, 1996 and Lighty *et al.*, 2000; Bhutiani *et al.*, 2021).Various carcinogenic components like PAHs remain attached to black carbon of PM, (IARC, 2010a and b). Particulate matter has been estimated to attribute approximately 3% of cardiopulmonary and 5% of lung cancer deaths globally (Cohen *et al.*, 2004). Recent study has indicated that annual$PM_{2.5}$ was responsible for 3.1 million deaths and around 3.1% of global disability-adjusted life years (Lim, 2012). Potential risks to human health from cooking fumes were observed to be higher due their emission at a relatively lower height thereby signifying the importance of control of cooking fume pollution. In present study attempt has been made to evaluate the

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indoor aerosols ($PM_{2.5}$) in the households located in residential, commercial and industrial sub urban areas Jammu District (J&K) during different seasons of the two year study period (2017-2019).

Material and Methods

Study area and Sampling sites

The study area, District Jammu of J&K, lies between $32^0 15^/$ and $37^0 17^/$ north latitude to $72^0 35^/$ and $80^0 20^/$ East longitude at the foothills of Himalayan region. The specific study area (Sub urban area) was divided into three zones i.e. 1) Residential zone (R), 2) Commercial zone (C) and 3) Industrial zone (I). Each zone was further demarcated into seven sites based on the type of fuel used for cooking and ventilation conditions of kitchen for the sampling of indoor air. The description of the sampling sites has been tabulated in Table 1.

Table 1: The sampling sites of the study area

Zones	Codes of the site	Cooking fuel and kitchen conditions	Specific Location
Residential	SURLE	LPG and exhaust in the kitchen	Khour
	SURLWE	LPG and without exhaust in the kitchen	Narayana
	SURLM	LPG and modular kitchen	Parwah, Marh
	SURLHE	LPG-Heater(Induction) and exhaust in kitchen	HalkaMarh
	SURLHWE	LPG-Heater(Induction) and without exhaust in kitchen	PatyaliChak
	SURLHM	LPG-Heater(Induction) and modular kitchen	Gajansoo
	SURC	Traditional Cooking Stove (Chullah)	Karloop
Commercial	SUCLE	LPG and exhaust in the kitchen	Gajansoo
	SUCLWE	LPG and without exhaust in the kitchen	GhouManhasan
	SUCLM	LPG and modular kitchen	Sari
	SUCLHE	LPG-Heater(Induction) and exhaust in kitchen	Padrore
	SUCLHWE	LPG-Heater(Induction) and without exhaust in kitchen	Deichak
	SUCLHM	LPG-Heater(Induction) and modular kitchen	Pounichak
	SUCC	Traditional Cooking Stove (Chullah)	Sarora
Industrial	SUILE	LPG and exhaust in the kitchen	Bawe Talab
	SUILWE	LPG and without exhaust in the kitchen	Patta
	SUILM	LPG and modular kitchen	Marjali
	SUILHE	LPG-Heater(Induction) and exhaust in kitchen	Shamachak
	SUILHWE	LPG-Heater(Induction) and without exhaust in kitchen	SukaPakhian
	SUILHM	LPG-Heater(Induction) and modular kitchen	Deharan
	SUIC	Traditional Cooking Stove (Chullah)	Bawe Talab

Indoor air sampling and collection of data

The sampling of indoor $PM_{2.5}$ was done thrice (once each in Kitchen, drawing room and bed room of two room accommodation and thrice in same one-room accommodation on three consecutive days) during the summer season (March-June), rainy season (July-October) and winter season (November-February) using Sioutas Personal Cascade Impactor with Leland Legacy Sampling Pump on Zefluor™ supported with PTFE filter paper of 0.5 micron pore size and 25 mm diameter for 24 hours at 9 lpm. Central Pollution Control Board (2014) prescribed the Gravimetric method was used. The filter paper was weighed usingMettler Toledo Microbalance Model MS105DU with a sensitivity of 0.01 mg. The determination ofthe $PM_{2.5}$ was made by the formula:

Conc. of $PM_{2.5}$ ($\mu g/m^3$) = $(W_1-W_0) \times 10^6$ / Volume of air

Where,
$W_1$and W_0 is Final and Initial weights of filter paper in mg.

All data was subjected to One-way ANOVA and Post Hoc Test using IBM SPSS Statistics Version 22 analysis after calculating average values with standard deviation.

Results and Discussion

The results of all the sites are given in table 2 to 5. The sites (i.e. SURLE, SURLWE, SURLM, SURLHE, SURLHWE and SURLHM) in in

Table 2: Seasonal variations in indoor $PM_{2.5}$ levels in residential zone sites of study area

SITE	$PM_{2.5}$ µg/m^3 during			
	Summer season	Rainy season	Winter season	Significance value (p) (One-way ANOVA)
SURLE	38.50±14.30 (25.45-58.10)	19.12±2.87 (14.12-21.29)	21.87±4.18 (16.66-27.77)	0.31
SURLWE	43.63±20.11 (26.89-70.60)	19.09±3.73 (16.20-25.23)	27.46±6.50 (21.06-36.57)	0.33
SURLM	34.14±13.58 (20.67-51.38)	15.96±2.11 (13.88-19.44)	18.81±2.49 (15.70-22.68)	0.34
SURLHE	40.99±16.21 (27.77-62.69)	19.74±6.08 (15.04-29.86)	31.32±6.76 (24.53-41.20)	0.32
SURLHWE	46.44±20.08 (30.09-72.68)	24.18±4.67 (19.21-31.48)	36.87±6.19 (30.09-45.83)	0.31
SURLHM	37.70±13.77 (24.65-56.48)	18.38±3.23 (14.00-22.45)	20.71±3.81 (16.20-26.38)	0.36
SURC	140.5±23.70 (141.57-183.92)	115.05±20.60 (119.56-149.87)	127.5±16.80 (133.44-164.53	0.39

Table 3: Seasonal variations in indoor $PM_{2.5}$ levels in Commercial zone sites of study area

SITE	PM2.5µg/m3 during			
	Summer season	Rainy season	Winter season	Significance value (p) (One-way ANOVA)
SUCLE	41.19±15.78 (28.89-62.26)	35.23±7.49 (28.24-45.60)	37.84±9.35 (30.09-50.92)	0.35
SUCLWE	48.11±21.44 (31.77-76.15)	36.83±7.64 (30.32-47.91)	40.31±10.48 (31.48-54.62)	0.31
SUCLM	35.59±8.58 (27.77-47.68)	28.97±4.94 (23.61-36.11)	32.17±5.57 (26.38-40.74)	0.29
SUCLHE	55.97±27.41 (34.72-92.12)	37.76±7.50 (31.48-48.37)	42.24±12.17 (32.87-59.25)	0.41
SUCLHWE	59.81±31.11 (37.74-100.23)	40.23±8.84 (32.40-52.08)	44.97±13.48 (34.25-63.88)	0.33
SUCLHM	41.12±15.24 (29.72-62.50)	32.78±7.18 (26.38-42.82)	35.37±7.19 (28.24-45.60)	0.36
SUCC	174.12±16.96 (147.29-181.21	144.93±20.80 (111.39-174.56	158.35±14.81 (141.20-181.21)	0.35

residential zone, sites (i.e. SUCLE, SUCLWE, SUCLM, SUCLHE, SUCLHWE and SUCLHM) Commercial zone and the sites (i.e. SUILE, SUILWE, SUILM, SUILHE, SUILHWE and SUILHM) in industrial zone were observed to be exhibit insignificant variations ($p>0.05$) in Indoor $PM_{2.5}$ except households at sites SURLM , SUCLM and SUILM with Modular kitchen exhibited significantly ($p<0.05$) lowest values of indoor $PM_{2.5}$ as compared with other types of households with non wood fuel burning practice.. Patel *et al.* (2017) while assessing spatio-temporal indoor particulate matter in households in Raipur, India also observed that PM concentrations in kitchen and adjoining rooms was effected with ventilation. Among the Residential Zone sites (i.e. SURLE, SURLWE, SURLM, SURLHE, SURLHWE and SURLHM), SURLHWE exhibited the highest value of 46.44µg/m^3 during summer season and SURLM exhibited the lowest value of 15.96µg/m^3 during rainy season. Among Sub-urban Commercial Zone sites (i.e. SUCLE, SUCLWE, SUCLM, SUCLHE, SUCLHWE and SUCLHM), SUCLHWE exhibited the highest value of 59.81µg/m^3 during summer season and SUCLM exhibited the lowest value of 28.97 µg/m^3 during rainy season and among Sub-urban Industrial Zone sites (i.e. SUILE, SUILWE, SUILM, SUILHE, SUILHWE and SUILHM), SUILHWE exhibited the highest value of 62.63µg/m^3 during summer season and SUILM exhibited the lowest value of 31.2µg/m^3 during winter season.

The critical analysis of indoor PM $_{2.5}$ of kitchens , drawing rooms and bedrooms in the sub urban households during study period (2017 -2019) revealed that all the kitchens, drawing rooms and bedrooms exhibited significantly ($p<0.05$) higher values during summer season followed by winter season and lower values during rainy seasons.The non-wood fuel burning households during summer seasons exhibited significantly ($p<0.05$) higher values of indoor PM followed winter season and lower values during rainy seasons during the two year study period .The present observation was contrary to that of Shukla and Sharma (2008) who observed the lowest concentration of PM_{10} during monsoon period and higher variability in summers in Kanpur because of higher wind speed in summers but present observation find support from the work of Kamath and Lokeshappa, (2014) who observed concentration of pollutants were more in summer in comparison to the pre monsoon and post monsoon seasons in Bangalore and that of Cheng and Wang-Li (2019) who reported $PM_{2.5}$

Table 4: Seasonal variations in indoor $PM_{2.5}$ levels in Industrial zone sites of study area

SITE	$PM_{2.5}$ µg/m^3 during			
	Summer season	**Rainy season**	**Winter season**	**Significance value (p) (One-way ANOVA)**
SUILE	47.89±20.10 (34.08-75.23)	36.87±8.38 (29.62-48.37)	38.11±10.61 (29.62-52.54)	0.32
SUILWE	56.38±26.53 (36.79-91.43)	38.77±9.04 (30.32-51.38)	41.12±12.82 (30.32-58.33)	0.36
SUILM	39.00±12.19 (29.56-56.71)	31.28±7.20 (24.53-41.43)	31.20±7.33 (24.53-41.43)	0.35
SUILHE	59.09±29.17 (37.03-98.37)	44.94±9.84 (36.80-58.33)	45.48±11.01 (36.80-60.87)	0.42
SUILHWE	62.63±30.86 (39.72-103.24)	48.95±12.68 (38.65-66.66)	48.71±12.39 (38.65-65.50)	0.39
SUILHM	42.89±15.18 (30.08-63.88)	34.56±6.71 (28.93-43.51)	36.33±8.78 (28.93-48.37)	0.35
SUIC	178.12±16.96 (149.29-181.21	164.12±16.96 (147.29-180.21	161.11±18.61 (121.54-159.96	0.39

Table 5: Year wise seasonal variations of Indoor PM $_{2.5}$ (µg/m3) levels in Study area

One-way ANOVA (zone and season wise) variations Significant ($p<0.05$)

Average Indoor aerosols (PM $_{2.5}$) level in Study area with wood and non wood burning for cooking during two year study period was calculated to be 93.71±59.98µg/m^3.

Site	Summer (Non-Wood fuel)	Summer (Wood fuel)	Rainy (Non-Wood fuel)	Rainy (Wood fuel)	Winter (Non-Wood fuel)	Winter (Wood fuel)	Two Year Study Period (Non-Wood fuel)	Two Year Study Period (Wood fuel)	Average Two Year Study Period
Residential	40.23±4.40 (20.67-72.68)	140.7 ±1.20 (141.57-183.92)	19.41±2.68 (13.88-31.48)	115.475±5.77 (119.56-149.87)	26.17±7.00 (15.70- 45.83)	127.49±8.41 (133.44-164.53	28.60±10.62 (13.88-72.68)	127.89± 12.62 (119.56-183.92)	78.24± 55.37 (13.88-83.92)
Commercial	46.96±9.42 (27.7100.23)	175.06±8.69 (147.29-181.21)	35.3±3.97 (23.61-52.08)	144.24±4.30 (111.39-174.56)	38.81±4.66 (26.38- 63.88)	158.76± 7.21 (141.20-181.21	40.35±5.98 (23.61-100.2)	159.35±15.41 (111.39-181.21)	99.85±66.01 (23.61-81.21)
Industrial	51.31±9.47 (29.56- 103.2)	179.24±6.61 (149.29-181.21)	39.22±6.60 (24.53- 66.66)	146.115±5.31 (147.29-180.21	40.15±6.34 (31.20- 65.50)	162.24±3.23 (121.54-159.96	43.56±6.72 (24.53-103.2)	162.53±16.56 (121.54- 181.21)	103.04± 66.13 (24.53-81.21)
Average	46.16 ± 5.58 (20.6- 103.2	165.00 ± 21.13 (141.5-183.92	31.31 ±10.49 (13.8-66.66)	135.27± 17.17 (111.39- 180.21)	35.04 ± 7.71 (15.70- 65.50	149.49 ± 19.13 (121.5-181.21)	37.50 ±7.87 (13.88-103.2)	149.92± 19.14 (111.39- 183.92)	93.71 ± 59.98 (13.88-83.92)

concentrations higher in summer and lower in winter in North Carolina.The study also revealed that Kitchen exhibited significantly ($p<0.05$) higher values of indoor $PM_{2.5}$ followed by Bedrooms and lower values at drawing room.The higher values of indoor PM $_{2.5}$ during summer seasons followed winter season and lower values during rainy seasons in all the wood fuel burning households were observed to be insignificant ($p>0.05$). The higher values of indoor PM $_{2.5}$ at industrial sites as compared with that of commercial sites followed by residential sites during all the seasons of two year study period were also observed to be insignificant ($p>0.05$).

All the households with non wood fuel burning for cooking exhibited average annual indoor PM $_{2.5}$ of 37.50±7.87 μg/m^3 which was observed to be below the annual limit of 40 μg/m^3 as prescribed by CPCB .whereas all types of households with wood fuel burning for cooking at study area exhibited annual indoor PM $_{2.5}$ of 149.92±19.14 μg/m^{3}which was observed to be above the annual limit of 40 μg/m^3 as prescribed by CPCB observation find solution by Chafe *et al.,* (2014) who suggested only by improving household cooking conditions ambient air quality would be improved. The present observation also support work of the Ojo *et al.,* (2015) who observed the mean indoor $PM_{2.5}$ using wood fuel to be 4584μg/m^3, 1657 μg/m^3, and 2414 μg/m^3 for the traditional, alternative mud brick stove and Envirofit G-series in Nepal respectively.

Statistical analysis revealed exhibited significantly ($p<0.05$) higher values (149.92±19.14 μg/m^3) of indoor PM $_{2.5}$ in households with wood fuel burning for cookingas compared with that (37.50±7.87μg/m^3) of households with non wood fuel burning for cooking at study area. Zhou *et al.* (2011) observed particulate matter due to cooking practices lowest in high socio-economic status households and highest in low socio-economic status households Moschandreas *et al.* (1980) observed higher indoor levels of respirable particulates, carbon-monoxide and organics due to wood burning in the households. This observation find support from the work of (Shrestha and Shrestha, 2005) who observed average PM_{10} levels three times higher in households using biomass fuels than those using cleaner fuels like LPG, Kerosene and biogas. Jiang and Bell (2008) also reported three times higher PM_{10} levels in rural kitchens as compared with that of urban kitchens and also that more than six times higher PM_{10} levels at the time of cooking than at time of non-cooking for rural kitchens.

Conclusion

Indoor $PM_{2.5}$ average values in wood fuel burning households of study area exhibited value (149.92±19.14 μg/m^3) almost four times higher than the value (37.50±7.87 μg/m^3) of non wood fuel burning households. Fuel wood burning added more $PM_{2.5}$ indoor pollutant. Year wise and Site Wise variations (except at Household with modular kitchen) exhibited insignificant ($p>0.05$) values as analysed by One way ANOVA and Post Hoc Test during Statistical Analysis of data by IBM SPSS Statistics Version22. Season wise variations exhibited significant ($p<0.05$) values as analysed by One way ANOVA and Post Hoc Test during Statistical Analysis of data by IBM SPSS Statistics Version 22.

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Conflict of interest

The authors declare that they have no conflict of interest.

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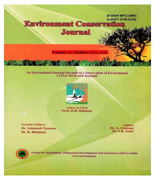

Fish antimicrobial peptides: at a glance

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ABSTRACT

Generally, antimicrobial peptides (AMPs) are considered as an important part of innate immunity, due to which they provide the first line of defence against various pathogens. Additionally, they also kill pathogens that show resistance towards many antibiotics. Fishes are regularly challenged by various pathogens which not only affect their health but the risk of becoming resistant to conventional antibiotics is also increasing. As fishes shows more dependence on innate immunity, AMPs can aid as important defensive weapon in fishes. In general, AMPs exhibit various multidimensional characteristics such as neutralization of pathogens (viral, fungal & bacterial), rapidly diffuse to the infection site, and other immune cells recruitment to the infected tissues. AMPs also show various biological effects such as immunomodulation, neutralization of endotoxin and angiogenesis induction. There are numerous AMPs that have been isolated from fishes but not fully characterized at molecular level. In this review we basically focus on approaches used to design new AMP, machine learning approach, current objectives of AMPs and future prospects.

Introduction

Various organisms develop antimicrobial peptide (AMP) as an important component of their innate immune response. Due to dependence of fishes on their innate immune system, antimicrobial peptides are considered major component as it forms the first line of defence (Hancock, 1997; Hancock & Scott, 2000). The AMP as part of innate immunity gives the advantage that they can function even without memory or high specificity. AMP helps in defencing the host by employing cytotoxicity on the attacking pathogenic microorganisms. In higher organisms they act as immune modulators (Zanetti, 2004). There are numerous pathogens in aquatic environment. Adaptive immune system is poorly developed in fishes either due to restricted classes of immunoglobulin or their functional diversion (Magnadottir, 2010). Antimicrobial peptide is also regarded as host defence peptides that constitutes innate immune system. By pore-forming "ionophoric" or disruptive "lytic" actions, it gives protection against viral, bacterial, fungal and other pathogenic infections (Smith *et al.,* 2010; Ageitos *et al.,* 2017). The site of secretion of antimicrobial peptides are mucus, saliva, circulatory system and those areas which are at high-risk pathogen targets (Noga *et al.,* 2010). Fish are considered as "gold mines" of AMPs whose immunomodulatory and antimicrobial activities have been extensively studied (Valero *et al.,* 2013; Shabir *et al.,* 2018). For the healthy growth of fishes, it is very important to

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give them suitable conditions especially under stressful environment in aquaculture. In stress conditions immunological conditions of fish reduces making it vulnerable to pathogens. The immune capacity of fish can be enhanced by many ways. First way is to examine the immune suppression of fish and take suitable measures when required. Another method to examine elevation in the expression of antimicrobial peptide earlier to stress incident leads to immune suppression of fish (Noga *et al.,* 2011b). PGLa, magainins and maganin 2 are the most studied AMPs that were structurally linear peptide and were extracted from the skin of an African frog (*Xenopus laevis*). Numerous functions showed by them are healing of wound, immunomodulation (Chakchouk *et al.,* 2014), function as chemokines and cause their production, lipopolysaccharide inhibition and initiate response of acquired immune system by recruiting antigen presenting cells (Niggemann *et al.,* 2014). There are AMPs which act against viral infections such as cecropin and mellitin blocks the production of cell associated HIV-1 by suppressing the expression of HIV-1 gene (Weisshoff *et al.,* 2014). There are 8164 entries of peptides in database ($CAMP^{R3}$) Collection of Anti-Microbial Peptides, mostly entries (74%) are from animals (Figure 1) (Waghu *et al.,* 2016). A database (DADP) Database of Anuran defence Peptides contributes the sequences of at least two thousand peptides (Novkovic *et al.,* 2012).

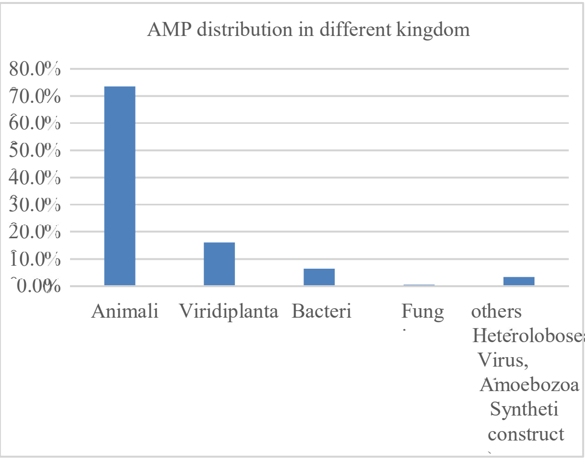

Figure 1: AMP distribution in different kingdoms on the basis of sequences in $CAMP^{R3}$ database. Source: (Rončević *et al.,* 2019).

Features on which the activity of AMPs depends

The major physiochemical properties on which the structure-function relationship of AMP depends are: hydrophobicity (Bahar & Ren 2013; Rončević *et al.,* 2017), charge (Walkenhorst *et al.,* 2013; López *et al.,* 2018), size (Hou *et al.,* 2011; Bahar & Ren 2013), amphipathicity (Edwards *et al.,* 2016; Mahlapuu *et al.,* 2016), solubility (Chen *et al.,* 2005), helicity (Huang *et al.,* 2010), sequence, and secondary structure (Tossi *et al.,* 2000). Although some amino acid that are highly conserved at their position between many antimicrobial properties. The AMPs activity is dependent on combination of various properties. Some properties of AMPs depend upon its interaction with the lipid bilayer membrane of target cells. For the better understanding between structure-function relationship, hence, need to recognize properties that are responsible for specificity and activity of AMPs. All these properties require to observed together since to get desired modification if one parameter change may change the other parameter.

Structural properties of AMPs

On the basis of secondary structure most AMPs can be characterized as following: alpha-helix, beta-sheet, loop and extended. Among them alpha-helix and beta-sheet are most usual (Powers & Hancock 2003). Till date the most studied AMPs are alpha-helical. Structurally in alpha-helix the two adjoining amino acids are at a distance of 0.15nm and the angle between them is 100 degrees. The residues that are not present in the alpha-helical AMP sequence are cysteine (Lewies *et al.,* 2015). (Brogden 2005). Examples- melittin, dermaseptin, and cercopins. In the formation of beta-strand the two beta-strand are linked with disulphide bond. (Bahar & Ren 2013; Pasupuleti *et al.,* 2012) It is cysteine residue that helps in the formation of disulphide bond and provide stability to the structure (Brogden 2005). (Lewies *et al.,* 2015 Due to disulphide the peptide acquires cyclic configuration and which is important for antimicrobial activity (Matsuzaki *et al.,* 1999). Examples- protegrin, defensins and droscomycin. Linear extended antimicrobial peptides are linear in shape without secondary structure (Seo *et al.,* 2012). There are some amino acids which are over expressed in them. The peptides are rich in arginine, proline, or histidine. Examples- apidaecin and indolicidin (Seo *et al.,* 2012). In loop antimicrobial peptide single disulphide bond is sufficient to acquire loop confirmation structure (Seo *et al.,* 2012). Structural modification and immobilization on the surface can be done easily in AMPs as they are made up of amino acids (Costa *et al.,* 2011). Synthetic peptides can be prepared with the help of recombination expression system (Ramos *et al.,* 2013) or by chemical synthesis (Wade *et al.,* 2012).

Synthesis of AMPs

Generally, AMPs are ribosomally synthesized and encoded by gene (in case of eukaryotes) or may accumulated by versatile enzyme named non-ribosomal peptide synthetase (NRPS) (Papagianni, 2003). Fungi and bacteria used the latter process (Finking & Marahiel, 2004) and integration of non-proteinogenic amino acid are permitted into the peptides and peptides are modified additionally with ring formation, hydroxylation, acylation and glycosylation (Walsh *et al.,* 2013; Hancock & Sahl, 2006). The known amino acids that are non-proteinogenic are ~ 500 at least, having added functional and structural properties that may help the activity of peptide significantly. Actually, in this manner the antibiotics of cyclic peptide vancomycin, gramicidin S and polymyxin B are prepared (Hancock & Sahl, 2006) and in their sequences all contain some non-proteinogenic amino acids (Walsh *et al.,* 2013). Mostly all forms of life, including bacteria produces peptides that are synthesized ribosomally, encoded by gene (Mahlapuu *et al.,* 2016; Waghu *et al.,* 2016). Often, the genes of various antimicrobial peptides are aggregated at an individual locus of chromosome, like in alpha and beta-defensins (Lai & Gallo, 2009) and may co-expressed. Moreover, they are generally expressed as an inactive precursor, having a region of signal peptide and pro-piece that function to inactive the mature peptide until it is transported to the infection site, where it is released proteolytically. That is the reason, propiece is generally anionic and mature peptide is cationic to complement each other. Mostly, N-terminal of AMP sequence is pro region, but in some instances C-terminal like for some plant and fish peptides (Patrzykat *et al.,* 2003). Thus, the potential of AMPs regulated by the level of expression as well as presence and abundance of suitable proteases at the right time to right place for peptide cleavage, majorly at dibasic cleavage sites (Lai & Gallo, 2009). The most common property of eukaryotic and prokaryotic proteins is signal peptide and require to enter secretory pathways (Von 1990). An important feature of AMPs is for a given class signal regions may be highly conserved than mature peptide (Patrzykat *et al.,* 2003). Majority of AMPs that are encoded by gene undergoes post-translation modifications, recently classified into more than 15 types like capping of C-terminal and N-terminal (amidation, pyroglutamic acid formation, acetylation), formation of disulphide bridge, glycosylation, phosphorylation, hydroxylation, halogenation etc.

Mode of action

The outer surface of eukaryotic cell is made up of zwitter ionic phospholipids namely sphingomyelin and phosphatidylcholine while the prokaryotic cell surface is made up of negatively charged teichoic acid or lipopolysaccharides (Dolis *et al.,* 1997). The primary mechanism for antimicrobial activity appears to be the electrostatic interaction of peptides with negatively charged molecules on the membrane. In some cases, the mode of action of AMPs in target cell by cell membrane translocation and inhibition of crucial cellular processes like cell wall synthesis, nucleic acid synthesis, protein synthesis, and enzymatic activities. The other factors were also important for the transportation of peptide through membrane like fluidity of the outer membrane, molecular architecture, negative charge concentration, charge and magnitude of the outside membrane (Kondejewski *et al.,* 1999). The membrane adsorption and insertion of antimicrobial peptides was regulated by membrane fluidity. On the basis of action mechanism AMPs mainly classified into membrane acting and non-membrane acting peptides. MPP (membrane permeabilizing peptides) are generally indicated by cationic peptides that are able to form transient pore on the membrane. The non-membrane permeabilizing peptides having potential to cross through the membrane without membrane permeabilization. There are some antibacterial peptides that form transmembrane pores on the cell membrane of target cells such as LL-37 (Henzler *et al.,* 2003), magainins (Hallock *et al.,* 2003), melittin (Yang *et al.,* 2001), and defensin. Some antimicrobial peptides are able to translocate through cell membrane and by inhibiting crucial cellular processes leads the cell to death such as, mersacidin (Brötz *et al.,* 1997), pyrrhocidin (Kragol *et al.,* 2001), indolicidin (Friedrich *et al.,* 2001), pleurocidin (Patrzykat *et al.,* 2002), dermaseptin (Patrzykat *et al.,* 2002), and buforin II (Park *et al.,* 2000). There are some antifungal peptides that shows their antimicrobial action through the production of reactive oxygen species such as, lactoferrin (Patrzykat *et al.,* 2002), histatin (Kavanagh & Dowd 2004), melittin (Park & Lee 2010), and papiliocin (Hwang *et al.,* 2011). AMPs encourage membrane damage by disruption of lipid bilayer, by formation of pores or by membrane thinning in target cells (Lohner & Prenner 1999). The mode of action of antimicrobial peptides was described through several models. The mechanism of cellular uptake of AMPs are classified into energy independent and energy dependent uptake mechanisms. The mechanisms of energy independent uptake involve carpet model, toroidal model, or barrel-stave model and mechanisms of energy dependent uptake involves micropinocytosis (Figure 2).

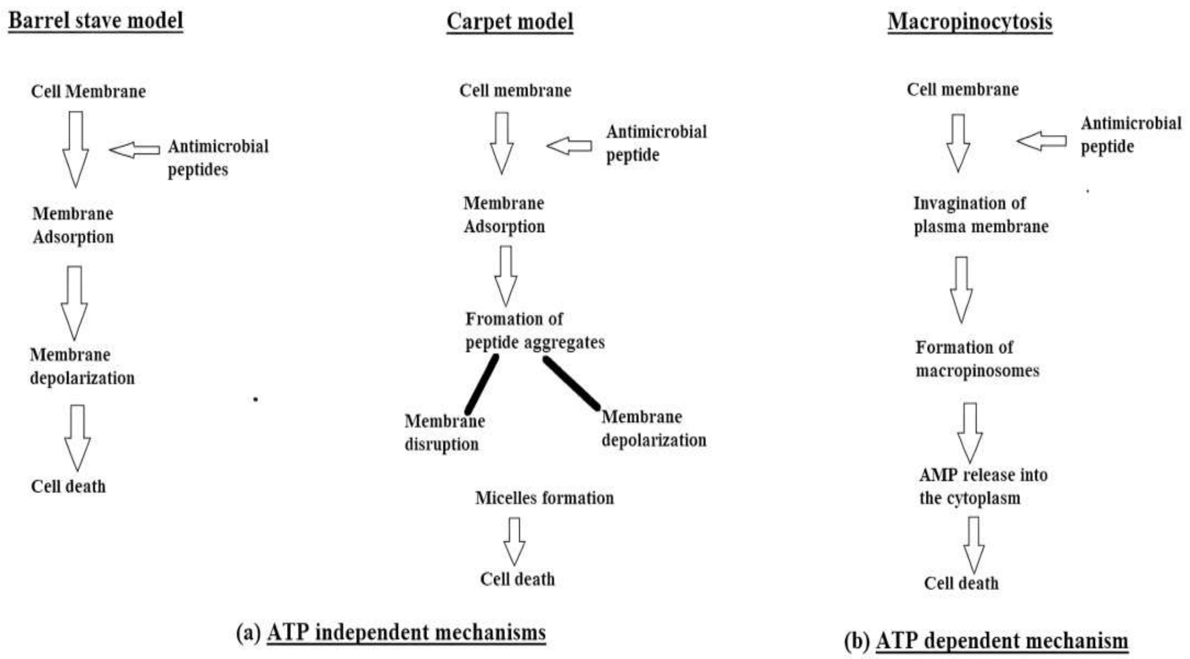

Figure 2: AMPs mode of action. (a) The mechanism that are independent of energy. (b) The mechanism that are dependent of energy (Source: Pushpanathan *et al.,* 2013).

Barrel-Stave model

The peptide monomer of AMPs gets accumulated on the surface of membrane in a perpendicular direction and acquires the structure of barrel-stave followed by membrane insertion (Yang *et al.,* 2001). Alpha-helical and beta-sheet peptides with hydrophobic surfaces are outward facing the barrel and interact with acyl chains of membrane, while hydrophobic surfaces are inward facing the barrel (Giuliani *et al.,* 2007) formed water filled pore in the transmembrane so that intracellular content released and resulting cell death. Examples, AMPs that follows this model are gramicidins and alamethicin (He *et al.,* 1996; Zhang *et al.,* 2001).

Carpet model

On the membrane surface the peptides get initially associated and establish a local carpet. After reaching threshold concentration, permeation of membrane was induced by the peptide that results in cell membrane destruction leads to microbial cells lysis (Oren & Shai 1998).

Toroidal pore model

The adsorption of AMPs takes place on the membrane in carpet form with perpendicular orientation they inserted into the membrane causing the membrane disruption (Matsuzaki *et al.,* 1997). Unlike two models, during insertion the peptides remain bound constantly to lipopolysaccharides of the membranes of bacteria. The peptides that are aggregated either after or prior binding with the surface of membrane induced depolarization of membrane and forms transmembrane pores of toroidal shape with the formation of micelle that leads to death of cell (Sengupta *et al.,* 2008).

Macropinocytosis

Macropinocytosis is the energy independent intake way of antimicrobial peptides, in which the formation of vesicle, macropinosomes, takes place by the inward folding of the membrane of target cell with peptide. Therefore, vesicle containing antimicrobial peptides gets discharge in cytoplasm and employ its antimicrobial potency (Madani *et al.,* 2011).

Post-translational modifications of AMPs

Several AMPs are synthesizing directly in their active forms but in some AMPs posttranslational modification is must for their functions. Various post-translational modifications are: proteolytic cleavage (Shinnar *et al.,* 2003), formation of disulphide linkage (Mangoni *et al.,* 1996), glycosylation (Oman *et al.,* 2011), amidation (Rifflet *et al.,* 2012), methylation (Hancock & Chapple 1999), addition of D-amino acids (Kreil 1997; Kamatani *et al.,* 1991), and phosphorylation (Goumon *et al.,* 1996).

Expressional regulation of AMPs: In animal

There are plenty of microbial infections are faced by living organism on regular basis and hence for recognition of pathogen as well as to defend attack of pathogen have developed a complex immune response. The recognition mechanism of microbes occurs within animals that aid them to discriminate between attacking pathogens (Lemaitre *et al.,* 1997). The recognition of pathogen takes place with the interaction between pattern-recognition receptors present on of host cell surface and molecular structures present on the pathogen (Medzhitov *et al.,* 1997).In non-chordates and chordates, the recognition of pathogen was done by many proteins having c-type lectin domains (Vasta *et al.,* 1999). PGRP (Peptidoglycan recognition proteins) are also takes part in identification of pathogen and are mostly conserved from insects to mammals (Kang *et al.,* 1998). There are several pathways that have been identified and mediate the gene expression of AMP (Imler & Hoffmann 2000). The signalling pathways of antimicrobial defence is highly conserved among fishes, insects and mammals (Beutler, 2000) form *Drosophila* a perfect design for understanding innate immune responses of animal. NF-kB like transcription factors mediates the AMP gene induction in *Drosophila* that comprises of three Re1 proteins: Relish, Dorsal and DIF (dorsalrelated immunity factor) and includes two pathways namely imd (immune deficiency) and Toll pathway (Figure 3) (Levashina *et al.,* 1998), which are homologous to mammalian TNFR (Tumour necrosis factor receptor) and TLR (Toll-like receptor).

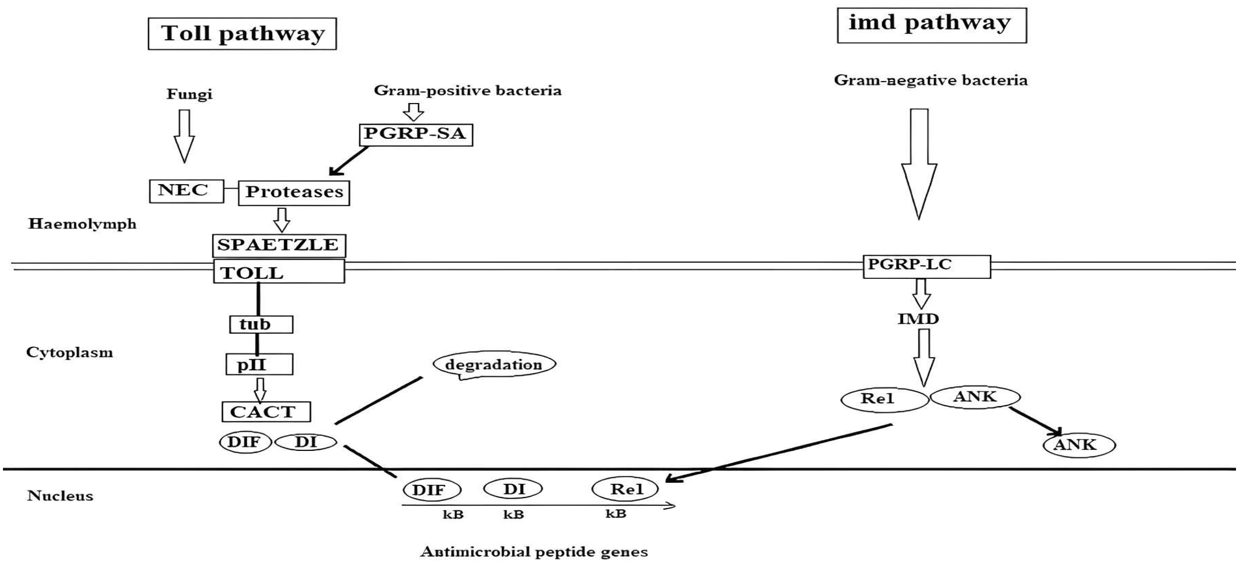

Figure 3: AMPs regulation in *Drosophila* by the pathway of Toll and imd (Source: Shabir *et al.,* 2018)

The dorsal and dorsal related immunity factor are controlled by Toll transmembrane receptor protein linked pathway (Rutschmann *et al.,* 2000), while the Relish is regulated by IMD (Immune deficiency gene). The gene expression of AMP is suppressed on mutations of these two pathways (Lemaitre *et al.,* 1996). The AMP encoding gene expression differs regarding distinct pathogens and is mostly depending on stimulation of Re1 and Toll pathways. Normally, IMD/Relish route is stimulated by the bacteria that are gram negative whereas Toll pathway is activated by the bacteria that are gram positive and fungi. For instance, the Drosomycin gene is induced by the bacteria that are gram positive and fungi which is IMD/Relish dependent signalling pathway whereas Drosomycin is regulated by Toll-DIF-Dorsal. On contrary, Diptericin is induced by the bacteria that are gram negative which depends upon Toll-DIF-Dorsal signalling pathway while Diptericin is regulated by IMD/Relish (Imler & Hoffmann 2000; Lemaitre *et al.,* 1996). The Toll pathway is very important for the survival after fungal infection, while the IMD pathway is necessary after the infection of gram-negative bacteria (Lemaitre *et al.,* 1995). NF-kB activity is also regulated by TNF signalling in mammals. In *Drosophila* and mammals, MAPK (mitogen activated protein kinase) pathway have been involved in gene regulation of AMP (Han *et al.,* 1998).

In fishes

The bacterial antigen of a typical *Aeromonas salmonicida* induces the piscidine gene in Atlantic cod (*Gadus morhua*) (Browne *et al.,* 2011). In the same way, numerous PAMPs like peptidoglycan and LPS induces the expression of beta-defensin genes in fishes (Casadei *et al.,* 2013). The cell walls of bacteria like peptidoglycan & LPS and poly I:C the viral synthetic analog might be manage as immunostimulant in fishes to activate pattern recognition pathway causing AMP expression and finally given immunity against pathogens in fish. Under stress conditions DAMPs (Damage-associated molecular patterns) releases that induces histone derived AMPs (Terova *et al.,* 2011). Imbalance iron levels or anaemia in biological system induces hepcidin in fish and in response to transferrin its expression changes (Chen *et al.,* 2008; Fraenkel *et al.,* 2009). Additionally, AMPs promotor region having binding site for many sequence-specific DNA-binding factor that establish their control by certain stimulation pathways and aiding their essential role in immunity and other biological functions (Shewring *et al.,* 2011; Katzenback 2015; Chaturvedi *et al.,* 2018).

Approach used to design new AMP Extraction and assay –guided isolation

Earlier, novel AMPs recognition required to operate many samples from similar species to acquire little amount of functional peptides. Homogenization of primary tissue was succeeded by the removal of peptide and many steps was involved to isolate crude peptide, especially by chromatographic techniques. There are cases, where AMP production stimulation was done with animals treated initially by noradrenaline or bacterial infection or electric shock (Giuliani *et al.,* 2010). The antimicrobial peptides were segregated by assay-guided fractionation and by the help of various techniques like mass spectrometry & Edman degradation sequence was determined. Magainin was isolated in this manner (Destoumieux *et al.,* 1997). Although, the approach is successful but time consuming and produces low yield (Figure 4).

Sequencing approach

The fast progress with reducing amount of sequencing techniques (high throughput sequencing), associated with effective and comparatively economic solid phase synthesis techniques, has unlock the hidden sequence data in genome and their functional testing, without the need of polypeptide isolation. For instances, the peptides of frog have been recognized by extracting the complete RNA and reverse transcription of mRNA on the basis of 3' poly-A tail. With the help of suitable vectors cDNA library was constructed and selection of positive clones and finally analysed with the help of nucleotide sequencing (Figure 4). QSAR approaches can involve the studies of virtual screening where construction of molecular descriptors of known active peptides is used based on their biophysical properties that are combined with various functional aspects. With the help of these descriptors the biological activity of a novel sequence is linked (Wang *et al.,* 2012). The important inference is that a mathematical function can be made that precisely relates physio-chemical characteristics with an observable outcome.

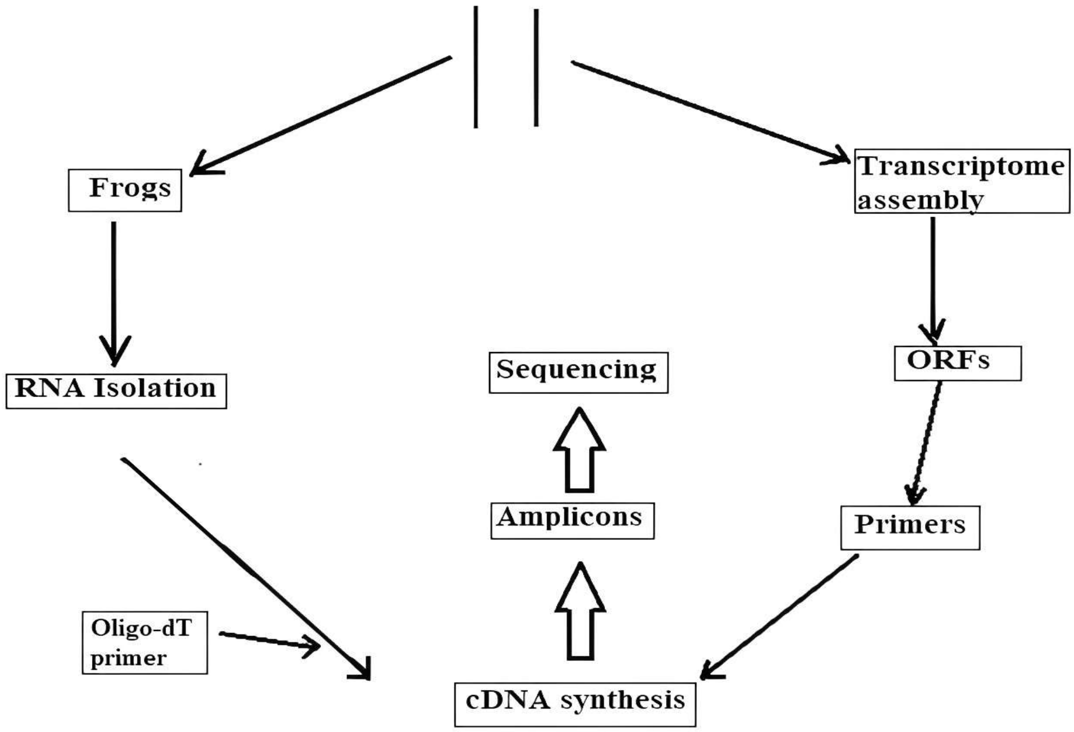

Figure 4: Diagram showing targeted DNA sequencing method (Source: Rončević *et al.,* 2019).

Quantitative Structure-Activity Relationship (QSAR) approach

After this statistical analysis is done to decide which combination of parameters or descriptor give an estimated functional value that relates to practically-determined values. Then QSAR design is validated on an external set of peptides (Figure 5) (Taboureau *et al.,* 2010; Veerasamy *et al.,* 2011). **Databases and tools of AMPs**

The steadily raising resistance of microbes against drugs force the researcher to develop antimicrobial agents. Past decade, numerous tools of AMP production and databases have been confirmed and are accessible online. There are databases that were developed to cover the sequences of AMP from many origins such as APD, ANTIMIC, AMSDb, YADAMP and CAMP. Some emphasize on particular families of AMP such as, EnzyBase (large lytic proteins), CyBase (cyclotides), Defensins (defensins), and THIOBASE (bacterial thiopeptides). There are some databases that assemble AMPs originated by shrimp (PenBase), fungi (peptaibols), bacteria (BACTIBASE and BAGEL), amphibians (DADP), and plants (PhytAMP). Swiss Prot database and AMPer are other tools of AMPs. Various computational techniques were established to hasten the process of classification and prediction of AMPs (Lin *et al.,* 2018). Quantitative structure-active relationship (QSAR) models were the earliest machine learning models that provide optimization and systematic screening of a peptide for experimental evaluation. These models operate on physico-chemical descriptors to find out the biological activity of a molecule and which is highly expensive and time consuming. Newly, machine learning approach is adopted because of their high speed, high efficiency and low cost. Following methods are involved for prediction power in a condition of supervised classification: hidden markov models (HMMs) (Fjell *et al.,* 2013), decision tree model (Lira *et al.,* 2013), neural network model (Veltri *et al.,* 2018), random forests (RFs) (Joseph *et al.,* 2012), nearest neighbor (Wang *et al.,* 2011) or k-nearest neighbour algorithm (Xiao *et al.,* 2013) and support vector machine (SVM) (Meher *et al.,* 2017).

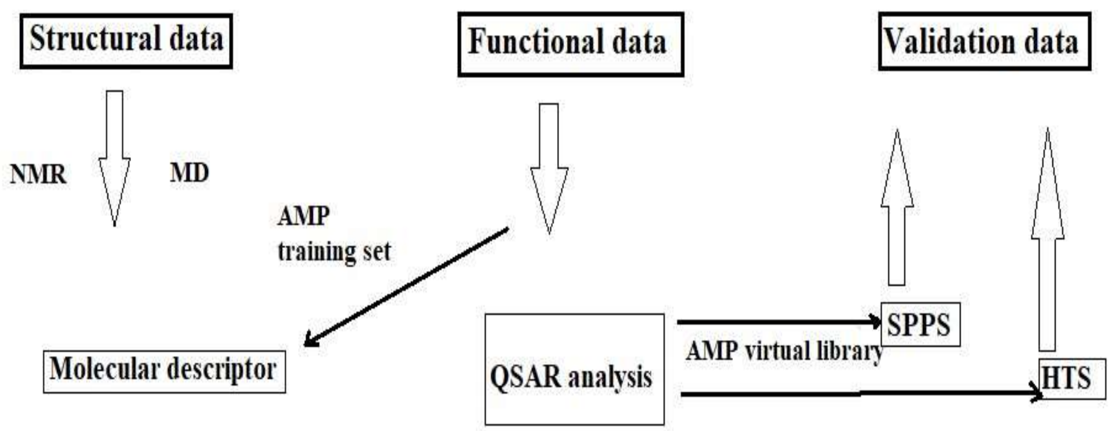

Figure 5: Outline of QSAR method (Source: Rončević *et al.,* 2019).

Machine Learning approach

Recently, "deep" network architecture for classification and chemical data analysis. To find out if the unknown sequence is AMP or not some predictors only use binary classifiers (Meher *et al.,* 2017; Wang *et al.,* 2011). For more detailed quantitative analysis, multiclass classifier is used. Four classes were identified to classify the antimicrobial activity of synthetic peptide with the help of decision tree model (Lira *et al.,* 2013). When we compare the sequences in database a common phenomenon observed was the occurrence of same sequence in different subclasses. Therefore, it is very crucial to establish a mechanism for quickly and precisely learning from multi-label datasets, to design novel and highly effective antimicrobial agents.

Current objectives of AMPs: Biofilms, Persister cells and drug resistant bacteria. As bacterial cells are directly target by the AMPs, they have potency to check antibiotic tolerant cells. Biofilms are the collective population of microorganisms that are immovable and able to grow on surfaces like medical implants and human tissues. Biofilms is responsible for causing almost 80 % bacterial infections in human (Harro *et al.,* 2010). Additionally, antibiotic resistance related to biofilm is also contributed stagnant biofilm cells (Mah & O'Toole 2001).However, there are some antibiotics that have been demonstrated to invade the matrix of biofilm (Dunne *et al.,* 1993), but their effectiveness is not shown against stagnant cells, mainly persister cells (Stewart & Costerton 2001).

Biofilm control

The electrostatic interaction between negatively charged matrix of biofilm and cationic peptides is the main difficulty of using AMPs against biofilms (Otto 2006). The other problem is the treatment of mature biofilms is highly challenging (Stewart & Costerton 2001). The coating of surface with AMPs has also preferred including free antimicrobial peptides as alteration of surface with antimicrobial peptides may help to lower the device related infections (Gao *et al.,* 2011). The biofilm matrix is supposed to create a diffusion barrier against some antimicrobial peptides (Lewis 2001). This barrier having negative charge and preserve the cells from antimicrobial agents that are positively charge and the diffusion of antimicrobial agents is reduced by the alginate in the matrix of biofilm (Shigeta *et al.,* 1997). Thus, AMPs must diffuse into biofilms and kill the cells of biofilms.

Persister control

Persister cells are dormant cells that found in any microbial populations and show tolerance to antibiotics (Lewis 2010). Though, for the survival of bacteria the integrity of membrane is must not the metabolic stages of cell and AMPs target mainly the cell membrane. Hence, they have great potency to kill persister cells.

Resistance to antimicrobial peptides
The mechanisms of resistance are basically of two types: inducible and constitutive resistance (Yeaman & Yount 2003). The constitutive resistance mechanisms include, formation of biofilm (Yeaman & Yount 2003)**,** electrostatic shielding (Friedrich *et al.,* 1999), and alter the potential of membrane during various cell growth stages (Yeaman *et al.,* 1998). The inducible resistance mechanisms involve acylation (Guo *et al.,* 1998), substitution (Lewis *et al.,* 2009),
modification (Gunn 2001) of molecules of the membrane, some proteolytic enzyme activation and efflux pumps and intracellular target alterations (Figure 6).

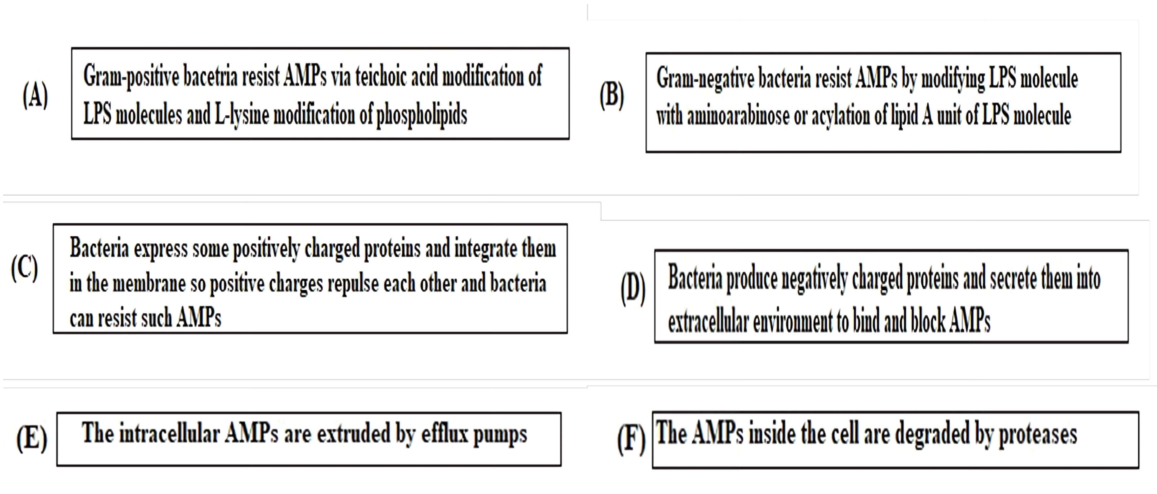

Figure 6: Diagram A to F showing the resistance mechanism of AMPs (Source: Bahar & Ren 2013).

AMPs categorization
Generally, in antimicrobial potency of AMPs enzymatic mechanisms are not involved (Phoenix *et al.,* 2013). For example, lysozyme is not considered as an antimicrobial peptide due of its large size (148 aa). It destroys the bacteria by enzymatic mechanism through dissociation of 1,4β-linkage in peptidoglycan chain (Kirby 2001).

Anticancer activity
There are certain amphiphilic alpha-helical antimicrobial peptides that reveal anti-cancerous characteristics as they have similar mode of action towards bacteria and cancer cell (Sang *et al.,* 2017). Their affinity to specific cell membrane linked glycoproteins describes the selectiveness towards cancerous cells. The AMP having cationic nature named Sapecin containing KLK motifs, interacting through negatively charged residues on the surface of host cell (Bednarska *et al.,* 2017). Fast and selectively cytotoxic activity (12µg/ml) was demonstrated by magainin-2 and their analogues against haematopoietic and rigid tumour cells (Deslouches & Di 2017). In contrary, against normal lymphocytes their cytotoxic activity was not understood even up to 200µg/ml.

Antiviral peptides
The neutralization of viruses by antiviral AMPs takes place with integration in the membrane of host cell or either in the viral envelop. It is shown in studies that antiviral AMPs target the enveloped RNA and DNA viruses (Horne *et al.,* 2005). AMPs causes instability of membrane by integrating into viral envelopes, so that viruses become unable to infect host cells (Sitaram & Nagaraj, 1999).
Antiviral antimicrobial peptides may inhibit the virus entry into the host cell capturing particular receptors on mammalian cells (Song *et al.,* 2001). For instance, for the attachment of Herpes Simplex virus (HSV) to the membrane of host cell heparin sulphate is very crucial (Wu & Spear 1989) and these are negatively charged molecules of glycosaminoglycan (Laquerre *et al.,* 1998). Lactoferrin is α-helical cationic peptide (Andersson *et al.,* 2004) may check the infection of HSV by linking to heparin molecules and checking virus-receptor associations (Jenssen *et al.,* 2004). There

are certain antiviral AMPs that confined in the cytoplasm and organelle by crossing the membrane of cell, leads to changes in the expression profile of gene in host cell, make it easier for host defence system to fight against viruses or check the gene expression of virus. The antiviral functions of beta-defensin (BD)-1 peptide was shown against VHSV (Viral hemorrhagic septicaemia virus) infection in rainbow trout (Falco *et al.,* 2008) and against NNV (Nervous necrosis virus) the antiviral functions are shown by epinecidin-1 from grouper and TH-5 from tilapia (Chia *et al.,* 2010).

Antiparasitic activity

The group of antiparasitic peptides is smaller in contrast with remaining groups of AMPs (Bahar & Ren, 2013). However, parasitic organism is multicellular, their mode of action includes killing of cells by compromising the integrity of cell membrane like other AMPs. *Trypanosoma brucei* and *leishmania* are the parasites against which antiparasitic peptides have been recognized (Jenssen *et al.,* 2006). The anti-parasitic functions in channel catfish was showed by a beta-hemoglobin peptide family AMP against *Ichthyophthirius multifiliis* (ich) that causes ichthyophthiriosis (Ullal & Noga 2010).

Antifungal activity

Despite differences in the cell wall of bacteria and fungus their mode of action in killing cells are same. Their mode of action of antifungal peptides (AFPs) involves disintegration of fungal cell causes lysis, osmotic stress, and finally cell death (Pushpanathan *et al.,* 2012). According to some research they may also intervene with intracellular machinery (Bahar & Ren 2013). Fungal cell wall is made up of chitin which binds selectively to antifungal peptides (Pushpanathan *et al.,* 2012). Striped bass secretes a peptide, piscidine-2, that act as fungicide by disrupting fungal membrane. Many AFPs that are derived from plant have been studied to acquire lectin like activity such as Ac-AFP1/2, Tu-AMP1/2, and Pp-AMP1/2. They alter the intracellular actin skeleton through binding fungal chitin. This disorganization of chitin has been found to compromise the morphology of fungal cell and integrity of membrane (Rautenbach *et al.,* 2016).

Antibacterial peptides

Mostly antibacterial AMPs are cationic AMPs that target the cell membrane of bacteria and causing the breakdown of lipid bilayer (Zhang *et al.,* 2001). As these AMPs have both hydrophobic and hydrophilic domains so they are amphipathic in nature. This property help AMPs to phospholipid group (hydrophilic region) as well as lipid component (hydrophobic region) (Jenssen *et al.,* 2006). In some research it was studied that certain AMPs kills the bacteria at low concentration without altering the integrity of membrane. These AMPs do not directly interact with membrane instead they block some pathways such as replication of DNA and protein synthesis to kill bacteria (Brogden 2005). For example, an AMP that dispersed into cells and attach to RNA & DNA without causing any damage to the membrane of cell is buforin II (Park *et al.,* 1998) other examples of such AMPs are apidaecin, pyrrhocoricin and drosocin. There is some example in which AMP have been demonstrate to kill the bacteria that are antibiotic resistant. For example, blocking the synthesis of cell wall can be done by an AMP (nisin) and an antibiotic (vancomycin). Although, a strain MRSA (Methicillin resistant *Staphylococcus aureus*) was found to be sensitive tom nisin, while resistant to vancomycin (Brumfitt *et al.,* 2002). There are two most common properties of AMPs are amphipathicity and cationicity (Bahar & Ren 2013; Mahlapuu *et al.,* 2016). There are numerous AMPs that are derived from food and have been found to act on the cell membrane of bacteria. For instance, the membrane of inner *Escherichia coli* changes rapidly by lactoferricin B have been showed by electron microscopy resulting its depolarization, disintegration of the transmembrane potential, outer membrane permeabilization (Théolier *et al.,* 2014). Majorly these peptides show salt sensitive antibacterial activity. For example, the activity of lactoferricin B is repressed if the addition of divalent cations (Mg^{2+}, Ca^{2+}, Fe^{2+}) in adequate amount takes place (Théolier *et al.,* 2013). Nearly all AMPs reveal antibacterial or bacterio-static functions against many strains of gram positive and gram-negative bacteria. The amino acids which are positively charged of these peptides binds to negatively charged molecule of the membrane of pathogens leads to the formation of pore causes degradation of membrane.

Multidisciplinary properties of AMPs

The various properties of antimicrobial peptides that are explained below (Figure 7).

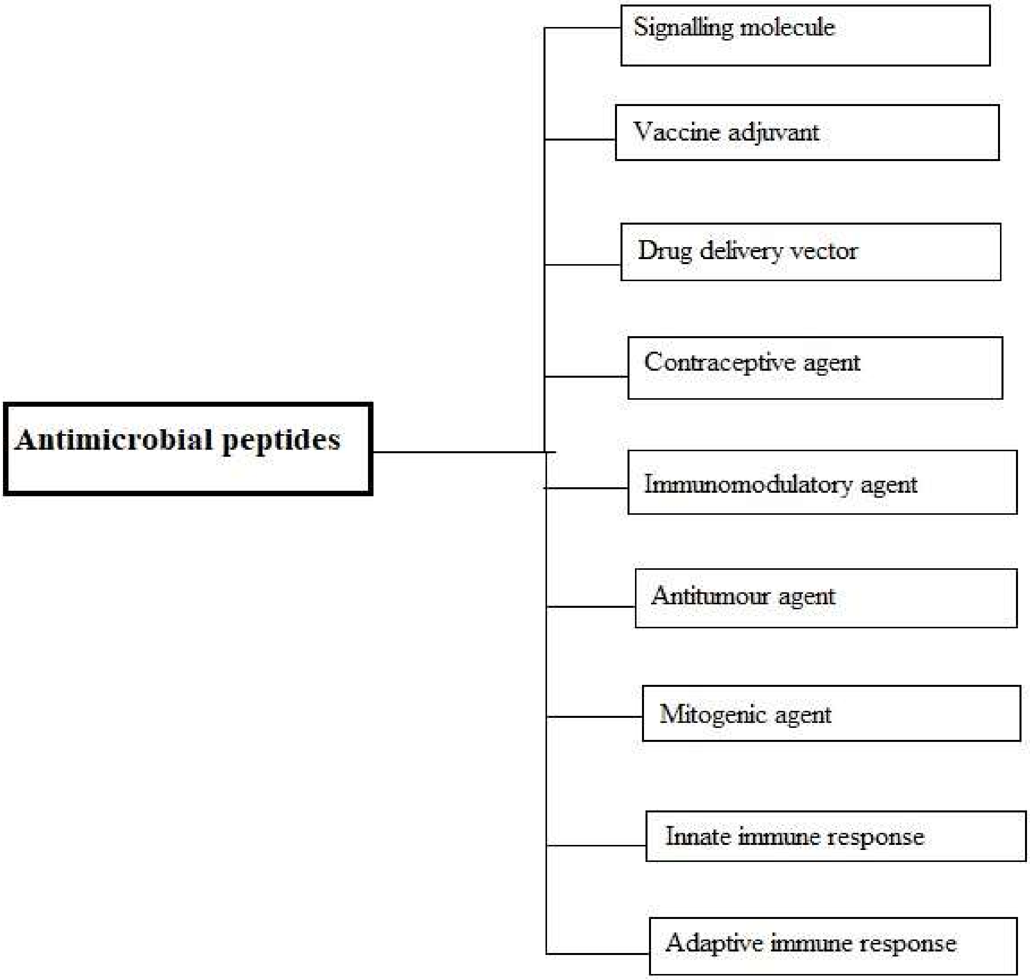

Figure 7: Layout of multidisciplinary properties of AMPs (Source: Pushpanathan *et al.,* 2013).

AMPs as signalling molecule

The immune system of many organisms produces short cationic AMPs known as Host Defence Peptide (HDP) that plays a crucial role in innate immunity (Steinstraesser *et al.,* 2011). As host defence many HDPs are involved in immune response modulation. They function as modulators of signal transduction pathways by affecting the activity of intracellular signalling targets like protein kinase. HDPs namely defensins are created by various cell types like small intestinal epithelial cells, lymphocytes, keratinocytes, neutrophils, cardiomyocytes, & tissue macrophages and are categorized into two groups such as alpha-defensins and beta-defensins. Defensins act as chemoattractant of immune cells, host cell receptor interaction, recruitment of neutrophils, activation of classical complement pathways, mobilization of immunocompetent T-cells as well as enhancer of cell adhesion (Hata *et al.,* 2008). The host defence AMP named LL-37 is synthesized by various cell types like mast cell, neutrophils, macrophages and monocytes that act as chemoattractant of mast cells and neutrophils, inhibit apoptosis of keratinocytes and neutrophils, promote induction of chemokine, angiogenesis, and stimulate differentiation of monocytes and proliferation of vascular endothelium. Moreover, it also shows anti endotoxin and anti-inflammatory effects (Steinstraesser *et al.,* 2011).

AMPs as vaccine adjuvants

Adjuvants helps to enhance the less-immunogenic potency of protein antigens or subunit vaccines (Kovacs *et al.,* 2009). There are some AMPs that function as inducers of proinflammatory cytokines such as IFN, TNF and COX-2. By encouraging differentiation of specific cell lineages, like dendritic cells, antigen-specific immunity is achieved, also cytokine expressions modulation changes the switch between Th1 and humoral Th2 polarization of adaptive immune responses (Kindrachuk & Napper 2010). Adjuvants utilized this property, and research focused with fish AMPs may promote the search toward alternative harmless vaccine adjuvants. According to some research, in the cell line of trout macrophage RTS11, upregulation of COX-2 and IL-1β with fish cecropin and pleurocidin analog peptide, CF17 (Chiou *et al.,* 2006). This explains that these compounds reveal the properties of adjuvants, and AMPs optimization with adjuvants will bring diversity in structure and choices while determining the adjuvants (Kindrachuk *et al.,* 2010).

AMPs in the development of inactivated vaccines

By inactivating pathogens, AMPs can be used as vaccines against particular pathogens. There are some problems like allergic reaction related with inactivated vaccines that are formalin based (Solomon 2008). Therefore, to reduce the aftereffects of formalin an alternative compound needs to be found to produce inactivated vaccines (Solomon 2008). As AMPs are developed from biological sources, they can be a bio alternative to formalin.

AMPs as drug delivery vector

Cell penetrating AMPs that are non-lytic were used as vector for drug delivery to manage and treat many diseases. There are some drugs that are large and hydrophilic in nature are unable to cross through the barriers of cell membrane. In such instances, the translocation property of AMPs helps them to enter into cells without causing any damage to the cell membranes were used as vectors for drug delivery (Henriques *et al.,* 2006). The most important property of AMPs to serve as delivery vector is that they must have the ability to cross the membrane of cell at very low concentration (micro molar) without any particular receptors and able to deliver the cargo such as drug into the interior of cell (J¨arver & Langel 2006). Antibacterial peptides such as pVEC, TP10, and LL-37 were involved in damage of bacterial membrane and function as CPP (cell penetrating peptides) without causing toxicity to the host cells of eukaryotes (Zhang *et al.,* 2010). There were analogues of AMP like buforin 2 and magainin, by membrane translocating mechanisms they enter into the carcinoma cells of human. The translocation of buforin2 analogue over the membrane takes place by passive mechanism which is less concentration dependent and with causing notable toxicity to carcinoma cells whereas for translocation of magainin 2 analogue the formation of transient pore is required as an intermediary step and leads to higher toxicity to carcinoma cells (Takeshima *et al.,* 2003).

AMPs as an active compounds of drug

Some study revealed that, for immunogenic drugs the fish AMPs can utilize as crucial compound. For example, on infection of *Vibrio vulnificus* the hybrid tilapia was tested for the defensive effects of pre-treating, co-treating and post-treating fish with TP3 and TP4 (Pan *et al.,* 2017). There is increased in survivability up to 95.3 % and 88.9 % after co-treatment with the pathogen and TP3 and TP4 while higher mortalities were observed after pre and post treatment (Pan *et al.,* 2017). However, the expression of immune related genes like *il1b, il6, il8, mcp8* was inhibited in all the treatment, and some AMPs, in co-treatment more pronounced effects were found, helps in the survival of fish, emphasizing the prospects of TP3 and TP4 to be utilize as antibacterial drug (Pan *et al.,* 2017).

AMPs as contraceptive agents for vaginal prophylaxis

In the reproductive tract of mammals there are many AMPs that are identified and they serve dual role in preventing sexually transmitted diseases as well as regulating fertility (Rana *et al.,* 2006). In the mucosal plug and vaginal fluid Lactoferrin was found, which checks fusion of virus and under acidic conditions its entry by binding and microbial membrane disruption. In seminal plasma, vaginal secretions and mucosal secretions Cathelicidin was found, that prevent the infection of microbes by neutralizing the polysaccharides of microbial cell. Magainins and dermaseptins are amphipathic, cationic alpha-helical peptides found in the skin extract of frogs *Xenopus laevis* and *Phyllomedusa sauvagei* that shows contraceptive activities against numerous sexually transmitted infections causing pathogen and HIV infections (Zairi *et al.,* 2009). Nisin having contraceptive effect by stopping the mobility of sperm without causing damage to the epithelial cells of vagina. Thus, might be used as unusual contraceptive microbicides (Gupta *et al.,* 2009).

Immunomodulatory effects

Immunomodulators are also regarded as immunosuppressant, immunoadjuvants, and immunostimulants. In immunotherapy the disease is treated by modulating the immune system of host. There are various targets on which immunomodulatory peptides or proteins acts like macrophages, T and B lymphocytes, NK cells and monocytes. The mode of action chiefly affects by the activation of macrophage, immunoglobulins and cytokines, phagocytosis stimulation, stimulation of NK cells and MAPK-dependent and NK-kB pathways activation (Figure 8).

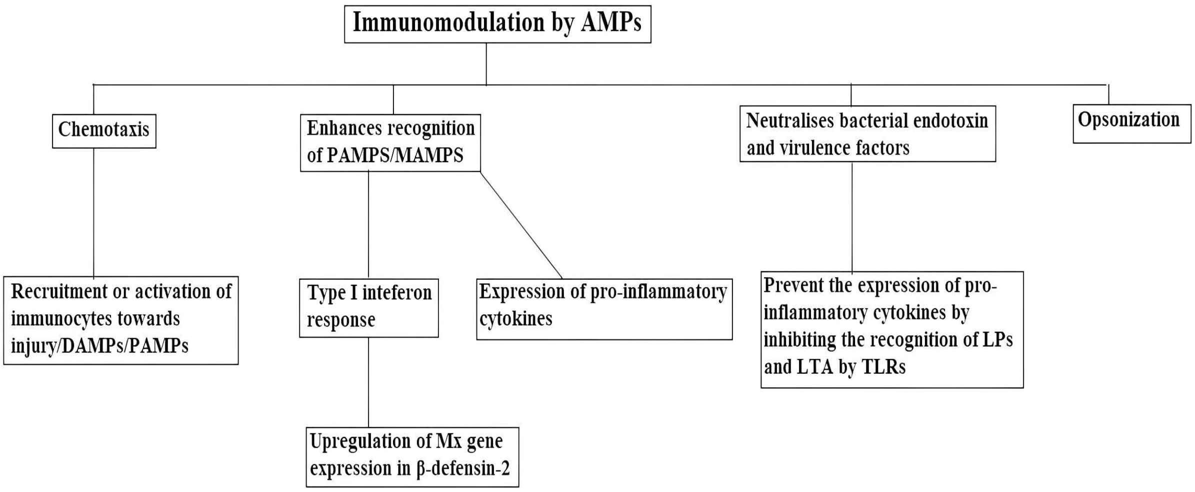

Figure 8: Immunomodulation by AMPs (Source: Chaturvedi *et al.,* 2018).

Future prospects: CRISPR-Cas and nutra ceutics

CRISPR-Cas system

Due to advancement in recent technologies the quality of synthesis of synthetic or recombinant peptides have increased and their expression levels also improved. Although, there are still challenging and important barriers. To overcome these problems, the improvement of target genome is an innovative technology. CRISPR (clustered regularly interspaced short palindromic repeat) system is the novel approach for pathogen-correcting genome and alters the target regions of pathogens (Gupta *et al.,* 2014). Cas (CRISPR associated proteins) are the mechanism that is adapted by the archaeal and bacterial immune system, which particularly identify genetic material of different pathogens that seek to infect them. By this approach they recognize earlier infections and hence resistance is developed against those infections by RNA-guided method (Pursey *et al.,* 2018). The two main objectives of producing antimicrobial peptides are: (1) to target pathogens by AMPs action. (2) the development of novel approaches to challenge against silent pathogens or avoid their progress and evading antimicrobial resistances (AMRs) (Greene 2017). The bacterial CRISPR-Cas system can be redesigned to attack rather than protect bacteria. Thus, guide RNAs may be able to target chromosomes that are essential for the survival of bacteria or responsible for virulence. Certainly, CRISPR-Cas approach has been modified and used with phagotherapy by encoding CRISPR-Cas9 targeting chromosomal genes of bacteria within the capsid of phage to treat resistances against *Staphylococcus aureus* and *Escherichia coli* (Citorik *et al.,* 2014). As bacterial adaptation got triggered due to the extensive use of antibiotics, hence antimicrobial resistance is the major issue to be solved. Therefore, priority should be given for combating AMR in aquaculture (Santos & Ramos 2018). CRISPR-Cas can be redesigned to target

AMR genes transported by a phage to resensitize bacteria to β-lactam antibiotics (Yosef *et al.,* 2015). These all innovations are based on the CRISPR-Cas tool emphasize higher probabilities to come in gene editing.

Nutraceutics

Food or parts of food, give health or medical aids, including the treatment and prevention of diseases is known as nutraceutics. Fishes are the biggest sources of nutraceutics (Chiesa *et al.,* 2016). Generally, AMPs observed as bioactive are firstly appear as precursors, after digestion a section is detached, indicating their useful effects on human health (Mohanty *et al.,* 2016). Many bioactive peptides of fish have been functioning as nutraceutics, though, they are hydrolysate of fish meal and as they are actually commercial products their impacts on human health have been determine, shows that they should cleared the clinical trials (Cheung *et al.,* 2015). Many AMPs of fish were earlier proposed as nutraceuticals, like epinecidin-1, tilapia piscidine 4 or tongue sole NKL 27, and grouper epinecidin-1 as they show an antibacterial function against known human pathogens (Cheung *et al.,* 2015).

Conclusion

AMPs are present as host defence molecules in prokaryotes as well as in eukaryotes. Fishes are regularly faced various pathogens which not only affects their health but also there is higher chances of becoming resistant to conventional antibiotics. Hence, in aquaculture AMPs may be a potential candidate for evolving therapeutic agents. Additionally, to the activity of microbicidal, AMPs also bears many other applications like immune modulators, signaling molecule, drug delivery vehicles, antitumor agents etc. The control of infection is still hindered by many challenges like high cost of manufacturing, low specificity, and animal cell toxicity. Even a small alteration can change the properties of AMPs but the results of these changes are still a challenging task. With the involvement of computational approaches, there is a better understanding of mode of action of AMPs and their activity. By combining machine learning molecular dynamics simulation and experiments it has been possible to design antimicrobial peptides from scratch. Machine learning not to directly discover and design AMPs with enhanced potency and antimicrobial efficacy, but rather to help glean understanding about the relationship between AMP sequences and function.

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Conflict of interest

The authors declare that they have no conflict of interest.

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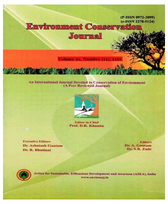

Management of agriculture through artificial intelligence in adverse climatic conditions

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ABSTRACT

Climate change has been a significant global challenge in recent years, resulting in adverse conditions for agricultural crops. Adverse climatic conditions, such as drought, flood, and extreme temperatures, have a significant impact on crop yields, resulting in food insecurity, economic losses, and environmental degradation. Agricultural experts have been working to develop innovative technologies to help farmers manage their crops better in adverse climatic conditions. One such technology is the use of Artificial Intelligence (AI) to model and manage agricultural crops. The main concern of this paper is to find the various applications of Artificial intelligence in agriculture to optimize irrigation and fertilizer application in adverse climatic conditions. By analyzing data on soil moisture levels and weather patterns, AI algorithms can determine the optimal timing and amount of irrigation and fertilizer application to maximize crop yield while minimizing water usage and fertilizer runoff. AI-based modeling and management of agricultural crops in adverse climatic conditions can help farmers improve crop yields, reduce costs, and mitigate the effects of climate change.

Introduction

The population of the globe is projected to reach over 10 billion people by the year 2050, which would result in an increase in agricultural production of up to 50% compared to 2013 despite modest economic growth. Currently, agricultural production accounts for around 37.7% of the total land area. Agriculture plays a significant role in creating jobs and raising the national income. It is actively participating in the economies of both rich and developing countries, making a considerable contribution to the prosperity of both. The income per person in rural areas has significantly increased as a result of the expansion of agriculture. It will thus be sensible and appropriate to place more attention on the agriculture sector. AI-based crop modeling involves collecting and analyzing large amounts of data from various sources, such as weather stations, soil sensors, and satellite images (Bochtis *et al.*, 2019). This data is then used to create models that can predict crop growth, yield, and quality. The models can also be used to simulate different scenarios, such as changes in weather patterns, irrigation schedules, and fertilization regimes, to identify the most effective strategies for managing crops in adverse climatic conditions. One example of AI-based crop modeling is the use of machine learning algorithms to predict crop yields in drought-prone areas. Researchers have developed a model that uses satellite images and weather data to predict crop yields with an accuracy of up to 90%. The model can help farmers plan their irrigation schedules, optimize their use of fertilizers and pesticides, and reduce water wastage, resulting in higher crop yields and lower costs. Another example is the use of AI-based tools to monitor crop growth and

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health. Researchers have developed algorithms that can analyze images of crops taken by drones or satellites to detect signs of stress, such as wilting or discoloration, which can be indicative of adverse climatic conditions. The tools can also identify nutrient deficiencies, pest infestations, and other problems that can affect crop yields. This information can be used to develop targeted interventions, such as precision irrigation (Huang *et al*., 2021)or pest control, to mitigate the effects of adverse climatic conditions. Fifty percent of the workforce in nations like India is employed in the agricultural sector, which contributes 18% of the GDP to the nation. Rural development will be boosted by agricultural sector growth, which will eventually lead to structural change (Mogili and Deepak, 2018).With the introduction of technology; several sectors have seen a drastic transition. Surprisingly, despite being the least digitalized sector, the research and commercialization of agricultural technology have gained traction. With the power to expand our perceptions and change the environment around us, artificial intelligence (AI) has started to play a significant part in daily life (Oyinbo*et al*., 2020). The fundamental idea behind AI is to create technology that works similarly to the human brain. Intelligent software and systems are created based on research into how the human brain functions, and how people learn, make decisions, and work together to solve problems. These intelligent gadgets, like the human brain, feed this software with training data and further provide us with the appropriate response for every legitimate input. AI encompasses a wide range of fields, including machine learning and deep learning (Sukhadia *et al.,* 2020).A system for harvest planning that couples crop assignment with vehicle routing. With the help of these new technologies, labor, which was formerly limited to a small number of industrial sectors, is now active in many other fields. In-depth fields including biology,linguistics, computer science, mathematics, psychology, and engineering are the foundation of AI.

Agricultural effects of AI

Agricultural crop production is heavily influenced by climatic conditions, and adverse weather events such as drought, heat waves, and floods can lead to significant crop yield losses. The increasing frequency and severity of these events due to adverse weather highlight the requirement for more efficient management practices to mitigate the negative impacts on crop production. Artificial intelligence (AI) has a chance to enhance crop management in unfavorable climatic conditions by providing accurate predictions and insights into crop growth and yield.The AI coordinates in many sectors and also manages the efforts of farmers who encounter a variety of obstacles including the various area in the agricultural sector such as crop establishment, weeding, crop monitoring, soil content sensing, irrigation, and crop output (Kim *et al.,* 2008). In order to supply high-value AI applications in the aforementioned industry, agricultural robots are constructed. The agriculture industry is in trouble as a result of the rising global population, but AI has the ability to provide a critical remedy. AI-based technical advancements allowed the farmers to increase output while using less input, enhance output quality, and ensure a quicker go-to-market for the produced crops. The typical farm is predicted to produce 4.1 million data points per day on average by 2050. The following list outlines the different ways that AI has benefited the agriculture industry.

Recognizing the topographical image

Autonomous UAVs and their applications, such as identification and surveillance, human body detection and geological area, search and rescue, and forest fire detection, have experienced an increase in attention in recent years. Spatial crop mapping and accuracy assessment using remote sensing and GIS also plays a very important role too (Nema *et al*., 2018)

Strengthen the workforce's skills

Farmers can gather enormous volumes of data from official and public websites, examine it all, and provide answers to a variety of confusing situations. It also offers us a more intelligent method of irrigation (Arvind *et al*., 2017) increasing the output for the farmers. As a result of artificial intelligence, farming will soon combine technological know-how with biological aptitudes, enhancing quality for all farmers while lowering losses and workload. According to one projection, two-thirds of the world's population would reside in cities, necessitating a reduction in the load placed on farmers. AI in agriculture may be used to automate a number of procedures, lower risks, and provide farmers with the information they need for simple and effective farming.

Production Optimization

The optimum output level for all crops is determined by variety selection and seed quality. Emerging technologies have enhanced the selection of hybrid seeds that are most suited for farmers' demands, as well as the optimum crop selection. It has been put into practice by gaining a knowledge of how seeds respond to varied climatic and soil conditions. By gathering this data, the likelihood of plant illnesses is decreased. We can now efficiently fulfill customer demands, market trends, and yearly results, enabling farmers to maximize crop returns.

Effectiveness of Chat bots

Chat bots are simply conversational virtual tools that automate communication with users. Chatbots have been made possible by artificial intelligence and machine learning. We can now comprehend natural language and communicate with users more individually thanks to it. Agriculture has made use of this facility by helping the farmers by supporting them in receiving answers to their queries, offering them guidance, and giving them various recommendations. They are mostly prepared for retail, travel, and media.

Advanced Farming AI System

Farmers and scientists may use AI-based models to forecast how unfavorable weather conditions will affect crop yield, allowing them to make educated decisions about planting, irrigation, and fertilization. These models are created by fusing information from several sources, such as satellite images, climate data, and previous crop yield information. Following the application of machine learning algorithms to this data, models that can precisely forecast crop growth and yield under various environmental circumstances are created. By using data analysis and predictions that have been validated, these technologies help and benefit farmers. There are sensor-based autonomous field monitoring agriculture robots providing data acquisition and wireless transmission (Hemalatha and Sujatha, 2015).Important agricultural data acquired by IoT devices and ML algorithms are processed and channeled through data science. Agriculture real-world applications of AI/ML and data science include:

Utilizing ML to eradicate weeds by identifying species of plants/crops; Predicting yield and quality evaluation; Predictive analytics for crop sustainability; Detecting crop infections and illnesses; intelligent harvesting and pricing choices. Reducing waste and satisfying expectations • cattle-herding robots that are autonomous. Various advancements in agriculture are being driven by AI/ML, some of which are listed below:

1. **Analysis of crops**

In order to combat drought and other detrimental environmental phenomena affecting crop yield, drone-based crop monitoring is practiced worldwide. Drone 3D imagery is being used to predict disease outbreaks, insect infestations, agriculture patterns and characteristics, and other things. Drones are also often used for crop pesticide spraying, for advanced precision farming (Natu and Kulkarni, 2016) since they can do the task five times faster than conventional gear, according to one analysis (Ahirwar *et al*., 2019). To avoid chemical pollution of groundwater, attention must be used. Using XRF analyzers to detect the mineral content of the grains, grain analyzers to establish traceability and digitalize the quality of the grain seeds, and ultra spectral analysis, for example, can improve the nutritional quality of the grains. Imaging might be used to predict how the nutritional content of leaves would vary as they develop.

2. **Importance of robotics in farming**

Increased productivity and increased yields in agricultural systems are both the results of the use of robots. Robotic weeding and spraying reduce the need for agricultural chemicals by around 90%. Robots are able to clear weeds without the assistance of humans since they can navigate field columns and rows. Generally using a laser beam and a camera for direction. In comparison to conventional approaches, using robots for plant transplantation results in more effective ones. The plucking of fruits and nuts is also done by robots.

3. Tracking devices through sensors

To monitor food for customers from the source, radio frequency identification (RFID) devices are utilized. For fresh and high-quality food, these sensors build from the first to last traceability trail for the yield, improving production compliance and dependability.The Crop OS platform created by Pairwise Plants is one illustration of an AI-based crop management model. It makes use of machine learning algorithms to forecast crop development and production based on genetic data and environmental elements like temperature, humidity, and soil moisture. Another illustration is the IBM Watson Decision Platform for Agriculture, which employs machine learning algorithms to offer insights into crop development, production, and disease management based on meteorological data, soil moisture, and other factors. This advanced technology also be used to optimize watering and nutrition enrichment applications in adverse climatic conditions. By analyzing the soil moisture levels and weather patterns, AI algorithms can determine the amount of irrigation ,optimal timing and fertilizer application to maximize crop yield while minimizing water usage and fertilizer runoff.

AI can help in the development of novel crop types that are more resistant to unfavorable environmental conditions in addition to enhancing agricultural management. Breeders may create new varieties that are more suited to these conditions by using AI algorithms to find features related to drought, heat, and other stress factors by analyzing genetic data from various crop kinds.

Conclusion

AI has the huge potential to revolutionize crop management in adverse climatic conditions by providing accurate predictions and insights into crop growth and yield. However, the successful adoption of these tools requires collaboration from service provider and partnerships between farmers, researchers, and technology developers to overcome the challenges of data availability, cost, and expertise. The implementation of AI-based models, however, for crop management under unfavourable climatic circumstances is still constrained by a number of issues, such as the availability and quality of data, the high cost of technology, and the requirement for specialized skills to design and utilize these models. To create user-friendly and affordable AI-based solutions for crop management, it is imperative that farmers, academics, and technology developers work together. Given that cutting-edge technology are primarily employed on sizable, well-connected farms, India's future usage of AI in agriculture will need to concentrate on ensuring broad access. The future of artificial intelligence, machine learning, and data science in farming will depend on expanding connection and outreach to even tiny farms in isolated regions throughout the world.

Conflict of interest

The authors declare that they have no conflict of interest.

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Impacts of hand sanitizer on human health and environment: a review

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ABSTRACT

Although the use of sanitizer starts around the middle of twentieth century but it becomes popular in early twenty first century (second decade of twenty first century during COVID-19 pandemic). To prevent the spread of COVID-19, World Health Organisation (WHO) recommended the use of sanitizers made up with different combinations of isopropyl alcohols, ethanol and hydrogen peroxides. Literature suggests some toxic effect of use and misuse of these components of sanitizers. Therefore, in the present study an attempt has been made to investigate the use of available hand sanitizers and their adverseeffects on human body as well as to collect and correlate the gathered information with their occupation which may further trigger the adverse effect of sanitizer on human being. Out of the total respondents, 98% are using but very few (2%) are not using any type of sanitizer. Age group 2 (16-25 years) is the largest user of hand sanitizer which shows the maximum awareness of this age group related to sanitation and hygiene. Data obtained suggests that males (57.5%) are more aware to sanitization in comparison to female (41.8%). Among the total respondents, house wives were very few (0.8%) which shows lack of awareness among them may be due to their busy schedule and household works. The obtained data revealed that education plays a key role inthe spread of sanitation and hygiene awareness. Most of the peoples are using the sanitizers of established brands. The data also revealed that 88% of the respondents were observing the various impacts on the body (49% skin dryness, 16% skin allergy 12% skin irritation and itching while 12% respondents are not sure about the impacts). Only 12% peoples responded that they are not observing any impacts of sanitizer. Besides this, different components used in sanitizers pose threat to the different spheres of the environment. Based on the findings of the present study, we can conclude that sanitizer is impacting the human health and environment in various ways. Therefore, there is a need of mass awareness regarding the use and disposal of disinfectants.

Introduction

COVID-19 virus was first identified as a human corona virus in 1965 which caused a common cold (Du *et al.,* 2020). The virus belongs to the same genus as severe acute respiratory syndrome corona virus (SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV, and was thus named SARS-CoV-2 by the International Committee on Taxonomy of Viruses, in 2020. SARS-CoV-2, the novel corona virus that causes COVID-19, was first detected in Wuhan, China, in late 2019 (Huang *et al.,* 2020). The persistent study of SARS-CoV-2 suggest that this virus was more stable on plastic and stainless steel than on copper and cardboard, and was detected up to 72 hours after application to these surfaces. The study carried out by Van Doremalen *et al.* (2020)

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highlighted that aerosol and fomite transmission of SARS-CoV-2 is possible, since the virus can remain sustainable and infectious in aerosols forhours and on surfaces up to days.Therefore, hand hygiene is very important as it maybe easily contaminated from direct contact with airborne microorganism such as SARS-CoV-2 droplets which may originate from coughs and sneezes. Predominantly in pandemic situations, it isessentially important to interrupt the transmission of the virus by the strict practice of proper hand sanitization. This can be accomplished by strict good hand hygiene and contact isolation (WHO,2020). The success of the hand sanitization simply depends on the use of effective hand disinfecting agents as sanitizer (Jing *et al.,* 2020). During the pandemic, using sanitizer was the basic necessities as with other basic daily needs. Therefore, it is vitalto know the effect of sanitizer on the human being as it was one of the basic requirement of human lifeduring this period. Yet to our knowledge, there has never been a comparison of the formulation and theadverse effect of a large number of brands of hand sanitizers. Therefore, the aim of the present study was to investigate the range of available hand sanitizers used by the society and the adverse effects on human body and environment. Attempt was also made to collect and correlate the gathered information with their occupation which mayfurther trigger the adverse effect of sanitizer onhuman being. The collected data can also be usedas reference for the further study.

WHO recommendation/guidelines for the formulations of hand rub (Hand Sanitizer)

Glycerol is one of the main ingredient used as humectant which reduce the loss of moisture, is cheap, easily available and miscible in water. Alcohol is non-toxic and do not promote allergy. Hydrogen peroxide is used as an antiseptic to eliminate the microorganisms that cause disease. Ingredients should be non-toxic in caseof accidental ingestion. A colorant may be allowed to add to the solution for differentiation it from other fluids, but it should not add toxicity, promote allergy, or interfere with antimicrobial properties. The addition of perfumes or dyes is not recommended due to safety as it may create allergic reactions (WHO, 2010). Except alcohols (96% ethanol or 99.8% isopropyl alcohol) WHO also recommended other ingredients including benzalkonium chloride as the active principal ingredient displayed excellent antibacterial activity, whereas others exhibited modest or poor activity in the assays performed (Chojnacki *et al.,* 2021; Aodah *et al.,* 2021).

Classification of Hand sanitizers

Specifically hand sanitizers can be categorized into three main classes: (1) Alcohol-based, (2) Alcohol-based supplemented = alcohol plus other antimicrobial agents and (3) Non-alcohol-based = majority of the product is water plus surfactant and antimicrobial agent (Jing *et al.,* 2020; Kumar and Das, 2021). Hand sanitizers containing 60–95% alcohol are most effective, other than this either lower or even higher concentrations are less effective, because water is essential to denature the proteins of virus. Further, pure alcohol or higher concentrations would evaporate too quickly to exert any germicidal effect (Meyers *et al.,* 2021). Therefore, mostly 60% to 80% concentrations are used for hand rubs. Alcohol-based hand rubs are available in the form of solutions (with low viscosity), gels and foams. However, most studies have suggested that gel-based formulations are rather less effective than solutions (Dharan *et al.,* 2003). It has also been emphasized that if a gel with lower activity is more frequently used, the overall outcome is expected to be better (Traore *et al.,*2007). Non-alcoholic hand sanitizers are safe in comparison to alcoholic due to use in very lowconcentrations (Jing *et al.,* 2020). In accord to the available literature and evidences on efficacy,tolerability and cost effectiveness, WHO recommends using an alcohol-based hand rub for routine hand antisepsis in most clinical and non- clinical situations. WHO also recommends the localproduction of the above given formulations as an alternative when suitable commercial products are either unavailable or too costly to afford (WHO, 2010).

Use of sanitizer and soap

Centres for Disease Control and Prevention (CDC) and WHO recommends the washing of hands with soap and water for at least 20 to 30 seconds or by alcoholic hand sanitizers (AHS) (comprised of either 80% ethanol or 75% isopropyl alcohol) frequently to reduce microbes (WHO 2020). Both CDC and WHO gives preference to AHS over soap and water due to its easy accessibility (Aodah *et al.,* 2021). People prefer to wash their hand frequently with sanitizers instead of soap and water due to easy availability, lack of water and time (Singh *et al.,* 2020).

Although in a study, Singh *et al.* (2020) concluded that use of sanitizer in conjunction with soap and water is much more effective. Emami *et al.* (2020) and Saha *et al.* (2021) also recommended hand washing with soap and water instead of AHS. AHS have proven to deliver rapid bactericidal activity towards bacterial pathogens as well as excellent virucidal activity toward both enveloped and non-enveloped viruses. AHS are active against influenza virus, severe acute respiratory syndrome corona virus (SARSCoV), middle eastern respiratory syndrome (MERS) virus, Zika virus, Ebola virus, and SARS corona virus 2 (SARS-CoV-2) (Kratzel *et al.,* 2020). In comparison to soap, sanitizers are not effective against all types of germs (ex- against non virus agents and *Clostridiumdifficile*) and in case of dirty and greasy hands (Vermeil *et al.,* 2019). A comparative life cycle assessment (LCA) of hand washing with soap (soap+water) and hand sanitizer (both ethanol and isopropanol based sanitizers) was carried out in UKto compare the environmental impact of increased levels of hand hygiene during the COVID-19 pandemic (Duane *et al.,* 2022). The isopropanol-based hand sanitizer had the lowest environmental impact in 14 out of the 16 impact categories used inthis study. It has been observed that all forms of hand hygiene have an environmental cost, and this needs to be weighed up against the health benefitsof preventing disease transmission. Whencomparing hand sanitizers to hand washing withsoap and water, this study found that using isopropanol based hand sanitizer is better for planetary health. However, no method of hand hygiene was ideal; isopropanol had a greater fossil fuel resource use than ethanol based hand sanitizer (Ghafoor *et al.,* 2021; Duane *et al.,* 2022).

Toxicity of Sanitizer ingredient

The ingredients and their possible impacts are presented in Table 1.

Table 1: Toxicity of sanitizer ingredient

SN	Component	Impacts
1	**Isopropyl alcohol**	Impacts on central nervous system, liver and kidney, drowsiness, ataxia, respiratory depression, irritation of mucous membranes and eyes, vomiting, pancreatitis, cold clammy skin, and hypothermia (Ghafoor *et al.,* 2021; Olson *et al.,* 2021).
2	**Propylene glycol**	Hyperosmolality, acute kidney injury, and sepsis-like syndrome (Zar *et al.,* 2007).
3	**Aminomethyl propanol**	Depends of quantity used. In excess concentration (more than 2% in mascara or any other cosmetic product) causes dermal irritation or allergic contact sensitization (Burnett *et al.,* 2007).
4	**EDTA (ethylene diamine tetra acetic acid)**	Depends of quantity used. Cytotoxic and weakly genotoxic, but not carcinogenic when used as chelating agent. Reproductive or developmental toxicity when used as an aerosolized cosmetic formulation (Lanigan and Yamarik 2002).
5	**Sodium Benzoate**	Oral, dermal or inhalation causes urticaria, asthma, rhinitis, or anaphylactic shock. The symptoms appear shortly after exposure and disappear within a few hours, even at low doses (Wibbertmann *et al.,* 2005).
6	**Octenidine dihydrochloride or triclosan (TCS)**	Immune disorders, ROS production, cardiovascular functions, reproductive and developmental defects in infants (Weatherly and Gosse, 2017), and skin allergy when used in dermal cream (Ridzwan and Zainudin 2017).
7	**Hydrogen peroxide (H_2O_2)**	Acute inhalation causes irritation to the nose, throat and respiratory tract. Dermal exposure to dilute solutions may cause whitening of the skin, slight gastrointestinal irritation (Moon *et al.,* 2006), portal vein thrombosis (Sung *et al.,* 2018), minor mucosal irritation, and vomiting, whilst more concentrated solutions can cause severe irritation and corrosion, severe burns, blisters, ulcers and permanent scarring (Colares *et al.,* 2019).
8	**Benzolkonium chloride**	Cytotoxic effect when used to sterilize soft contact lenses also decreases the vision tremendously (Gasset 1977).
9	**Ethanol**	Respiratory arrest, arrhythmia, hypothermia, hypotension, hypoglycemia, ketoacidosis (Wilson *et al.,* 2015), and skin irritation or contact dermatitis (Lachenmeier 2008).

Most of the ingredients have impacts on humanbody. Only very few ingredients show quantitative impacts. Toxicity of ingredients also depends on formulations and combinations (Mahmood *et al.,* 2020). Hand sanitizer containing the impurities of benzene, acetal and acetaldehyde cause differenttype of disease in human beings such as eye and skin irritation, cancer, and irritation in upperrespiratory tract (FDA, 2021; Cohen *et al.,* 2021). Dermal contact with ethanol is responsible for skin irritation and allergic reaction especially who are sensitive to ethanol and those are with skindisorders including fissures (Mahmood *et al.,*2020). Higher number of hand-skin problems were particularly reported among health care workers during the COVID-19 pandemic, which was associated with the increased frequency of hand sanitizer use (Altunisik *et al.,* 2020). Such incidences of hand-skin problems included mainly the dryness of the skin and other conditions such as redness, burning pain and itching. Moreover, alcohol is highly flammable and can result in fire hazards if used near fire or exposed to high temperatures. Hydrogen peroxide toxicity depends on its concentration used in the AHS. A low concentration (3%) of hydrogen peroxide may cause mild irritation of the eyes and skin when used externally and when ingested, may result in irritation of the mouth and the gastrointestinal tract,and may also result in air embolism in rare cases (Watt *et al.,* 2004; Ghannoum *et al.,* 2014). Excessive use of AHS may result in a rise of other viral diseases and antimicrobial resistance due to the selection of resistant strains, particularly for bacteria.

Materials and Methods

Data was collected via online mode using google form as it was contactless method of data collection during the pandemic. Different age groups, gender and occupational peoples such as students including research scholars, teaching faculties, health workers and house wives were targeted for the study. Collected data was based on the Questionnaire which was answered by each group of peoples (Table 2). Previous studies for literature survey purpose was searched on various research platforms such as Research gate, Google scholar, Sci hub and in the archive section of various journals using different keywords. The data obtained was segregated as per the age and divided into five age groups (5-15, 16-25, 26-35, 36-45, 46 above). The data of brands of sanitizer was divided into six brand such as brand A(Dettol), brand B (Lifebuoy), brand C (Himalaya), brand D (Savlon), brand E (Mix use), and brand E (others).

Table 2: Questionnaire used during the study

SN	Questions
1	Have you used sanitizer?
2	What is your age?
3	Mention your gender?
4	Mention your occupation?
5	Mention the brand of sanitizer used
6	Mention the quantity (Approximate) of sanitizer used per month?
7	Mention the bad impact observed on the Body?

Results and Discussion

Impacts on human health

The study was performed on the peoples of age group from 5 years to above 45 years, ignoring the genders and occupations. The results obtained during the study period are given in Table 3 and Figure 1 to 4.

Table 3: Number of respondent as per their age group and sex (n=129)

Age group	Number of respondent	%	Sex	Number of respondent	%
5 to 15	1	0.8	Male	0	0.0
			Female	1	0.8
16 to 25	86	66.7	Male	46	35.7
			Female	40	31.0
26 to 35	32	24.8	Male	25	19.4
			Female	7	5.4
36 to 45	5	3.9	Male	1	0.8
			Female	4	3.1
46 above	5	3.9	Male	2	1.6
			Female	3	2.3

It is come out in the study that 98% peoples frequently used sanitizer in total; only 2% never used it as shown in the graph. From the age group 5to 15 years only 0.8% respondents are using the sanitizer. The respondent belongs to age group 16- 25 years are the key users of sanitizers which is 66.7%. The second large percentile (24.8%) from 26-35 years, further decrement is continuing as 3.9% for 36-45 years and above 46 years respectively (Figure 1). This has been shown in the graph that the male users of sanitizers are maximum to age group 16-25 and 26-35 years' as 35.7% and 19.4% respectively, rest male respondent do not have significant contribution as 1.6% (45 above years) 0.8% (between 35-45 years) and 0.0% (5-15 years). In female category, the highest respondents are age group 16-25 years (31%) and the second highest 26-35 years (5.4%). Similarly, with male trends, the female related to 36-45 (3.1%), above 46 years (2.3%) and below 15 years is 0.8%. It is clear from the data (Table 3) that the smallest percentage (0.0% and 0.8%) belong to 5-15 years age group in both male and female and slightly above (3.1% and 2.3%) in both male and female of age group of 36-45 years and above 46 years respectively.

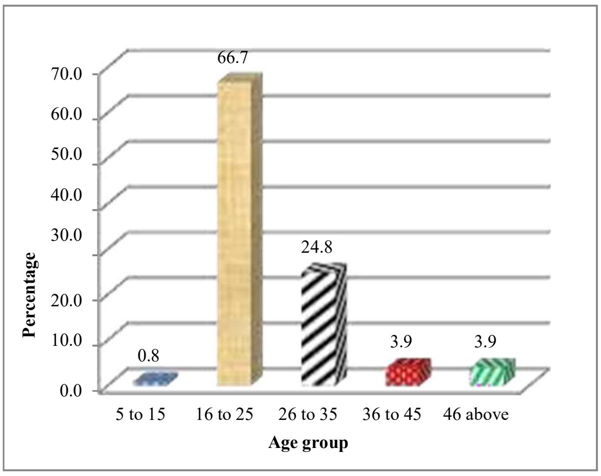

Figure 1: Percentage of respondent using the sanitizer as per their age group

By profession maximum users of sanitizers are students (71.3%) and minimum are house wives (0.8%). Nearly similar percentage as 11.6% and 10.9% correspond to teaching profession and private job respectively, rest (5.4 %) belong to the professions other than teaching and private jobs (Figure 2). It has been find out in the study that among the various brands of sanitizer brand A was more popular and has been used extensively by48% respondents. Other brands such as brand B, brand C, brand D were used by 12.6%, 6.3% and 6.3% respondents respectively. Some respondents (11%) used more than one brands as per the availability labelled as mix in this study. Other thanthese known brands, 17.3% respondents used other brands of sanitizer which may not be very popular or local brands as shown in Figure 3.

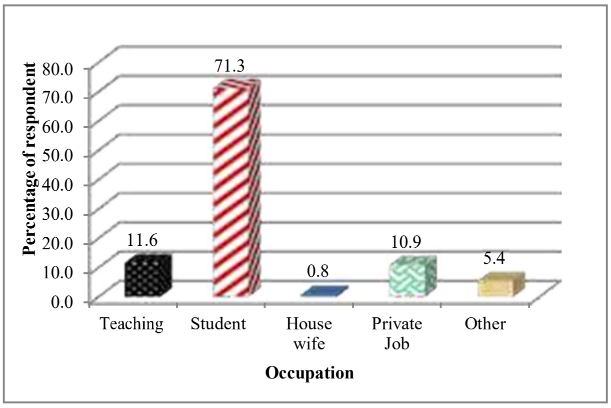

Figure 2: Percentage of different occupation of respondent

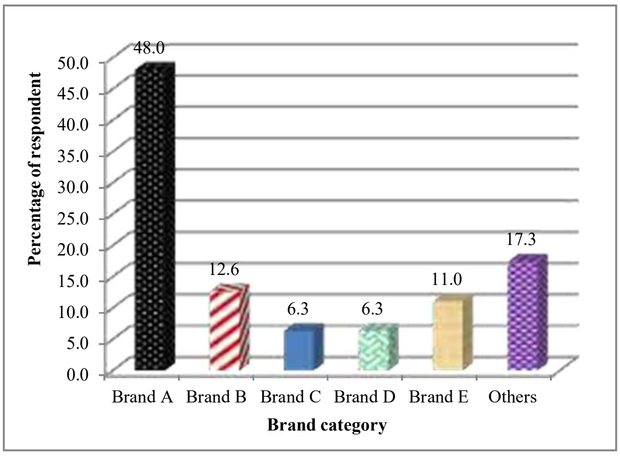

Figure 3: Percentage of different brand user among the respondent

Although, frequent use of hand sanitizer is necessary to maintain the hand hygiene and safety but some adverse effects were also observed by the number of peoples who were using hand sanitizer frequently. The data obtained shows that peoples are facing extreme hand dryness (49%), skinallergy (16%) and irritations or itching (12%). Ithas been find out in the study that 12% of therespondents were not aware of any direct visualimpact of sanitizer on their body. The rest numbers of peoples (12%) do not have any negative effecton their hand and other body parts (Figure 4).

It has been find out that awareness related to theuse of sanitizer increased as the age of respondents increased till the age of 35 years and above this age, a decrement is observed. It is summarised from the study that the respondent belongs to young age (under 15 years) are too young to understand the

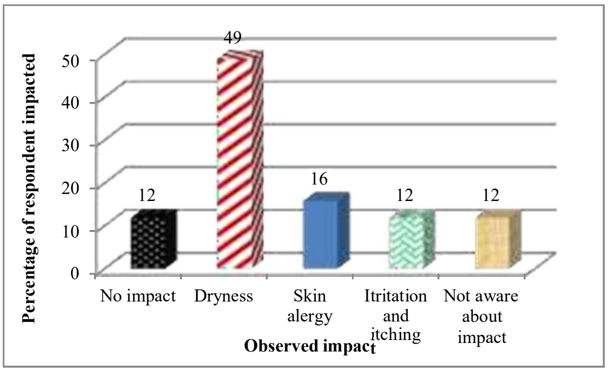

Figure 4: Different impact and their percentage in the respondent

importance of hygiene or may not have easy excess of sanitizer. On the other hand, the people belong to the age group between 16-25 years are more aware of hand hygiene and are maximum users. The moderate (24.8%) responses were received from age group 36-45 years. The people belong to 36 to 45 years and above are the least users of sanitizers may represent their indolence or ignorance. The male user of sanitizer is 57% in comparison to female (43%) which is 14% lesser than male users. Very less percentage of the older age male andfemale between 36-45 years and above 46 years uses hand sanitizers . It seems that either both male-female users in this category are not aware to sanitizer or cannot afford due to additional cost ofit.When the data obtained was analysed as per their professions, it was found that students are moreactive towards sanitisation and use the hand sanitizer more frequently. On the other hand, house wives are the least users of sanitizers they account too busy to perform house related responsibilities which results ignorance of hand hygiene or possiblyuse soap instead of sanitizer. This is also true that only few numbers of house wives were taking part in the study, therefore the given data cannot show the real picture of the society. It is also come out in the study that the peoples related to teaching profession and private jobs are nearby equally aware towards hand sanitization as it could be the result of their quality education. Only 5.4 % respondent relate to the jobs other than private job such as farmers, mechanical workers, shop holders and technicians etc. Again the low percentage indicate that hand hygiene is more promised in educated class of peoples and they used thesanitizer more frequently in comparison to non or less educated peoples.

Most of the respondents use the sanitizers of most popular brand such as Dettol, Savlon, Lifebuoy,and Himalaya. Some respondents use sanitizers ofmore than one brand may be due to lack of awareness regarding the composition and unavailability of the same brand in nearby market due the hike in the demand of sanitizers during COVID-19 pandemic. When the impacts of hand sanitizers were analysed, it was find out that the fragmented data does not seem very effective although, all together 88% peoples facing somekind of envisaged or non-envisaged problems after frequent use of sanitizer, only 12% do not have any significantly visual effect on them. Alcohol based sanitizers are responsible for skin dryness (cutaneous xerosis), hand dermatitis, irritant contact dermatitis (ICD), rarely allergic contact dermatitis (ACD) (Emami *et al.,* 2020; Beiu *et al.,* 2020; Aodah *et al.,* 2021), and elimination of helpful flora (Weaver, 2005) due to the impurities of acetaldehyde, acetal, benzene, and methanol (Cohen *et al.,* 2021). Now a day the use of orange extracts in perfumes and fragrances have been increased. However, it causes phytophotodermatitis on the exposure of sunlight (Lee *et al.,* 2022). Bakkar *et al.* (2021) and Abo-Zeid *et al.* (2022) worked on some lipid based sanitizers and concluded them as future alternative of AHS.

Impacts on Environment

The hand sanitizers which we considered and discussed in the present study are mainly based on ethanol, isopropyl alcohol and hydrogen peroxides. Sanitizers have different impacts on the environment based on their main components(Mahmood *et al.,* 2020). Ethanol is widely used in research and development laboratories, industries, academic laboratories and at home (Pendlington *et al.,* 2001). Bioaccumulation and bio concentration factor in fatty tissues for ethanol is very less due its high anticipated metabolic rate (HSDB, 2012). Aquatic organism are more likely to impacted by ethanol exposure due to its direct impact on dissolved oxygen concentration (NEIWPCC, 2001).Terrestrial organisms are less likely to be impacted by ethanol exposure because it either evaporates orpenetrates into the soil and water (Mahmood *et al.,* 2020). Ethanol spills on soil or in water impacts the invertebrates and microbial population (MassDEP, 2011).

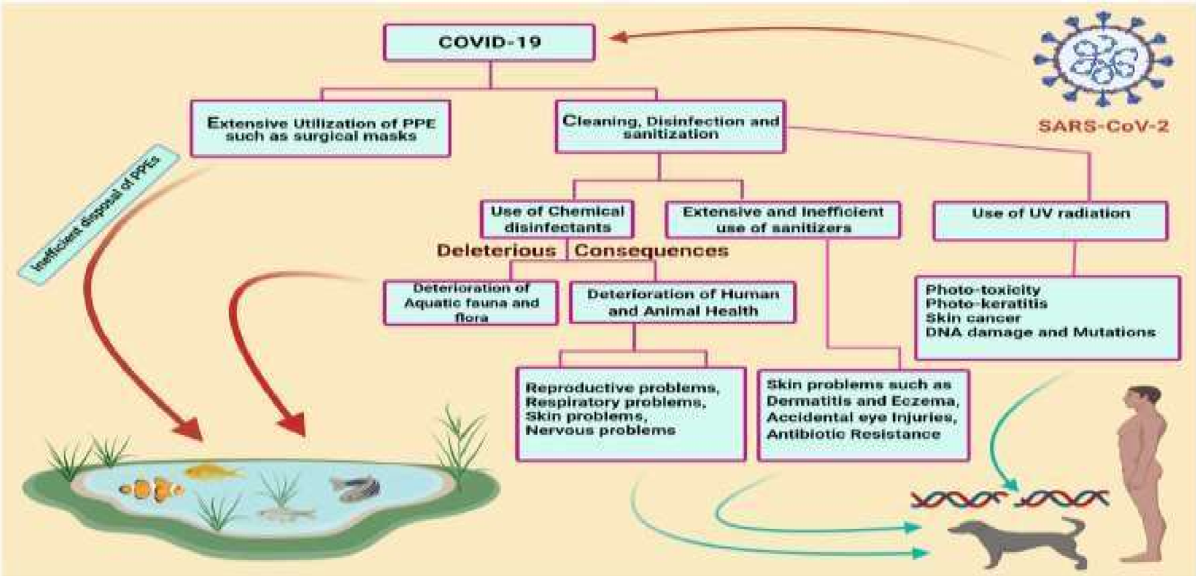

Figure 5a: Impacts of sanitizers on human health and Environment (adapted from Dhama *et al.,* 2021).

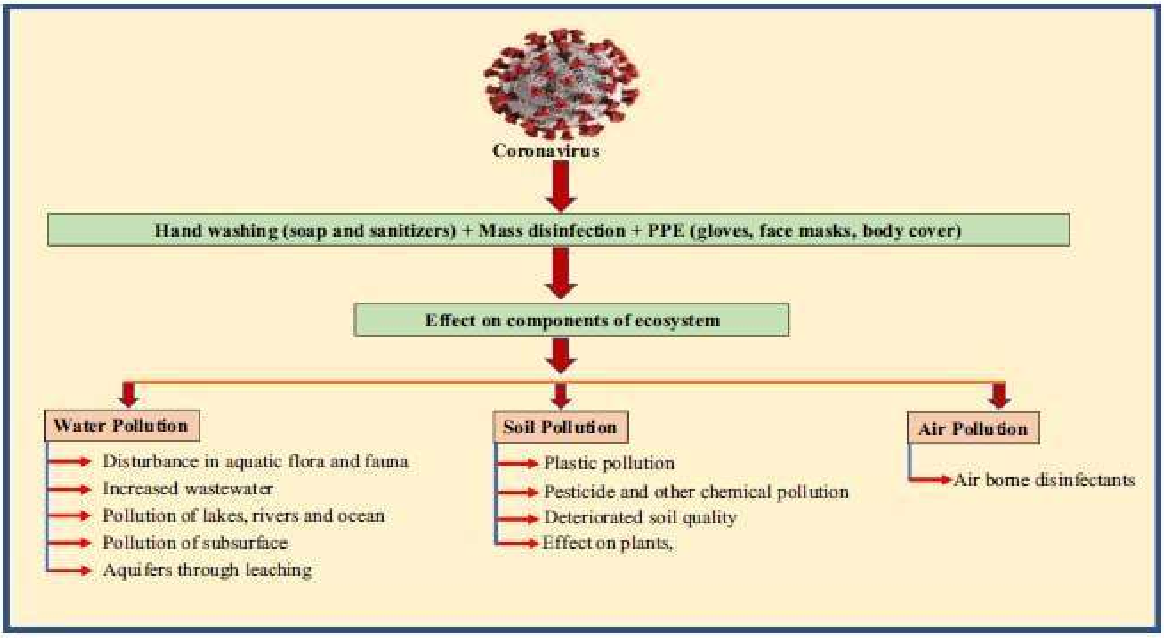

Figure 5b: Impacts of sanitizers on different components of Environment (adapted from Kumar *et al.,* 2021)

Wildlife shows different impacts on the exposure of different concentrations of ethanol USEPA ECOTOX Report, 2011; Nabi *et al.,* 2020).Minute quantities of isopropanol were reported in drinking water without any detectable limit (HSDB,2012) but larger spills of isopropanol on ground can contaminate ground water due infiltration through the upper strata of soil (Atolani *et al.,* 2020).

Isopropanol is highly biodegradable and non-persistent in the atmosphere. Like ethanol, isopropanol also deplete the dissolved oxygen content of aquatic bodies and ultimately impacts theaquatic life (BABEC, 2001). In the formation of ground level ozone and photochemical smog, no role of isopropyl was reported in the literature. Hydrogen peroxide reacts with other compound with a very fast speed and therefore exists in the environment for a short duration and therefore probabilities of impacts are very less (ATSDR, 2002). Due to fast degradation in soil and water, there are very less probability of accumulation in food chains (Mahmood *et al.,* 2020). Besides these, sodium hypochlorite, hypochlorous acids and other chlorine disinfectant pose threat to aquaticorganism (China Ministry of Ecology and Environment 2020; Sedlak, 2011; Subpiramaniyam, 2021). The disinfection practices such as washingof hospital, house, and laboratory floor, streets, and market places generated a huge amount of wastewater containing the sufficient quantity of disinfectant released directly or indirectly in rivers, lakes and ponds without any treatment (Geller *et al.,* 2012; Bashir *et al.,* 2020). Chlorine after reacting with organic matter produced organic chlorine which persists in the environment and posethreat to aquatic flora and fauna (Emmanuel *et al.,* 2004; Bhat *et al.,* 2021). Disinfectant when enters in wastewater impairs with the microbial life and therefore impacts the efficiency of wastewater treatment plant (Dhama *et al.,* 2021; Kumar *et al.,* 2021).

Conclusion

The present study was carried out to investigate the range of available hand sanitizers and the adverse effects on human body and environment. Attempt was also made to collect and correlate the gathered information with their occupation which may further trigger the adverse effect of sanitizer on human being. Most of the respondents were using the hand sanitizer and among them very few were aware about the composition of the sanitizer. Very few respondents of the age group 5-15years were using the sanitizer may be due to lack of awareness or reach of sanitizers. In comparison to males (57.5%), less awareness regarding the use of sanitizers were observed in females (41.8%) of different age groups. In females, only 0.8% of the house wives were using the sanitizers which maybe due to their busy schedule in household works. Maximum awareness level regarding sanitizer use was observed in age group 2 (16-25years) followed by 3 (26-35years) 4 (36-45y ears) 5 (46years) and above) and 1(0-15years). Sanitation and hygiene is more promised in educated class of respondents andthey used the sanitizer more frequently in compare to none or less educated respondents. 73.2% of the respondents were aware about the brands and using certain fixed brand while 11% of the respondents were using more than one brand simultaneously and 17.3% of the respondents were only using the sanitizer regardless of their brand. 76% of the respondents reported some sort of impacts on their body. Based on literature and the findings of the present study, we can conclude that the componentsof the sanitizer and other disinfectants are affecting the humans in various ways and also causing the disturbances in different components of the environment and therefore disturbing the natural functioning of the environment. Thus there is a need of strict regulations on the manufacturing units regarding the composition of sanitizers. Also, there is a need of spreading the awareness among the common peoples regarding the use of sanitizer and the impacts of their components on the body so that they can be more aware while selecting the sanitizers based on their components and impacts aswell as in disposal of various disinfectants.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Humanitarian assistance in cases of natural disasters and the 2023 earthquake in Turkey and Syria

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ABSTRACT

Natural disasters represent a grave danger that threatens the countries of the world as a whole due to the enormous losses that result from them at all human, economic, social, and environmental levels, which justifies the urgent need for international cooperation in providing humanitarian and relief assistance to the victims affected by these disasters. The importance of research related to the legal scope of humanitarian aid in cases of natural disasters appears with the increase in the frequency and intensity of disasters, the growing losses resulting from them, and the insufficiency of the material or technical capabilities to rescue the victims. Hence, there is a need for a regulating and binding law for the international community that is more stringent and enjoys the spontaneity and speed of the initiative in the moment of disaster. The recent events of the massive earthquake that struck southern Turkey and northern Syria revealed a significant gap in the mechanisms of dealing with the disaster after it occurred, which doubled the human and economic losses in particular. This is especially true with transparency and objectivity in presenting the dimensions and limits of the disaster through media coverage and social media.

Introduction

Natural disasters threaten humanity around the world, cause severe damage to lives and properties, and exceed the capacity of the states because of their adverse effects on human health and safety, and the resulting destruction of property and damage to infrastructure and services, which leads to economic and social turmoil for the affected country and requires the solidarity of society to confront it (Fatoni *et al.,* 2022). To confront them, the international community must unite to avert these disasters, face their difficulties, and expedite relief for those needing aid and recovery from their devastating effects.Furthermore, emphasis must be placed on the need to provide the appropriate environment to prepare for disasters and reduce their risks, in addition to diligent and persistent work to provide humanitarian assistance in cases of natural disasters, taking into account the application of the principles of humanity, neutrality, integrity, and the approval of the concerned country must also be obtained under the principle of respecting the sovereignty of the affected country and not interfering in its internal affairs. This will be detailed in the research later.

This research sheds light on the basic principles on which efforts to provide humanitarian assistance should be based, especially humanity, integrity, and neutrality. This is while respecting the rules of traditional international law, on top of which is the sovereignty of states and non-interference in their internal affairs. The research focuses on the extent of the affected country's responsibility to protect the people on its territory. It also deals with the consequences of the devastating earthquake that struck Turkey and Syria recently to identify the extent to which countries and international organizations are committed to their duty to provide humanitarian aid to the victims of the earthquake.

The main objective of the research is to define the importance of the need for a legal framework that calls on the international community to overcome the gaps and to come up with innovative new mechanisms that contain this type of natural disaster. Unfortunately, the countries directly

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concerned with the problem may fail to note the sudden losses due to their lack of capabilities, the enormity of the disaster, or their inaction and complacency, which requires concerted international efforts and speedy enforcement to achieve the desired feasibility. The research problem is manifested in the insufficient financial and logistical capabilities in the countries afflicted by natural disasters to advance the sudden consequences of devastating disasters on a large scale (The Organization for Economic Co-operation and Development, 2015). This necessitates an urgent need for immediate humanitarian aid from other countries and international organizations. However, aid delayed for days in this disaster to save those affected is useless. After all, those trapped under the rubble may be killed by hunger, thirst, and psychological stress if they do not die from their wounds (United Nations Office for the Coordination of Humanitarian Affairs A, 2023).

The research summarizes legal mechanisms that can be implemented to minimize the effects of natural disasters and meet the needs of those affected in the best way by answering the following main questions:

a) How effective are the efforts made in the local and international humanitarian response related to the 2023 earthquake in Turkey and Syria?

b) Moreover, how can we ensure the most effective and timely delivery of humanitarian assistance?

The descriptive method was used to investigate the issue of humanitarian aid in cases of natural disasters from a theoretical perspective by defining the concept of disasters and their impact on human rights and revealing the legal principles that govern the provision of aid (Manjunatha, 2019). In addition, the analytical approach was used by analyzing the relevant international legal texts, including international treaties, resolutions, international legal documents, and official reports issued by the relevant international institutions. It also used the comparative approach to provide a brief comparison of the earthquake that struck Turkey and Syria and how to deal with it and respond to it at the national and international levels, to finally reach a set of recommendations aimed at improving the reality of humanitarian aid and achieving the desired goal of its provision.

The Concept of Natural Disasters and their Impact on Human Rights

Natural disasters are catastrophic events that occur suddenly and unexpectedly, such as earthquakes, volcanic eruptions, floods, hurricanes, and other natural hazards such as landslides, tsunamis, floods, and droughts that affect the daily lifestyle. As a result, people direly need national and international assistance to cover their needs for protection and housing, food, medical care, and other needs (United Nations Statistics Division, 2011). Alternatively, it is the occurrence of a severe disruption in the life of a society, which constitutes a widespread threat to human life, health, property, or the environment, whether that defect results from an accident, natural cause, or human activity, and whether it is sudden or develops because of complexlong-term processes (Tampere Convention, 1998). Consequently, natural disasters cause a severe disruption in the life of society, as they result in massive human, material, economic or environmental losses on a large scale, which exceed the ability of the affected society to face them by relying only on its resources (Inter-Agency Standing Committee, 2008).

In 2021, about 432 natural disasters occurred in different parts of the world. In this way, hurricanes, floods, and other natural disasters caused 10,492 deaths and an estimated economic loss of US$ 252 billion (The Centre for Research on the Epidemiology of Disasters, 2022). Furthermore, the Disaster Risk Reduction Report issued in 2022 indicated that the average annual economic losses from disasters have doubled over the past three decades, with an estimated increase of about 145%. This equates to an average of $70 billion annually in the 1990s to more than $170 billion annually at the end of 2022 (United Nations Office for Disaster Risk Reduction, 2002).

Affected people suffer from the adverse effects of disasters, foremost of which is the displacement of large numbers of people, or they are forced to migrate from their homes in search of safety and shelter. In 2021, the number of internal displacement cases recorded worldwide reached 38 million, and 23.7 million were caused by natural disasters (Internal Displacement Monitoring Centre, 2022).

Disasters constitute a significant threat to people’s health, as they lead to deaths and serious physical injuries. Meanwhile, health risks are exacerbated by the damage to medical facilities, in addition to the mental and psychological problems that disaster victims are exposed to in their mental and psychological health resulting from crises and psychological trauma associated with the loss of loved ones and property (Giorgadze *et al.,* 2011).

Among other problems associated with disasters, we mention the long-term environmental effects related to the decline in agricultural production, drinking water pollution, and damage to infrastructure such as schools, hospitals, religious facilities, and roads. We further note that the effects of disasters are exacerbated in developing countries more than others, whether in terms of loss of life or economic loss, which calls for immediate and long-term help (The Third UN World Conference on Disaster Risk Reduction, 2015).

However, disasters are linked to food security because they cause damage to crops, livestock, and livelihoods and exacerbate hunger and malnutrition. For example, the average level of undernourishment in countries highly exposed to climate disasters increased by three percentage points compared to other countries (United Nations Office for Disaster Risk Reduction, 2022).

Disasters often affect education because of the resulting death, displacement, disease, and health problems for students and teachers. Disasters also lead to the destruction of educational facilities and school buildings, in addition to affecting the income of parents of students and losing their sources of livelihood, which negatively affects their ability to spend on their children and thus declines their academic achievement and production (Kousky, 2016).

The Humanitarian Principles Governing the Provision of Humanitarian Aid in Cases of Natural Disasters

Natural disasters are similar to armed conflicts in that both represent a severe challenge to the international community and lead to humanitarian crises that require a humanitarian response on the part of the affected country or regional and international efforts according to the size of the disaster and the resulting losses and the extent of the affected country's ability to respond. This is to ensure the survival of disaster victims, meet their basic needs, reduce their exposure to danger, and carry out their rescue, rehabilitation, and assistance (Human Rights Council, 2014). From this viewpoint, human suffering is the same. As such, the principles related to international humanitarian law that govern international and non-international armed conflicts apply to the management of natural disasters, represented by these humanitarian principles:

The Principle of Humanity

The principle of humanity is considered the cornerstone of protecting people under the provisions of international human rights law. The principle is intended to treat all those affected by natural disasters humanely and equally in a way that guarantees to save their lives, respect their rights and dignity, and alleviate their suffering in all circumstances. (Rubin & Dahlberg, 2017). The human suffering of disaster victims in afflicted countries must be addressed, respected, and protected from any brutal or humiliating treatment (International Review of the Red Cross, 1993). It should also pay special attention to the needs of the most vulnerable, such as women, children, the sick, the elderly, and refugees (Office for the Coordination of Humanitarian Affairs, 2007). In other words, giving priority when providing humanitarian aid can be discriminatory if it is based on the extent of the need of the affected people. All international governmental and non-governmental organizations that provide humanitarian assistance related to rescue, relief, and rehabilitation must carry out this assistance without discrimination between the victims and with purely humanitarian motives (United Nations General Assembly,1988).

Integrity Principle

Humanitarian assistance in disaster situations must be provided fairly and objectively, with no considerations in assisting based on race, religion, color, gender, or any other criteria. This principle has been mentioned in many international documents related to the legal readiness to deal with natural disasters. For example, the Guidelines on the Right to Humanitarian Assistance stipulate that humanitarian assistance must be provided to all people in dire need of it impartially and with no discrimination or prejudice (International Review of the Red Cross, 1993).

The Impartiality Principle

This principle stems from the importance of all persons enjoying fundamental rights and freedoms equally with other persons, where humanitarian assistance related to natural disasters is supposed to be provided with no discrimination on the grounds of race, religion, color, sex, political opinion, social class, or any other consideration. Instead, the priority in providing aid must be based on the extent of need (International Federation of Red Cross and Red Crescent Societies, n.d.).

Therefore, it is unacceptable for humanitarian assistance in natural disasters to be based on discrimination. All states must respect the rights of people on their lands and be subject to their jurisdiction with no discrimination (International Covenant on Civil and Political Rights, 1966).

Based on the preceding, humanitarian assistance must not involve any form of discrimination, which leads to excluding those in need of assistance or prioritizing those who do not deserve it based on discriminatory criteria.

The Principle of Neutrality

The principle of neutrality refers to the impartial nature of actions related to the response to disasters, where the interest of the affected people must be placed at the center of attention of humanitarian aid efforts, in addition to refraining from taking any actions or positions of a political, religious or ideological nature that constitute interference in the internal affairs of the affected countries. The principle of neutrality must be above all consideration for all those who provide humanitarian assistance (United Nations General Assembly,1988).

Providing Humanitarian Assistance in Cases of Disasters and The Principle of Non-interference in Internal Affairs

The principle of non-interference in the internal affairs of states is one of the most well-established principles in international law, as it is the basis for ensuring the international order. Furthermore, the principle represents the embodiment of legal means for protecting the sovereignty of states and ensuring their independence. Since the establishment of the United Nations Organization in 1945, the principle of non-interference has emerged strongly in the Charter, Article 2(7) of the Charter states that "Nothing contained in the present Charter shall authorize the United Nations to intervene in matters which are essentially within the domestic jurisdiction of any state or shall require the Members to submit such matters to settlement under the present Charter, but this principle shall not prejudice the application of enforcement measures under Chapter VII."

Based on respect for the state's territorial sovereignty and non-interference in its internal affairs, the duty to protect and provide relief to persons affected by natural disasters rests primarily with the affected state (International Review of the Red Cross, 1993). Therefore, this state must care for its victims and secure their basic needs. Accordingly, it draws up plans for humanitarian assistance within its territory and is concerned with organizing, coordinating, and implementing it (United Nations General Assembly, 1988). The affected country also must develop and implement effective measures to deal with the disaster, control its internal conditions, and mitigate the severity of the disaster, in cooperation with relevant international and regional organizations and bodies, to protect the lives of people in its territory and protect its national wealth and infrastructure from the effects of the disaster (United Nations General Assembly, 2008). It must be considered that the affected state's fulfillment of its responsibility to assist the affected and protect them effectively requires the support of the public authorities through a mixture of official and voluntary work efforts. The essential role played by civil society organizations, such as the National Societies of the Red Cross and the Red Crescent Movement, is highlighted here (Sphere Association, 2018).

In emergencies resulting from natural disasters, the situation usually exceeds the capabilities of the affected country. Consequently, countries must cooperate to provide aid and assistance and coordinate relief operations with the disaster-stricken country (United Nations General Assembly, 1990). When the affected state cannot provide adequate humanitarian assistance to victims under its jurisdiction or effective control, it may seek assistance from competent international organizations or other states. Although the responsibility for preventing the risk of natural disasters and taking effective measures to reduce them rests with the state on whose territory the disaster occurred, international cooperation and solidarity in relief in cases of natural disasters rest with all states and international governmental and non-governmental organizations alike (World Conference on Disaster Reduction, 2005). On this basis, the affected state bears the responsibility in the first place to protect its citizens from disaster. At the same time, states and international organizations can offer or provide humanitarian assistance to the afflicted country, provided that the sovereignty of the country receiving the aid is considered, to avoid considering it an illegal interference in the internal affairs of the affected country (United Nations General Assembly, 1988).

Thus, humanitarian operations are subject to the state's consent and approval. However, in the event of an apparent failure of the state to play its role in protecting its citizens, or the state's arbitrary refusal and non-acceptance of the entry of humanitarian relief operations into its territory without a serious reason or legal justification for this refusal, The United Nations can intervene through its agencies to provide relief, despite the lack of consent of the concerned country, considering that depriving civilians of humanitarian relief and not preserving their fundamental rights is a threat to international peace and security. So, it is the duty of the affected state not to consider humanitarian assistance provided by other states or organizations to victims as interference in their internal affairs and not to refuse assistance without justification if such refusal could lead to a humanitarian disaster or endanger the fundamental rights of the victims. In such a case, the United Nations and other competent global and regional organizations may take appropriate coercive measures to urge the affected country to comply with the rules of international law andto enable it to deliver its aid and alleviate the suffering of the victims (International Review of the Red Cross, 1993). Whether the humanitarian aid is related to providing relief to the survivors and those affected by the disaster, providing them with immediate assistance, securing decent places to stay for those who have lost their homes, or if the assistance is related to the later stages during rehabilitation and reconstruction.We conclude that the relief offered by states and international and humanitarian organizations cannot be considered illegal interference in the state's internal affairs if humanrights are seriously violated, and the life or health of the victims is seriously endangered.

The Position of International Law Regarding the Providing of Humanitarian Aid in Cases of Natural Disasters

Obtaining humanitarian assistance from natural disasters is considered one of the most important rights guaranteed by international law, which ensures that people affected by disasters will be entitled to the right to life, health, protection, and other rights necessary for their survival and well-being(International Review of the Red Cross, 1993).Several international policies, agreements, and guidelines emphasize the obligation of states and regional and international organizations to take the necessary measures to protect those affected by natural disasters and avoid and mitigate the human suffering resulting from there. This is through providing humanitarian assistance immediately and directly or in the post-disaster recovery phase and the need to rebuild infrastructure and provide housing for affected survivors.

International cooperation in providing the necessary humanitarian assistance in disaster situations is one of the well-established duties enshrined in the Charter of the United Nations, which is related to the promotion of friendly relations and cooperation between states in solving international economic, social, or humanitarian problems, including the provision of assistance to victims of disasters, especially food, medicines and medical care. At the same time, it is the responsibility of the countries near the place of the disaster to provide assistance and participate as much as possible to facilitate the delivery of humanitarian aid to the victims, especially in areas difficult to reach (United Nations General Assembly,1988). After all, disaster risk reduction requires sharing responsibilities among all concerned governments and sectors, each according to its circumstances, in a way that respects and promotes human rights and protects people's lives, property, and sources of livelihood, as well as environmental and cultural resources (The Third UN World Conference on Disaster Risk Reduction, 2015).

The charters relating to humanitarian assistance within the scope of managing natural disasters are based mainly on the International Bill of Human Rights, represented in the Universal Declaration of

Human Rights, the International Covenant on Civil and Political Rights, and the International Covenant on Economic, Social, and Cultural Rights. This is because the right to humanitarian assistance is closely linked to the right to life stipulated in all the aforementioned international documents. Many international agreements stipulate the importance of international cooperation in strengthening the capacity of the affected country in cases of natural disasters. For example, the Tampere Convention clarified mechanisms for providing telecommunications resources to mitigate disasters and facilitate cooperation in relief operations. Also, it specified how to provide international assistance in telecommunications, following the principles of the United Nations for humanitarian assistance, the nature of the natural disaster, and the ability of the affected country to prepare for it or respond to it (Tampere Convention, 1998).

In the United Nations' goal to provide support and humanitarian assistance to the affected countries in cases of disasters, it has established the United Nations Office for the Coordination of Disaster Relief Operations, which is responsible for coordinating international assistance in cases of natural disasters, facilitating access to disaster areas, organizing needs assessment tasks and collecting resources. In addition, many international and regional bodies are concerned with supporting the efforts of countries facing natural disasters, such as the United Nations Development Program, the United Nations High Commissioner for Refugees, the World Food Program, and the United Nations Children's Fund. These include the International Council of Charities, African Humanitarian Action, the Asian Network for Disaster Reduction and Response, and the Inter-American Network for Disaster Mitigation.

The Sphere Handbook Humanitarian Charter and Minimum Standards in Humanitarian Response “Sphere Guide” are among the most critical initiatives related to humanitarian standards that should govern disaster response. These documents aim to improve humanitarian work quality at the level of all sectors related to planning, managing, and implementing humanitarian responses and taking the necessary steps to mitigate human suffering from disasters. This, in turn, ensures that the affected population obtains protection and security, receives aid, lives in dignity, and participates in decisions related to their recovery (Sphere Association, 2018). The Gender Manual in Humanitarian Action includes the procedures to be followed by humanitarian actors to ensure that the specific gender needs, priorities, and capacities of different women, girls, men, and boys are considered in a way that ensures the promotion of gender equality in all aspects of humanitarian response, targeting assistance to the neediest groups, and monitoring the impact of assistance programs on the beneficiaries. The ultimate goal is to improve those programs' levels and facilitate disaster-affected communities' recovery (Inter-Agency Standing Committee, 2018).

The Sendai Framework outlined priorities to prevent new disaster risks and reduce the risks of existing natural disasters. These priorities are understanding, monitoring, and assessing disaster risks, enhancing effective response by transferring, exchanging experiences and technical knowledge, and investing in disaster risk reduction. In addition, the goal is to increase the ability to confront all concerned institutions and sectors and strengthen early warning systems and disaster preparedness. This would improve the understanding of the causes of disasters and thus enhance the capabilities to deal with them and rebuild better in the stages of recovery, rehabilitation, and reconstruction (The Third UN World Conference on Disaster Risk Reduction, 2015).

The February 6 Earthquake and the Assessment of National and International Responses to it

On February 6, 2023, southern Turkey and its neighboring regions in Syria were subjected to devastating earthquakes. One earthquake, which had a magnitude of 7.8 on the Richter scale, was classified as one of the ten deadliest earthquakes in the twenty-first century (Cohen, 2023). According to official statistics, the earthquake and the resulting aftershocks caused widespread damage, including killing about 41,000 people, injuring 108,000 others, displacing hundreds of thousands due to the collapse or damage of thousands of buildings, and destroying infrastructure facilities, including water pipes, telephone, and electricity lines, as well as the Internet, and the destruction of roads, railways, bridges, and dams (Turkey-Earthquake: Emergency Situation Report, 2023).

Since the first hours of the earthquake, the Turkish government declared a level four emergency in the affected areas for three months, which enabled it to provide flexibility in taking the necessary measures and actions to protect the public interest, confront the disaster, contain its effects, and recover from it. Furthermore, it sent rescue teams to the affected areas immediately to search for survivors under the rubble and transfer them to hospitals, informed the residents to refrain from entering the damaged buildings, provided them with alternative shelter, and called for the mobilization of all national forces. Beyond this, it also called on other countries and international organizations to extend a helping hand and provide international assistance to support rescue efforts (Al Jazeera, 2023). Indeed, many countries rushed to provide in-kind and cash assistance, medical equipment and staff, search and rescue teams, and a group of governmental and non-governmental organizations joined to provide humanitarian support to the Turkish government. For example, the World Health Organization sent medical teams to treat the injured, supported health workers, and provided health services to nearly 400,000 people affected by the earthquake. More actions include supporting 120,000 surgeries and strengthening mental health services and psychosocial support (WHO Foundation, 2023). The United Nations Development Program also pledged its assistance in repairing damaged water facilities, managing, removing, and recycling debris, paving the way for deliveries of vital supplies and food, and to start restoring livelihoods and returning to everyday life by supporting the Turkish authorities in providing essential social services, and protection of damaged cultural heritage monuments (United Nations Development Programme, 2023). At the same time, the World Food Program provided nearly one million general food aid, meals, and ready-to-eat rations to those affected by the earthquake (World Food Programme, 2023).

Despite the global and regional relief response to the emergency caused by the earthquake in Turkey, many volunteers were involved in the medical relief operations, and field hospitals were built. The difficulty of rescue operations and the complication of vital international aid deliveries due to bad weather, a blizzard, and three airports out of service due to the earthquake, given that Turkey hosts the most significant number of refugees worldwide (Al Jazeera, 2023). The response to the emergency resulting from the earthquake in Turkey also showed the need to provide earthquake monitoring centers to reduce its risks and the need for more corporation and coordination to provide services promptly (Responding to the Türkiye–Syria Earthquake, 2023).

On the Syrian side, the Syrian government announced taking urgent measures to deal with the state of emergency resulting from the earthquake. It urged citizens to provide assistance and alleviate the impact of the humanitarian disaster as much as possible. However, the Syrian authorities did not officially declare an emergency in the affected areas. On the other hand, the areas under the influence of the Syrian authorities have received support from some countries and international humanitarian organizations, especially allied countries such as Iran and Russia or friendly countries such as Iraq and Algeria, as land and air convoys loaded with food, relief, and medical supplies were sent to them (United Nations Secretary-General, 2023).

Despite the concentrated humanitarian efforts in the Syrian territories, the humanitarian situation was complicated in the areas affected by the earthquake, especially with the displacement of tens of thousands of those affected who lost their homes in the cities of Aleppo, Hama, and Lattakia, and there are not enough safe shelters for them. In addition, water pumping stations and sanitation facilities were seriously affected because of the earthquake (United Nations Office for the Coordination of Humanitarian Affairs B, 2023). This led to the spread of mental and psychological health disorders and increased disease cases after the earthquake, whether outbreaks of infectious diseases such as lice and scabies, various infections, and respiratory diseases due to cold weather. This was due to the lack of resources and the urgent need to provide baby food, medicines, equipment, and medical supplies (Responding to the Türkiye–Syria Earthquake, 2023).

Turning to the areas outside the control of the Syrian authorities and under the control of the opposition, in the north-west of the country, which is the area most affected by the earthquake on the Syrian territory, the Civil Defense Forces known as the White Helmets took over the response to the earthquake and declared north-west Syria a completely disaster-stricken area after the earthquake that hit it. This resulted from the catastrophic situation resulting from the large numbers of dead, injured, and stranded people due to the collapse of buildings and sharp cracks in them because they are not durable and do not meet the conditions for resistance to earthquakes amid a lack of capabilities and services and the lack of shelters, food insecurity, stormy and freezing weather conditions, and low temperatures. The Civil Defense Forces called on all local forces to mobilize their cadres. They called on international humanitarian organizations to intervene quickly to relieve the afflicted and meet their needs (Mistou, 2023). Indeed, aid was provided, but it was somewhat late due to supplies, financing, and delivery difficulties. After all, the Syrian authorities delayed delivering aid to the affected people in need. The first UN aid convoy entered the affected areas in northwestern Syria three days after the earthquake (United Nations Secretary-General, 2023). There has been the politicization of the process of distributing humanitarian aid and its delivery to areas under the control of the opposition. In this way, the Syrian government insists that any foreign aid should be sent through it, with allegations that some relief materials and food aid parcels are sold on the black market instead of delivered to the earthquake victims (Khatib, 2023).

This has complicated relief efforts in what can be considered a violation of the principles of humanitarian work, which are supposed to be based on the principles of humanity, neutrality, and integrity. This is especially the case if we consider that most of the Syrians affected by the earthquake, who reside in southern Turkey in the Syrian areas under the control of the opposition, were displaced initially due to the country's crisis for 12 years.

Thus, it seemed as if there was tolerance for the collective suffering of the Syrians, whose humanitarian needs, according to the United Nations, have reached their highest level since the outbreak of the conflict nearly 12 years ago (United Nations Office for the Coordination of Humanitarian Affairs C, 2023). The humanitarian catastrophe that befell Turkey and Syria as a result of the earthquake highlighted the failure of the international community to assume its responsibilities in the aftermath of the devastating earthquake disaster. Considering the growing needs of the affected people in the two countries, the slowdown in the provision of aid, and the delay in the arrival of first aid and participation in operations to save lives at risk convoys to the region to the earthquake victims in Syria. It is important to remember that the earthquake was associated in Syria, in particular with deteriorating living conditions, poor infrastructure, a shortage of health personnel, and the lack of necessary medical care for those affected by the earthquake or those suffering from chronic diseases who could not find the required treatment for them.

Conclusion

Despite the importance of the efforts made within the scope of the international humanitarian response related to facing the dangers of the 2023 earthquake in Turkey and Syria and limiting its losses. We have felt the mobilization of the affected country, Turkey, with all its capabilities to note the problem that struck the country, taking all possible measures to protect the affected people. This enabled it to limit the losses and reduce them despite the severity of the disaster, helped by the support it received from the international community. On the contrary, the local and even international response to the earthquake in Syria is considered insufficient. After all, the support provided was not commensurate with the enormity of the damage caused by the devastating earthquake and the difficulties in delivering aid. This, in turn, necessitates the urgent need for more international cooperation at all social, economic, and health levels. To ensure the provision of the most significant amount of adequate humanitarian assistance and to work to reduce the effects of the earthquake. The research recommends:

- Conclusion of a binding international agreement concerned with protecting those affected by natural disasters, mobilizing immediate assistance, and organizing international relief efforts to build the necessary capabilities to prevent, respond to, and mitigate natural disasters.
- Integrating precautionary plans for managing natural disasters into countries' development plans. Such as providing early detection techniques for natural disasters and warning

residents to forestalled their effects in the event of their occurrence.

- Training the population to perform the necessary first aid methods in disasters to help reduce the possibility of heavy losses of life and property.
- Ensuring effective follow-up with local authorities, the international community, and humanitarian organizations assume their relief responsibilities while respecting the human rights and dignity of those affected by natural disasters.
- Facilitating the arrival of humanitarian aid, organizing its distribution process, and providing relief equipment, rescue teams, and human cadres trained in dealing with natural disasters.
- Providing early detection techniques for natural disasters and warning residents in the event of their occurrence to reduce damage to them and their property.
- Increasing community awareness of dealing with natural disasters and developing clear plans for evacuating buildings in emergencies.
- Adopting strict laws for the construction of housing and facilities in Turkey and Syria to avoid the recurrence of the humanitarian catastrophe associated with the February 6 earthquake.
- Providing urgent essential services such as health, food, water, sanitation, and urgent housing for people displaced by the earthquake in Turkey and Syria and supporting livelihoods and rehabilitation in the areas affected by the earthquake.

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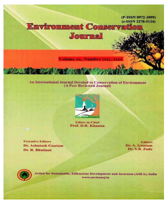

Microclimate analysis of high-density urban residential open enclosures: A case of Thane, India

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ABSTRACT

Urban heat island produces a significant impact by modifying the microclimate in urban areas. To ensure good quality of life with a safe and healthy built environment, the floor space index (FSI) can be an effective tool. It helps to control the urban densities and shape the morphology of the built environment. Taking the case of typical residential development in a densely populated Indian city, an attempt has been made to explore the relationship between FSI and the microclimate of such open spaces that perform like an open enclosure (OE). Adopting the simulation pathway in ENVI-met, a mathematical relationship is established between an important tool used by urban planners and the variables of the microclimate in a typical urban enclosure. The observations indicate that FSI has a strong negative correlation with air temperature and mean radiant temperature. Evaluation of physiological equivalent temperature reveals a similar relationship with FSI, demonstrating a temporal transposition of the trend for a particular FSI range of 2.5-3.5 in the late evening and early morning hours due to high humidity levels and reduced wind speeds. The study will help the planners to prognosticate the microclimatic variables while working out the data-based, logical and well-evaluated future development control regulations.

Introduction

According to the '2018 Revision of World Urbanization Prospects', by the year 2050, around 68% of the world's population will live in urban areas and India alone will add around 416 million people to the overall urban dwellers across the globe. Due to the swelling urban densities, the quality of life in urban areas is greatly compromised. The formation of urban heat islands (UHI) has deteriorated the urban environment by modulating the microclimate of the cities. In the recent past, studies related to UHI and urban microclimate have gained much importance worldwide. UHI is the resultant warming of ambient air temperatures (T_{air}) in the urban areas as compared to its rural counterparts due to a variety of factors that include reduced vegetation, increased material-low albedo surfaces, trapping of radiations due to multiple reflections, urban morphologies, reduced windspeeds and anthropogenic emissions. The fabric of cities has evolved over a while, either organically or with partial/piecemeal interventions or even entirely as a greenfield development. In all cases, the tools used by planners play an important role in shaping our cities. The configuration and orientation of streets, built volumes and the properties of the surfaces are directly linked to the urban climate (Shareef & Abu-Hijleh, 2020). In the process of urbanisation, the cities have transformed into concrete jungles, and small pockets of open spaces that are left within the developed areas are

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the only places that function as relief zones providing fresh air to breathe, enriching the quality of life, for the people living in their proximity. But due to the presence of UHI the microclimate of these open spaces too, is greatly altered which in turn affects the functionality/use of the open spaces for which they were intended. Improper building bylaws and open space requirements are responsible for heat gain from all sides of the building (Thakur & Sanyal, 2016). The role of urban planners is crucial for achieving good air quality and thermal comfort (OTC). According to the international standard of thermal comfort (ISO 7730:1994), the factors affecting thermal comfort can be classified into two groups, namely, climatic and personal factors. Climatic factors include air temperature (T_{air}), radiation (T_{mrt}), humidity and wind speed (WS), whereas personal factors are the rate of metabolism of the human body and insulation due to clothing.The studies conducted in the past to understand the relationship of the elements of the cityscape, with the microclimate can be categorized under the following major heads. Large-scale urbanization has resulted in the loss of green cover in the cities which in turn is responsible for the loss of habitat for many species, increase in the pollution of natural resources and deterioration of the quality of life of the city dwellers (United Nations). Vegetation helps to mitigate the impact of climate change in urban areas (Tumini& Rubio-Bellido, 2016). It helps to control the microclimate by decreasing T_{air}, increasing humidity and modulating the wind ventilation (Nasir *et al.*., 2015). The tree cover ratio is found to be negatively related to daytime UHI (Lin *et al.*., 2017). Roadside plantations can cool the microclimate near building facades by up to 0.25-1.28^{0}C (Chatzinikolaou *et al.*., 2018). Urban geometry plays an important role in formulating the microclimate of built and unbuilt areas by modifying the duration of exposure of surfaces to the solar radiations, in terms of sky view factor (SVF)(Jamei *et al.*., 2017). Shading reduces the amount of light that reaches building surfaces and pavements, reducing the reflected light, thermal radiations and surface temperatures(Erell *et al.*., 2011). Shading is a function of aspect ratio that can be achieved by placing the buildings in close proximity and orienting streets north-south (Emmanuel *et al.*., 2007). A case study of a superblock in Jakarta concluded that the comfort conditions surrounding a high-rise building were worse with minimal diurnal variations as compared to the central park area (Donny Koerniawan, 2015). Urban areas are characterized by increased material surfaces. These surfaces absorb more radiation and are responsible for increased ambient T_{air}, depending on their material and colour (Mehrotra *et al.*., 2021). Therefore, optimization of material selection can substantially improve thermal comfort(Kakoniti, Georgiou, Marakkos, Kumar, &Neophytou, 2016). High albedo materials in a high-density commercial area, were found to reduce surface temperature up to 5-6°C (Emmanuel *et al.*., 2007). Using reflective materials on pavements helps to improve thermal conditions (Santamouris *et al.*., 2012). But the benefit is reduced due to the reflected solar radiations being trapped in the built environment, thereby, increasing T_{air} and cooling loads.(Chatzidimitriou &Yannas, 2016; J. Yang, Wang, & Kaloush, 2015). Thermal comfort conditions are negatively related to the sky view factor of high albedo material surfaces(Salata, Golasi, Vollaro, &Vollaro, 2015). Several microclimate simulation and prediction software tools with different levels of complexity are available. In a comparative study, ENVI-met was found to be most suitable to simulate thermal comfort indices, based on the fundamental laws of fluid dynamics and thermodynamics. It is a user-friendly tool to predict meteorological parameters and evaluate design strategies in outdoor spaces (Albdour&Baranyai, 2019). It has been used by several researchers in the past. A study conducted by Kakon *et al.*. (2009) to investigate the impact of development rules on the microclimate of urban canyons in Dhaka, (Kakon *et al.*., 2009), and another study by Du *et al.*. (2018) examined the influence of building heights and porosity on the wind comfort, around various building configurations in Hong Kong, (Du *et al.*., 2018), and study by Stocco *et al.*. (2021) to evaluate various design alternatives of an urban square in Mendoza Argentina to achieve the best conditions of thermal behaviour and comfort (Stocco *et al.*., 2021) are some of the similar studies done in the past using ENVI-met as the simulation tool. Using measured data as full forcing ENVI-met produces realistic results with good accuracy (Ketterer&Matzarakis, 2015; X. Yang, Zhao, Bruse, & Meng, 2013). Development Control Regulations (DCR as per local authority) applies various tools to control the development in urban areas and thereby maintain an equitable distribution of infrastructure facilities, and open spaces and ensure a healthy built environment for all. It is important to understand the relationship of various tools of DCR with the microclimate of built and unbuilt areas of the city fabric. The relationship shall provide inputs to optimize the urban morphology in the development plans and formulate mitigation strategies as DCR thereby reducing UHI (Smith & Levermore, 2008; Yahia & Johansson, 2013). As per the local DCR of the study area, the provision of open space is a function of the area of the land parcel, available for development, therefore as the permissible floor space index (floor space index or FSI is the ratio of the total built-up area divided by the plot area available for the development. It is also known as FAR or floor area ratio at many places) increases a greater number of floors are added in the same building and layout plan, to consume the full FSI available. The research aims to understand and analyse the relationship of the floor space index (FSI) with the variables of the microclimate of a typical OE, taking the case of the current trend of housing development in the study area of Thane city.

Material and Methods

The methodology for the present research is based on the simulation method adopted by many researchers using ENVI-met software(Yahia & Johansson, 2013), (Kakon *et al.*., 2009), (Chatzidimitriou & Yannas, 2004). The 3D models of various scenarios of the case studies with varying FSI were constructed. The validation of the models is done by comparing the simulation results of the 3D model of the existing scenario with the microclimate variables recorded on-site in the case studies. The simulation is performed using meteorological data from the nearest weather station. Further, the readings of the variables which constitute the microclimate are recorded at various locations in the 3D models. Statistical analysis of the results of the simulation is conducted to understand the relationship of each microclimatic variable with the FSI. The mean values of the data recorded at various receptors (the number of receptors placed are; CS1-7, CS2 & CS3-9 each) were used to establish a correlation with the FSIand derive the regression equations for each variable for future applications. In this study, thermal comfort is evaluated based on two climatic factors, specifically, air temperature (T_{air}) and mean radiant temperature (T_{mrt}), along with wind speed (WS) and relative humidity (RH). In a tropical climate, physiological equivalent temperature (PET) is the most commonly used OTC index to assess the value of thermal comfort in outdoor spaces(Fong *et al.*., 2019; Koerniawan, 2017). Therefore, to understand the response of an average person to the thermal environment, PET is used for evaluation.

Study area

The study area lies in Thane (Maharashtra), the most populous district of India, and is currently facing an influx of population as a result of its locational advantage, of adjacency to the megacity of Mumbai. The need to accommodate more people in the limited area of the city boundaries has resulted in increased FSI (also called Floor area ratio FAR) and reduced vegetated unbuilt areas. In high-density residential development, there is a trend of providing central open spaces surrounded by peripheral built structures on all sides. This works as an enclosure for the central open space and the morphology of the surrounding built structures plays an important role in modulating the microclimate and usability of the central open areas. In the first phase 50 housing societies (Figure 2) based on visual analysis of the formation of OE in the layout plans, were selected, mostly located off-Ghodbunderroad, which is the main focus of the new development. Further, for the second phase, keeping 20.99% cut/void in the perimeter of the enclosure as the criteria 20 societies were selected, out of which 3 societies (Figure 1) from the middle range were finalized for simulation and analysis, the required physical parameters of the selected case studies are as given in Table 1.

Climate: Thane has a tropical monsoon type of climate that borders on a tropical wet and dry climate. The monthly mean temperature varies from 22°C to 36°C. In winter, the temperature can fall to

Figure 1: Location plan of 50 Housing Societies selected in Phase 1 (Source: Google Earth Satellite Image 2021)

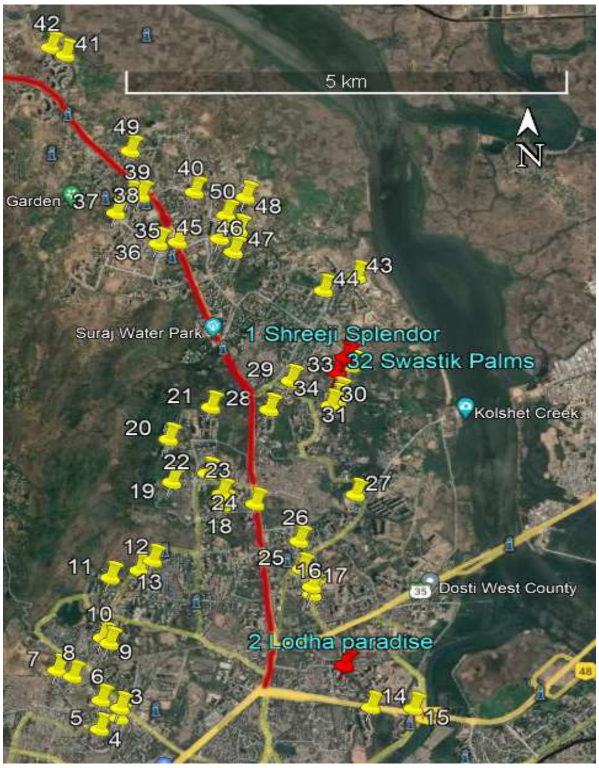


Table 1: Statistical data related to the selected case studies

CS. No.	Total area in sqm	Central open space in sqm	Open enclosure (OE) dimensions		
			Area	Perimeter	Cut/Void
CS1	22968	2853	4984	315	44
CS2	19600	3712	7888	356	48
CS3	21868	2688	6216	316	52

12 °C at night, while in summer it can rise to over 40°C at noon. The monthly average RH ranges from 53% to 90%. The annual range of WS lies between 1 to 3 m/s. Since the impact of UHI is more prominent in summer, therefore, 3rd May 2021, which lies in peak summer, is selected for on-site data recording and simulation.

Model Validation

The existing scenario (Figure 2) with OE and existing vegetation of 3 case studies, was constructed in ENVI-met spaces using common parameters of, dx =2.0m, dy =2.0m, base dz =3.0m, where 3m of dz, represents the floor-to-floor height as per the prevailing practice in multi-storied housing complexes. The grid cell dimension of 2m x 2m was used to achieve optimum time for simulation and also maintain a realistic level of detail for the ENVI-met model (Salata *et al.*., 2016) with vertical grid generation as equidistant and the lowest cell split into 5 sub-cells. The material for walls and roofs was taken as default wall and default roof with moderate insulation. The discrete model geometry of individual cases is as per Table 2. The microclimate was simulated using full forcing of climatic data; for air temperature, relative humidity, direct SW radiations, diffuse radiations, wind speed and direction retrieved from historic weather data for the selected day for Thane, retrieved from https://www.meteoblue.com, accessed on 12th September 2021 and using IND_Mumbai.430030_ISHRAE.epw, available on https://energyplus.net/weather. The results of the simulation for T_{air} and humidity were validated with the on-site observations. The microclimate variables of T_{air} and RH were recorded using a dry and wet bulb hygrometer (Table 3). The percentage of RH was calculated with the help of an online interactive psychrometric chart at flycarpet.net, for the corresponding dry and wet bulb temperature (DBT and WBT) readings. The T_{air} and RH were recorded at 11:00, 14:00, 17:00 and 22:00 at one of the receptor locations in each case study (Table 3), at a height of 1.5m from ground level, in the OE and validated with the on-site readings which were recorded at the same location, with the help of hygrometer.According to the correlation coefficient (Table 4) for the simulated and on-site readings of T_{air} and RH, it is concluded that the simulation results have a fairly strong correlation with the on-site observations, with slight variations, which can be attributed to the difference in geometries and other morphological details between the model constructed in ENVI-met and on-site scenario. In the model constructed, only the vegetation, ground surface finishes and built volumes are considered.

Construction of scenarios

To understand the impact of changed FSI regulations on the microclimate of central open space in a typical layout, four scenarios (Figure 3) were constructed for each case study, in ENVI-met spaces, with the same layout plan, grid dimensions,

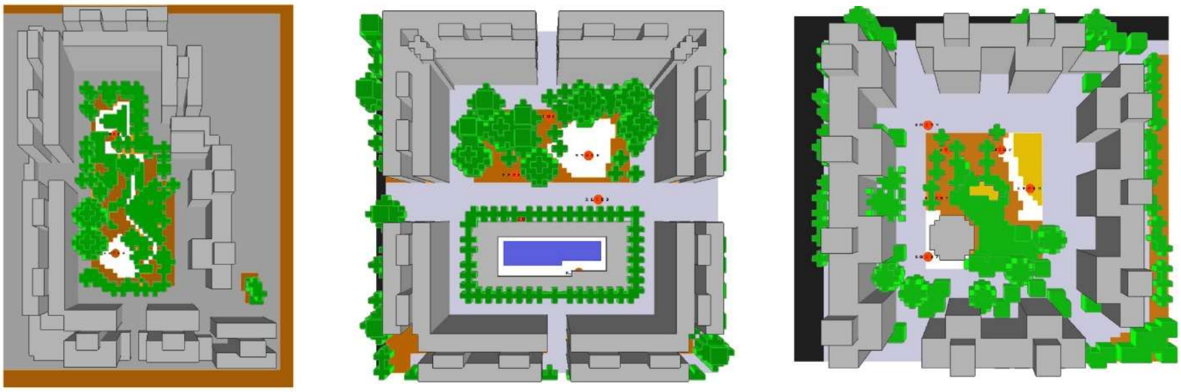


Figure 3: 3D- models of the existing scenario of case studies 1, 2 and 3 (from left to right) constructed in ENVI-met spaces 4.4.6

Table 2: Discrete parameters of 3D-model geometry for case studies

	Grid dimensions	Core xy domain size	Nestin g grids	Highest building in the domain	Height of 3D model
CS1	66 x 87 x 30	132.00m x 174m	5	30m	90m
CS2	70 x 70 x 30	140.00m x 140m	7	28m	90m
CS3	77 x 71 x 30	154.00m x 142m	7	30m	90m

Table 3: On-site hygrometer readings for CS1, CS2 & CS3, on 3rd May 2021

Time of recording	Case study 1		Case study 2		Case study 3	
	DBT	WBT	DBT	WBT	DBT	WBT
11:00	34	28	34	26	33	27
14:00	35	28	35	28	34	27
17:00	35	30	35	30	34	29
22:00	33	29	33	25	29	26

Table 4: Comparison of on-site recorded data with simulation results of the existing scenario

Time of recording→			11:00	14:00	17:00	22:00	Correlation coeff.
CS1	T_{air} in ^{o}C	Simulation	39.12	39.62	34.67	26.94	R=0.727
		Recorded	34	35	35	33	
	RH%	Simulation	28.72	33.55	37.82	82.49	R=0.806
		Recorded	63.3	58.6	69.3	74.1	
CS2	T_{air} in ^{o}C	Simulation	38.60	39.50	34.78	26.89	R=0.754
		Recorded	32	35	35	29	
	RH%	Simulation	30.81	33.40	37.59	82.68	R=0.762
		Recorded	62	58.6	69.3	72.2	
CS3	T_{air} in ^{o}C	Simulation	28.56	32.42	36.82	26.92	R=0.759
		Recorded	33	34	34	29	
	RH%	Simulation	28.56	32.42	36.82	82.51	R=0.899
		Recorded	62.6	57.9	68.8	78.7	

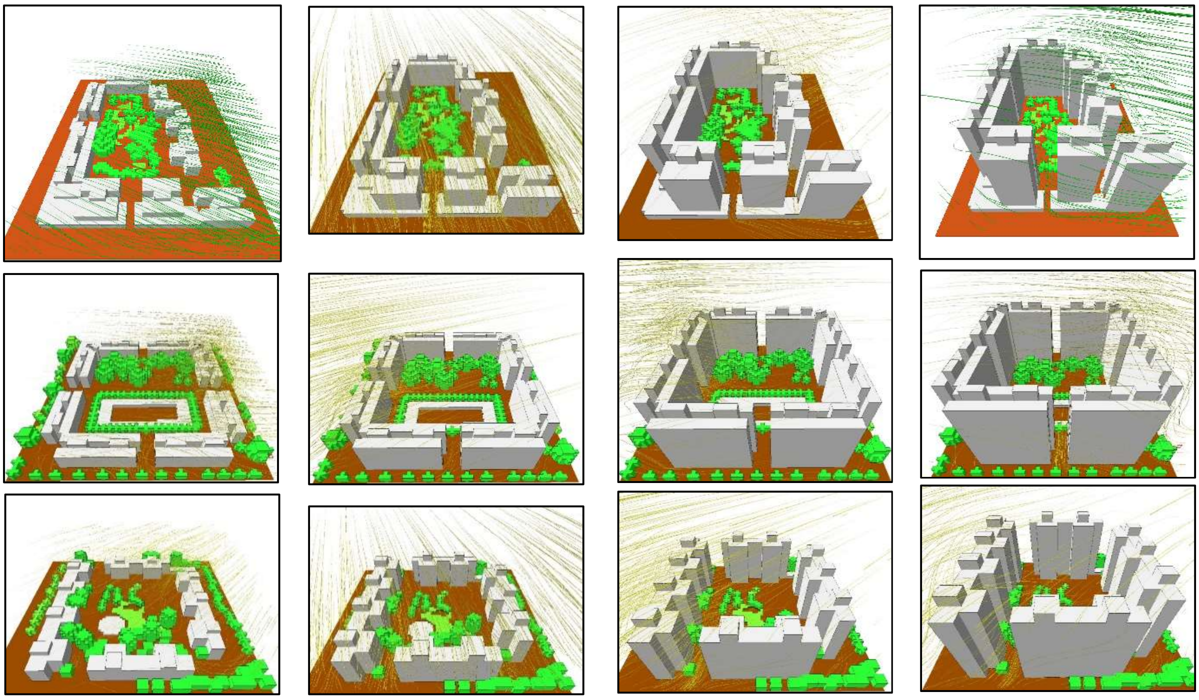


Figure 3: Physical models created in ENVI-met Spaces 4.4.6 for case studies CS1(Top row), CS2 (Middle row), CS3 (Bottom row) and their FSI scenarios respectively, SCN 1,2,3&4 (left to right)

core xy domain size, nesting grids and grid cell dimensions as in existing scenario. The overall height of the built structures under various scenarios is as per the observed trend of development which is a reflection of local firefighting regulations. The physical parameters of various scenarios are as per Table 5. The 3D models of all the scenarios were simulated for 24 hours, starting at 00:00 hrs. All the variables were evaluated at the x/y cut at level k=2 in ENVI-met, which is at 1.5 m from ground level and corresponds to the average height at which the microclimate is experienced. The OTC index of PET was calculated in Bio-met with the values for the average Indian male of 35 years, a height of 1.65m and a weight of 70kg. Clothing value was taken as 0.5clo considering light summer clothing, walking speed as 1.21 m/s and work metabolism of 80W of light activity. For analysis, daytime is considered as between 06:00 to 19:00 as per the sunrise and sunset on simulation day (Figure 4).The simulation was done in ENVI-met core, using full forcing with the climatic data identical to the existing scenario. Simulation results were then analysed in Leonardo and Bio-met.

Table 5: Physical parameters of constructed FSI scenarios

Case study →				**CS1**	**CS2**	**CS3**
Total land under development in sqm →				16400	19600	16600
Building footprint in sqm →				4196	4908	4252
Scenario	**FSI**	**No. of floors**	**Max. height**	**BUA**	**BUA**	**BUA**
SCN1	0.75	4	12	16605	19845	16808
SCN2	1.5	8	24	33210	39690	33615
SCN3	2.5	13	39	55350	66150	56025
SCN4	3.5	18	54	77490	92610	78435

Note: The actual built-up areas under the scenarios may vary depending upon the detailing and type of architectural spaces created on the individual floor plates. The floor-to-floor height is considered as 3m

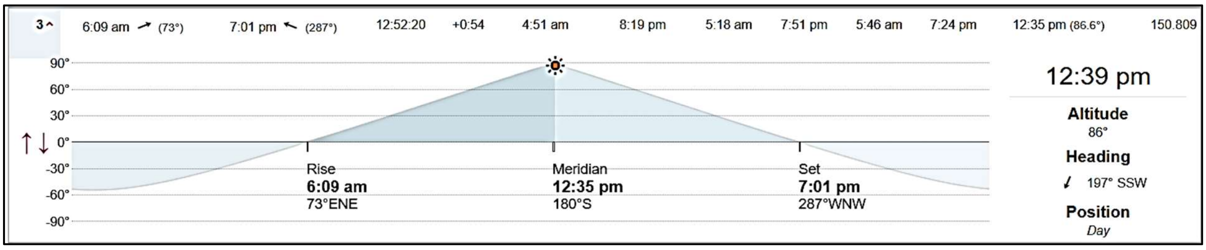


Figure 4: Sun positions on 3rd May 2021 retrieved from www.timeanddate.com

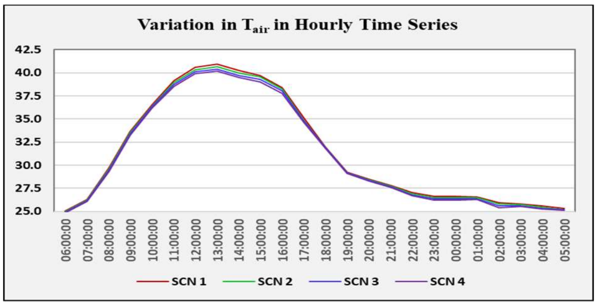
Results and Discussion

The simulation output was recorded at the strategically located receptors, which represent the overall microclimate of the OE. The selected parameters of T_{air}, T_{mrt}, PET, RH and WS, were noted and the mean values of all receptors were considered for further statistical analysis. The values of all the variables were found distorted for the initial and the last hour of the simulation, hence they were reproduced via the interpolation method for analysis. The relationship of microclimatic variables is first analysed individually with the FSI and then a comparative study was conducted to understand the relative impact of FSI on these parameters. A summary of per-floor variation in the microclimatic parameters corresponding to FSI represented by the scenarios is presented in Table 6.

FSI vs T_{air}(Figure 5): Simulation results revealed that during the entire day the impact of FSI on $T_{air}$was maximum at 14:00 hrs (Figure 6) when the overall reduction in mean $T_{air}$of SCN 4 was found to be -0.77^{0}C less as compared to SCN 1.

Table 6: Summary of per-floor variation in microclimate parameters against FSI scenarios

Category→	FSI 0.75 to 1.5	FSI 1.5 to 2.5	FSI 2.5 to 3.5	FSI 0.75 to 3.5
Change in category →	Low-rise to Mid-rise	Mid-rise to Small high-rise	Small to Big high-rise	Low rise to Big high-rise
No. of floors →	4 to 8 floors	8 to 13 floors	13 to 18 floors	4 to 18 floors
		DAY TIME		
ΔT_{air} ^{0}C	-0.043	-0.029	-0.029	-0.033
ΔT_{mrt} ^{0}C	-1.327	-0.816	-0.637	-0.898
ΔPET ^{0}C	-0.480	-0.346	-0.269	-0.357
ΔWS	-0.029	-0.015	-0.003	-0.014
ΔRH%	-0.021	0.011	0.057	0.018
		NIGHT TIME		
ΔT_{air} ^{0}C	-0.030	-0.022	-0.020	-0.023
ΔT_{mrt} ^{0}C	-0.109	-0.049	-0.050	-0.067
ΔPET ^{0}C	-0.070	0.019	-0.001	-0.014
ΔWS	0.018	-0.023	-0.014	-0.008
ΔRH%	0.405	0.107	0.097	0.189



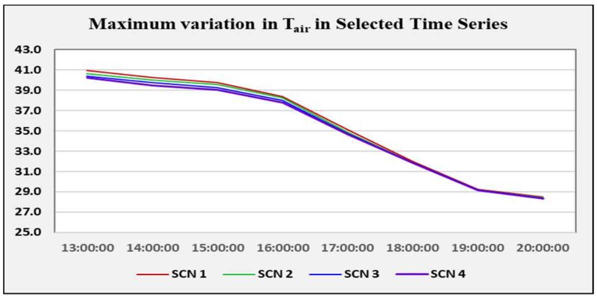


Figure 5: (Left) Chart showing the variation of Mean values of T_{air} at all receptor locations, for hourly time series and Figure 6: (Right) Chart showing maximum variation in T_{air} in selected time series

The minimum impact of FSI was seen in the evening at 19:00 hrs. when the mean T_{air} of SCN 4 was -0.12^{0}C less than SCN 1. The maximum impact of –0.51^{0}C in the nighttime was noted at 2:00 hrs. Overall, for an FSI increase of 2.75 (i.e., from 0.75 in SCN 1 to 3.5 in SCN 4), in the daytime, the average reduction in T_{air} was-0.46^{0}Cand in the nighttime, the average decrease in T_{air} was found to be -0.32^{0}C. Therefore, the impact was more pronounced in the daytime. There is a statistically significant relationship (r= 0.99, p= 0.005) between FSI and T_{air} in ^{0}C. Overall, FSI is found to have a strong negative correlation with T_{air}. A similar observation was made by Emmanuel *et al.*, in their study related to the H/W ratio. It was observed that the higher H/W ratio tends to decrease T_{air} (Emmanuel *et al.*., 2007). Since higher FSI results in a high H/W ratio, therefore, it will also tend to lower T_{air}. Another study concluded that the high-density urban morphology if well planned can lead to improvement in thermal comfort in tropical climates (Xue *et al.*., 2017). Since high FSI is associated with high density, therefore, the present study is in agreement with the findings of previous research. Impact of UHI on T_{air} (Figure 7): Study of the scatter plots of change in T_{air} (ΔT_{air}) of the simulation results against the T_{air} of the inflow boundary condition of weather data, for 24 hrs time series, it is found that when the inflow T_{air} is higher, ΔT_{air} is also more. Therefore, when the UHI is more prominent at the city level, ie. the inflow $T_{air}$is higher, its impact will be further pronounced (R^2 = 0.54) at the micro level, especially when the type of development is low rise and midrise. When the typology of development changes from midrise to high-rise the impact of high UHI is lesser (R^2 = 0.48) on the microclimate. Hence the study supports the argument that the higher FSI helps to reduce the impact of UHI by lowering the T_{air}. FSI vs T_{mrt} (Figure 8): It was observed that during the entire day the maximum impact of FSI on T_{mrt} was noted at 16:00 hrs. (Figure 9), when it was found to be -26.22^{0}C less in SCN 4 as compared to SCN 1.

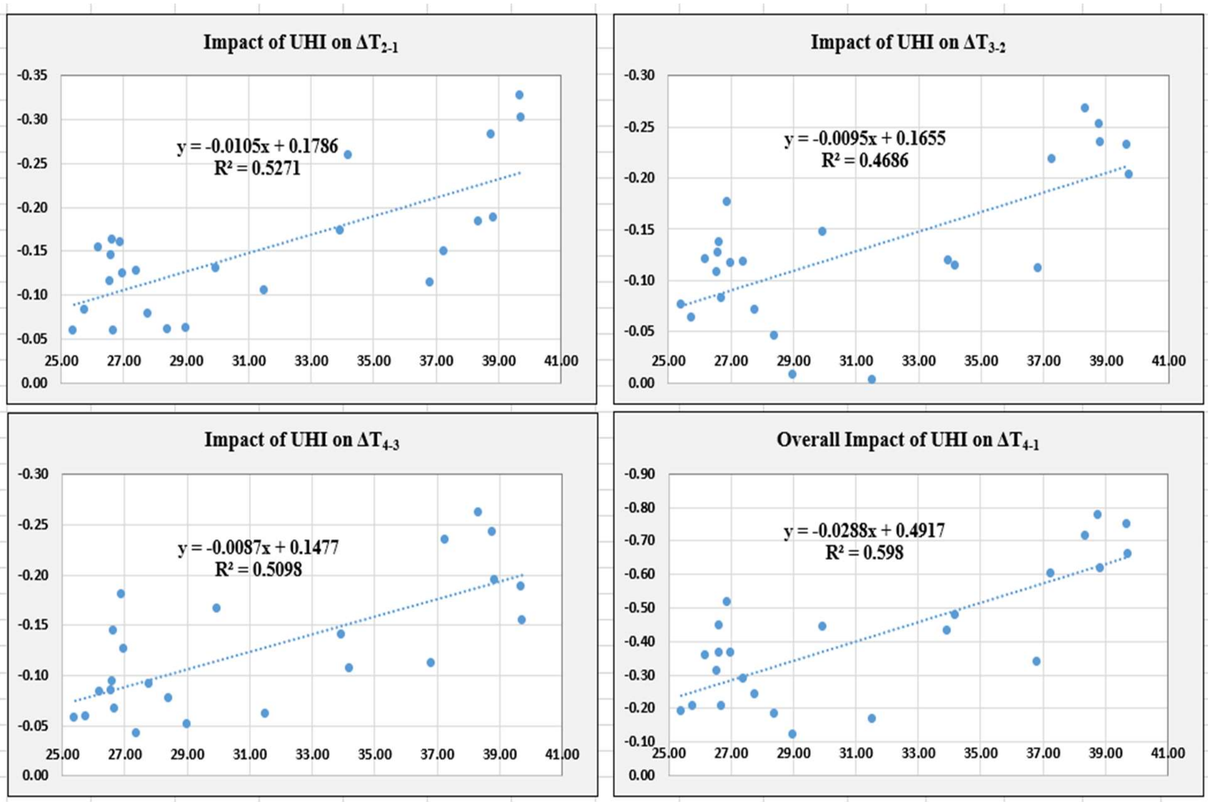
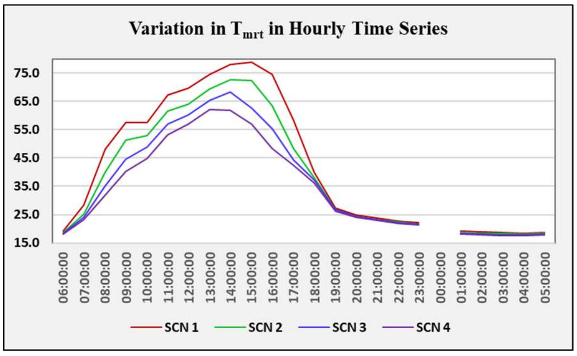


Figure 7: Scatter plots showing the impact of UHI on T_{air} (clockwise from the top left) change in SCN 1 to SCN 2, SCN 2 to SCN 3, SCN 1 to SCN 4 and SCN 3 to SCN 4

The minimum impact of FSI was observed at 23:00 hrs. when the values of SCN 4 were found to be -0.69⁰C less than the value in SCN1. In the nighttime, a maximum reduction of -1.21⁰C in T_{mrt} was observed at 2:00 hrs., from SCN 1 to SCN 4. It was observed that, for an increase of 2.75 in the FSI, the average reduction in T_{mrt} was -12.57⁰C in the daytime, whereas, in the nighttime, the average reduction was -0.93⁰C. There is a statistically significant relationship (r= 0.98, p= 0.015) between FSI and T_{mrt} in ⁰C. Therefore, T_{mrt} is strongly negatively correlated toFSI, the impact being maximum at 16:00 hrs. In the absence of direct radiation at nighttime, the overall impact is much less. The results of the present study were found to be in close agreement with a similar study conducted to understand the impact of building regulations in Chennai(Salal Rajan & Amirtham, 2021). To compare the values in both cases, a reduction in $T_{mrt}$with the SVF was calculated. It was found that in the present study for every 0.1 reduction in SVF, the T_{mrt} was reduced by -12.48°C, whereas in the case of the previous study, for a reduction of 0.1 in SVF the T_{mrt} was reduced by 11.65°C. Since Mumbai and Chennai both are coastal cities separated by a latitudinal distance of around six degrees, have similar climatic conditions. FSI vs WS (Figure 9): In the statistical analysis it was observed that the resultant windspeed is highest in SCN1 when the building heights are 12m and the FSI was 0.75.



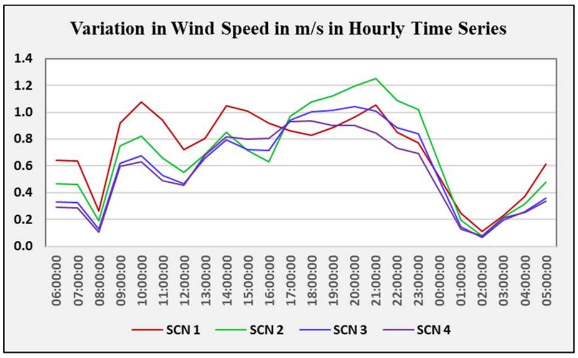
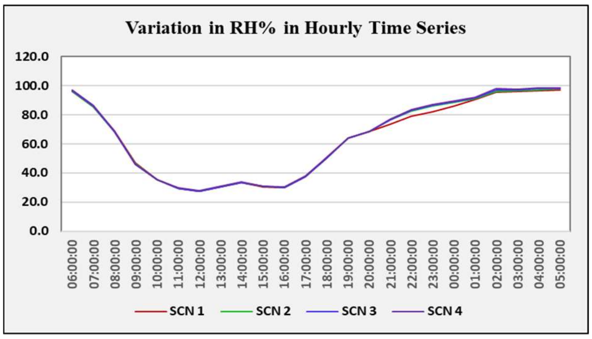


Figure 8: (Left) Chart showing a scenario-wise variation of Mean values of T_{mrt} at all receptor locations, in hourly time series and Figure 9: (Right) Chart showing scenario-wise comparative WS variation in hourly time series

As compared to the wind inflow boundary condition measured at 10 m, the WS measured at 1.5 m was much less. It was observed that as the FSI increases from SCN1 to SCN4 (Figure 9) the windspeed reduction is significant. The maximum impact of FSI in terms of WS modulation was observed at 11:00 hrs when the WS is reduced by -0.455m/s from SCN1 to SCN4. During the entire day, the minimum impact was observed at 19:00 hrs. when the reduction in WS was -0.17m/s. Further observing the variation in windspeed among scenarios it was found that when the building typology changes from low-rise to mid-rise there is a decrease in wind velocity from 17:00 hrs. till midnight when the inflow boundary conditions of WS is highest in the entire day. This trend is negligible when the building typology changes from mid-rise to high-rise. Further when the typology of the building changes from high-rise to big high-rise the reduction in wind speed is observed in the afternoon till 16:00 hrs and then a marginal reduction between 2:00 to 3:00 hrs in the late night. A similar trend of the reduction in wind speed was reported in a previous study in severe cold regions of China (Jin *et al.*., 2017). Considering the average change in the WS it was noted that the average reduction of -0.09 m/s in WS was the maximum between SCN2 and SCN3 when the building typology changed from mid-rise to high-rise, which supports the findings of a previous study that the midrise enclosed layout shows the worst performance in terms of wind speeds (Ma & Chen, 2020). FSI vs RH (Figure10). It was observed that the variation in RH across the constructed FSI scenario is nominal. However, there is an average increase of 1.25% RH from SC1 to SCN4, ranging from a maximum of +5.3% at 23:00 hrs. to a minimum of +0.02% at 19:00 hrs. Across the scenarios, the maximum increase in RH was noted between SCN1 and SCN2 when the typology of development changed from low-rise to mid-rise. The average increase in RH in the daytime is less than in the nighttime. As per the analysis, the average increase in RH in the daytime is 0.26% as compared to the average increase of RH of 2.64% in the nighttime. FSI vs PET(Figure11): The urban form has a noticeable impact on PET (Othman &Alshboul, 2020). From the analysis, it is seen that as the FSI increases from scenario SCN1 to SCN4, the comfort condition improves which is demonstrated by lower mean values of PET. The maximum impact is observed at 16:00 hrs. when the PET in SCN4 is 10.03^{0}C lower as compared to SCN1, whereas the least impact was observed at 21:00 hrs. when the PET in SCN4 was higher by 0.013^{0}C than in SCN1. The impact of increased FSI is found to be more significant in the daytime with a reduction of -4.99^{0}C in the average values of PET from SCN1 to SCN4 as compared to the nighttime reduction of -0.19^{0}C.



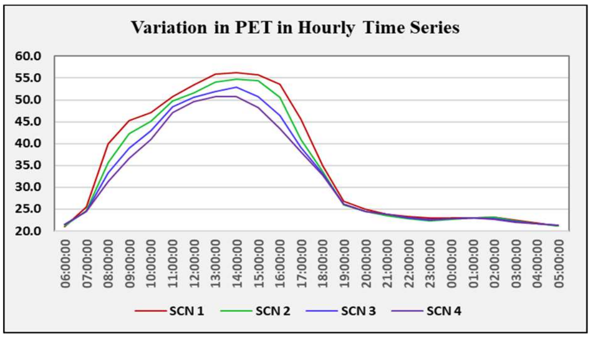
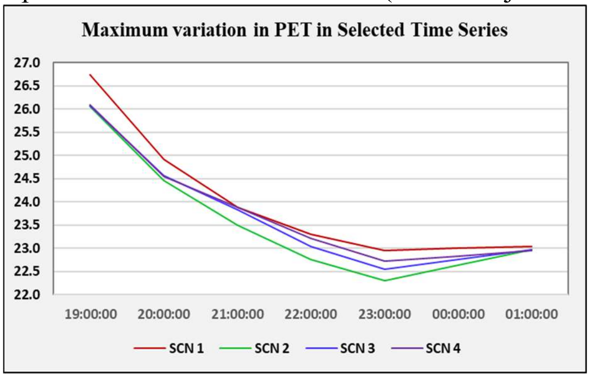


Figure 10: (Left) Scenario-wise comparative RH% variation in hourly time series and Figure 11: (Right) Scenario-wise comparative PET variation in hourly time series

Transposition of trend (Figure 12): Taking a closer look at the trend of change in PET across the scenarios it is found that in a selected time slot from 19:00 hrs. to 01:00 hrs. there is a transposition of trend in the scenario curves. The thermal comfort conditions in terms of mean PET values in SCN3 and SCN4 become worse in this time slot. In other words, when the building typology changes from mid-rise to high-rise, a dip in thermal comfort is observed which worsens as the typology further changes from high-rise to big high-rise (Figure 14).Further to understand the impact of UHI on the thermal comfort in OE, a scatter plot (Figure 13) of inflow boundary conditions of T_{air} with the ΔPET among scenarios was worked out. From the scatter plots it was concluded that the correlation of the two variables was stronger in the higher FSI scenario which implies that the impact of increased T_{air} due to the UHI effect will be intensified and the thermal conditions in the OE will worsen as the height of the built structures increases. Therefore, the study supports the previous research that the aspect ratio has a strong effect on PET values; an increase in aspect ratio leads to lower daytime PET values though this reverses at night (Chatzidimitriou & Yannas, 2016).Temporal heat stress: Considering 35^{0}C PET as the threshold value(Matzarakis&Amelung, n.d.), the duration of heat stress was analysed to understand the performance of various scenarios. In the SCN1 and SCN2 with low-rise and mid-rise built structures, the heat stress starts at 8:00 hrs. and continues till 17:00 hrs. It was further observed that there was a relief in the form of reduced duration of heat stress and a reduction in the highest recorded PET. In SCN3 and SCN4 the heat stress starts at 9:00 hrs and continues till 17:00 hrs. There is an average decrease of -4.99^{0}C in the PET recorded between SCN1 to SCN4. The results of the present research are also in agreement with previous research which concluded that the increase in FSI and building heights reduces the duration of heat stress and improves comfort conditions (Salal Rajan & Amirtham, 2021).



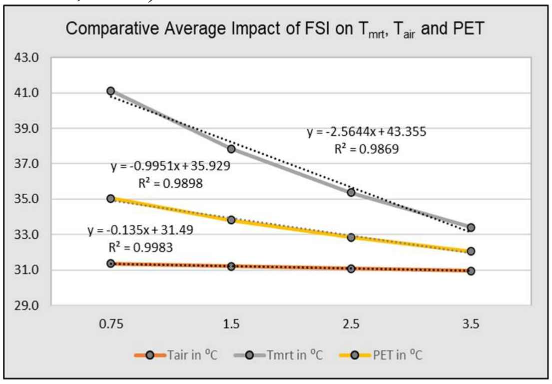


Figure 12: Chart showing the transposition of trend in the selected time slot and Figure 14: Comparative analysis of the regression of FSI on the average values of T_{air}, T_{mrt} and PET, on the simulation day

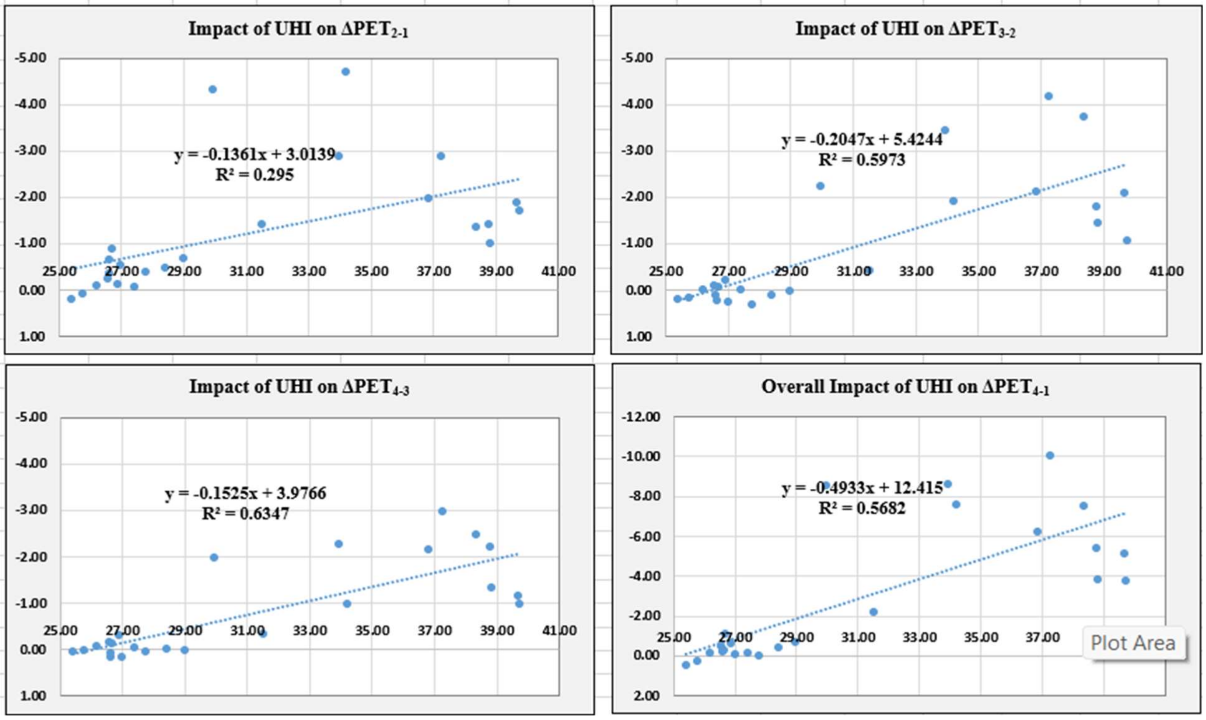


Figure 13: Scatter plots showing the impact of UHI on PET. (clockwise from the top left) change of SCN 1 to SCN 2, SCN 2 to SCN 3, SCN 1 to SCN 4 and SCN 3 to SCN 4

Further, the study of per-floor change in microclimate variables (Figure15) reveals that in the daytime the maximum impact is seen in T_{mrt} when the type of development changes from low rise to mid-rise, which gradually decreases as the building category changes to small and big high-rise. Since the observations are made at 1.5 m above ground level for all the categories, which corresponds to the height at which the microclimate is experienced, the impact of change in building height beyond SCN3 gradually decreases. Change in T_{air} is not significant as compared to $T_{mrt.}$ Change in PET is significant in the category shift of low rise to mid-rise, which becomes minor in the next category and slightly picks up in the big high-rise category. This change in the trend of PET is ascribed to the increased WS and RH%. In Figure15 it is seen that in the third category the trend of change in WS and RH is positive which means that these two parameters are increasing with the additional floor heights in the higher FSI category. This helps to maintain the comfort conditions similar to the previous category in terms of similar PET levels, despite the increase in T_{air} and T_{mrt}. In the nighttime, the maximum impact is seen in T_{mrt} in the category change of low to mid-rise which slows down in the next category shift and again becomes steeper in the big high-rise category. The trend of thermal comfort conditions in terms of PET reverses after midrise, which is more prominent at nighttime and becomes worse in the high-rise. The overall magnitude of change in T_{mrt}(r=0.98, p=0.015) was more as compared to the T_{air}(r=0.99, p=0.005).In high FSI, the duration of heat stress was reduced in the daytime. The high FSI shows better thermal comfort as measured in

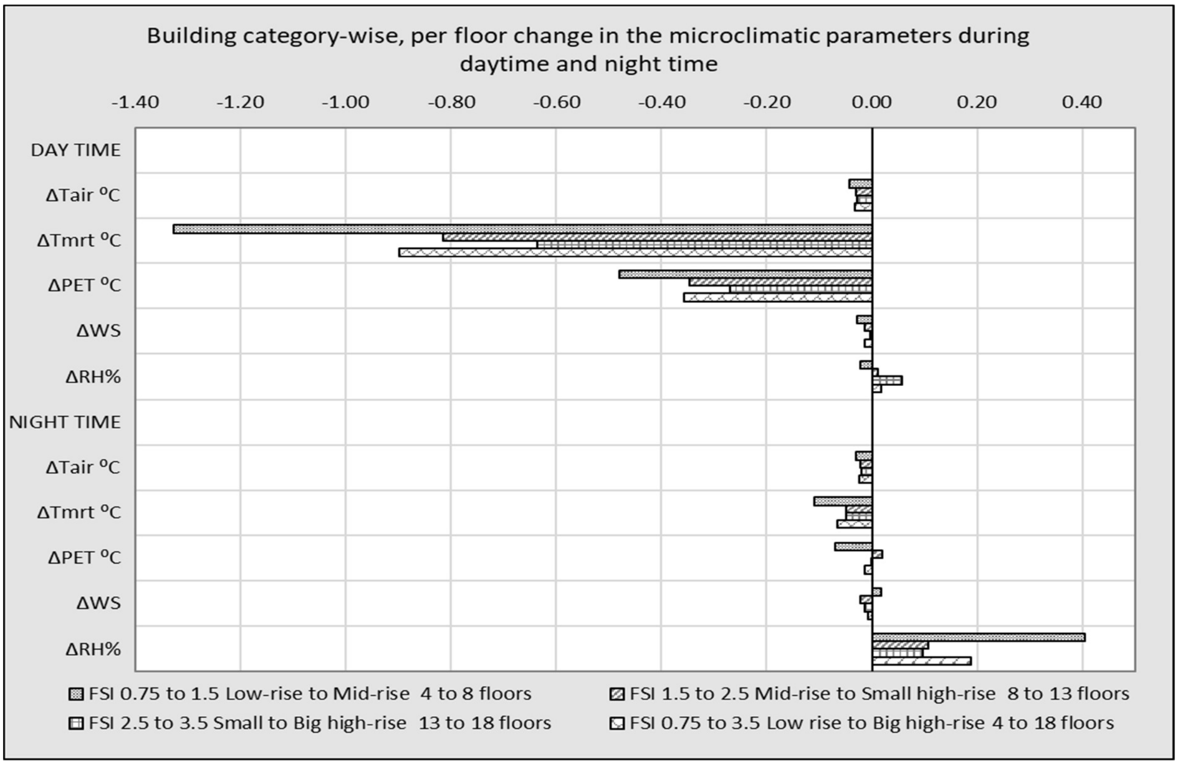


Figure 15: Building category-wise, per floor change in the microclimatic parameters

terms of PET, in the daytime but the trend was reversed in the late evening hours ie. from 20:00 to midnight. In this time slot, the PET was higher with the increase of FSI. There is a statistically significant relationship (r= 0.98, p= 0.013) between FSI and PET in ^{0}C. The analysis of WS and RH shows that a high FSI beyond 2.5, is more desirable due to the increased wind speed and relative humidity.

Conclusion

It is concluded that the scenarios with higher FSI reported low air temperature and mean radiant temperature throughout the day. Also, in the high FSI scenario, it is found that the comfort conditions in the open space improved with the increase in WS and RH which can be attributed to the better shading and stack effect. Therefore, the present study concludes that higher FSI construction in the cities is more desirable as compared to low-density low FSI construction. Open spaces which are surrounded by buildings must be planned carefully to allow comfortable wind movements and maximize the shading effect. The regression equations (Figure 15) of each variable were derived considering the average values for the entire day. It will help planners to predict the impact of the proposed FSI on the thermal comfort conditions of the open spaces enclosed by the built structures and achieve the desired values. The study concludes that the low FSI (up to 1.0) performs worst on account of thermal comfort, mid-FSI development (ranging from 1.0-2.5) is less desirable and high FSI development is found to be the best form of urban development. Since the boundary conditions and the morphological details of every research study done previously are different, therefore the results could not be compared directly. This study is specific to a particular type of layout in which the central open space is surrounded by the buildings at the periphery forming an open enclosure. It is applicable for the tropical monsoon type of climate and specific range of FSI. Therefore, the impact of changing FSI may not be similar in other parts of the world with other types of climates. Further, there is a need to conduct similar studies with a greater number of FSI scenarios in different layouts to generalize the findings.

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

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