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CONTENTS

RESEARCH/REVIEW PAPERS

1. Performance of Guava- Based Hortipastoral System with Different Soil and Moisture Conservation Techniques 177-186
Sunil Kumar, Avijit Ghosh, Amit Kumar Singh A.Ahmed, A.K. Shukla, A.K. Rai , H.V. Singh and R.V. Kumar
2. Isolation of Characterization of Pseudomonas Bacteria Causing Leaf Blight Disease of Wheat (*Triticum Aestivum* L.) 187-190
Naresh Kumar and Umesh Mishra
3. Influence of Iba and NAA on Regeneration of Stem Cuttings of Sweet Lime (*Citrus Limettioides* Tanaka) 191-197
Esha Jaiswal, V.K. Tripathi, Himanshu Trivedi and Rahul Kumar
4. A Critical Study on Operational Feasibilit Development of Aluminium Dross to Treat Pollutants of The Aqueous Environment : Waste Reuse for Resource Recovery 198-208
Rekha Yadav
5. Genetic Analysis of Yield and Quantitative Traits in Common Bread Wheat (*Triticum Aestivum* L.) 209-214
Anil Kumar and Sanjeet Kumar
6. A Critical Study on Remediation of Fluoride Contaminated Water Using Encapsulated Active Growing Blue-Green Algae (*Phormidium* Sp.) 215-221
Rekha Yadav
7. Influence of Integrated Nutrient Management on Growth, Yield and Quality of Aonla Cv. NA-7 222-227
Sachin Kumar, V. K. Tripathi, Manuj Awasthi and Saurabh Tiwari
8. Effect of Various Organic Sources of Nutrients on Growth, Yield and Quality of Wheat (*Triticum Aestivum* L.) 228-232
Pankaj Kumar Bagri, S.S. Bhadauria, Vikash Singh, Uma Shankar Bagri and Rajenrda Patel
9. Estimation of Total Fungi Count (TFC) in Dairy Product Samples by Standard Plate Count (SPC) Technique 233-236
Rekha Yadav

10. Estimation of Genetic Divergence in Tomato (*Solanum Lycopersicum* L.) Genotypes 237-241
Mohit, Bijendra Singh, Satya Prakash, Vipin Kumar, R.S. Sengar, L.K. Gangwar, Chetan Chauhan, Jagraj Singh
11. Analysis of Leghaemoglobin, Chlorophyll and Protein Content in the Selected Legume Plants (*Vigna Radiata* L. and *Vigna Mungo* L.) 242-247
Krishna Kumari, Shefali Poonia, Purushottam and D.K. Sachan
12. Physiological Characters Associated with Water-stress Tolerance under Pre anthesis Water-stress Conditions in Wheat 248-254
Arun Kumar
13. Biotechnological Stratagems for the Production of Microbial Bioplastics and its Commercial Applications 255-260
Aakansha Goswami and B. Singh
14. Constraints Faced by Hortipreneurs in Kumaon Hills of Uttarakhand 261-267
Kritika Pant, Arpita Sharma Kandpal, V.P. Singh and Virendra Kumar
15. A Review on Spoilage and Preservation of Milk and Milk Products 268-274
Rekha Yadav



PERFORMANCE OF GUAVA- BASED HORTIPASTORAL SYSTEM WITH DIFFERENT SOIL AND MOISTURE CONSERVATION TECHNIQUES

Sunil Kumar, Avijit Ghosh, Amit Kumar Singh *A.Ahmed,
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Abstract

A long term (2008-2016) field experiment was conducted to evaluate the performance of guava based hortipasture system in semi-arid rainfed situation. The experiment consisted 12 treatments i.e. 2 cultivars (Shweta and Lalit) with pasture (*Cenchrus ciliaris* + *Stylosanthes hamata*) under 6-treatment viz., vegetative barrier, staggered trenches (2m x 0.50m x 0.50m) , stone mulch in basin (locally available stone in 50 cm radius) , guava + pasture (without soil and water conservation) , sole pasture and sole guava. The cultivar Lalit performed better growth in term of height, canopy spreads and yield (20 percent higher) as compared to Cv. Shweta. Staggered trenches produced higher plant growth as compared to control. The stone mulch in basin recorded minimum growth and yield with both cultivars. Over the nine years the average yield of pasture production with staggered trenches was higher (7.0 t DM/ha) followed by vegetative barriers (6.4 t DM/ha). The fruit quality was significantly influenced with staggered trenches and vegetative barriers. The pasture production in association with tree was little higher (6.1 t DM/ha) as compared to sole pasture (6.0 t DM/ha). The average crude protein yield with staggered trenches was also higher (527.0 kg/ha) followed by vegetative barriers (485kg/ha). The crude protein yield in association with tree was little higher (463.4kg/ha) as compared to sole pasture (397.4 kg/ha).

Key words: Guava cv. Lalit and Shweta, *Cenchrus ciliaris* , *Stylosanthes hamata*, staggered trenches, vegetative barriers, stone mulch in basin, sole guava, sole pasture

Introduction

In India, about 60 percent of net sown area is rainfed, which contributes to 40 percent of the total food production. It supports 40 percent of human and 60 percent of live stock population. Thus areas crop husbandry is a big gamble in these areas due to rainfall uncertainty. Under such a situation, incorporation of fruit trees along with animal husbandry in common farming system is advisable to improve lively hood, income and nutritional security of the farmers. Due to

increasing demographic pressure along with land degradation has lead to a shortage of green and dry fodder, resulting in poor livestock rearing. Consequently hortipasture system is now considered to be the most ideal strategy to provide food, nutrition and income security to the people living in rainfed areas. It is socially accepted, ecologically feasible and economically viable for class IV and V type of land in which fruit trees are grown in association with grasses and legumes (21).

Guava (*Psidium guajava* L.), the “apple of tropics,” is one of the most common fruits of India. It is a rich source of Vitamin C (150-350mg/100g edible pulp), pectin and fair source of vitamin A, iron, calcium and phosphorus. It has national productivity of about 11.3 t/ha, with production potential reaching 20 t/ha in Madhya Pradesh where maximum areas are rainfed (Shikhamany, 2004). Guava occupies about 1,15,000-1,62,000 ha area in the country (22). Mrig Bahar is very much suitable for rainfed regions. That provides quality and quantity fruits in winter with available natural moisture in soil. Two open pollinated coloured seedling selections namely Lalit (CISH-G-3) and Shweta (CISH-G-4) developed by Central Institute of Subtropical Horticulture, Lucknow were taken in this experiment (Vision, 2030). *Stylosanthes hamata* (Caribbean stylo) is regarded as the most important range legume for the humid to semi-arid tropics of India. It is extensively utilized in pastoral, agropastoral and silvipastoral systems for animal production. Due to its ability to restore soil fertility, improve soil physical properties and provide permanent vegetation cover, it plays a vital role in the development of wastelands in India (4). *Cenchrus ciliaris* (Buffel grass) is also most suitable species for arid and semi-arid region having rainfall range from 125-1250 mm (30).

Establishment of hortipasture system is quite difficult task in rainfed region because of moisture stress. In initial stage of establishment fruit plants require watering. But providing regular irrigation is neither practically possible nor economically feasible in rainfed regions. Harvesting of rainwater and *in-situ* moisture conservation is the only viable alternative to artificial irrigation. However, shaping the surface in the immediate vicinity of the trees so that runoff collects at the root zone can also enhance availability of moisture for long duration. Better survival and establishment of fruit trees in rainfed and dry lands requires proper micro-site improvement in the pits in addition to lifesaving water during summer in the initial years (20). Management of micro-catchment *in-situ* gave better establishment and production in ber under arid situation (11). (29) also reported that mulching in mango crop gave higher yield in addition to conserve moisture simultaneously and also regulate soil

temperature. Keeping these facts in view an experiment was conducted to evaluate the performance of guava cultivars under hortipastoral system with different soil and water conservation measures.

Materials and Methods

Study was conducted at central research farm (longitude 25° 26' 08" N, latitude 78° 30' 21" E and altitude 216 m above msl) of the Institute, Jhansi during 2008–2016. Grafted plants of Guava cultivars Shweta and Lalit were planted during October, 2008 at 6 x 6m in 1m³ pits filled with soil + 30 kg farmyard manure + recommended termiticide. The soil was clay loam; contain 38.55% clay, 32.5% sand and 29.5% silt with around 2 m depth. The initial soil composition was poor in available N (140-148 kg/ha), P (3.8-4.1 kg/ha) and K (110.7 kg/ha), low in organic carbon (0.42%), neutral in pH (6.54). The plot size was 12m x 36m having 12 trees (6 trees Shweta and 6 trees Lalit) /plot (6m X 6m). The new seedlings of Anjan grass (*Cenchrus ciliaris*) were transplanted in July, 2007 at 100 cm row- to- row and 50 cm plant to plant and Caribbean stylo @ 4 kg /ha were sown in line between 2 rows of grass under guava tree and similar practice was adopted for pure pasture block. The experiment consisted 12 treatments i.e. 2 cultivars (Shweta and Lalit) with pasture (*Cenchrus ciliaris* + *Stylosanthes hamata*) under 6-treatment viz., vegetative barrier (*P. maximum* was raised at 6m) in between the row of guava plant, staggered trenches (2m x 0.50m x 0.50), stone mulch in basin (locally available stone in 50 cm radius), guava + pasture (without soil and water conservation), sole pasture and sole guava replicated thrice under RBD and data were analyzed statistically as per (13). The weather parameters rainfall (mm) and rainy days during the experiment period were collected from meteorological observatory of central research farm of IGFR, Jhansi. The data on fruit tree growth was recorded in the month of November and yield was recorded every year from mid November to January and calculated in totality as per treatment of randomly selected and marked three plants. The pasture was harvested at top a height of 12 cm from soil surface. The pasture yield was recorded with one cut in first year and 2 cuts

in second year onward. Fresh sample (500 g) were collected randomly from each plot and dried in hot-air oven at 70 °C till constant weight for estimation of dry matter and crude protein. Available soil moisture was taken by simple fresh soil weight and oven dry basis at monthly intervals from October to February each year of experiment at 0-15 and 15-30 cm depth. The weather parameters (Fig. 1 and 2) viz., rainfall (mm), rainy days during monsoon period, was collected from meteorological observatory of Central Research Farm of IGRI, Jhansi during the experimental period (July- October). The physico-chemical characteristics like fruit weight, fruit volume, fruit size, T.S.S. (total soluble solids), glucose, acidity (%) and ascorbic acid, Total sugar, reducing sugar and non-reducing sugar were also recorded using standard methods at the time of harvest in year 2009-2016 and average data were analyzed.

Results and Discussion

Plant growth: The average plant height was observed higher in cultivar Lalit as compared with cultivar Shweta during each consecutive years of experiment. Among the moisture conservation measures the staggered trenches performed significant effect on plant height and with Lalit and Shweta followed by vegetative barrier and sole guava and minimum plant height was observed with stone mulch in basin with both cultivars in all the consecutive years (Table 1). The average collar diameter was recorded higher in cv. Shweta as compared with Cv. Lait. Among soil moisture measures the staggered trenches promote maximum collar diameter as compared to vegetative barrier and sole guava and guava + pasture without moisture conservation measures. The minimum collar diameter was recorded with stone mulch in basin (Table 2). There was no much difference in canopy spread with both the cultivars. However, the canopy spread was little higher with Cultivar Cv.Lalit (Table 3). The stone mulch in basin gave minimum growth of tree with both the cultivars. It might be due to the stone mulch done not allowed to any cultural practices after October and onward. As well as in Bundelkhand region

temperature gone above 44 °C and stone itself become too hot as a result the plant growth with stone mulch in basin was minimum. The differences in plant growth was might be due to difference in available soil moisture with different moisture conservation measures. Similar finding was reported by (19) in pomegranate, (14) in custard apple, (3) in ber and (24) in bael and (23) in tamarind. (18) also reported positive effect of different *in-situ* moisture conservation in Cashew (*Anacardium occidentale* L.) as it affects mean soil moisture content, growth of plants, yield of cashew for the first 5 harvests years.

Fruit yield: The fruiting started from second year of plantation (October, 2008) and in the first year of fruiting (2009-10). Over the eight fruiting years cultivar Lalit produced 20 percent higher fruit yield than Shweta. It gave 23.47, 16.39, 17.22, 23.03, 16.66, 15.07, 6.70 and 20.6 percent higher yield respectively from first, to eight years of fruiting (Table 4). The fruit yield under rainfed condition also depends on rainfall quantity and distribution. (12) reported that grafted aonla start fruiting after 4th year of plantation and after 6 year it may produce maximum fruit 60– 80 kg /tree under rainfed situation. Similarly (8) also reported that aonla started fruiting in fifth year of plantation and maximum production of aonla in eighth year of plantation and under rainfed situation and fruiting also depends on rainfall pattern and distribution. Among moisture conservation measures scattered trenches gave significantly higher yield followed by vegetative barrier and sole guava during constitute year of experiments (Table4). The average production was higher with scattered trenches (6.9 t/ha) followed by vegetative barriers (6.0t/ha) and Guava+ pasture without soil and moisture conservation. The average minimum fruit production was recorded with stone mulch in basin. It is also might be due to the stone mulch done not allowed to any cultural practices after October and onward. As well as in Bundelkhand region temperature gone above 44 °C and stone itself become too hot as a result the plant growth with stone mulch in basin was minimum (Cv. Lalit 4.9 and Cv. Shweta 4.5t/ha).

(19) also reported that yield of pomegranate plantation influenced with *in-situ* moisture conservation in 7 years old plantation with in-situ moisture conservation and different management practices. (14) also noticed result in same line in custard apple. (3) also reported that guava yield related with cultivars along with pruning and moisture conservation practice. (7) also reported that bunding of orchard gave significantly higher yield of ber fruit which might be due to sufficient moisture regime during fruit growth and development phase. (3) reported that moisture conservation measure 8 years old ber orchard increased, fruit yield and quality of ber. He also reported that the maximum fruit size weight and yield were found from the plant under black polythene mulch and paddy straw was found best natural mulch.

Fruit Quality: The physical attribute of fruit also affected with moisture conservation measure and it was recorded higher with staggered trenches followed by vegetative barriers, and sole guava. The lowest values was recorded with stone mulch in basin. The *in-situ* moisture conservation might be helpful in conserving soil moisture by reduction of evaporation loss of water from soil and more absorption of nutrient. Similarly (14) highest fruit length and width was noticed in custard apple with circular basin with organic mulching. The cultivars Shweta showed significantly higher T.S.S. (12.87 ° Brix) as compared to Lalit (11.12) (Table 6). The staggered trenches of *in-situ* moisture conservation practices gave significant effect followed by vegetative barrier and Stone mulch. The minimum T.S.S. was recorded under T₄ with both the cultivars. The maximum fruit acidity (Table 6) percentage was observed in cultivar Lalit (0.28 %) as compared to cultivar Shweta (0.27 %) & Lalit (0.27%). Staggered trenches produced significantly lower acidity percentage with both cultivars as compare to Vegetative barrier, stone mulch in basin, and control. The cultivar Lalit produced significantly higher ascorbic acid content (177.77 mg/100g pulp) as compared to Shweta (176.3 mg/100g pulp). Staggered trenches treatment produced significantly higher ascorbic acid content as compared to Stone Mulch in basin and control (T₄). The difference between staggered trenches and vegetative barrier was found statistically at par.

The cultivar Lalit showed higher total sugar (7.99 %) as compared to Shweta (7.80 %). In this regard staggered trenches gave significantly higher total Sugar percentage as compared to stone mulch in basin and control with both the cultivars. The cultivars Lalit and Shweta showed likely to be similar reducing and non-reducing sugar values. Staggered trenches produced significantly higher non-reducing Sugar as compare to vegetative barrier, stone mulch in basin, and control (T₄). (10) reported that there were significant variation with regard to yield, vitamin C, TSS and acidity in different cultivars of guava. Similarly (26), (6), (28) and (27) also reported findings in same line.

Pasture production: The pasture production was increased up to fourth year latter on it showed decline trend it might be due to reduction in *Stylosanthes hamata* population as well as due to reduction in *Cenchrus ciliaris* population damaged during fruit harvesting (Table 7). The dry matter yield varied significantly with different moisture conservation measures in all the consecutive years of experiment. Staggered trenches produced significant higher dry matter yield followed by vegetative barrier over control (T₄) in all the consecutive year. Over the nine years of experiment the average yield of pasture production with staggered trenches was higher (7.0 t DM/ha) followed by vegetative barriers (6.4 t DM/ha) as compared to control (T₄). The minimum pasture production (5.3 t DM /ha) was recorded with stone mulch in basin. The pasture production in association with tree was little higher (6.1tDM/ha) as compared to sole pasture (6.0tDM/ha).

(7) also reported bunding as moisture conservation measure improved forage production in ber based hortipasture system, while (24) and (7) reported that staggered trenches gave significantly higher forage yield in Bael (*Aegle marmelos*) and Tamarind (*Tamarindus indica*) based hortipasture system respectively, similarly (15) reported that staggered trenches gave significantly higher forage yield in *Hardwickia binata* based silvipasture system. The pasture production in association with tree was higher as compared to sole pasture crop. The probable reason may be that manure and fertilizers applied to guava tree were also utilized by sown pasture

component for these there was no physical barrier between root system of tree and pasture and thus, better availability of nutrients compensated for the lost area. (21) reported earlier increasing trend of forage production in Jujube -based hortipasture system and (8) and (7) in Aonla based hortipasture system.

Crude protein: The crude protein yield was also influenced significantly by various treatments in each year of experiments (Table 8). It showed similar pattern as in pasture production because it is totally dependent on dry matter yield. It was significantly higher with staggered trenches followed by vegetative barriers, sole pasture and control. The crude protein production with stone mulch was lower than control but statically at par. Average crude protein yield of nine years with staggered trenches was higher (527.0 Kg/ha) followed by vegetative barriers (485kg/ha) as compared to control (435.6 kg/ha). The minimum crude protein production (406.0 /ha) was recorded with stone mulch in basin. The crude protein yield in association with tree was little higher (463.4kg/ha) as compared to sole pasture (397.4 kg/ha). (15) reported that the crude protein yield was directly dependent on forage yield and combinations.

Soil moisture dynamics: Normal date of onset of monsoonal rainfall at Jhansi is around 25th June of each year. Long-term annual rainfall of Jhansi is around 870 mm (standard deviation 247 mm) out of which about 90% and 4% of the total rainfall occurs during monsoon (June-September) and winter (December-February) respectively. Monsoon rainfall occurs in Jhansi due to south-west mausim wind whereas; few rainfall events in winter season occur due to western disturbances. In this experiment, monthly soil moisture data at 0-15 cm and 15-30 cm depth soil profile was measured from October to February using gravimetric method for the period 2008 to 2016. The purpose is to assess the effectiveness of the *in-situ* soil moisture conservation measures in conserving the overland runoff as well as the net rainfall generated from guava tree and moisture variation in dry periods (Oct-Feb) after the

cessation of monsoonal rainfall. The average long-term (2008-2016) monthly variation of soil moisture in weight basis in different treatments is shown in figure 1. It shows that staggered trenches is the most efficient *in-situ* technique in conserving the soil moisture followed by vegetative barriers. It was found that average monthly soil moisture (including both the soil profile data) was highest in staggered trenches (7.42%), lowest in stone mulch in basin (6.31%) and follows the order as staggered trenches (7.42%)>vegetative barriers (6.67%)>sole guava (6.41%)>sole pasture (6.36%)>guava + pasture(6.33%)>stone mulch in basin (6.31%). Hence, staggered trenches were conserved and capable to store significantly higher moisture (on an average 17.5% more) in comparison to stone mulch in basin. On the other hand, it was observed that soil moisture stored in 15-30 cm soil profile was higher than soil moisture content in 0-15 cm depth for each treatment. Most of the roots of grasses are concentrated in 0-20 cm depth whereas; maximum concentration of feeding roots of guava is mostly concentrated up to 30-45 cm depth (5). It signifies that soil moisture present at 0-15 cm and below 15 cm depth will be mostly used by grasses and guava respectively to fulfill needs for their metabolic requirements. It was observed after analyzing the average monthly soil moisture data for all treatment that soil moisture store in 15-30 cm depth is about 9% more than moisture store in 0-15 cm depth. The reason for highest conservation of soil moisture in staggered trenches and vegetative barriers attributed to highest capturing of overland flow and net rainfall. The average infiltration capacity which was measured with double ring infiltrometer for the experimental plots was found to be 6.4 mm h⁻¹. Whenever a rainfall event having intensity more than the infiltration capacity occurs, the water to be stored in soil profile is the overland flow (runoff water coming from the upstream treatment) and net rainfall (stemflow and through fall generated from each guava tree). The reported (Ahmed *et al.*, 2015; Ahmed *et al.*, 2017) net rainfall amount was more than 85% of the incident rainfall for *Morus alba* and *Grewia optiva* trees which are having similar morphological features like guava.

[illegible]

Figure. 1. Rainfall (mm) of experimental site from July to October 2008-2016

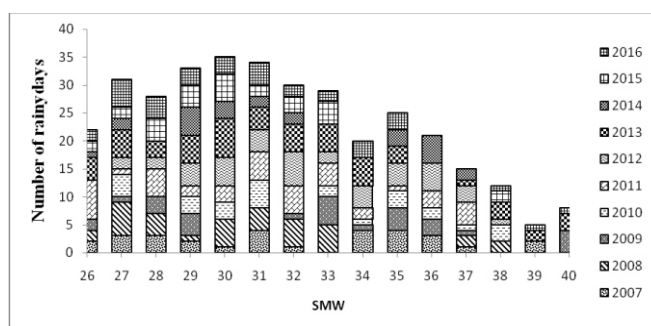


Figure.2. Rainy days of experimental site from July to October 2007-2016

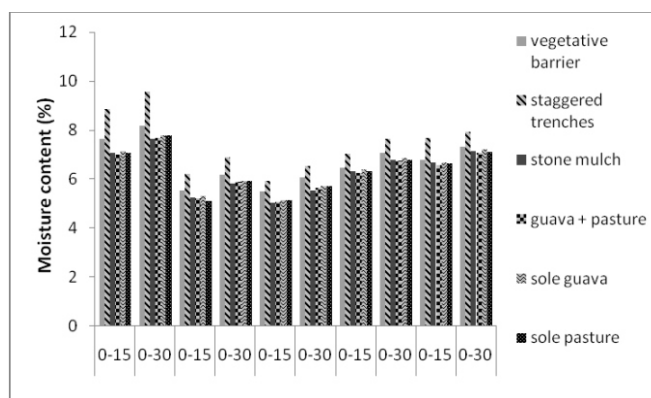


Table 1: Effect of moisture conservation and grass combination on height (cm) of guava cultivars

[illegible]

Mean	173.66	152.36	244.8	244.8	267.6	265.7	284.4	281.1	312.1	293.7	358.1	312.0	405.8	384.0	403.9	365.5	452.2	416.0
CD at 5%	NS	NS	11.6	8.6	13.6	11.8	14.7	10.7	16.2	10.3	7.50	2.31	8.55	4.67	11.01	11.88	5.12	8.05
SD M ($\pm$)			3.63	2.69	4.26	3.70	4.06	3.35	5.07	3.22	2.35	0.72	2.68	1.42	3.05	3.36	1.60	2.52

T₁- Vegetative barrier, T₂- Staggered trenches, T₃ Stone mulch in basin, T₄ Guava+ pasture without soil and moisture conservation, T₅ sole guava, T₆ sole pasture

Table:2. Effect of moisture conservation and grass combination on collar diameter (cm) of guava cultivars

Treatment	2008		2009		2010		2011		2012		2013		2014		2015		2016	
	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta
T ₁	3.9	3.6	5.3	5.6	6.1	6.8	8.3	8.0	8.6	8.5	10.2	10.1	12.0	11.9	13.2	12.8	13.7	13.2
T ₂	3.9	3.9	5.9	5.7	6.6	6.4	8.3	8.0	8.7	9.0	10.5	10.3	12.1	12.3	13.3	13.8	14.5	14.3
T ₃	3.7	3.8	5.3	5.4	6.2	6.1	6.2	7.0	7.1	7.8	8.6	8.4	8.4	11.2	9.4	11.7	10.2	11.9
T ₄	3.6	3.8	5.2	4.6	5.9	5.9	7.2	7.0	8.1	8.3	9.6	9.6	10.9	11.5	11.6	11.9	12.1	12.4
T ₅	3.9	4.0	5.3	5.3	6.5	6.6	7.3	7.9	7.8	8.2	9.8	9.7	11.3	11.2	12.1	12.6	12.5	13.1
T ₆	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	3.8	3.8	5.4	5.3	6.3	6.4	7.5	7.6	8.1	8.4	9.7	9.6	10.9	11.6	11.9	12.5	12.6	12.9
CD at 5%	NS	NS	0.04	0.03	0.36	0.42	0.09	0.07	0.10	0.06	0.23	0.09	2.48	1.68	1.61	2.48	1.54	1.79
SD M ($\pm$)			0.01	0.00	0.11	0.13	0.02	0.05	0.03	0.01	0.07	0.02	0.77	0.52	0.50	0.77	0.48	0.56
			2	1		1	9	0	2	8	2	8	7	7	5	8	4	4

T₁- Vegetative barrier, T₂- Staggered trenches, T₃ Stone mulch in basin, T₄ Guava+ pasture without soil and moisture conservation, T₅ sole guava, T₆ sole pasture

Table:3. Effect of moisture conservation and grass combination on Canopy spread (Cm) of guava cultivars

Treatment	2009		2010		2011		2012		2013		2014		2015		2016	
	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a	Lalit	Shwet a
T ₁	271.9	266.8	300.3	266.8	377.2	356.2	440.3	430.3	430.7	409.7	510.3	540.2	450.3	459.2	470.2	490.6
T ₂	292.5	276.4	306.9	276.4	410.3	358.3	440.2	440.5	460.3	450.3	559.7	550.3	470.1	520.8	470.0	529.4
T ₃	267.7	264.8	246.1	264.8	291.0	299.1	370.2	380.3	310.0	310.5	370.3	440.0	370.6	400.2	400.3	460.0
T ₄	257.9	187.1	294.4	187.1	345.2	307.2	410.3	420.0	420.2	409.5	440.4	479.6	410.5	460.4	470.7	480.3
T ₅	262.4	232.1	306.2	232.1	357.4	330.5	420.2	420.2	388.0	420.3	490.1	479.5	430.2	460.1	460.3	490.2
T ₆	0	0	0	0	0	0	-	0	-	-	-	-	-	-	-	-
Mean	270.5	245.4	290.8	245.4	356.2	330.3	416.2	418.3	401.8	400.1	474.2	479.9	426.3	460.1	454.3	490.1
CD at 5%	4.5	6.2	17.1	6.2	6.8	7.2	7.32	7.6	7.50	5.8	12.72	8.54	11.36	10.19	6.94	5.91
due to Moist ure conser vation														3		
SD M ($\pm$)	1.41	1.94	5.35	1.94	2.13	2.25	2.26	2.38	2.35	1.81	3.99	2.677	3.56	3.19	2.17	1.85

T₁- Vegetative barrier, T₂- Staggered trenches, T₃- Stone mulch in basin, T₄- Guava+ pasture without soil and moisture conservation, T₅- sole guava, T₆- sole pasture

Table:4. Effect of moisture conservation and grass combination on fruit yield of guava cultivars

Treatment	2009		2010		2011		2012		2013		2014		2015		2016		Mean	
	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta	Lalit	Shw eta
T ₁	2.96	2.35	5.98	5.07	3.76	3.13	6.73	5.25	6.9	5.6	9.2	7.5	10.33	9.6	11.3	9.4	7.1	6.0
T ₂	3.21	2.54	6.67	6.2	4.51	4.07	7.53	6.03	7.8	6.4	10.1	8.6	11.63	10.26	13.4	11.2	8.1	6.9
T ₃	2.82	2.29	5.18	4.21	2.96	2.27	4.92	4.14	4.3	4.5	5.8	6.0	6.1	6.62	7.2	5.9	4.9	4.5
T ₄	2.52	2.1	5.20	4.57	2.99	2.63	5.85	4.80	6.1	5.1	8.2	6.8	8.99	8.23	9.7	8.1	6.2	5.3
T ₅	2.71	2.21	5.35	4.35	3.13	2.68	6.23	5.18	6.5	5.5	8.7	7.5	9.9	9.3	10.5	8.6	6.6	5.7
T ₆	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	2.84	2.30	5.68	4.88	3.47	2.96	6.25	5.08	6.3	5.4	8.4	7.3	9.39	8.8	10.42	8.64	6.6	5.7

CD at 5% due to Moisture conservation	0.14	0.13	0.52	0.34	0.46	0.39	0.53	0.41	0.50	0.43	1.922	1.084	2.214	2.183	1.844	1.63		
SD M ($\pm$)	0.043	0.40	0.16	0.10	0.14	0.12	0.16	0.12	0.15	0.13	0.604	0.340	0.787	0.776	0.578	0.512		

T₁- Vegetative barrier, T₂- Staggered trenches, T₃- Stone mulch in basin, T₄- Guava+ pasture without soil and moisture conservation, T₅- sole guava, T₆- sole pasture

Table 5: Effect of moisture conservation and grass combination on physical quality of guava cultivars

Treatment	fruit weight (gm)		fruit length (cm)		fruit width (cm)		fruit volume (cc)	
	L	S	L	S	L	S	L	S
T ₁	134.27	156.90	6.03	7.29	7.64	8.47	125.30	190.20
T ₂	142.70	168.50	6.84	7.85	8.70	9.15	167.21	222.90
T ₃	127.67	150.67	5.40	6.62	7.17	7.73	101.82	115.80
T ₄	117.60	138.50	4.82	6.47	6.54	7.62	77.27	149.27
T ₅	122.6	148.30	5.80	6.72	6.93	7.26	82.62	136.55
Mean	128.97	152.57	5.78	6.99	7.39	8.04	110.84	162.94
CD at 5% due to Moisture conservation	13.25	14.65	1.03	1.63	0.59	0.61	10.56	11.06
SD M ($\pm$)	3.89	4.30	0.30	0.47	0.17	0.17	3.10	3.25

T₁- Vegetative barrier, T₂- Staggered trenches, T₃- Stone mulch in basin, T₄- Guava+ pasture without soil and moisture conservation, T₅- sole guava, T₆- sole pasture

Table 6: Effect of moisture conservation and grass combination on chemical quality of guava cultivars

Treatment	TSS (O Brix)		Fruit Acidity (%)		Ascorbic acid (mg/100g pulp)		Total sugar (%)		Reducing sugar (%)		Non-reducing sugar (%)	
	L	S	L	S	L	S	L	S	L	S	L	S
T ₁	11.40	13.50	0.29	0.25	183.50	185.80	8.50	7.90	5.32	5.36	3.18	2.56
T ₂	12.90	15.20	0.26	0.22	193.20	191.9	8.80	8.3	5.65	5.66	3.15	2.63
T ₃	10.60	12.50	0.28	0.30	171.90	168.7	7.70	7.70	5.03	5.04	2.67	2.66
T ₄	10.30	11.47	0.30	0.29	168.67	167.5	7.60	7.40	5.01	4.94	2.50	2.45
T ₅	10.40	11.69	0.27	0.28	171.6	167.6	7.35	7.56	5.04	5.01	2.63	2.56
Mean	11.12	12.87	0.28	0.27	177.77	176.3	7.99	7.80	5.21	5.2	2.8	2.6
CD at 5% due to Moisture conservation	1.05	1.06	0.02	0.03	11.6	12.9	0.23	0.26	1.07	1.05	0.16	0.15
SDM ($\pm$)	0.30	0.31	0.017	0.009	3.41	3.79	0.067	0.076	0.31	0.31	0.047	0.044

T₁- Vegetative barrier, T₂- Staggered trenches, T₃- Stone mulch in basin, T₄- Guava+ pasture without soil and moisture conservation, T₅- sole guava, T₆- sole pasture

Table 7: Effect of different Soil Water Conservation measures on Pasture yield (tDM/ha)

Treatment	2008	2009	2010	2011	2012	2013	2014	2015	2016	Mean
T ₁	4.4	8.2	8.1	7.7	7.3	5.7	6.3	4.9	5.3	6.4
T ₂	6.6	8.45	8.7	8.3	7.6	6.1	6.6	5.2	5.5	7.0
T ₃	3.6	7.3	6.8	6.5	5.9	4.7	5.1	4.1	4.3	5.3

T ₄	3.9	6.1	7.9	7.2	6.6	5.1	5.5	4.6	4.9	5.7
T ₅	-	-	-	-	-	-	-	-	-	-
T ₆	4.7	6.6	6.4	6.3	6.3	4.7	5.1	3.9	4.2	6.0
Mean	4.6	7.5	7.9	7.4	6.8	5.4	5.8	4.5	5.0	6.1
CD at 5% due to Moisture conservat ion	0.21	0.71	0.33	0.26	0.34	3.2	1.44	0.630	0.878	
SD M (±)	0.06	0.22	0.10	0.08	0.11	1.003	0.453	0.224	0.275	

T₁- Vegetative barrier, T₂- Staggered trenches, T₃- Stone mulch in basin, T₄- Guava+ pasture without soil and moisture conservation, T₅- sole guava, T₆- sole pasture

Table 8: Effect of different Soil Water Conservation measures on crude protein yield (kg/ha)

Treatment	2008	2009	2010	2011	2012	2013	2014	2015	2016	Mean
T ₁	378.4	697.0	664.2	600.6	532.9	399.0	434.7	318.5	339.9	485.0
T ₂	561.0	705.6	704.9	655.7	554.8	433.1	448.8	338.0	341.0	527.0
T ₃	309.6	605.9	557.6	507.0	436.6	333.7	351.9	274.9	275.2	406.0
T ₄	335.4	512.4	655.7	568.8	495.0	351.9	379.5	303.6	318.5	435.6
T ₅	-	-	-	-	-	-	-	-	-	
T ₆	404.2	516.0	518.4	478.8	459.9	333.7	351.9	249.6	264.6	397.4
Mean	397.7	607.4	620.1	526.2	495.8	370.3	403.7	308.7	307.8	
CD at 5% due to Moisture conservat ion	28.16	32.01	38.07	33.8	31.05	27.63	26.09	22.01	20.02	
SD M (±)	8.28	9.41	11.20	9.94	9.13	8.12	7.67	6.41	6.47	

T₁- Vegetative barrier, T₂- Staggered trenches, T₃ Stone mulch in basin, T₄ Guava+ pasture without soil and moisture conservation, T₅ sole guava, T₆ sole pasture

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ISOLATION OF CHARACTERIZATION OF PSEUDOMONAS BACTERIA CAUSING LEAF BLIGHT DISEASE OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Leaf scourge of wheat may be a bacterial infection caused by a few pathovars of *Pseudomonas syringae*, such as *P. syringae* pv. *syringae* and *P. syringae* pv. *atrofaciens*. These microbes can decrease the abdicade and quality of wheat and deliver poisons that are destructive to people and creatures. In this consider, we disconnected and distinguished *Pseudomonas* microscopic organisms from necrotic wheat leaf injuries utilizing culture-based and atomic strategies. We utilized Wilbrink's-boric acid–cephalexin semi-selective medium to choose for *Pseudomonas* microscopic organisms and PCR to increase the 16S rRNA quality for sequencing and phylogenetic investigation. We moreover tried the opposing potential of fluorescent pseudomonads confined from the phyllosphere of wheat against *Fusarium* and *Alternaria* organisms, which are other wheat pathogens. We found that 40 out of 175 fluorescent pseudomonads appeared hindrance of parasitic development in double culture measures, and 20 of them had the phlD quality encoding the anti-microbial 2,4-diacetylphloroglucinol.

Introduction

India produces about 107 million tons of wheat (*Triticum aestivum* L.) annually on about 105 million ha situated in the north, southwest, central west, and southeast regions. The wheat is one of the crops in Guilan province.

Pseudomonas syringe is a Gram-negative, rod-shaped bacterium that infects wide range of plants, including wheat .pseudomonas leaf blight of wheat, also known as bacterial blight halo blight, is caused by *pseudomonas Syringae* Pv. *Syringae*. this disease is prevalent in wheat -growing areas of the world, including north America ,Europe, and Asia .It causes significant yield losses ,reduces the quality of grain ,and affect the overall health of the plant. In this essay, we will discuss the symptoms, pathogen, epidemiology, management, and prevention of pseudomonas leaf blight of wheat .

Wheat is one of the most important cereal crop grown globally, and it plays a vital role in the food security of Millions of people worldwide. However, wheat production is often affected by various biotic

and abiotic stresses, among which, diseases caused by bacterial pathogens are one of the significant challenges.

Pseudomonas Syringae is a gram-negative bacterium that causes leaf blight, a severe disease of Wheat, Characterized by the necrotic lesion on leaves, stems and heads. The disease can significantly reduce the yield and quality of wheat, thereby affecting the economic returns of farmers. Moreover, the bacterium is highly adaptable, making it difficult to control using conventional management strategies.

Leaf Blight Disease Cycle:

- (i) **Perennation:** The pathogen is considered to be seed-borne (**Kumar and Arya (1973)**) have confirmed the seed- borne nature of perennation of the fungus; the pathogen can survive as conidia on the seed surface or as deep seated mycelium inside the seed coat.
- (ii) **Primary Infection:** Whenever infected seeds, which are the source of pathogen perennation, are sown in the field, they develop seedlings and subsequently these

seedlings get infected by the pathogen inoculum and become diseased.

- (iii) **Secondary Infection:** When the primary infection is established on young wheat plants, the pathogen starts speculating usually in darkness and the conidia so produced serve as the source of secondary infection.

Material and Methods

Plant Collection: The present investigation was done during the timescale of 2022-2023 at Umesh Kumar leaf blight of wheat Research Laboratory in department of Agronomy.

Sample collection: Leaves of *Tritium Aestivum* were collected from horticultural research Centre, J.S University Shikohabad Firozabad 283135 (U.P). The leaves were washed thoroughly with tap water to remove any dirt or dust particles and then washed with 70% ethanol for disinfection (for 1 to 3 minutes) then washed with distilled.

Isolation: *Pseudomonas Syringae* is isolated from samples by using an enrichment procedure. It is confirmed through use of selective and differential media. Nutrient-agar medium (NA)

Beef extract 5 g, Peptone 10 g, Agar- agar 20 g, and Distilled water 1000 ml.

Nutrient broth medium Peptone 10 gm, Beef Extract 5 gm, NaCl₂ gm, Distilled water 1000 ml. Peptone, beef extract and NaCl were dissolved in 1000 ml distilled water and adjusted pH 7.2. Two liters of medium was poured 500 ml per conical flask, plugged and autoclaved at 15 lbs/sq. inch pressure for 15 minutes. This medium was used to grow antagonistic bacterium *Bacillus subtilis* (S-12) to control citrus canker disease.

Glucose broth Meat extracts 3 g, Peptone 10g, Glucose 5g, Distilled water 1000 ml.

Medium for motility test Peptone 10g, Sodium chloride 5 g, and Agar- agar 5 g, and Distilled water 1000 ml Nutrient gelatine Meat extracts 3 g, Peptone 10 g, Gelatine 150 g, Distilled water 1000 ml.

Working cultures were obtained from lyophilized strains that were revived, and stored on nutrient agar slants at 5-7°C for periods of less than 3 wk.

Bacterial strains were classified on the basis of physiological and biochemical tests and host of origin. Bacterial strains defined as *P. Syringae* were fluorescent on king,

Material and methods the study was conducted in 2004 at Montecillo and Chapingo, both located in the State of México. Montecillo is located at 19°29' N and 98°53' W, 2250 m altitude, climate C (i) B/2(a'), annual average temperature of 14.6°C and 558 mm average annual rainfall. Chapingo is situated in the same coordinates as Montecillo, but its climat.

Procedure for isolation of native *Pseudomonas* isolates:

1. After the collection of samples, they were air dried between the sheets of paper at room temperature.
2. From each sample, 1 cm of leaf tissue (infected part) was excised with a sterile blade,
After washing, the tissue was again air dried using What man filter paper.
3. Now the excised tissue part was sequentially passed through 70% alcohol for 20 seconds, 0.01% mercury chloride for 20 seconds and thrice in sterile double distilled water for 40 seconds.
4. After washing, the tissue was again air dried using what man filter paper.
5. Isolation of native *Pseudomonas* isolates was made from the paste formed by crushing the excised plant tissue in sterile water using mortar pestle.
6. lapful of paste formed was streaked onto Nutrient agar plate and was incubated at 28°C for 72 hours.
7. After incubation period the suspected colonies were picked and further streaked on YDC agar and was incubated at 28°C for 24 to 48 hours.

Purification and Preservation:

A single bacterial colony isolated from nutrient agar medium which showed the characteristic features of native *Pseudomonas* isolates were picked up and streaked on sterilized plates containing sterilized nutrient agar to find pure culture of isolated bacteria. Then the pure culture

was preserved by transferring single pure colony to nutrient agar slants and nutrient broth and was kept 24 hours at 28°C. Then it was kept inside refrigerator at 4°C for future use. Also, the isolates were labelled properly for easy recognition and further laboratory purposes.

Identification and Characterization

Biochemical test

1. **Gram Stain** A Gram stain is a laboratory test that checks for bacteria at the site of a suspected infection or in certain bodily fluids. A medical laboratory scientist processes the gram stain, which gives relatively quick results, so healthcare providers can know if bacteria are present and, if so, the general type. This can help guide further identification tests and treatment options.

Under a gram stain, different kinds of bacteria change one of two sets of colours (pink to red or purple to blue) under a special series of stains and are categorized as gram –negative or gram-positive, accordingly. Gram staining works by differentiating bacteria by the chemical and physical properties of their cell walls.

Triple Sugar Iron (TSI) agar test: Microorganisms utilize glucose and lactose or sucrose ferment and produce hydrogen sulphide (H₂S). It contains 10 parts of lactose: 10 parts of sucrose: 1 part of glucose and peptone. Triple sugar iron agar test is used to determine whether Phenol red and ferrous sulphates serves as an indicator for acidification of medium and H₂S production respectively.

Citrate utilization test: The ammonium salts (NH₄H₂PO₄) as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. The simon's citrate medium contains citrate as the sole carbon source and is inorganic. Growth is indicative of utilization of citrate, an intermediate metabolite in the krebs cycle. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns

the bromothymol blue indicator in the medium from green to blue above pH 7.6.

Result and Discussion

Biochemical characteristics of *Pseudomonas*

S.N	TEST	RESULT
1.	GRAM STAINING	Negative (-ve)
2.	CITRATE UTILIZATION TEST	Negative (-ve)
3.	OXIDASE TEST	Positive (+ve)
4.	CATALASE TEST	Positive (+ve)
5.	MOTILITY TEST	Positive (+ve)
6.	TRIPLE SUGAR IRON (TSI)	Positive (+ve)
7.	NITRATE REDUCTION TEST	Positive (+ve)
8.	INDOLE TEST	Negative (-ve)
9.	GELATIN LIQUEFACTION TEST	Positive (+ve)
10.	UREASE TEST	Negative (-ve)
11.	SIM TEST	Positive (+ve)

1. **SIM test:** Hydrogen sulphide production

Sim medium contains ferrous ammonium sulfate and sodium thiosulfate, which together serve as indicators for the production of hydrogen sulfide. Hydrogen sulfide production is detected when ferrous sulfide, a black precipitate, is produced as a result of ferrous ammonium sulfate reacting with H₂S gas. In which, observed that bacterium of SIM test indicated liberation of H₂S. The bacterium showed positive reaction for this test.

2. **Triple Sugar Iron (TSI) Test:**

The TSI agar test helps differentiate between different bacterial species based on their ability to ferment sugars and produce gas and hydrogen sulfide (H₂S). *Pseudomonas* species typically show an alkaline/alkaline reaction, meaning they do not ferment sugars and do not produce gas or H₂S.

3. **Catalase test**

Pseudomonas bacteria are catalase positive, meaning they produce the enzyme catalase. The catalase test involves adding hydrogen peroxide to a bacterial culture, and if catalase is present, it will break down hydrogen peroxide into water and oxygen, resulting in the formation of bubbles.

Test and biochemical and characterization of *Pseudomonas*.

Summary & Conclusion

In the present study, pathogen native *Pseudomonas* isolates were isolated from the wheat causing leaf Blight, showed characteristics of the genus *Pseudomonas*. Out of total 21 samples 13 isolates showed typical pale-yellow colour, round mucoid, small to medium size colony on nutrient agar medium. These were further transferred to yeast dextrose agar medium, and after 24 hours' incubation at 28°C, typical yellow colour, small, round, and mucoid colony were observed. These isolates were identified as S4, S7, S9, S13, S14, S17, S18, S20 and S21. The morphological characteristics of these 13 suspected isolates for *Pseudomonas* isolates reported that 09 were gram negative small rod-shaped bacteria which shows the pink colour after gram staining.

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INFLUENCE OF IBA AND NAA ON REGENERATION OF STEM CUTTINGS OF SWEET LIME (*CITRUS LIMETTIODES* TANAKA)

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Abstract

The present experiment entitled “Influence of IBA and NAA on regeneration of stem cuttings of Sweet Lime (*Citrus limettioides* Tanaka)” was undertaken in the nursery, Department of Fruit Science, College of Horticulture, C. S. Azad University of Agriculture and Technology, Kanpur during the year 2022-23. The experiment was laid down in C.R.D. and incorporated nine treatments, each being replicated thrice. Two sets of PGRs viz., Indole-3-butyric acid (IBA), 1-Naphthaleneacetic acid (NAA), each of four levels (500, 1000, 1500 and 2000 ppm) including a control were tested against the regeneration potential of stem cuttings of sweet lime. The results of the experiment signify that IBA @ 2000 ppm was influential in invigorating the shoot and root traits of sweet lime cuttings and resulted in the earliest sprouting (23.13 days) with maximum number of sprouts (7.67), percentage of sprouted cuttings (70.87%), length of sprout (10.98 cm), diameter of sprout (4.44 mm), number of leaves (20.93), length of leaves (6.35 cm), width of leaves (4.11 cm), fresh weight of shoots (24.17 g), dry weight of shoots (4.81 g), number of roots (26.27), length of roots (14.76 cm), diameter of roots (4.00 mm), percentage of rooted cuttings (70.88%), survival percentage (62.85%), fresh weight of roots (5.22 g) and dry weight of roots (1.31 g).

Key Words: IBA, NAA, Root growth, Sweet lime, Stem cutting, Shoot growth, Survival.

Introduction

Sweet lime (*Citrus limettioides* Tanaka) is a fruit tree appertaining to the family Rutaceae and is categorized as a member of the acid group under the genus citrus. It is primarily a diploid with a chromosome complement of $2n=18$, and finds its home in the North Eastern states of India. The fruits are rich in ascorbic acid but low in sugar content and possess 0.1 % citric acid. The bland and insipid taste of the fruit is attributed to the lack of acidity, with citric acid content being six times less than other members of the acid group. The fruits can be consumed fresh from the tree as well as squeezed for juice extraction. The species has been also exploited as a rootstock in past, with trees being grafted on sweet lime exhibiting elevated vigour and reduced acidity of the fruit.

The exogenous application of plant growth hormones promotes root initiation and early root formation. Auxin, a classical plant hormone, regulates various aspects of growth and development. It is the most extensively used phytohormone utilized for the propagation of plant material due to its ability to initiate the formation of adventitious roots. Auxins promote the accumulation of osmotic solutes by increasing the permeability of the plasma membrane and by reducing the wall pressure. It also aids in the expansion and synthesis of the cell wall and prompts the cells to divide, thus favouring root initiation and growth. Keeping all these in view, the present experiment on “Influence of IBA and NAA on regeneration of stem cuttings of Sweet Lime (*Citrus limettioides* Tanaka) were carried out under north Indian plains.

Materials and Methods

The present experiment was conducted in the nursery, Department of Fruit Science, C.S. Azad University of Agriculture and Technology, Kanpur, during the rainy season of the year 2022-23, under the agro-climatic and edaphic conditions of Central Uttar Pradesh. Four genuine Sweet Lime trees were utilized for the procurement of the cuttings. Branches free from any malady, *viz.*, visible pest infestation and pathogen attack were utilized for the preparation of the stem cuttings. Mature shoots having girth of about 1.0 cm, were used to prepare cuttings of length 20-25 cm, bearing a minimum of 4-5 buds. A straight horizontal cut, perpendicular to the cutting's axis was made at the proximal region, whereas, an oblique sloping cut was made at the upper end of the stem cutting. The experiment was laid out in C.R.D. design and incorporated nine treatments, *viz.*, control (T₁), IBA @ 500 ppm (T₂), IBA @ 1000 ppm (T₃), IBA @ 1500 ppm (T₄), IBA @ 2000 ppm (T₅), NAA @ 500 ppm (T₆), IBA @ 1000 ppm (T₇), IBA @ 1500 ppm (T₈), and IBA @ 2000 ppm (T₉), with three replications. The basal portion of each cutting was then supplied with PGR solutions of their desired concentrations for 30 seconds (quick dip method) and then planted in polybags containing soil as the rooting medium. A hole was made in the soil inside the poly bag with the help of a stick before the insertion of cuttings. The cuttings were watered immediately after planting with a hand sprinkler. The parameters under study included days to sprouting, number of sprouts per cutting, percentage of sprouted cuttings, length of sprouts, diameter of sprouts, number of leaves per cutting, length of leaves, width of leaves, fresh weight of shoots, dry weight of shoots, number of roots, length of roots, diameter of roots, percentage of rooted cuttings, survival percentage, fresh and dry weight of roots were recorded using standard techniques. Statistical analysis of the data was carried out as suggested by Panse and Sukhatme (1985) and the results were tested at 5% significance.

Results and Discussion

Days to sprouting

Earliest sprout initiation (23.13 days) was witnessed under IBA @ 2000 ppm (T₅), which was at par with treatments of IBA @ 1000 ppm (T₃) and 1500 ppm (T₄) which required (24.47 and 24.07 days) respectively for sprout initiation. Cuttings under the control group exhibited maximum delay in sprout emergence (32.33 days) which was found to be significantly inferior to other treatments. The earliness in sprout initiation attributed to the enhancement of auxin content within the cuttings as a consequence of the exogenous application of NAA and IBA, which subsequently led to rapid cell division and thus faster sprout initiation (Table-1). The experimental outcome coincides with the studies of (11) in sweet lime, and (8) in pomegranate.

Number of sprouts per cutting

Maximum number of sprouts (7.67) was attained through the supply of IBA @ 2000 ppm (T₅), followed subsequently by T₄ which produces sprouts 7.47 (Table 1). All the treatments demonstrated significant variations relative to control (T₁) which produced the lowest number of sprouts (5.33). The maximization in number of sprouts attributed due to auxin-induced enhancement of physiological activities of the cells, like improved permeability of the cell to water, and the rise in osmotic pressure of cells which eventually led to their elongation, enlargement and division. The results are concurrent with the reports of (11) in sweet lime and (3) in acid lime.

Percentage of sprouted cuttings

A perusal of the data presented in Table 1 indicates the provision of IBA and NAA at all levels ranging from (500 to 2000 ppm) significantly impacted the success rate of sprouted cuttings over control which only produced (52.93%) sprouting. The maximum percentage of sprouted cuttings significantly (70.87%) were recorded under IBA @ 2000 ppm (T₅) pursued closely by IBA @ 1500 (T₄) ppm which resulted in (70.11%) sprouting success. The enhancement in the percentage of sprouted

cuttings considered a direct consequence of increased sprout number resulting from auxin treatment relatively higher concentrations, auxin tends to favour the division of the cells at the site of sprout initiation, which eventually leads to a higher sprouting percentage. The findings are in alignment with the works of Gnawali *et. al.*, (2022) in Eureka lemon, and Seiar (2016) in pomegranate.

Length of sprouts

It is evident from the data specified in Table 1 that the application of treatment (T₅) *i.e.*, IBA @ 2000 ppm was successful in eliciting a significantly superior outcome in regard to sprout length (10.98 cm) in comparison to remaining treatments and control. The second longest sprout was observed under T₄ (10.11 cm) which was followed closely by T₃ (9.73 cm). Treatment T₁ *i.e.*, control (8.05 cm) was found to be subordinate to every other treatment except T₆ (8.35 cm) and T₇ (8.43 cm) which were non-significant in their influences when compared within themselves. The provision of auxins, positively impacted the rooting traits thus leading to a rise in nutrient absorption, and consequently lengthened the sprouts, additionally. Auxins led to the linear extension of the cells and consequently lengthened the sprouts. Similar results were elucidated by (1), and in kagzi lime, and (20) in mulberry.

Diameter of sprouts

Significantly thicker sprouts (4.44 mm) were found under the influence of IBA @ 2000 ppm (T₅) which was significantly superior to other treatments, barring T₄ which exhibited sprout girth of 4.32 mm (Table 1). Cuttings treated with PGR exhibited significantly thicker sprouts over control which were responsible for the development of the thinnest sprout (2.50 mm) under observation. It deciphered that the quantitative augmentation in leaf and root number due to the assimilation of photosynthates was accountable for the amelioration of sprout diameter that shoot diameter excelled under the influence of auxins due to spike in the activity of cells and enhanced photosynthesis on the application of the PGRs.

The findings are congruent with those of (4) and, (13) in lemon.

Number of leaves

The exogenous provision of IBA and NAA significantly impacted the number of leaves present per cutting, as evident from the data prescribed above in Table 1. The extensive analysis of data demonstrates the ability of IBA @ 2000 ppm (T₅) to effectively producing the significantly maximum number of leaves (20.93) which was followed by (T₄) IBA @ 1500 ppm which produced (20.87) leaves. Cuttings devoid of any PGR *i.e.*, the control (T₁) resulted in the minimum number of leaves (15.00). Auxins foster the development of leaf buds which later manifests as a greater number of leaves per cutting the auxins were influential in instigating profuse rooting, which expedited the absorption of minerals and water, thus favoured leaf production in plenty. The current results are in harmony with the experiments of (17) in acid lime and (11) in lemon.

Length of leaves and width of leaves

The treatment of cuttings with IBA and NAA contributed to elongation in the length of the leaves as observed in Table 1. The use of IBA @ 2000 ppm (T₅) significantly produced maximum expansion in the length of leaves (6.35 cm) followed by IBA @ 1500 ppm (6.23 cm). The only T₆ *i.e.*, NAA @ 500ppm (5.25 cm) was detected to be non-significant in comparison to control (4.89 cm) which resulted in the shortest length of leaves. The expansion in the width of the leaves was significantly higher in IBA @ 2000ppm T₅ (4.11 cm) over the remaining treatments barring T₄ (3.98 cm). The PGRs treated cuttings were successful in creating significant improvement in leaf width in comparison to the control which displayed the narrowest leaf (3.22 cm). The concerned phytohormones were pivotal in aiding the enhancement of leaf area and subsequently, the length and width of the leaf as insinuated by proposed that auxins regulated the process of favourable translocation of photoassimilates to the leaves, which ultimately led to their expansion. Similar observations are evident in the works of (22) in mulberry and (5) in Guava.

Fresh weight of shoots

Cutting treated with IBA @ 2000 ppm (T₅) played a decisive role in significantly maximizing the fresh weight of shoots (24.17g) over other treatments barring T₄ IBA @ 1500 ppm which registered a weight of 23.45 g (Table 1). The control (T₁) displayed the minimum fresh weight (19.15 g) among all the treatments under consideration. The lengthening and thickening of the sprouts coupled with an increased number of leaves in PGRs treated cuttings was accountable for the increased fresh weight of spouts. The higher weight accorded to the development of numerous sprouts in cuttings administered with NAA and IBA in contrast with control which generated relatively fewer sprouts. The reports are in harmony with the works of (2) in pomegranate and

(7) in fig.

Dry weight of shoots

A perusal of data under Table 1 reveals that IBA @ 2000 ppm (T₅) was the most effective treatment for significantly improving the dry weight (4.81 g) of shoots, pursued closely by IBA @1500 ppm i.e., T₄ (4.69 g). The treatment control which was responsible for the lowest (3.83 g) dry weight of the shoots. Auxins triggered the enhancement in the number of sprouts and leaves in cuttings which eventually caused increased carbon fixation within the plants, therefore such cuttings accumulated dry matter at higher rates, which is evident from their increased dry weight. The results are in alignment with the studies of (5), (21) in sweet orange and (10) in plum.

Table 1. Influence of IBA and NAA on shoot growth parameters

Treatments	Days to sprouting	No. of sprouts	Percentage of sprouted cuttings	Length of sprouts	Diameter of sprouts	No. of Leaves	Length of leaves	Width of leaves	Fresh weight of shoots	Dry weight of shoots
T ₁	32.33	5.33	52.39	8.05	2.50	15.00	4.89	3.22	19.15	3.83
T ₂	24.93	6.87	66.44	9.29	3.95	19.53	5.70	3.81	21.97	4.39
T ₃	24.47	7.40	67.85	9.73	4.20	20.33	6.03	3.87	22.74	4.54
T ₄	24.07	7.47	70.11	10.11	4.32	20.87	6.23	3.98	23.45	4.69
T ₅	23.13	7.67	70.87	10.98	4.44	20.93	6.35	4.11	24.17	4.81
T ₆	28.00	6.20	62.20	8.35	3.56	17.40	5.25	3.63	20.54	4.09
T ₇	26.87	6.47	63.42	8.43	3.69	17.67	5.42	3.68	20.75	4.12
T ₈	26.73	6.60	64.15	9.20	3.80	18.60	5.50	3.74	21.05	4.17
T ₉	26.40	7.20	67.16	9.57	4.07	19.93	5.76	3.75	22.27	4.42
S.E.(d)±	0.678	0.259	1.314	0.203	0.080	0.393	0.186	0.063	0.410	0.094
C.D. at 5%	1.436	0.549	2.783	0.430	0.169	0.831	0.393	0.133	0.868	0.200

Number of roots

Data in Table 2 reveals that the maximum increment in number of roots (26.27) was observed by exposing the cuttings to solution of IBA @ 2000ppm (T₅) closely followed by IBA @ 1500 ppm (T₄) and IBA @ 1000 ppm (T₃) which produced (26.00 and 25.67 roots respectively). The untreated cuttings (T₁) produced the minimum number of roots (18.93). Auxins bear the potential to regenerate the roots in stem cuttings and therefore act as a rooting hormone. They alter the elasticity of the cell wall, thus enabling enhanced water uptake by them in the process, which eventually leads to rapid cell division and subsequent proliferation of the root system. The formation of roots in larger number can be ascribed to the activation of the cambium by growth regulators. Treatment of cuttings with auxins converts complex carbohydrates into simple sugars, which promotes the initiation of new cells and enhances the respiration rates in the regenerating cells of the root primordium. Similar results have been obtained by (9) in avocado.

Length of roots

It is clearly evident from the data mentioned in Table 2 that the application of IBA @ 2000 ppm led to significant improvement in the length of roots (14.76 cm) closely followed by T₄ (IBA @ 1500 ppm) which exhibited (14.05 cm) long roots. All treatments were statistically superior relative to the control which produced the shortest roots (10.98 cm). The length of the roots observed in PGRs supplied cuttings can be attributed to the biosynthesis of enzymes which initiate the enlargement of cells. Additionally, auxin favoured partitioning of the sugars towards the root formation zone, thus triggering the enlargement of cells leading to the elongation of the roots. The results are in conformity with the findings of (1) and (19) in peach.

Diameter of roots

Formation of the thinnest root (2.07 mm) could be attributed to control, and that, the remaining treatments were statistically at par in comparison (Table 2). The thickest roots (4.00 mm) were reported under IBA @ 2000 ppm (T₅) closely followed by (3.82 mm) was recorded with T₄ (IBA @ 1500 ppm). Auxins instigate the enlargement of cells by increasing its permeability to water and minerals, and increasing the elasticity of the cell wall to promote the expansion of the cell, thus leading to an increase in the diameter of the cuttings. The experimental outcome is in accordance with the findings of (4) and (13) in lemon.

Percentage of rooted cuttings

Data provided under Table 2 indicates that treatment of sweet lime cuttings with IBA @ 2000 ppm (T₅) displayed significant superiority in rooting (70.88%) relative to all other treatments. Following up next was IBA @ 1500 ppm (T₄) and IBA @ 1000 ppm (T₃) displaying (68.23% and 67.71%, respectively) success in rooting and found to be non-significant influences when compared within themselves. The control (T₁) with (53.15%) rooting elicited the significantly poorest rooting response. The exogenous provision of auxin to the cuttings, improved the free auxin content within them, which subsequently led to increased mobility and usage of carbohydrate and nitrogen reserve of the plants, thus increasing the

percentage of rooted cuttings. It is proposed that the initiation of adventitious roots requires an expenditure of energy this energy is released from hydrolysis of starch into sugars within the cuttings, which further stimulates the meristematic activity of root cells, eventually leading to the formation of adventitious roots in cuttings. The findings of the experiment are in line with the research works of (3) in acid lime, and (16) in karonda.

Survival percentage

A thorough evaluation of the data under Table 2 reveals that administration of IBA @ 2000 ppm (T₅) ensured maximum improvement in the survivability of rooted cuttings (62.85%), pursued closely by IBA @ 1500 ppm (62.48%). The poorest survivability rates were accorded to untreated cuttings (43.55%). The proliferation in number of roots and their elongation due to the provision of auxins facilitated the uptake and adsorption of minerals and water from the soil and thus enhanced the chances of survival in the field. The earlier rooting of the cuttings and development of profuse root and shoot system in auxin-treated cuttings ensured their survival over untreated cuttings which displayed relatively poorer morphological features. The better survival rates attributed to a higher C/N ratio and increased carbohydrate and sugar accumulation within the cutting, these factors were favourably improved due to provision of IBA and NAA to the stem cuttings. The results of the current investigation coincide with reports of (1) and, (20) in mulberry.

Fresh weight of roots

Detailed inspection of the data under Table 2 indicates that IBA and NAA modulated the fresh weight of the roots, with IBA @ 2000 ppm (T₅) imparting the maximum recorded fresh weight (5.22 g) closely followed by IBA @ 1500 ppm (5.09 g) while the lowest weight was attributed to untreated cuttings (4.23 g). The Supply of auxins, particularly NAA and IBA to the cuttings, modified the cell's permeability in favour of enhanced water and mineral build-up within them, thus ensuring the formation of a robust root system, which inevitably accounted for the increment in the fresh weight of root. The increased number as well as the girth and length of

roots achieved through auxin treatment was also influential in positively affecting their fresh weight. The results are in alignment with the research work of (11) in sweet lime, and (5) in kagzi lime.

Dry weight of roots

Dry weight of the roots was impacted by the provision of growth regulators with IBA @ 2000 ppm (T₅) yielding the significantly maximum recorded dry weight (1.31 g) pursued subsequently by IBA @ 1500 ppm (1.27 g). The treatment control (T₁) registered significantly minimum dry weight (0.97 g) of roots (Table 2). There was an improvement of 35.05% in IBA @ 2000 ppm (T₅) treated cuttings relative to control (T₁), this might be due to the stimulating effect of auxin on cell division which fostered the emergence of adventitious roots, further proteins and carbohydrates accumulated in larger quantities in auxin treated cuttings which could be responsible for the increased dry weight. The results are in conformity with the findings of and (2) in pomegranate.

Table 2: Influence of IBA and NAA on root growth parameters

Treatments	Number of roots	Length of roots	Diameter of roots	Percentage of rooted cuttings	Survival percentage	Fresh weight of roots	Dry weight of roots
T ₁	18.93	10.98	2.07	53.15	43.55	4.23	0.97
T ₂	24.87	13.24	3.57	64.64	57.82	4.69	1.21
T ₃	25.67	14.02	3.69	67.71	60.91	4.85	1.24
T ₄	26.00	14.05	3.82	68.23	62.48	5.09	1.27
T ₅	26.27	14.76	4.00	70.88	62.85	5.22	1.31
T ₆	23.07	12.25	3.00	58.35	53.86	4.49	1.12
T ₇	23.47	12.51	3.07	60.14	54.48	4.55	1.17
T ₈	24.20	13.01	3.34	62.67	54.81	4.62	1.20
T ₉	24.67	13.57	3.49	66.18	59.53	4.73	1.23
S.E. (d)	0.506	0.423	0.092	1.233	0.950	0.115	0.027
C.D. @5%	1.071	0.896	0.195	2.611	2.012	0.243	0.056

Conclusion

The results of the current investigation establish the superiority of IBA@2000 ppm in causing the earliest sprouting and development of maximum number of sprouts, percentage of sprouted cuttings, length of sprout, diameter of sprout, number of leaves, length of leaves, width of

leaves, fresh weight of shoots, dry weight of shoots, number of roots, length of roots, diameter of roots, percentage of rooted cuttings, survival percentage, fresh weight of roots and dry weight of roots. Taking into account the aforesaid results, IBA 2000 ppm could be endorsed to horticulturists for fast multiplication of true to type sweet lime for a large scale plantations.

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A CRITICAL STUDY ON OPERATIONAL FEASIBILITY DEVELOPMENT OF ALUMINIUM DROSS TO TREAT POLLUTANTS OF THE AQUEOUS ENVIRONMENT : WASTE REUSE FOR RESOURCE RECOVERY

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Abstract

The need to reduce eutrophication in water bodies and the depletion of phosphate rock reserves have prompted the search for sequestration and recovery of phosphate from alternative sources, such as effluent. In this investigation, aluminium dross (AD), an industrial waste encapsulated in calcium alginate (Ca-Alg) beads, was used for phosphate removal. The removal of phosphate was evaluated both in batch and continuous operation modes. In addition, two prototype scale studies utilizing actual municipal wastewater have been conducted: (i) constructed wetlands (CWs) followed by Ca-Alg-AD beads based fixed bed and (ii) Ca-Alg-AD beads based free water surface (FWS) CW assembly. The bulk adsorption study revealed that 96.86% of the initial 10 mg/L phosphate concentration was removed in 70 minutes. Multiple characterizations, were performed in order to comprehend the influence of surface properties on the phosphate adsorption behavior. In addition, data analysis indicates that phosphate adsorption on Ca-Alg-AD beads follows a pseudo-kinetic model of second order and is best described by the Freundlich isotherm with regeneration capacity up to five consecutive adsorption-desorption cycles. In a 30-day pilot scale experiment, constructed wetlands (CWs) followed by a Ca-Alg-AD bead-based fixed bed system removed an average maximum of 85.04 percent phosphate while simultaneously removing 93.4 percent ammonium (in a regenerated column). In addition, the Ca-Alg-AD bead-based FWS CW assembly exhibited a maximal phosphate removal of 79.856 % and a simultaneous ammonium removal of 65.4521 %. The algal growth control study demonstrated that the innovative Ca-Alg-AD-based FWS CW assembly is effective in remediating and upgrading eutrophic lentic water bodies such as lakes and ponds. The results validate the applicability of Ca-Alg-AD bead-based systems in the field. Ca-Alg-AD beads have been conclusively demonstrated to be effective for the simultaneous remediation and recovery of phosphate and ammonium from actual municipal wastewater and are therefore suitable for use as a wastewater treatment technology.

KEY WORDS: Phosphate Rock, Feasibility, Remediation, Phosphate and Ammonium.

Introduction

Phosphorous (P) is essential to all life forms on Earth. Extracellular enzymes typically convert organic phosphorous forms to inorganic orthophosphates (PO_4^{3-}), the only form of phosphorus that autotrophs can assimilate [1]. Consequently, high levels of orthophosphates can

accelerate the production of autotrophs in water bodies, especially cyanobacteria and algae, resulting in eutrophication (1). Also, as the number of autotrophs in water bodies has increased, high respiration rates have been observed, resulting in anoxia or hypoxia and the extinction of aquatic life (2). To prevent

eutrophication, total phosphate concentrations in water bodies must be regulated. (3) The United States Environmental Protection Agency (US-EPA) recommends that the total phosphate concentration in effluent discharge be between 0.1 and 1.0 mg/L. Although the source of increased phosphate concentration in surface water can be natural, such as phosphorus-bearing bedrock, it is generally assumed that concentrations above 0.05 mg/L are anthropogenic (4). Soil erosion, industrial and municipal effluent discharge, and agricultural phosphate fertilizers can contribute to anthropogenic activities [5]. Ironically, the modern agricultural sector relies heavily on phosphorus (as fertilizer) derived from phosphate bedrock, a nonrenewable resource that is swiftly depleting. Recent estimates project that global reserves will deplete in 50 to 100 years [6]. Never before has it been so urgent to discover new and sustainable ways to meet future phosphate demand. On the other hand, phosphate in sewage water (contributed by human waste (2 g P/person/day), food waste, washing bars, and detergents, etc.) is being reported more frequently [7], while our sewage treatment systems are ill-equipped to remove it, resulting in the eutrophication of water bodies. It is of the utmost importance to develop technologies to not only remove but also recover this phosphate as a resource in order to help bridge the gap between agricultural demand and supply and to maintain food security and productivity.

Although natural materials are readily available, they lack effective adsorption capacity, whereas industrial by-products such as calcium silicate hydrate, steel slag, iron fillings, and steel by-products have demonstrated phosphate removal potential (8), in contrast to several natural minerals such as limestone, zeolite, and calcite. It can also be concluded that the phosphate-absorption capacity of industrial byproducts was one order of magnitude greater than that of natural materials. In addition, the availability of industrial byproducts in large quantities facilitates its use as an adsorbent for phosphate removal.

This study concentrates on the potential for phosphate removal by aluminum dross (AD), a byproduct of the aluminum industry. It is estimated that the aluminium industries in India

produce 75,000 tons of AD per year, the majority of which is used to manufacture firecrackers, low-quality blocks, and low-purity chemicals [9]. Phosphate removal and recovery from an aqueous environment is an example of a high-value application where massive quantities of AD must be utilized. Several metal oxides, including aluminum oxides and hydroxides, have demonstrated a specific affinity for phosphate and, consequently, an outstanding adsorption capacity for phosphate removal (10).

A recent study described the use of unprocessed AD for phosphate removal from aqueous solution and demonstrated a phosphate adsorption capacity ranging from 2.312 to 3.467 mg-P/g. However, maximal phosphate adsorption was observed at pH values of 12.0 and 3.0 (11). Later, raw AD has been investigated as an adsorbent for Congo red (CR) dye removal from aqueous solution and results revealed 100% of CR removal with initial CR concentration of 20 mg/L and 40 mg/L using 0.4 g of AD at a high pH value i.e., 9 (329). Nonetheless, it can be inferred readily from the aforementioned raw AD studies that it can only function effectively in highly basic or highly acidic solution conditions, thereby limiting its application in the natural environment. Also, the use of unprocessed AD powder as an adsorbent prevents its even distribution in water due to density differences, limiting the contact of AD with water and complicating the AD recovery process from water.

In this investigation, therefore, raw AD was used to create a novel material for phosphate removal and recovery from an aqueous environment. In the present study, calcium alginate aluminium dross (Ca-Alg-AD) beads were developed by encapsulating aluminium dross with sodium alginate and calcium chloride. The encapsulation of AD was anticipated to have the following advantages: (i) on-site treatment, (ii) high selectivity for phosphate, (iii) efficient contact of AD with aqueous environment contaminants, and (iv) simple separation of AD from water after phosphate adsorption. In addition, the current innovation investigated the performance of encapsulated AD beads (Ca-Alg-AD beads) for phosphatic nutrient removal and recovery from an aqueous environment under varying conditions, such as (i) batch and continuous mode operation,

and (ii) the application of Ca-Alg-AD beads for phosphate removal and recovery in a real-world setting consisting of (a) constructed wetlands (CWs) followed by a Ca-Alg-AD This will validate (i) whether the treated water is suitable for discharge, and (ii) whether Ca-Alg-AD beads have sufficient potential to treat polluted water bodies efficiently. According to the authors' knowledge, this is the first comprehensive investigation of Ca-Alg-AD adsorbent for nutrient removal and recovery from wastewaters.

Materials and Methods

Preparation of calcium alginate- aluminium dross bead

In order to create Ca-Alg-AD beads, 0.2 g of sodium alginate powder was dissolved in 20 mL of DDW using a magnetic stirrer at 100 °C to assure homogeneity and prevent agglomeration. A translucent and viscous sodium alginate solution was obtained after 10 minutes of agitation. Then, for the preparation of sodium alginate- AD solution, 0.4 g of ground (mortar and pestle) AD powder was added to sodium alginate solution and stirred continuously (without heating) to ensure proper blending. Later, Ca-Alg-AD beads were formed by continuously dripping (via a burette) sodium alginate-AD solution into 0.2 M CaCl₂ solution at a constant rate. The crystals were left in CaCl₂ solution at 4 degrees Celsius for 24 hours. During this procedure, the beads harden into Ca-Alg-AD beads that are stable. The beads were then rinsed multiple times with DDW, and the average size of the beads was determined using optical stereomicroscopy. As a control for our experiments, a distinct batch of Ca-Alg beads was prepared without the addition of AD.

Pilot scale application of Ca-Alg-AD beads

Two distinct CWs investigated the application at the pilot scale level: (i) CW followed by Ca-Alg-AD beads based fixed bed system in continuous mode operation for actual municipal wastewater treatment, and (ii) Ca-Alg-AD beads based FWS CW assembly imitating polluted lentic systems such as lakes or ponds (eutrophic water bodies).

Artificial wetlands followed by a Ca-Alg-AD bead-based fixed bed system

A system consisting of constructed wetlands (CWs) followed by a Ca-Alg-AD bead-based fixed bed system (CW-Ca-Alg-AD; Fig. 7-2a) was designed. CW column was constructed with a 60 cm x 11 cm (height x diameter) PVC tube with apertures at 10 cm, 33 cm, and 54 cm from the top. The base of the column was crowned with a circular PVC base. The CW facility at CSIR-IMMT in Bhubaneswar, India, was loaded with siliceous garden gravels measuring 5-8 mm in diameter. The CWs were fed by actual municipal secondary wastewater every day with wastewater collected from a CWs facility in CSIR-IMMT, Bhubaneswar, India. Wastewater was collected weekly, 2 L of it was added to a bucket daily, and the remainder was retained at 4 °C. The secondary wastewater was supplied to the CWs with the aid of a peristaltic pump (Watson Marlow Pvt. Ltd., UK), which was operated in an upflow motion via a 2-cm-diameter PVC conduit located in the middle of the CW column. The CW column was planted with *Canna indica* species collected from CW's facility at CSIR-IMMT and positioned 15 cm from the top. The uprooted *Canna indica* had 18.4 cm long roots, 69.5 cm long shoots, and weighed 78 g. From the apex of the CW column, treated sample was gathered. Fig. 7-2a depicts the overall configuration of a filled vertical upflow CW column.

As depicted in Figure 7-2a, the Outlet of the CW column was connected to the base of the Ca-Alg-AD glass column. The Ca-Alg-AD column was constructed using a glass tube (200 mm in length and 30 mm in diameter). The column was filled to a height of 200 mm with Ca-Alg- AD crystals weighing 2 g (representing the AD content of the beads). Three sample apertures were located 3 cm, 10 cm, and 20 cm from the bottom of the column. After completion of empty bed contact time (EBCT), duplicate samples were collected from the uppermost inlet and analyzed further. Thus, three distinct wastewater samples were collected: (i) initial wastewater (0 hr), (ii) CW treated sample from the top of CW column (i.e., after 24 hr of HRT), and (iii) Ca-Alg-AD bead based fixed bed system treated sample collected after

24.56 hr. In addition to testing for reactive soluble phosphorous, each sample was also analyzed for ammonium and chemical oxygen demand (COD). All investigations were conducted at a constant temperature of 30 ± 2 degrees Celsius.

To further investigate the potential of a Ca-Alg-AD bead-based fixed bed system, a 0.5 M NaOH solution was used to regenerate the used beads from the column (abbreviated as regenerated Ca-Alg-AD). In order to regenerate the Ca-Alg-AD column, it was disconnected from the CW column and further, with the aid of a peristaltic pump, NaOH solution was passed in continuous mode to the Ca-Alg-AD beads fixed bed column for one day to achieve regeneration. After that, SDW was passed through the column for two days to remove the retained NaOH. After regeneration, the CW column was reconnected to the Ca-Alg-AD column and COD, ammonium, and phosphate removal were evaluated. Table-1 contains exhaustive data, including numerous CW and Ca-Alg-AD column parameters.

Additionally, simultaneous ammonium removal was obtained in the Ca-Alg-AD bead-based fixed bed system, and additional experiments were conducted to comprehend this phenomenon. Ca-Alg-AD beads and control Ca-Alg beads were exposed to 50 ml of 13 mg/L ammonium and a mixture of 25 mL of mg/L ammonium and 25 mL of 10 mg/L phosphate in separate conical flasks. After 70 minutes of contact, all samples were analyzed for their phosphate and ammonium concentrations.

Table -1. Details of different parameters utilized in CWs followed by Ca-Alg-AD beads based fixed bed system

Constructed wetlands		Ca-Alg-AD bead bed parameters	based fixed
Flow rate	2 L	4 ml/min	226.70 cm ³
Void volume		Total volume	
Hydraulic retention time (HRT)	1 day	Adsorbent height	20 cm
Working height	50 cm	Adsorbent mass	6 gm
Working volume	4749.3 cm ³	Empty bed contact time	56.67 Min

Ca-Alg-AD beads based FWS CW assembly

A Ca-Alg-AD bead-based FWS CW assembly was constructed in a PVC container of 54 cm in

height, 34 cm in diameter, and 46 L in volume. The PVC container had three apertures located at depths of 3 cm, 27 cm, and 51 cm. A 23 cm x 20 cm basket with 8-10 mm holes was suspended inside the container using a steel rod, as shown in Figure 7-2b. Using a soldering machine, perforations were drilled into the outer container to support the basket on steel rods. Inside the receptacle, 12 kg of siliceous gravel with a diameter of 12 to 15 mm were placed, and then *Canna indica* was planted. Gravels are only used to sustain the *Canna indica* plant life. The uprooted *Canna indica* had a root length of 21.5 centimeters, a stalk length of 64.5 centimeters, and a mass of 78 grams. With the assistance of a receptacle, 25 packets of Ca-Alg-AD beads containing 11 g of AD in calcium alginate beads were hung. As depicted in Figure-2, these packets were composed of a synthetic net (1.2 mm in diameter) and suspended with 1 mm insulated wire. In addition, the packets were suspended at varying heights in order to maintain the homogeneity of the Ca-Alg-AD beads within the container, as shown in Figure-2. Locally collected effluent containing nutrient pollutants and organics that cause eutrophication in water bodies was fed to a Ca-Alg-AD bead-based FWS CW assembly. Without treatment with Ca-Alg-AD nanoparticles, the collected water demonstrated rapid algal growth within fourteen days. The sample was collected every alternate day from the Ca-Alg-AD beads-based FWS CW system (S-2, S-4, S-6) and the effluent was changed every 6 days from the time of influent filling. Using the same sampling and inoculation technique, 15 experimental cycles were conducted. For homogenous sample collection, water was decanted from a pail through the bottommost inlet of the container and thoroughly mixed prior to sample collection. In addition to testing for reactive soluble phosphorous, each sample was also analyzed for ammonium and chemical oxygen demand (COD). All investigations were conducted at a constant temperature of 29 ± 2 degrees Celsius.

In addition, an experiment was devised to determine whether or not FWS CW assembly treated samples could stimulate algal growth. The purpose of this experiment was to determine how Ca-Alg-AD bead-based FWS CW treated water acts upon discharge to promote algal growth or is retained in lentic system-like situations to

promote algal growth. In order to obtain mother culture for the algal experiment, 5 mL of water containing algae was collected from a local facility and combined with 500 mL of BG11 solution. Furthermore, in order to generate mother culture, algae from natural wastewater were adopted on purpose to mimic the natural/practical system. After 10- 12 days of algal growth in BG-11 solution, the UV-Vis spectra (Cary 100, Agilent technologies, USA) of the algal culture were observed repeatedly to ensure that the algal culture attained an absorbance value of 0.9 at a wavelength of 689 nm. In addition, 50 mL of the mother culture was centrifuged and the supernatant was removed to obtain an algae particle. This pellet was rinsed multiple times with DDW before being combined with 75 mL of Ca-Alg-AD beads-based FWS CW assembly-treated water in a conical flask. In addition, the formation of algal pellets was repeated multiple times to produce flasks in triplicates for the initial (I-0), second day (S-2), fourth day (S-4), and sixth day (S-6) conditions, as well as a negative control (NC-DDW, or algal pellet dissolved in 75 mL of double-distilled water). The aforementioned procedure made it possible to inoculate each sample with an equal amount of phytoplankton. For appropriate mixing and algal growth, all experimental conical flasks and the mother culture were kept on a rotatory shaker (Spinix Orbital shaker MC-02, Tarson, India) at 140 rpm. The entire experiment was also illuminated by a light source (85 W, Spiral, Crompton Greaves Ltd.) for 12 hours per day, from 6 am to 6 pm. An automatic on-off timer (HPA digital timer, Australia) was used to sustain the on and off cycles. The absorbance of each algal conical flask was then measured at 689 nm using a UV-Vis spectrophotometer (Cary 100 UV-Vis, Agilent Technologies, USA) for up to 25 days after the beginning of the experiment.

Results and Discussion

Characterization Studies

Phosphate-adsorbing functional groups on the surface of Ca-Alg-AD nanoparticles were identified through FTIR analysis. Fig.1, depicts the FTIR spectra of Ca-Alg-AD beads prior to and after phosphate adsorption. The spectra of these materials displayed significant displacements of

some characteristic transmittance bands, as well as variations of intensity within the 400 cm^{-1} to 4000 cm^{-1} range. A broader peak at 3460 cm^{-1} was ascribed to -OH stretching vibration, which may be related to water bending vibration (12). However, FTIR spectra of Ca-Alg-AD beads after phosphate adsorption/absorption revealed a weaker band at 3460 cm^{-1} , which can be ascribed to the replacement of -OH with phosphate. Moreover, the band at 1404 cm^{-1} is attributed to the asymmetric stretching vibration of -COO, which shifted from 1404 cm^{-1} to 1395 cm^{-1} after phosphate adsorption, as depicted in Figure. This transition in spectra to a weak band suggests that the COO-group and phosphate interact during adsorption/absorption (14). Similar peak modifications are also observed at 1593 cm^{-1} , which corresponds to the ester group (-COO) present in the typical structure of calcium alginate [15]. The peak became weaker and displaced to 1584 cm^{-1} following phosphate adsorption. In addition, the band observed between 1000 and 1100 cm^{-1} corresponds to the phosphorous-oxygen (P-O) stretching region. The broadening of the band at 1015 cm^{-1} in Ca-Alg-AD beads upon phosphate adsorption was attributed to asymmetric P-O bond vibrations and the presence of phosphate (16). After interaction with dissolved phosphate, FTIR peak shifting and the appearance of new peaks indicate that phosphate interacts with functional groups present in Ca-Alg-AD beads.

Thermogravimetric analysis (TGA) is a crucial criterion for evaluating the relative stability of a material in relation to a change in temperature. The TGA curves for Ca-Alg beads, Ca-Alg-AD beads, unprocessed AD, and differential thermal analysis (DTA) of Ca-Alg-AD beads over the temperature range of 25°C to 1000°C are depicted in Figure -1b. TGA thermograms depict a progressive and sequential pattern of weight loss. Fig.1c, Ca-Alg beads exhibit the greatest mass loss of 70% compared to Ca-Alg-AD beads (35%), and unprocessed AD powder (9%). Initial mass loss at temperatures 200°C could be attributed to water evaporation in the bead matrix/powder (17). This loss was greater for Ca-Alg raw beads (20%) and

Ca-Alg-AD beads (10%) than for raw AD powder (2.5%), due to more water entrapped in the beads matrix than in the raw pulverized AD. The same is reflected in the DTA curve, which displays an abrupt exothermic peak near 200 °C. In addition, weight loss between 200°C and 300°C for Ca-Alg raw beads (25%), and Ca-Alg-AD beads (13.5%), can be attributed to the degradation of glycosidic bonds present in the linear copolymer alginate matrix $((C_6H_8O_6)_n)$. On the other hand, an abrupt exothermic peak around 300 °C in the DTA plot of Ca-Alg-AD beads can be attributed to the synergistic decomposition of glycosidic bonds and residual organic matter. 15% more mass loss in Ca-Alg-AD beads between 300 and 500 degrees Celsius is due to the continued decomposition of glycosidic bonds. No significant mass loss was observed after 500 °C, which could be attributed to the slower rate of decomposition in Ca-Alg-AD beads due to the presence of AD compared to AD-deficient raw Ca-Alg beads, which is supported by a lower concentration of alginate in Ca-Alg-AD beads compared to Ca-Alg raw bead. Since unprocessed bead experienced a 26% mass loss at temperatures greater than 300 °C, this corresponds to the increased carbonization of the alginate bead matrix in an Argon atmosphere. In addition, both Ca-Alg-AD beads and raw AD powder follow a similar weight loss profile, which guarantees the encapsulation of raw AD powder and its stability within the bead matrix.

Figure 1c depicts the structural morphology and elemental composition of Ca-Alg beads and Ca-Alg-AD beads before and after phosphate adsorption. Before adsorption, the structure of the Ca-Alg-AD particle is observed to be rough and porous. This type of surface morphology may facilitate phosphate adsorption on the Ca-Alg-AD bead surface. However, after adsorption (Fig. 1c(iii)), the surface of Ca-Alg-AD beads became coarser and non-porous compared to before adsorption, which may be attributed to the

precipitation of aluminium phosphate via the ligand complexation mechanism.

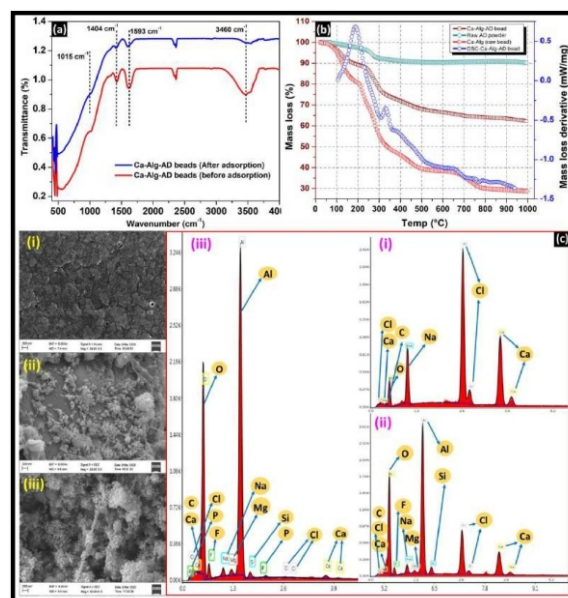


Fig. 1. Represents (a) FTIR spectra of Ca-Alg-AD before and after phosphate adsorption, (b) Thermogravimetric analysis of Ca-Alg-AD, raw AD powder, and Ca-Alg (control) and DTA of Ca-Alg-AD bead and (c) SEM micrograph of (i) Ca-Alg bead (control), (ii) Ca-Alg-AD bead before adsorption, and (iii) Ca-Alg-AD bead after adsorption and their EDX graph, respectively

The observed a similar morphology and mechanism when using Zinc-Aluminium stratified double hydroxide for phosphate adsorption. In elemental analysis (EDX) of raw Ca-Alg beads (Fig. 7-3c(i)), the presence of only C, Ca, Cl, Na, and O was observed, whereas in Ca-Alg-AD (Fig. 7-3c(ii)), the presence of several new elements (such as F, Na, Mg, and Al) was observed due to the encapsulation of AD particles within the bead. The presence of Cl in both the Ca-Alg bead and the Ca-Alg-AD bead suggests the presence of chlorides in the interlayer of beads, which could aid in phosphate removal through the ion exchange mechanism by substituting chlorides with phosphates in Ca-Alg-AD beads. Furthermore, Al was quantified at the maximum weight percent of 27.16 in Ca-Alg-AD beads, confirming that AD was adequately loaded within the beads. In addition, the incorporation of an extra element P in Figure 7-3c(iii) confirms the phosphate adsorption on the surface of Ca-Alg-AD beads.

Mechanism of phosphate adsorption on Ca-Alg-AD beads

SEM-EDX analysis confirmed the presence of metals such as Al and Mg at high atomic weight percentages in Ca-Alg-AD beads, and these metals played a significant role in phosphate removal [20]. One of the plausible mechanisms for phosphate adsorption is ion exchange between phosphate and interlayer anions, as suggested by SEM-EDX and analysis of the residual liquid sample after adsorption. In order to acquire insight into the mechanism of phosphate adsorption/absorption on Ca-Alg-AD beads, complementary analyses such as FTIR analysis were utilized. In FTIR investigations, the presence of P-O bond vibration at 1015 cm^{-1} after phosphate adsorption further confirmed inner-sphere complexation in Ca-Alg-AD beads via ligand exchange via metals. Besides, in FTIR spectra, no peaks were observed for O-P-O bond vibration, and thus mononuclear monodentate inner-sphere complexation can be concluded. In general, phosphate ions form inner sphere complexation with metal hydroxides as a consequence of ligand complexation. Moreover, in acidic conditions, an increase in pH upon phosphate adsorption or uptake was caused by the liberation of OH⁻ following the occurrence of the ligand exchange mechanism [3]. Additionally, favorable phosphate adsorption/absorption or anion adsorption/absorption can occur when the pH_{pzc} of Ca-Alg-AD is greater than the pH of the solution, i.e. when pH is less than 8.6., at this pH, Ca-Alg-AD bead surfaces were positively charged, resulting in electrostatic attraction between phosphate anion and Ca-Alg-AD bead surfaces. Three plausible mechanisms can be inferred: ion exchange, electrostatic attraction, and ligand complexation. These multiple mechanisms are also consistent with the Freundlich isotherm, which suggested that multiple adsorption/uptake mechanisms were involved in the phosphate adsorption on Ca-Alg-AD beads.

Pilot Scale Studies

Pilot scale studies utilize actual wastewater for the studies and thus variation in influent wastewater concentration of nutrient can be observed in these studies.

Constructed wetlands followed by Ca-Alg-AD beads based fixed bed system for real municipal wastewater treatment

The percentage of phosphate and ammonium removal during the actual treatment of municipal wastewater in constructed wetlands followed by a Ca-Alg-AD bead-based fixed bed system. The duration of the first cycle of the test was 30 days. The phosphate removal rate was 85.04%

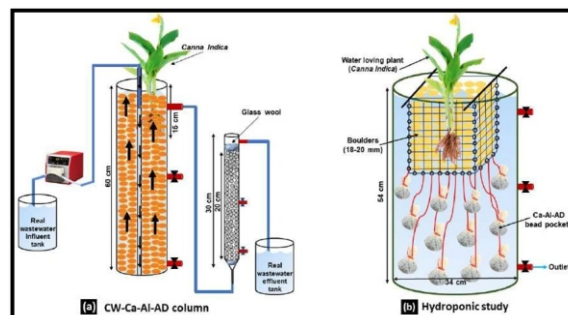


Fig. 2. The schematic diagram of pilot scale studies (a) CW followed by Ca-Alg-AD beads fixed bed column and (b) Ca-Alg-AD beads based FWS CW assembly.

percent after eight days of operation, whereas it diminishes to 42.56.032% within 14 days and further to 23.79.155% within 23 days. At the end of 30 days, phosphate removal dropped to 9.49.142 percent. High phosphate removal during the first eight days of operation may be attributable to the high surface area afforded by a fixed bed of Ca-Alg-AD beads, thereby permitting the availability of a large number of binding sites. However, a decrease in phosphate removal with time can be attributed to the saturation of phosphate-adsorbing active sites [7]. In this study, it was determined that low phosphate removal is achieved in CWs, i.e., an average of 12.79.8.10%, which may be attributed to (i) less adsorption capacity of filter material (siliceous gravels) used in CWs and (ii) less phosphorous uptake as a nutrient and adsorption in roots by *Canna indica* vegetation, as it was not well flourished. However, the application of CWs prior to the Ca-Alg-AD bed prevented the destruction of beads by suspended particles in actual municipal effluent. In Fig. 3a, it can also be observed that phosphate removal was affected by phosphate concentration in solution, i.e., high phosphate removal was observed when phosphate concentration was high and vice versa.

Even in continuous mode, the Freundlich isotherm proved to be the best suit for phosphate adsorption on Ca-Alg-AD based on batch isotherm results.

In previous studies, several adsorbents have been examined for their phosphate and ammonium removal efficiency such as iron scraps with main phosphorous adsorption by FeOOH (iron oxide), shale as a substrate for simultaneous ammonium and phosphate removal by horizontal subsurface flow CWs, ceramsite and magnetite as substrates for phosphorous treatment of swine wastewater and various other substrates (5). Despite the fact that these studies replaced the CWs substrate with the adsorbent rather than using the adsorbent as a tertiary treatment removal process, this could increase the complexity of the system due to the fact that plants also follow complex mechanisms and processes. In addition, after adsorbent saturation, it will be difficult to remove all adsorbent from the CW system alongside plants. In addition, while opting for adsorption as a tertiary treatment process, the independent effect could be visualized and a greater understanding of mechanisms and removal efficiency could be developed. Encapsulated AD beads have an advantage over raw AD powder because the former will not clog the CW system, neither when used as a substrate replacement in a CW column nor in a distinct tertiary column, whereas the latter could be conducive to clogging.

Ammonium removal was also examined via the CW-Ca-Alg-AD column depicted in Figure-3b. CW typically eliminates ammonium through nitrification, plant assimilation, and adsorption onto substrates. The nitrification process occurs under aerobic conditions, which in the present study can be supplemented by (i) atmospheric oxygen diffusion and (ii) root exudates of planted vegetation, which can release oxygen into the CW column. In the CW column, ammonium removal averaged 9.10 8.9%, indicating low nitrification along with low nutrient assimilation by *Canna indica* vegetation and negligible adsorption over the siliceous gravel substrate. This could be due to the low adsorption capacity of the filter material (siliceous gravels) used in CWs, the low uptake by the *Canna indica* vegetation because it was not

fully flourished, and the low dissolved oxygen availability in the CW column as a result of both atmospheric diffusion and root exudates from non-flourished vegetation, thereby inhibiting nitrification. Further, in Fig. 3b, the average ammonium removal in the Ca-Alg-AD bead-based fixed bed system was observed to be 29.32% 1.69 % after 30 days of operation, which can be ascribed to, firstly, ammonium stripping from the column due to a pH greater than 8. Observed a comparable proportion (i.e., 27.4%) of ammonium depletion at a pH of 8.5. Another reason could be the high concentration of exchangeable Mg^{2+} and exchangeable Na in the Ca-Alg-AD bead, which can replace the ammonium in the effluent via ion exchange. In addition, the chemical oxygen demand (COD) in the influent was determined to be 40 20 mg/L, the majority of which was removed in the CWs column; therefore, the data is not displayed.

In addition, the Ca-Alg-AD bead-based fixed bed system was regenerated with 1 M NaOH to determine its potential. The study on the regenerated CW-Ca-Alg-AD beads-based fixed bed system was titled regenerated CW-Ca-Alg-AD beads-based fixed bed system. In which the CW column remained unchanged during the regeneration investigation. As shown in Fig. 3c and d, the removal of phosphate and ammonium is greater in the CW than in the 1st cycle: 51.42 25.64% and 30.92 15.74%, respectively. This may be due to (i) uptake of nutrients by the fully-grown flowering *Canna indica* (18), (ii) adsorption of phosphate and ammonium in the newly grown and dense roots of *Canna indica*, and (iii) bacterial conversion of ammonium (nitrification) by nitrifying biofilm development in CWs due to sufficient dissolved oxygen supplied by root exudates of *Canna indica* vegetation (14). Due to the fact that plants store phosphorus primarily in their roots rather than their foliage [379,380], phosphorus could be returned to the CW column. To avoid these situations, CW effluent is treated with a Ca-Alg-AD bead-based fixed bed column that has been regenerated.

As shown in Fig. 3c, after the regeneration of a Ca-Alg-AD bead-based fixed bed system, initial phosphate removal was 55% and decreased to 4% after 35 days of operation. This decline in removal

also appears to be dependent on the concentration of phosphate in the influent, as readily observed in Fig. 3c. Since the initial days of column operation received a high influent phosphate concentration, 6.33 mg/L, the influent phosphate concentration decreased to as low as 0.46 mg/L by the end of column operation. Thus, the higher the phosphate concentration in the influent, the greater the phosphate removal, and the lower the phosphate concentration in the influent, the lower the phosphate removal.

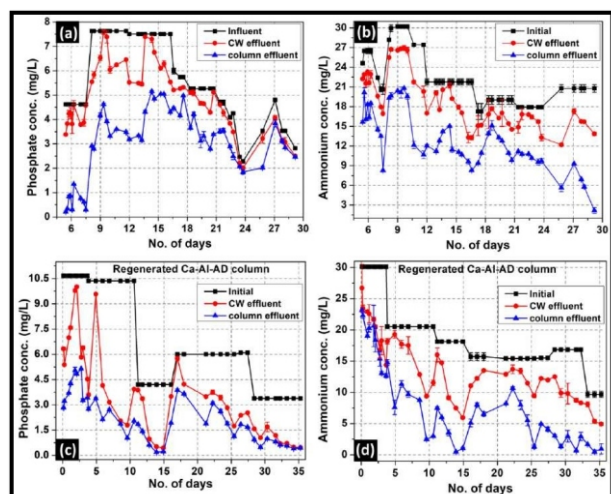
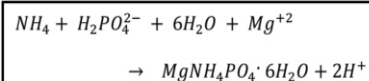


Fig. 3. Illustrates (a) and (b) phosphate and ammonium removal with CW followed by Ca-Alg-AD column respectively, (c) and (d) phosphate and ammonium removal with CW followed by regenerated Ca-Alg-AD column respectively.

Therefore, a continuous column's phosphate elimination decreased significantly following regeneration. Nevertheless, batch study results revealed a high adsorption capacity after regeneration, indicating that column regeneration is less effective than batch regeneration. Accordingly, it can be deduced that a batch-type regeneration process consisting of evacuating the column and then regenerating it could be utilized. In contrast, ammonium removal in the regenerated Ca-Alg-AD column increased from 4.23 to 93.44% over time (Figure -3d). This can be explained by the results achieved from the ammonium experiment carried out to comprehend the simultaneous removal of ammonium with phosphate with Ca-Alg-AD beads. Ca-Alg-AD nanoparticles are only ineffective at removing ammonium from ammonium-containing solutions,

according to the results. In contrast, the mixture of ammonium and phosphate in Ca-Alg-AD beads removed both ammonium and phosphate with final concentrations of 4.54 mg/L and 0.139 mg/L, respectively, from an initial concentration of 13 mg/L ammonium and 10 mg/L phosphate after 70 minutes of contact time. Thus, simultaneous ammonium removal can be attributed to the ion exchange mechanism of ammonium with Ca-Alg-AD beads cations (Mg^{2+} and Na^{+}) and this will ultimately increase the concentration of Mg^{2+} cation in the solution, which can form struvite with ammonium and phosphate ion present in the solution, as shown in Eq. Similar outcomes while investigating the simultaneous removal of phosphate and ammonium from eutrophic fluids using natural calcium-rich attapulgite.



CW followed by Ca-Alg-AD beads based fixed bed system and CW followed by regenerated Ca-Alg-AD beads based fixed bed system have demonstrated effective functioning and application for simultaneous phosphate and ammonium removal from actual municipal wastewater. Therefore, Ca-Alg-AD bead-based fixed beds can be used in the actual world for tertiary wastewater treatment.

Ca-Alg-AD based FWS CWs for remediation and up-gradation of eutrophic lentic water bodies

Ca-Alg-AD bead-based FWS CWs were investigated to determine their potential for remediation and up-gradation of eutrophic lentic water bodies such as lakes, ponds, etc. The hydraulic retention time (HRT) was maintained at 6 days for the Ca-Alg-AD bead-based FWS CW system. The arrangement of Ca-Alg-AD beads-based FWS CW system for phosphate and ammonium removal is depicted in Figure 7-1b. Ca-Alg-AD based FWS CWs have removed an average of 33.75 11.43, 61.082 13.71, and 79.18 8.56 percent of phosphate over the course of 15 cycles of continuous experimentation. In contrast, ammonium removal on the second, fourth, and sixth days was 26 9.92%, 45.45 20.19%, and 65.45 21.01%, respectively.

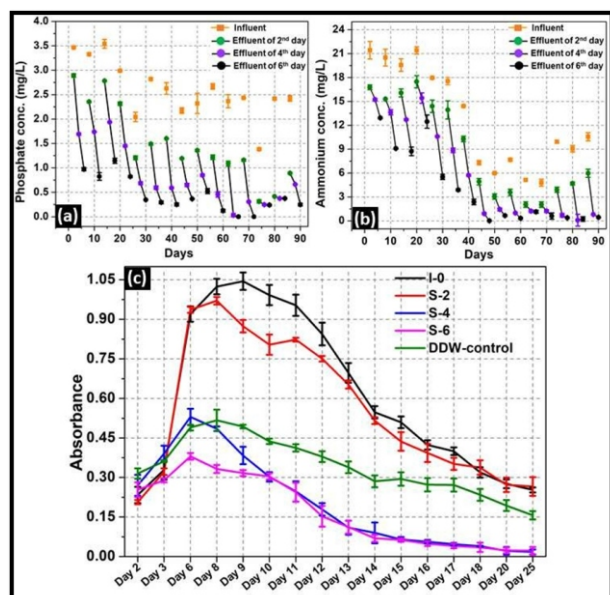


Fig. 4. Illustrates (a) phosphate removal (b) ammonium removal in innovative Ca- Alg-AD beads based FWS CW system and (c) algal growth study, where I-0 shows initial and S-2, S-4, and S-6 denote 2nd, 4th, and 6th day treated samples.

The highest increase in ammonium and phosphate removal can be observed on the second day, indicating that Ca-Alg-AD beads are actively interacting with fresh real effluent due to the presence of a large number of adsorption sites and a high solution concentration gradient. However, the availability of adsorption sites decreases over time. The results indicate that removal of ammonium and phosphate increases as HRT increases. In addition, the removal of nutrients increases over time, as shown in Figure-4a and b. The observed results may be attributable to the presence of vegetation, which may have played an important role in nutrient removal via (i) adsorption by the densely grown roots of *Canna indica* (9), (ii) nutrient assimilation via roots, and (iii) nutrient loss due to evapotranspiration (10). In addition, very low ammonium and phosphate concentrations can be observed in the influent; however, high ammonium and phosphate removal efficiencies can still be observed, suggesting that Ca-Alg-AD bead is a suitable adsorbent for eutrophic waters with relatively low concentrations of pollutants. Additionally, the chemical oxygen demand was not prioritized due to the minimal COD concentration in the influent.

In addition, an experiment was conducted to

determine the ability of Ca-Alg-AD bead-based FWS CW treated samples to inhibit algal growth. The results are depicted in Figure 7-9c, which demonstrates that algal cells can sustain transient growth of algae in DDW for the first few days (up to the eighth day), after which the growth begins to decline. It may be due to nutrient discharge from decomposing matter. These results demonstrate that S-4 and S-6 can inhibit algal growth by removing nutrients to levels of N/P4 and N/P2.5%, respectively. The high N/P ratios of 10 and 8.5, respectively, are corroborated by the high growth of I-0 and S-2 [2,3,8,3]. Ca-Alg-AD beads-based FWS CW is able to halt algal growth, it can be concluded. Consequently, Ca-Alg-AD beads based FWS CW are prospective for the remediation and up-grading of eutrophic lentic water bodies such as lakes, ponds, etc.

Conclusion

Ca-Alg-AD beads have demonstrated their capability for phosphate removal from synthetic solutions and simultaneous phosphate and ammonium removal from real wastewater in batch and column experiments. Both surface adsorption and intraparticle diffusion were found to be rate-limiting processes in the complex phosphate adsorption on Ca-Alg-AD beads. Yoon and Nelson's model is the best fit for describing the dynamic behavior based on the breakthrough curve obtained through continuous packed bed column experiments at a fixed flow rate. The plausible mechanism for phosphate removal was deduced based on FTIR and SEM-EDX results to be mononuclear monodentate inner sphere complexation, electrostatic attraction, and ion exchange with Ca-Alg-AD beads. In contrast, ammonium removal may result from the formation of struvite and ion exchange between interlayer cations in Ca-Alg-AD beads and ammonium. Ca-Alg-AD as adsorbent was benefited in terms of (i) selective affinity towards phosphate, (ii) functioning in a wide pH range (2-9), (iii) efficient adsorption capacity and removal even at low phosphate concentrations, (iv) easy separation/recovery from the treated aqueous environment without using any chemical or additional energy input (v) efficient contact with phosphate pollutant because of beads' even

distribution in water (vii) encapsulated in biopolymer, which is an eco-friendly material and can be directly reused as fertilizer; (vi) effective phosphate recovery for five consecutive adsorption-desorption cycles; and (viii) phosphate recovery success for five consecutive adsorption-desorption cycles. Despite this, it was determined that the bulk method of material regeneration was more effective than regeneration in a continuous column. Simultaneous phosphate and ammonium removal from actual municipal wastewater treatment has been demonstrated to function and be applicable on a pilot-scale basis. In addition, the treated water of the Ca-Alg- AD beads-based FWS CW system, namely S-4 and S-6, was found to be incapable of algal growth due to a lack of nutrients and was therefore deemed safe for discharge into rivers. This ensures that the FWS CW assembly is adaptable for use in lake restoration, remediation, and upgrading of eutrophic lentic water bodies.

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GENETIC ANALYSIS OF YIELD AND QUANTITATIVE TRAITS IN COMMON BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

The wheat breeding experiment was conducted at agriculture research farm of Kulbhaskar Ashram P.G. College, Prayagraj (U.P.) during Rabi Seasons of 2020-21 and 2021-22 to study of the Genetic Analysis of Yield and Quantitative Traits in Common Bread Wheat (*Triticum aestivum* L.). 24 parents of wheat; C-306, Unnat Halna, PBW-226, PBW-502, UP-2382, PBW-550, DBW-16, DBW-17, WH-542, WH-711, HD-2687, WCW-95-3, WCW-95-2, WCW-98-41, PBW-175, UP-2425, Raj-3765, HD-2329, PBW-590 and UP-2338 were used as line while WH-1021, PBW-343, Halna and PBW-373 were used as tester. All the parents and their eighty (80) F₁s, obtained by crossing in line x tester were evaluated during Rabi Seasons of 2020-21 and 2021-22 for grain yield per plant and related traits which were analyzed in randomized blocks design with 3 replications.. On the basis of overall performance parents PBW175, PBW373, UP2425, UP2338 and PBW502 were found good general combiner for yield per plant and gluten content with some other characters also which may improve yield as well as chapatti quality. Cross combinations PBW226xPBW373, PBW502xPBW343, WH542xhalna were common for grain yield per plant and gluten content which can improve chapatti quality with grain yield.

Key words: Common bread wheat (*Triticum aestivum* L.), Line x Tester, General combining ability and Specific combining ability

Introduction

In wheat yield levels have been improved substantially through intensive and concerted breeding efforts hence further yield advances seem more difficult necessitating the application of newer breeding approaches. An effect prevailing in the breeding material is necessary to decide the kind of breeding procedure to be chosen for better exploitation of the genetic potential of different plant traits in a crop. An understanding of the genetic factors that govern the plant traits is necessary because breeding for yield depends largely upon genetic manipulation of the components along with yield. These characters are polygenic and exhibit additive and non-additive

genetic variation. Parents with significant GCA effects are useful for exploitation through conventional crossing programme and those with significant SCA effect are suitable for hybrid variety development. The concept of combining ability is considered to be a landmark in the development of efficient and effective breeding strategy in different crop plants. The knowledge of combining ability together with *per se* performance of parents, hybrids and heterotic response helps the breeder in selecting suitable parents and crosses for their use in systematic breeding programme (7). By resorting to this technique, the breeders come out with the best general combining parents and specific cross combination for future exploitation.

Materials and Methods

C-306, Unnat Halna, PBW-226, PBW-502, UP-2382, PBW-550, DBW-16, DBW-17, WH-542, WH-711, HD-2687, WCW-95-3, WCW-95-2, WCW-98-41, PBW-175, UP-2425, Raj-3765, HD-2329, PBW-590 and UP-2338 were used as line while WH-1021, PBW-343, Halna and PBW-373 were used as tester to produce eighty crosses in a line x tester fashion design. Seeds of parents and F1 plants were sown in two rows with spacing 25 cm between rows and 15 cm between plants according to the randomized blocks design with 3 replications during rabi seasons of 2020-2021 and 2021-22 at agriculture research farm, Kulbhaskar Ashram P.G. College, Prayagraj (U.P.). Fifteen competitive plants in parents and F1's were sampled randomly. Observations were recorded on fifteen randomly selected plants from each plot for characters like, plant height, length of spike, number of tillers per plant, total number of spikelets per spike, biological yield per plant, harvest index, number of grain per spike, 1000 grain weight, dry gluten content and grain yield per plant while days to 50% flowering and days to maturity were recorded on plot basis. The analysis of variance for combining ability was carried out according to method outlined by (3).

Results and Discussion

Analysis of variance for combining ability (Table 1) revealed highly significant differences among parents as well as their progenies for most of the important traits. Present study has revealed the presence of mainly non-additive genetic variance with respect to all the characters. The mean degree of dominance $(\hat{\sigma}^2_s/\hat{\sigma}^2_g)^{0.5}$ was found greater than unity for all the traits indicating the involvement of over dominance. In earlier studies, role of non-additive genetic variance were reported by (6) and (4) for yield per plant and other traits.

The concept of combining ability is considered to be a landmark in the development of efficient and effective breeding strategy in different crop plants. The knowledge of combining ability together with *per se* performance of parents, hybrids and heterotic response helps the breeder in selecting suitable parents and crosses for their use in systematic breeding programme (7). By

resorting to this technique, the breeders come out with the best general combining parents and specific cross combination for future exploitation.

General Combining Ability Effects

For days to 50% Flowering, parents PBW-590, WH-1021, PBW-550, C-306 and UP-2425 were identified as a good general combiner while PBW-502, UP-2425, PBW-343, DBW-17 and HD-2687 were identified as best parents on the basis of *per se performance*. For days to maturity, parents WCW-95-3, PBW-343, HD-2687, WCW-98-41, and PBW-175 were identified as a good general combiner while parents PBW-542, UP-2425, PBW-175, Raj-3765, and PBW-373 were identified as best parents on the basis of *per se performance*. For plant heights, parents HD-2687, UP-2382, WH-711, UP-2338, DBW-16 were identified as a good general combiner while parents UP-2382, HD-2687, WH-711, WH-1021, PBW-590 were identified as best parents on the basis of *per se performance*. For length of spike, parents WCW-95-2, PBW-373 were identified as a good general combiner while parents UP-2338, WCW-98-41, UP-2425, DBW-17, and PBW-343 were identified as best parents on the basis of *per se performance*. For tillers per plant, parents HD-2329, PBW-373 were identified as a good general combiner while parents PBW-343, WCW-98-41, DBW-17, UP-2425, and WH-1021 were identified as best parents on the basis of *per se performance*. For spikelets per spike, parents HD-2329, DBW-16, WCW-95-2, PBW-590, PBW-373 were identified as a good general combiner while parents UP-2338, UP-2425, PBW-343, DBW-17, WH-1021 were identified as best parents on the basis of *per se performance*. For biological yield per Plant, parents Unnat Halna, PBW-502, HD-2687, PBW-226, WCW-98-41 were identified as a good general combiner while parents Halna, C-306, Raj-3765, Unnat Halna, UP-2425 were identified as best parents on the basis of *per se performance*. For harvest index, parents UP-2382, DBW-16, HD-2687, WH-711, Raj-3765 were identified as a good general combiner while parents WH-1021, PBW-343, UP-2338, DBW-16, UP-2382 were identified as best parents on the basis of *per se performance*. For

grain number per spike, parents Unnat Halna, C-306, HD-2329 were identified as a good general combiner while parents UP-2338, PBW-590, PBW-343, UP-2425, WH-1021 were identified as best parents on the basis of *per se* performance. For 1000 grain weight, parents C-306, WH-542, PBW-502, WCW-95-3, Raj-3765 were identified as a good general combiner while parents HD-2687, PBW-550, PBW-175, WH-542, DBW-17 were identified as best parents on the basis of *per se* performance. For gluten content, parents PBW-175, PBW-373, UP-2425, UP-2338, PBW-502 were identified as a good general combiner while parents PBW-373, DBW-16, Raj-3765, HD-2687, WCW-95-3 were identified as best parents on the basis of *per se* performance. For grain yield per plant, parents HD-2329, WCW-95-2, DBW-16, UP-2382, Raj-3765 were identified as a good general combiner while parents UP-2338, PBW-343, WH-1021, DBW-17, UP-2425 were identified as best parents on the basis of *per se* performance.

A close examination of the Table reveals that the line Line C-306, DBW-16, HD-2329 combines well for the highest number of characters (six each) followed by HD-2687 with five characters, PBW502, WCW-95-2 and PBW-590 with four characters each and the lines Unnat Halna, WH-542, WH711, WCW-98-41, PBW175 and UP2425 with three characters each, PBW226, UP2382, WCW-95-3, Raj-3765 and UP-2338 with two characters each while line DBW-17 was good general combiner for none of the characters. Tester PBW 373 combines well for the highest number of characters (eight) followed by Halna (three), PBW-343 (three) and WH-1021 (two).

The estimates of gca effects of the parental lines, including lines and testers, for different characters revealed that none of the parental lines excelled in gca effects either for all the characters studied or at least for all the important yield components studied. This suggested the use of multiple parent participation through multiple crossing to affect substantial improvement in yield

and its components. These results were closely confirms with the findings of (6, 2, 4 and 8).

Specific Combining Ability Effects

Specific combining ability represents the dominance and epistatic type of gene action and it can be related with heterosis. If crosses showing high sca effects involve parents which are also good general combiner, these crosses could be exploited by simple methods like pedigree selection. In the present investigation, none of the crosses expressed good specific combining ability for all the traits under study. Out of 80 crosses combinations, nine best crosses were chosen on the basis of sca effects, *per se* performance and gca effects. Unnat Halna x WH1021 was found good for tillers per plant, biological yield per plant, grain number per spike, 1000 grain weight and grain yield per plant. PBW-226xPBW-373 was found good for harvest index, 1000 grain weight and grain yield per plant. PBW-502xPBW-343 was found good for spikelets per spike, biological yield per plant, grain number per spike, gluten content and grain yield per plant. DBW-17xPBW-343 was found good for spikelets per spike, biological yield per plant, grain number per spike and grain yield per plant. WH-542xHalna was found good for days to maturity, tillers per plant. Biological yield per plant, grain number per spike, gluten content and grain yield per plant. WH-711xPBW-343 was found good for tillers per plant, grain number per spike, 1000 grain weight and grain yield per plant. WCW-95-3xHalna was found good for tillers per plant, spikelets per spike, biological yield per plant, grain number per spike and grain yield per plant. Raj-3765xWH-1021 was found well for length of spike, tillers per plant, spikelets per spike, biological yield per plant, harvest index, grain number per spike, 1000 grain weight and grain yield per plant. UP-2338xWH-1021 was found good for days to 50% flowering, spikelets per spike and biological yield per plant.

Out of 80 cross combination, on the basis of sca effects and *per se* performance five crosses viz; Unnat Halna x WH-1021, PBW-502xPBW-343, WH-542xHalna, WCW-95-3xHalna, Raj-3765xWH-1021 were found superior. On the other hand, Unnat Halna x WH1021 was common for grain yield per plant. Crosses PBW-226xPBW-373, PBW-502xPBW-343, WH-542xhalna were common for gluten content and grain yield per plant which can improve chapatti quality with grain yield. These cross combination may be expected to be utilized in heterosis breeding programme and developing genotypes having broad genetic base by multiple crossing programme. Similar findings were reported by (5 and 1).

Conclusion

On the basis of above findings it is concluded that grain yield and its components were mainly controlled by both additive and dominance components with predominance of later therefore heterosis breeding will be suitable for the utilization of the present material. Parents C306, DBW16, HD2329 and HD2687 and crosses Unnat Halna x WH-1021, PBW-502xPBW-343, WH-542xHalna, WCW-95-3xHalna and Raj-3765xWH-1021 were found superior in the present study therefore these parents should be utilized in breeding programme along with superior crosses.

Table 1 Analysis of variance for combining ability for twelve characters in common bread wheat (*Triticum aestivum* L.) (Mean data of 2 years)

Source of variation	DF	Days to 50% Flowering	Days to maturity	Plant heights	Length of spike	Tillers per plant	Spikelets per spike	Biological yield per plant	Harvest index	Grain number per spike	1000 grain weight	Gluten content	Grain yield per plant
GCA (Line)	19	26.7333	28.7532	386.9845**	0.6980	0.4549	16.1472	230.7319**	236.0914**	11.7697	41.0287	0.6360	9.9753
GCA (Tester)	3	212.611**	164.9042**	186.6491	2.10**	1.668**	15.0340	147.6700	235.5598	4.8183	7.88682	6.1512*	9.4888
SCA (Line x Tester)	57	38.9502**	24.3223**	155.3714**	0.6380*	0.513**	13.8694**	95.6833**	99.6992**	17.0395**	36.9557**	0.81512**	11.2834**
Error	158	1.2496	0.7334	6.6527	0.4315	0.1299	1.9446	1.0170	12.0453	2.78861	0.3258	0.02249	2.4219

Table 2 Estimates of components of variance, degree of dominance, additive and dominance component and heritability (narrow sense) for 12 characters in common bread wheat (*Triticum aestivum* L.) (Mean data of 2 years)

Components	Days to 50% Flowering	Days to maturity	Plant heights	Length of spike	Tillers per plant	Spikelets per spike	Biological yield per plant	Harvest index	Grain number per spike	1000 grain weight	Gluten content	Grain yield per plant
σ^2_g (males)	2.101	2.328	31.754	0.025	0.029	1.194	19.15	18.384	0.58	3.36	0.051	0.599
σ^2_g (females)	3.518	2.735	3.012	0.028	0.026	0.22	2.44	3.668	0.00	0.12	0.102	0.112
σ^2_g (pooled)	3.282	2.667	7.802	0.028	0.026	0.382	5.22	6.12	0.097	0.66	0.094	0.193
σ^2_s (f x m)	12.475	7.833	49.811	0.08	0.134	4.015	31.58	28.07	4.081	12.1	0.264	2.833
σ^2_A	6.564	5.334	15.604	0.056	0.053	0.765	10.459	12.241	0.194	1.322	0.187	0.386
σ^2_D	12.475	7.833	49.811	0.080	0.134	4.015	31.583	28.07	4.081	12.10	0.264	2.833
σ^2_A/σ^2_D	0.526	0.681	0.313	0.699	0.393	0.190	0.331	0.436	0.048	0.10	0.708	0.136
$(\sigma^2_D/\sigma^2_A)^{0.5}$	1.379	1.212	1.787	1.196	1.595	2.291	1.738	1.514	4.583	3.02	1.188	2.709
h^2_n	33.57	39.68	23.15	20.75	23.57	14.19	24.69	26.91	3.30	9.69	40.80	9.30

Table 3 Ranking of desirable parent on the basis *per se* performance and gca effect for different characters in common bread wheat (*Triticum aestivum* L.). (Mean data of 2 years)

S.No.	Character	Best parent <i>per se</i> performance in desirable direction	Best general combiner based on significant gca effect in desirable direction
1	Days to 50% Flowering	PBW-502, UP-2425, PBW-343, DBW-17, HD-2687	PBW-590, WH-1021, PBW-550, C-306, UP-2425
2	Days to maturity	PBW-542, UP-2425, PBW-175, Raj-3765, PBW-373	WCW-95-3, PBW-343, HD-2687, WCW-98-41, PBW-175
3	Plant heights	UP-2382, HD-2687, WH-711, WH-1021, PBW-590	HD-2687, UP-2382, WH-711, UP-2338, DBW-16,
4	Length of spike	UP-2338, WCW-98-41, UP-2425, DBW-17, PBW-343	WCW-95-2, PBW-373
5	Tillers per plant	PBW-343, WCW-98-41, DBW-17, UP-2425, WH-1021	HD-2329, PBW-373
6	Spikelets per spike	UP-2338, UP-2425, PBW-343, DBW-17, WH-1021	HD-2329, DBW-16, WCW-95-2, PBW-590, PBW-373
7	Biological yield per Plant	Halna, C-306, Raj-3765, Unnat Halna, UP-2425	Unnat Halna, PBW-502, HD-2687, PBW-226, WCW-98-41,
8	Harvest index	WH-1021, PBW-343, UP-2338, DBW-16, UP-2382	UP-2382, DBW-16, HD-2687, WH-711, Raj-3765
9	Grain number per spike	UP-2338, PBW-590, PBW-343, UP-2425, WH-1021	Unnat Halna, C-306, HD-2329
10	1000 grain weight	HD-2687, PBW-550, PBW-175, WH-542, DBW-17	C-306, WH-542, PBW-502, WCW-95-3, Raj-3765
11	Gluten content	PBW-373, DBW-16, Raj-3765, HD-2687, WCW-95-3	PBW-175, PBW-373, UP-2425, UP-2338, PBW-502
12	Grain yield per plant	UP-2338, PBW-343, WH-1021, DBW-17, UP-2425	HD-2329, WCW-95-2, DBW-16, UP-2382, Raj-3765

Table 4. Best cross combination on the basis of sca effect and *per se* performance for yield and other related traits in common bread wheat (*Triticum aestivum* L.) (Mean data of 2 years)

S.No.	Crosses	sca value (Grain yield)	Per Se Peformance (Grain yield)	gca of parent		For other character
				P1	P2	
1	Unnat Halna x WH1021	4.948**	29.533	0.379	-0.489*	Tillers per plant, Biological yield per plant, Grain number per spike, 1000 grain weight, Grain yield per plant, Phenol colour reaction.
2	PBW226 x PBW373	2.395*	26.773	-0.744	0.426*	Harvest index, 1000 grain weight, Grain yield per plant.
3	PBW502 x PBW343	2.909**	26.993	-0.483	-0.129	Spikelets per spike, Biological yield per plant, Grain number per spike, Gluten content, Grain yield per plant.
4	DBW17 x PBW343	3.277**	26.033	-1.811*	-0.129	Spikelets per spike, Biological yield per plant, Grain number per spike, Grain yield per plant.
5	WH542 x Halna	2.247*	27.827	0.691	0.191	Days to maturity, Tillers per plant. Biological yield per plant, Grain number per spike, Gluten content, Grain yield per plant.
6	WH711 x PBW343	1.946*	25.913	-0.599	-0.129	Tillers per plant, Grain number per spike, 1000 grain weight, Grain yield per plant.
7	WCW-95-3 x Halna	2.767**	28.16	0.504	0.193	Tillers per plant, Spikelets per spike, Biological yield per plant, Grain number per spike, Grain yield per plant.
8	Raj3765 x WH1021	4.233**	29.207	0.767	-0.489*	Length of spike, Tillers per plant, Spikelets per spike, Biological yield per plant, Harvest index, Grain number per spike, 1000 Grain weight, Grain yield per plant.
9	UP2338 x WH1021	2.689	25.4	-1.496*	-0.489*	Days to 50% flowering, Spikelets per spike, Biological yield per plant, Phenol colour reaction.

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A CRITICAL STUDY ON REMEDIATION OF FLUORIDE CONTAMINATED WATER USING ENCAPSULATED ACTIVE GROWING BLUE-GREEN ALGAE (*PHORMIDIUM SP.*)

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Abstract

Fluoride concentrations in potable water that exceed the prescribed limit are always problematic. Encapsulating blue-green algae, *Phormidium sp.* in live, active form permits the formation of a novel type of bio-sorbent that perpetually generates new active biosorption sites due to the blue-green algae's growth within the calcium alginate beads. In addition, encapsulation facilitates the removal of biosorbent from treated water. The experiment was carried out with modulation of parameters such as biosorbent dose (3- 6 gm), initial fluoride concentration [F⁻] (2-5 mg/L), and pH (3.5- 4.5) and then further optimized employing response surface methodology (RSM). In addition, a biosorption study reveals that an initial [F⁻] of 3 mg/L removes 60% of fluoride. In addition, the Freundlich model was the best-fitting isotherm model for the biosorption study. In addition, first-order kinetics was observed throughout the entire investigation, and it was determined that both intra-particle diffusion and surface biosorption contributed to the rate-determining step. In addition, characterization techniques such as scanning electron microscopy (SEM)-Energy dispersive X-ray (EDX) and Fourier transform infrared spectroscopy (FTIR) confirmed the role of algal biomass in fluoride removal.

Key Words: Remediation, Fluoride, Contaminated Water, Encapsulated, Blue-Green Algae.

Introduction

Fluoride-contaminated potable water negatively impacts the health of millions of people. According to WHO (1), the standard fluoride concentration [F⁻] in drinking water range between 0.5 and 1.5 mg/L. A fluoride concentration in potable water of > 1.5 mg/L or even 0.5 mg/L can cause health problems. Although fluoride is naturally present in groundwater, an increase in its concentration above 1.5 mg/L in river bodies as a result of natural processes such as volcanic eruption and mining or industrial discharge can be harmful to human health (2). Ingestion of greater than 1.5 mg/L of fluoride can cause mottling of teeth, and higher concentrations, 3-6 mg/L, can cause skeletal fluorosis. In addition, more than

twenty industrialized and developing countries have fluoride concentrations above the WHO-recommended level (3). Include competitive nations such as India, the United States, China, Argentina, Mexico, the United Kingdom, Norway, and South Africa [4]. Several physicochemical processes for defluoridation of potable water, such as membrane filtration, electrolysis, precipitation, ion exchange, and biosorption, have been proposed and implemented over time (5). Some of these processes, such as ion exchange, membrane filtration, and electrolysis, have operational limitations and disadvantages and require expensive maintenance and operation. Additionally, coagulation and precipitation processes generate secondary toxic sediment that

requires additional treatment [6]. However, over time, adsorption has become the most practical and effective method for defluoridation due to its low initial start-up costs, straightforward design strategy, minimal sludge production, operational flexibility, and ease of maintenance (7). The fundamental criteria for selecting any adsorbent for fluoride removal applications are reusability, regeneration viability, operational simplicity, and high adsorption capacity (8). Clay (9), bone charcoal (10), brick powder (11), activated alumina (12), coconut shell carbon (13), tamarind seeds (14), rice husk (15), calcite (13), fly ash (16), alum sludge (17), natural zeolites (18) and countless other inexpensive sorbents have been extensively reviewed for their fluoride removal efficiency. Nonetheless, biosorbents such as algal and fungal detritus are gaining prominence due to their wide availability and cost-effectiveness (19). *Spirogyra* IO2 (20), *Anabaena fertilissima* (21), *Spirogyra* IO1 (3), *Chlorococcum humicola*, *Pleurotus ostreatus* 1804 (22), *Chlorella vulgaris* (24), and *Pleurotus eryngii* ATCC 90888 (5) are reportedly used for fluoride removal. However, the limitations of the aforementioned biosorbents stem from their preparation from inactive, nonliving biomasses of species.

There is negligible research associated with algal biomass as a defluoridation agent (6). Therefore, the current investigation is focused on utilizing immobilized algal biomass for fluoride removal. The fundamental concept behind the encapsulation of blue-green algae was the feasibility of on-site treatment with ease in the segregation of algal biomass from the water after biosorption. Therefore, blue-green algae of the genus *Phormidium* were chosen for this study (7) because they are abundantly available in aquatic environments. The study employs optimally grown encapsulated algae prior to bloom formation in order to prevent the inherent danger of cytotoxin release (8). Furthermore, no human illness has been linked to the cyanotoxins produced by blue-green algae *phormidium* sp., rendering it safe for use in the treatment of drinking water (9). In the event that contaminants are released, the system can also be used for non-drinking water purposes, such as reducing the fluoride concentration in irrigation water (for agricultural purposes) or other applications. In addition, the

abundance of cyanobacteria in nature heightened the importance of the present research. Consequently, we investigated fluoride removal in batch studies with varying contact time, algal dose, and pH using response surface methodology (RSM) and optimization of the aforementioned factors.

Materials and Methods

Preparation of stock solution

A 100 mg/L fluoride stock solution was made by dissolving 221 mg of sodium fluoride -NaF (Merck Pvt. Ltd., AR grade) in 1 liter of milliQ water. Using Milli-Q (18.2 M.Ω·cm) water, 2 mg/L, 3 mg/L, 4 mg/L, and 10 mg/L of fluoride were obtained through serial dilutions of a 100 mg/L fluoride stock solution. Due to the fact that the greatest reported groundwater [F⁻] concentration was 5.0 mg/L, the highest fluoride concentration in this study was deemed to be 10 mg/L (300).

Preparation of live immobilized algal beads

Biomass of the blue-green algae *Phormidium* sp. was cultured and enriched for approximately one year (at 25(2) °C and 85 W visible light source) in BG-11 medium with a pH of 6.8. BG-11 medium was having composition as MgSO₄ · 7H₂O, 0.075 g; Na₂CO₃, 0.02 g; (NH₄) [Fe₅(C₆H₄O₇)₂], 0.006 g; EDTA, 0.001 g; C₆H₈O₇, 0.006 g; K₂HPO₄ · 4 · 3H₂O, 0.04 g; NaNO₃, 0.6 g; CaCl₂ · 2H₂O, 0.036 g and trace metal mix 1mL (297). In addition, flasks were aerated and maintained on an orbital shaker for continuous shaking (Spinix Orbital shaker MC-02, Tarson) for optimal algal growth. Subsequently, a sodium alginate solution of 2% w/v (i.e., 6 g of sodium alginate per 300 mL of Milli-Q water) was prepared by agitating constantly at 50 °C until a homogenous solution was obtained. Once the homogeneous solution was obtained, it was cooled down to room temperature followed by the addition of algal culture (i.e., moist wt.; 3.5% w/v). Following this, the algal biomass was thoroughly rinsed five times with milli-Q water and excess water was removed prior to weighing. Thus, a CaCl₂ solution of 0.2 M (AR grade, Fisher Scientific Pvt Ltd.) was formulated for further immobilization. CaCl₂ solution was used to entrap algal biomass in sodium alginate polymer and 4°C was maintained overnight for

bead hardening. The following day, the formed beads were kept in BG-11 medium for an additional three months to promote algal growth.

In addition, the prepared beads were observed to be of uniform dimension (i.e., 2.7–3.0 mm in diameter) and shape, each containing approximately 4 mg of entrapped algal biomass. Since the experiment was conducted in a laboratory, two LED bulbs (85 W, Spiral, Crompton Greaves Ltd.) were affixed to provide blue-green algae, phormidium sp. with sufficient light for healthy growth. In addition, calcium alginate particles were used as an experimental control (i.e., without algal entrapment).

Table- 1. Represents Box–Behnken design matrix for three variables with coded value of variables and observed response (predicted and experimental).

Run	A : pH	B: Initial F Concentration (mg/L)	C: Beads concentration (gm)	Final F Concentration (mg/L)/actual Value	Predicted Value
1	-1	-1	0	1.56	1.35
2	1	1	0	3.38	3.36
3	0	1	-1	2.95	3.12
4	0	1	1	2.25	2.52
5	0	0	0	2.22	1.93
6	1	0	-1	1.67	1.74
7	1	0	1	2.29	2.26
8	0	-1	-1	.8140	.8729
9	0	-1	1	1.06	1.22
10	1	-1	0	.7420	.7410
11	-1	0	-1	1.97	2.25
12	0	0	0	2.26	1.93
13	0	0	0	2.05	1.93
14	0	0	0	1.49	1.93
15	-1	1	0	2.69	2.38
16	0	0	0	2.18	1.93
17	-1	0	1	1.29	1.47

Biosorption studies

The prepared biosorbent (around 3-month-old algal beads) was extracted from the BG- 11 medium and rinsed several times with milli-Q water. After complete surface water loss, the biomass of algal beads was weighed and then added to 12.5 mL of different concentrations of fluoride solutions in a 30 mL round-bottomed tube according to the Box–Behnken design of RSM modeling. 24 hours were spent using an orbital shaker (Spinix Orbital shaker MC-02, Tarson) to stir round-bottomed containers at 60 rpm and 25 °C at room temperature. After filtering the

samples with Whatman filter paper (Grade 1), the remaining concentration of fluoride in the vials was determined. The fluoride concentration was analyzed at 570 nm using the SPADNS procedure, as specified in the APHA protocol.

Bio-sorbent characterization

Utilizing a PerkinElmer Frontier-94609 spectrophotometer, Fourier transform infrared (FTIR) spectroscopy was used to characterize algal particles (before and after treatment) and the control. SEM-EDX (model- EVO 18, Zeiss) was used to analyze surface morphology and elemental composition. Initially, samples were desiccated at 50 °C for two hours, then coated with gold using a sputtering procedure.

Experimental design and data analysis

In the present study, RSM, a multivariable technique, was used to optimize the parameters (3, 4). For the biosorption investigation, the pH, biosorbent dose (gm of beads), and initial [F⁻] were studied and varied within the ranges 3.5–5.5, 3–6 g, and 2–5 mg/L, respectively. According to Table 6-1, a total of 17 experiments were conducted based on the Box–Behnken design matrix.

In addition, regression, graphical, and statistical analysis was performed on the experimental response using Stat-Ease, design Expert Version 11 software, United States. In RSM, the two most common designs are central composite and Box–Behnken (BBD) design. Box–Behnken design, a well-known method for optimization studies, was implemented for this research. In addition, this method is suitable for investigating the interaction of multiple parameters with a minimum number of experimental runs.

Results and Discussion

Effect of different parameters on fluoride removal RSM was used to investigate the effect of initial [F⁻], biosorbent dose (alginate beads encapsulating live and active algal biomass), and pH on fluoride removal, and the results are depicted by 3D and contour plots (Fig. 1a, b, and c). With increasing biosorbent dose and pH modulation, a decrease in the final [F⁻] was observed, as depicted in Fig. 1. The 3D diagram depicted in Figure 1a (drawn with an initial [F⁻] of

3.5 mg/L; a biosorbent dosage of 3.6 g; and a pH of 5.4) illustrates the reduction in $[F^-]$ to 1.85 mg/L. However, when pH is decreased to 3.5 and the biosorbent dosage is increased to 5.99 g, while maintaining the same initial $[F^-]$, the final $[F^-]$ is reduced to 1.46 mg/L (a 21% increase in fluoride removal). The rationale for increased fluoride removal with increasing biosorbent dose may be dependent on the availability of additional unoccupied binding sites on the biosorbent surface, which exhibits a positive charge at the experimental pH [5]. The saddle point on contour plots denotes the local maxima for two variables; consequently, the optimal values for biosorbent dose and pH parameter can be determined by locating the saddle point or by examining the maxima points.

In the case of a higher initial $[F^-]$, however, the importance of pH was also observed. Fig. 1b depicts the correlation between pH and defluoridation capacity at various initial $[F^-]$. The graphs illustrate that at pH 5.0 and an initial $[F^-]$ of 2.0 mg/L, the maximum fluoride removal, i.e. 50 %, was attained.

However, simply lowering the pH value by 0.5 (i.e., from 5 to 4.5) and simultaneously increasing the initial $[F^-]$ to 3.5 mg/L maintains a removal rate of 41.34% (i.e., a decrease in $[F^-]$ value to 2.05 mg/L), which is consistent with the RSM model values.

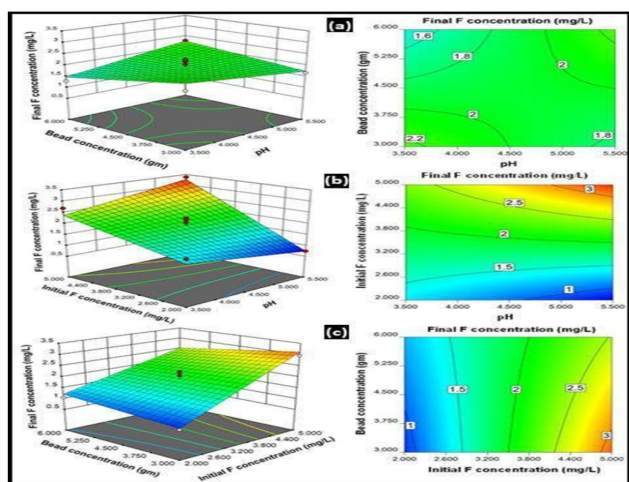


Fig. 1. Shows the impact of multiple parameters on final $[F^-]$, wherein (a) bead dosage and pH; (b) changing initial $[F^-]$ and pH; (c) initial $[F^-]$ and biosorbent dosage.

Defluoridation by means of *Pleurotus ostreatus* 1804; a waste fungus, Neem charcoal (7), and *Padina sanctae-crucis* algae (8) has been reported to follow a similar pattern. In addition, Fig. 1c demonstrates that as the biosorbent dose is increased, the final $[F^-]$ decreases, indicating an increase in fluoride removal efficacy. This could be due to an increase in the number of biosorption sites as a consequence of a higher biosorbent dose. Moreover, through contour plot, maximal fluoride removal of 70.48% can be observed at an initial $[F^-]$ of 3.5 mg/L with 4.5 gm of biosorbent amount.

Statistical Analysis

Two statistical analyses were conducted to determine the defluoridation capability of live encapsulated algal biomass. Tests were conducted to optimize the model for fluoride biosorption using a sum of squares sequential model and model summary statistics (9). the results obtained from both statistical analyses. Except for the cubic model, which was determined to be aliased by nature, the 2-factor interaction (2FI) model discloses the maximal R^2 and adjusted R^2 value. Consequently, 2FI was appointed to conduct additional analysis. Moreover, R^2 and adjusted R^2 values for defluoridation via algal biomass were found to be close to 1, indicating a close relationship between model predicted values and experimental values (10,11).

Removal kinetics - effect of time, initial concentration, and pH variation

Initial $[F^-]$ concentrations of 2, 3, 4, 5, and 10 mg/L were varied throughout the kinetic experiment. As the contact time increases, the defluoridation capacity increases, as shown in Fig. 6-2a. In addition, the defluoridation efficiency for an initial $[F^-]$ of 2–3 mg/L was 50–60% after 90 hours of contact. Nonetheless, when the initial $[F^-]$ was increased from 3 mg/L to 10 mg/L, the efficiency decreased to 35%–45% with equal contact time. Similarly, results for defluoridation using neem charcoal have been reported [304]. The proliferation of active sites may be one of the factors contributing to the decline in removal efficiency (2).

Various kinetic models, such as the first, second-order kinetic model, and the intra-particle diffusion model (12,13), were used to examine biosorption kinetic data in greater detail.

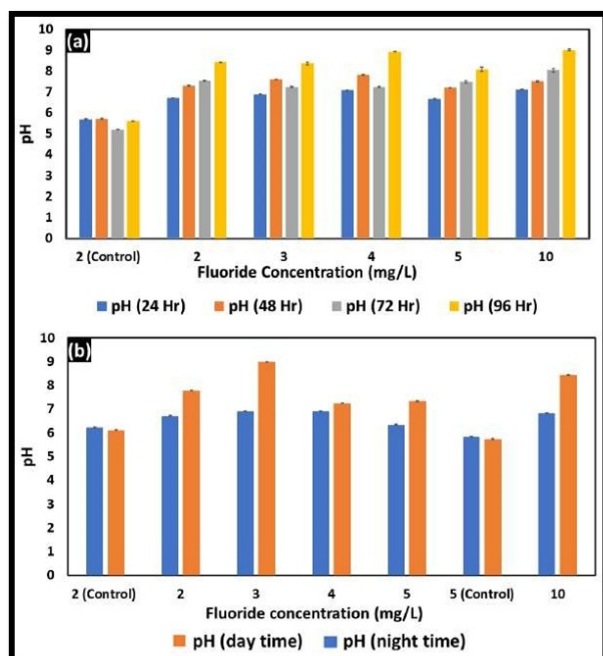


Fig. 2. Depicts (a) pH variations with initial $[F^-]$ of 2-10 mg/l, wherein 2 and 5 mg/l were also considered for control experiment at distinct time intervals, and (b) pH variations observed in daylight and night time.

SEM-EDX and FTIR Analysis

As shown in Figure 3, the SEM-EDX depicts the morphology and surface texture of the biosorbent, as well as its elemental composition. In SEM images, a thread-like structure is clearly visible after algal entrapment (Fig. 3b), and in beads with fluoride adsorption, Cyanobacterium, Phormidium Sp. growth is pronounced (Fig. 3c). Similar SEM morphology changes were reported when adsorption studies were conducted over the cyanobacterium Phormidium parhydematicum (7). Peaks on the EDX graph indicated that nitrogen, carbon, oxygen, and phosphorus (N, C, O, P) are the most abundant elements in algal biomass. N and P maxima were not observed in the control due to the absence of blue-green algae entrapment. Gao et al. (8) documented a similar conclusion. In addition, the presence of Ca and Na peaks may be attributable to the presence of

sodium alginate and calcium chloride in the particle composition. On the fluoride-adsorbed algal bead, fluoride concentrations of approximately 1.47 percent by atomic weight and 1.94 percent by weight were detected, confirming fluoride biosorption over the biosorbent surface.

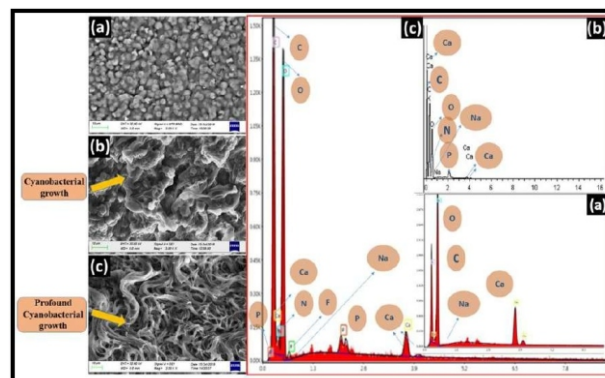


Fig. 3. SEM-EDX micrograph represents the morphology of surface, wherein (a) control beads (without algal entrapment); (b) untreated entrapped algal beads; (c) fluoride adsorbed algal beads.

In addition, an FTIR spectrum was acquired to identify the functional groups responsible for fluoride removal on the encapsulated algal beads. For various functional groups, the obtained spectra disclose peak shifts with increases and decreases in transmittance between 400 cm^{-1} and 4000 cm^{-1} . This spectrum confirmed the presence of functional groups such as alkenes, hydroxyl, alkanes, and amines (Fig.4) on the surface of the encapsulated algal bead, indicating the presence of abundant fluoride biosorption sites. In addition, the presence of an intense peak between 3500 cm^{-1} and 2900 cm^{-1} in Fig. 4 indicates that the -OH and $-\text{NH}_2$ stretching peaks overlap. In addition, the peaks at 2850 cm^{-1} and 2923 cm^{-1} confirm the presence of $(-\text{CH}_2)$ aliphatic groups. Furthermore, the 1035 cm^{-1} band indicated the C-O stretching vibration of carboxylic acids and alcohols (3). Peaks at 1520 cm^{-1} and 1462 cm^{-1} indicate N-H bending within the amine group. After biosorption of fluoride, $-\text{NH}_2$ peak shifting was observed, with peaks at 1520 cm^{-1} shifting to 1499 cm^{-1} and 1462 cm^{-1} shifting to 1426 cm^{-1} . The shifting of the peak could be an indication of interaction between F^- ions and the encapsulated algal biomass (2,3).

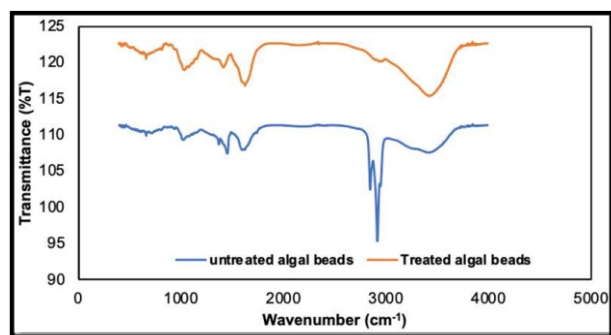


Fig. 4. Illustrates FTIR spectra of fluoride treated and untreated algal beads

Conclusion and Environmental Implications

This study is the first of its kind to estimate the biosorption capacity of fluoride through immobilized live blue-green algal beads *Phormidium* sp., which have been shown to be effective biosorbents for defluorisation. As indicated by RSM, the 2FI (2- factor interaction) model was able to interpret the experimental results clearly in this study. As a result of the best fit of the Freundlich isotherm in the biosorption experiment, chemisorption was inferred to be the primary removal mechanism for fluoride. In addition, a kinetics investigation demonstrated that 60% fluoride removal efficiency could be obtained with an initial $[F^-]$ of 3 mg/L, a contact time of 90 h, a pH of 4.5, and an adsorbent dose of 4.5 g. The biosorption of fluoride followed a first-order kinetic model, but the entire mechanism was found to be complex. In the rate-determining step, both surface biosorption and intraparticle diffusion modes contributed. Box–Behnken appears to be an appropriate model for optimizing the experimental parameters for defluoridation via biosorption technique, based on recent research. In addition, SEM-EDX and FTIR analyses confirmed the biosorption of fluoride on the biosorbent surface. This research demonstrates that the encapsulated algal bead could be used as a cost-effective and environmentally benign material for defluoridation of drinking water. In spite of the fact that the present research establishes a proof of concept, additional research is necessary to evaluate the efficiency of biosorbent in real ground wastewater and the influence of co-existing ions on the fluoride removal process, which could lay the

groundwork for pilot scale implementation of the present technology. Nevertheless, we achieved very promising results as a starting point for further promising implementation in the direction of low-cost defluoridation.

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INFLUENCE OF INTEGRATED NUTRIENT MANAGEMENT ON GROWTH, YIELD AND QUALITY OF AONLA CV. NA-7

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Abstract

An experiment was carried out in the Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, (U.P.), India during 2022, to study the influence of integrated nutrient management on growth, yield and quality of Aonla cv. NA-7, using nine treatments viz., T₁: 100% RDF (Control), T₂: 75% RDF + 2kg Vermicompost, T₃: 75% RDF + 4kg Vermicompost, T₄: 75% RDF + 2kg Vermicompost + 100g *Azospirillum*, T₅: 75% RDF + 4kg Vermicompost + 100g *Azospirillum*, T₆: 75% RDF + 2kg Vermicompost + 100g *PSB*, T₇: 75% RDF + 4kg Vermicompost + 100g *PSB*, T₈: 75% RDF + 2kg Vermicompost + 100g *Azospirillum* + 100g *PSB*, T₉: 75% RDF + 4kg Vermicompost + 100g *Azospirillum* + 100g *PSB* which were replicated thrice in Randomized Block Design (RBD). Observations on plant morphological characters and yield attributes were recorded at maturity of twenty-one years old aonla trees. Maximum fruit set (40.25%), fruit retention (32.61%), fruit yield (160.72kg/per plant) with maximum fruit length (3.95cm), fruit width (4.80cm), fruit weight (48.21g), fruit volume (44.99cc), pulp weight (46.26g) along with minimum fruit drop (67.39%), seed weight (1.95g). As far as the quality characters are concerned the highest TSS (12.91°Brix), total sugars (6.29%), ascorbic acid (559.27mg/100g pulp), TSS: acid ratio (9.28) with minimum titratable acidity (1.39%) were found with application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₉). These observations suggested that for getting better fruiting, substantial higher yield with qualitative fruits, the plants of aonla should be treated with an integrated dose of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* in the plains of Northern India.

Key Rords: Aonla, Integrated Nutrient Management, Vermicompost, *Azospirillum*, *PSB*, Growth, Yield, Quality.

Introduction

Aonla (*Phyllanthus emblica* L.) a member of family Euphorbiaceae, is one of the most important minor fruit crop in our country. This fruit is highly nutritious and is the richest source of vitamin-C (400-1300mg 100-1 g fruit pulp), is also known as Indian gooseberry, Malacca tree, Amritphal, Scared tree and sometime called Myrobalan tree. It is one of the important indigenous fruits of the Indian subcontinent which is native to the subtropical region of South East Asia, particularly central and southern India.

Aonla is also naturally found in other tropical countries of the world viz., Ceylon, Cuba, Puerto Rico, Hawaii, Florida, Iran, Iraq, Java, West Indies, Trinidad, Pakistan, Malaya, and China. In India, aonla fruit is spreading very fast in the semi-arid regions of Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Karnataka, Tamil Nadu, the Arawali hills in Haryana, the Kandi area in Punjab, and Himachal Pradesh. The area under aonla cultivation in the state of Uttar Pradesh, particularly in Pratapgarh, Raebareli, Varanasi, Jaunpur, Sultanpur, Kanpur Nagar, Etawah, Agra, and Mathura districts of Uttar

Pradesh is increasing day by day due to its prospects under wasteland circumstances, particularly on salt-affected soils. Aonla appears in the Vedas, Ramayana, Charak Samhita and other classical Indian works that mentioned valuable its fruits are as food, medicine, and hair color. The aonla is prized for its high nutritional content, therapeutic characteristics, processing into the variety of value-added products like; preserves, pickles, candy, and herbal medicines. The fruit are also used as a blood purifier, antioxidant, laxative, liver tonic, and antibiotic and very effective remedy for diarrhea, jaundice, cough, cold, asthma, diabetes, and chronic dysentery. It is an essential ingredient in the ayurvedic remedies of Triphala, Chavanprash, and others (6).

Soil type, fertility and nutrient management play important roles in obtaining higher growth and yields of aonla. Now a days, using various chemical fertilizers results regularly harms the soil, pollutes the land and degrades the quality of the fruits, to solve these issues, the implementation of integrated nutrient management techniques is the best way in the present scenario. By using these techniques, we can maximize resource efficiency and increase crop production. Due to poor physical properties of soil, it becomes very hard during dry season and crops suffer due to deficiency of major plant nutrients. With the discovery of an aerobic N-fixer clostridium and nonsymbiotic free living aerobic organism *Azospirillum* and PSB it was made possible to sustain productivity of different non-legumes under normal pressure of oxygen. Vermicompost is being commercialized as potting mixture for different fruit plants and for raising rootstocks for grafting and budding. Vermicompost provides essential nutrients to the plants and allows plants to quickly absorb nutrients. The growth characters were significantly higher in plants amended with enriched vermicompost systematic studies have been made so far to determine the requirement of manures and fertilizers in an integrated manner for aonla in the state of Uttar Pradesh. Hence, the present investigation was undertaken to study the influence of different organic and biofertilizer combination with chemical fertilizers for better fruiting and higher return of aonla fruit in the

plains of Northern India.

Materials and Methods

A field trial was conducted during February 2022 December 2022 on twenty-one-year-old aonla trees in the Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur- 208002 (U.P.). The trial was laid out in Randomized Block Design with nine treatments viz., T₁: 100% RDF (Control), T₂: 75% RDF + 2kg Vermicompost, T₃: 75% RDF + 4kg Vermicompost, T₄: 75% RDF + 2kg Vermicompost + 100g *Azospirillum*, T₅: 75% RDF + 4kg Vermicompost + 100g *Azospirillum*, T₆: 75% RDF + 2kg Vermicompost + 100g PSB, T₇: 75% RDF + 4kg Vermicompost + 100g PSB, T₈: 75% RDF + 2kg Vermicompost + 100g *Azospirillum* + 100g PSB, T₉: 75% RDF + 4kg Vermicompost + 100g *Azospirillum* + 100g PSB) and each treatment was replicated thrice. Observations on fruiting characters viz., fruit set, fruit retention, fruit drop, and yield attributes such as fruit weight, fruit length, fruit width, fruit volume, seed weight and biochemical attributes i.e., TSS, total sugars, ascorbic acid, and titratable acidity were recorded at harvest maturity as per methods suggested in (1). The experimental data were analysed statistically with analysis of variance method given by (11).

Results and Discussion

Fruit set (%): The maximum fruits set (40.25%) was recorded under T₉ (75% NPK + 4kg Vermicompost + 100g *Azospirillum* + 100g PSB) followed by T₈ (75% RDF + 2kg Vermicompost + 100g *Azospirillum* + 100g PSB), Whereas, the minimum fruit set (24.13%) was recorded under control (T₁) treatment (Table 1). This increased fruit set during present experimentation period might be due to improvement in the emergence of a greater number of female flowers per shoot and reduced fruit drop resulting in higher number of fruits per shoot. These findings agree with (19, 21) in strawberry, (20) in aonla; (15) in papaya.

Fruit Drop (%): In the present investigation, the minimum fruit drop (67.39%) was recorded when the application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g PSB (T₉) followed by T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g PSB). While, the

maximum fruit drop (83.42%) was recorded under T₁ (control) treatment, which received the 100% RDF of NPK (Table 1). The reduction of fruit drop might be associated with the higher photosynthesis, translocation of photosynthates and plant growth promoter particularly auxin. These findings are agreed with (20 and 22) in strawberry.

Fruit Retention (%): The maximum fruit retention (32.59%) was recorded with T₉ which plants treated with 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*, followed by T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*), whereas, the minimum fruit retention (16.56%) was recorded under (T₁) control i.e., 100% RDF of NPK (Table 1). The combination of NPK, vermicompost, and bio-fertilizers (*Azospirillum* and *PSB*) are very useful in supplying proper nutrition which might result in achieving higher fruit retention during present experimentation period. The supply of nutrients and their proportion right from starting of the experiment to the harvesting of the crop induced more flowering and retention of fruit due to production and supply of photosynthates at critical requirements. These findings agree with (4) in strawberry, (20) in aonla.

Fruit yield (kg): The maximum fruit yield (160.72kg/plant) was recorded with application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₉) closely followed by T₈ (75% NPK + 2kg vermicompost + 100% *Azospirillum* + 100g *PSB*), whereas, the minimum fruit yield (132.44kg/tree) was recorded under control (T₁) treatment (Table 1). The yield was better in the treatment combination of NPK, vermicompost and bio-fertilizers, which may probably be due to better growth and development of the plants and vermicompost provides more C/N ratio and more essential nutrients found which required for better metabolic activities in the plant and increase protein and carbohydrate synthesis and finally increased the fruit yield. The present findings are in conformity with the earlier work by (4); (2) in strawberry and (25 and 21) in strawberry.

Fruit length (cm): In the present investigation the fruit length, significantly affected by different integrated dose of NPK,

vermicompost and biofertilizer (*Azospirillum* and *PSB*). The maximum fruit length (3.95cm) was recorded with the application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₉), followed by treatment T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*) and lowest fruit length was recorded under (T₁) control (Table 1). The maximum fruit length gain is due to greater mobility of the metabolites into the developing fruits, which acted as strong metabolic sink as compared to the treatments involving higher dose of NPK, vermicompost and bio-fertilizers (*Azospirillum* and *PSB*). These findings were agreed with (8) in strawberry, (5) in ber.

Fruit width (cm): The maximum fruit width (4.80 cm) was recorded with T₉ treatment which was plants treated with application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* followed by (75% RDF + 2kg Vermicompost + 100g *Azospirillum* + 100g *PSB*) (T₈), whereas, the minimum fruit width (4.15 cm) was recorded under control (T₁) treatment (Table 1). Nitrogen leads to increased fruit width with the nitrogen increased the efficiency of metabolic process of the plants and encouraged the growth of the plant and consequently increases the fruit size and fruit weight. The integration of organic and biofertilizers sources of nutrients greater mobility of nutrients to the developing fruits which act as strong metabolic sink. Similar, results were also obtained by (9) and (22) in the banana, (13) in guava; (3) in acid lime.

Fruit weight (g): The maximum fruit weight (48.21g) was found with T₉ (75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*) followed by treatment T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*), while, lowest fruit weight was recorded under control (T₁) treatment (Table 1). The maximum fruit weight gain was due to greater mobility of the metabolites into the developing fruits, which act as strong metabolic sink as compared to the treatments involving higher dose of NPK, vermicompost and bio-fertilizers. There was increase in average fruit weight due to the integration of organic and biofertilizers sources of nutrients which accelerated mobility of photosynthesise from source to sink as influenced

by the growth hormones. These finding was agreed with (4) in strawberry and (3) in acid lime.

Fruit volume (cc): The maximum fruit volume (4.80cc) was recorded under T₉ (75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*) followed by application of 75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₈), whereas, the minimum fruit volume (4.15cc) was recorded under T₁ (control) treatment (Table 1). The increasing of fruit volume might be due to improved fertilizer use efficiency with application of NPK, vermicompost, and bio-fertilizers to help in increase fruit volume and it is highly correlated with dry matter content and balanced level of hormones. These finding was agreed (16) in aonla; (17) in phalsa.

Pulp weight (g): The highest pulp weight (46.26g) was found under T₉ treatment which was plants treated with 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* followed by T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*) was recorded under T₁ (control) treatment (Table 2). Nitrogen increased the efficiency of metabolic process of the plants and increased the growth of the plant and consequently increased the fruit size and fruit weight than increasing pulp weight. These finding was agreed with (18) in aonla (9) and (22) in the banana.

Seed weight (g): The minimum seed weight (1.95g) was recorded under T₉ treatment, plants which received the doses of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* followed by T₈ (75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*), whereas, the maximum seed weight was recorded under T₁ (control) treatment, which plants received the doses of 100% RDF of NPK (Table 2). This might be due to the maximum gain fruit size and fruit weight, these effect on the minimum seed weight. The similar results were also reported by (12) in sapota; (5) in ber.

Total Soluble Solids (°Brix): The TSS (12.91°Brix) was recorded highest under T₉ (75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*) followed by application of 75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₈), whereas, the minimum T.S.S. (11.13°Brix) was recorded under T₁ (control)

treatment, which plants received the 100% RDF of NPK (Table 2). The TSS contents with application of *Azospirillum* and *PSB* may be due to the quick metabolic transformation of starch and pectin into soluble compounds and rapid translocation of sugars from leaves to the developing fruits. The vermicompost play important role of the plant nutrient and plant hormones, which are directly or indirectly involved in enhancing fruit quality, these results also corroborate the findings of (18) in aonla, (8), (7) in strawberry.

Titrateable acidity (%): Based on present investigation, the minimum titrateable acidity (1.39%) was recorded under treatment T₉ (75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*) followed by 75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₈), while, maximum acidity (2.10%) was recorded under treatment (T₁) control (Table 2). The titrateable acidity decreases due to the increasing of total soluble solids and total sugars. The results reported by (13) in phalsa; (1) in acid lime are in agreement with the present findings.

Total sugars (%): The maximum sugars (6.29%) were recorded with T₉ (75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB*) followed by application of 75% RDF + 2kg vermicompost + 100g *Azospirillum* + 100g (T₈), whereas, minimum sugars (5.48%) were recorded under (T₁) control (Table 2). This might be due to the application of balanced dose of NPK, vermicompost along with bio-fertilizers. The Nitrogen content has various energy sources like; amino acids and amino sugars which increase the sugar level in fruit. The similar results were also reported by (9) and (22) in the banana, (12) in sapota; (13) in phalsa, (7) in strawberry.

Ascorbic acid (mg/100g of pulp): Significantly influenced of ascorbic acid with the application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* (T₉) gave highest ascorbic acid content (559.27 mg/100g of pulp) followed by T₈ (75% NPK + 2kg vermicompost + 100g *Azospirillum* + 100g *PSB*), while, the lowest ascorbic acid content (530.67mg/100g of pulp) under control (Table 2). The possible reasons for increased ascorbic acid may be due to the conversion of soluble sugars into ascorbic acid. The increased level of NPK, vermicompost and

biofertilizers are induced the functioning of number of enzymes in the physiological process, vermicompost and biofertilizers supply major and some essential micronutrient and proper supply of nutrients to stimulates the photosynthesis, carbohydrate and sugar metabolisms which improve the quality and ascorbic acid in fruit. Similar trends were also observed by (14) in phalsa; (27) in mango.

Conclusions

Based on the findings of present study, we can conclude that application of NPK, vermicompost and biofertilizers on aonla fruits resulted in significant improvement of fruiting and yield behaviour, which ultimately leads to biochemical parameters of aonla. The application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* is more effective compared to control and remaining other treatments. It appears from the investigation that application of 75% NPK + 4kg vermicompost + 100g *Azospirillum* + 100g *PSB* per tree is beneficial for increased fruiting as well as improving yield and yield attributing characters and biochemical characters as improving the quality of Aonla cv. NA-7 in the plains of Northern India.

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EFFECT OF VARIOUS ORGANIC SOURCES OF NUTRIENTS ON GROWTH, YIELD AND QUALITY OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

A field experiment was conducted during winter seasons of 2020-21 and 2021-22 at the Research Farm, RVSKVV College of Agriculture, Gwalior (M.P.) to study the effect of organic sources of nutrients on growth, yield and quality of wheat. The treatment receiving VC 5 t/ha + Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10t/ha resulted in maximum growth parameters (plant height 88.77 cm, leaves 60.60 /plant, grains 40.22 /earhead, length of earhead 11.91 cm, weight of earhead 2.40 g and 1000-seed weight 41.05 g), grain yield (41.82 q/ha), straw yield (61.70 q/ha), harvest index (40.40 %), starch content (73.33 %) and protein content (14.22 %). The second best treatment was F_{10} = VC 5 t/ha + Panchagavya 15 lit./ha + FYM 10 t/ha, closely followed by Jeewamrit 500 lit./ha + VC 5 t/ha + FYM 10 t/ha and then Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10 t/ha. All the parameters were decreased significantly under treatments receiving single or two organic sources of nutrients.

Key words : Organic sources, nutrients, wheat and quality

Introduction

Wheat (*Triticum aestivum* L.) is the most important food grain crop having 9-10% protein and 60-80% carbohydrates. Wheat is a heavy nutrient feeder and leads to large withdrawal of plant nutrients from soil. The soil properties and health is decreasing day by day due to non-addition of organic manures upto desirable extent. The indiscriminate use of inorganic fertilizers is believed to cause deterioration of soil texture, structure, hindrance of microbial activities, ground water pollution and finally decreased soil fertility and production. On the other hand, the use of organic manures, vermicompost biofertilizers and recently available growth promoters like Panchagavya, Jeewamrit improve soil texture, structure, humus, colour, aeration, water-holding capacity, microbial activities, nutrient use efficiency and thereby increase production and reduce environmental

hazards (3, 7). Soil organic matter is generally one of the most important criteria of soil properties. The indicators of soil quality can be categorized into four general groups; visual, physical, chemical and biological properties that can be measured by monitoring changes in the soil. Biological indicators include measurement of macro and microorganisms and their activities. Some parameters of biological indicators for screening of soil quality are soil organic carbon, dehydrogenase activity, microbial biomass, carbon and nitrogen (15).

Panchagavya and Jeevamrit are the plant growth-promoting substances containing beneficial microorganisms which promote growth and yield of crops. Microorganisms are well activated in the soil by the addition of both these liquid products. The microorganisms such as nitrogen fixer *Azotobacter*, *Azospirillum* and phosphorus-solubilizing bacteria. *Pseudomonas*

fluorescence and potash-solubilizing bacteria like *Bacillus silicus* are present in the cow dung that is used to prepare Jeevamrit (4). These organic solutions are the recent inclusion in the integrated nutrient management system. The information on their effect on the productivity and quality parameters of wheat is lacking for the gird region of M.P., hence the present research was taken up.

Materials and Methods

The field experiment was conducted during *rabi* seasons of 2020-21 and 2021-22 at the Research Farm, RVSKVV College of Agriculture, Gwalior (M.P.). The soil of the experimental field was clay loam having pH 7.3 to 7.4, electrical conductivity 0.32 to 0.37 dS m⁻¹, organic carbon 4.8 to 5.4 g kg⁻¹, available-N 196 to 219 kg ha⁻¹, available-P₂O₅, 16.0 to 18.5 kg ha⁻¹ and available-K₂O 254 to 274 kg ha⁻¹. The rainfall received during the winter months was 161.2 and 98.6 mm in both the years. The 12 treatments comprised of F₁ = control, F₂ = FYM 10 t/ha, F₃ = Jeewamrit 500 lit./ha, F₄ = Vermicompost 5 t/ha, F₅ = Panchagavya 15 lit./ha, F₆ = Jeewamrit 500 lit./ha + FYM 10 t/ha, F₇ = VC 5 t/ha + FYM 10 t/ha, F₈ = Panchagavya 15 lit./ha + FYM 10 t/ha, F₉ = Jeewamrit 500 lit./ha + VC 5 t/ha + FYM 10 t/ha, F₁₀ = VC 5 t/ha + Panchagavya 15 lit./ha + FYM 10 t/ha, F₁₁ = Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10 t/ha, and F₁₂ = VC 5 t/ha + Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10t/ha. The experiment was laid out in randomized block design with three replications. Wheat var. HI-1544 was sown between 5th to 12th November in both the years @100 kg seed ha⁻¹ in rows 22.5 cm apart. An uniform dose of N₁₂₀ P₆₀ K₄₀ was applied through urea, single superphosphate and muriate of potash, respectively in all the treatments. Before sowing, the seeds were first treated with thirum fungicide @3 g kg⁻¹ seed. Wheat was grown as per recommended package of practices. The crop was harvested on 14th March, 2021 and 24th March, 2022. The protein content in grain was determined by multiplying the percent N content in grain with 6.25 (A.O.A.C., 1997). Carbohydrate content in seed was estimated by the acid hydrolysis process and titration with Fehling's solution (12).

Results and discussion

The data (Table 1) indicate that the application of four types of organics in combination (F₁₂) resulted in significantly maximum plant height (87.77 cm), leaves 60.6/plant and dry matter accumulation (7.03 g / plant). The highest vegetative growth parameters under F₁₂S treatment may be owing to the fact that the combined input of VC 5 t/ha + Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10t/ha provided maximum availability of multinutrients in addition to the improvement of physico-chemical and biological properties of the soil. The cumulative effect of four organics provided the rich nutrient value with a lot of beneficial soil microorganisms and made soil porous, friable with improved water infiltration and moisture retention. Moreover, vermicompost, FYM, Jeewamrit and panchagavya have got different nutrient contents, variation in decomposition of organic residue, C:N ratio and nutrient release pattern under the existing agro-climatic conditions.

The treatments receiving three types of organics, F₉ (Jeewamrit 500 lit./ha + VC 5 t/ha + FYM 10 t/ha), F₁₀ (VC 5 t/ha + Panchagavya 15 lit./ha + FYM 10 t/ha) and F₁₁ (Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10 t/ha) recorded almost equal vegetative growth parameters which were of course lower than that of F₁₂ treatment receiving four types of organic sources of nutrients. This was but natural because of differences in the addition of multi-nutrient, and multi-advantages. Similar was the case with two organics applied as in F₆, F₇ and F₈ treatments in comparison to three organics applied. The another most interesting finding is that 5 t vermicompost /ha proved more better than 10 t FYM/ha. Similarly, panchagavya @ 15 lit. /ha was found superior than Jeewamrit applied @ 500 lit./ha prepared solution of Panchagavya and Jeewamrit are the plant growth promoting substances containing beneficial microorganisms which promote growth and yield of crop. The present results corroborate with the findings of (6, 11, 16 and 17).

Yield-attributes

The same treatment F_{12} augmented the yield attributing parameters upto maximum extent where the effective tillers were 112.85/m row length, spikes 46.05/plant, grains 40.22 /earhead, length of earhead 11.91/cm, weight of earhead 2.40 g and 1000-seed weight 41.05 g. the second, third and fourth best treatments having applied three types of organics were F_{10} , F_9 and then F_{11} , respectively (Table 1 and 2). The higher yield attributes from F_{12} , F_{10} , F_9 and then F_{11} may be owing to higher increase in dry mater production and its effective partitioning to the economic sink. The increased supply of multi-nutrients and microbial population might have increased multi-role activities in plant and soil which, in turn, resulted in greater accumulation of carbohydrates, protein and their translocation to the reproductive organs (yield-attributes). The results are in consonance with those of (5, 13, 14, 9 and 10).

Productivity and quality

Higher grain yield with good quality is the prime objective of most of the agricultural research. Crop yield is a complex process which is

resultant of partitioning of photosynthates from source to the sink which depends upon various growth and yield-attributes. Vermicompost with FYM along with Jeewamrit and panchagavya exerted cumulative effect upon the maximum productivity of wheat. Accordingly the treatment F_{12} took a lead in this respect where grain was found significantly higher (41.82 q/ha), but straw yield 61.70 q/ha, respectively. This was followed by the treatments receiving three types of organics (F_{10} , F_9 and F_{11}). All these treatments also produced quality grain where the starch and protein contents were 73.23 and 14.22 %, respectively. The response from combined functions of multi-organics in improving starch and protein contents in grain may be attributed to their significant role in regulating the photosynthesis, root enlargement and better microbial activities and more synthesis of starch and protein as a result of associated metabolism (8, 14 and 7). These results are in close conformity with observations made by (2), (17), (16), and (11). The results concluded that the combined applicaiton of Jeewamrit + Panchagavya + vermicompost + FYM proved the most beneficial on the productivity and quality of wheat for gird region of Madhya Pradesh.

Table 1. Growth Parameters and yield attributes of wheat as influenced by organic sources of nutrients.

	Treatments	Plant height (cm)	Leaves/plant	Dry matter/plant (g)	Effective tillers/m row length	Spikes/Plant	Grains/earhead	Length of earhead (cm)
F_1	Control	64.15	36.92	3.32	68.80	33.93	28.28	7.33
F_2	FYM 10 t/ha	71.00	44.23	4.76	91.80	40.05	35.09	9.39
F_3	Jeewamrit 500 lit/ha	69.08	40.96	3.72	83.45	38.16	32.17	9.06
F_4	Vermicompost (VC) 5t/ha	72.60	46.97	5.18	96.45	42.07	36.08	9.41
F_5	Panchagavya 15 lit./ha	74.11	44.58	4.86	89.60	38.28	34.97	9.46
F_6	Jeewamrit 500 lit./ha + FYM 10 t/ha	75.01	48.28	5.56	95.50	40.96	36.3	9.63
F_7	VC 5 t/ha + FYM 10 t/ha	76.45	49.05	5.78	98.60	43.22	36.97	9.91
F_8	Panchagavya 15 lit./ha + FYM 10 t/ha	78.29	48.60	5.70	97.45	42.09	36.29	9.68
F_9	Jeewamrit 500 lit./ha + VC 5 t/ha + FYM 10 t/ha	84.00	56.66	6.45	103.25	44.14	38.11	10.06
F_{10}	VC 5 t/ha + Panchagavya 15 lit./ha + FYM 10 t/ha	85.04	58.76	6.57	108.65	44.89	38.35	10.77
F_{11}	Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10 t/ha	81.33	52.45	6.24	99.65	43.92	37.09	9.82
F_{12}	VC 5 t/ha + Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10t/ha	87.77	60.60	7.03	112.85	46.05	40.22	11.91
S.E.M$\pm$		0.29	0.27	0.15	0.98	0.61	0.85	0.06
CD (P=0.05)		0.87	0.78	0.43	2.83	1.76	2.45	0.17

Table 2.Yield attributes, yield and grain quality of wheat as influenced by organic sources of nutrients

	Treatments	Weight of earhead (g)	1000-seed weight (g)	Seed yield (q/ha)	Straw yield (q/ha)	Harvest index (%)	Starch content (%)	Protein Content (%)
F ₁	Control	1.82	33.62	31.64	50.21	38.94	66.72	12.22
F ₂	FYM 10 t/ha	1.98	37.04	36.72	53.73	40.62	67.73	12.85
F ₃	Jeewamrit 500 lit/ha	1.88	36.26	33.61	52.78	38.92	68.20	13.10
F ₄	Vermicompost (VC) 5t/ha	2.03	37.98	38.06	56.72	40.49	70.53	13.25
F ₅	Panchagavya 15 lit./ha	1.94	36.26	34.83	53.30	39.54	68.00	13.19
F ₆	Jeewamrit 500 lit./ha + FYM 10 t/ha	2.03	37.44	36.69	58.97	38.37	70.35	13.32
F ₇	VC 5 t/ha + FYM 10 t/ha	2.13	37.74	38.86	58.18	40.31	71.52	13.54
F ₈	Panchagavya 15 lit./ha + FYM 10 t/ha	2.08	37.27	37.65	59.43	38.80	71.91	13.41
F ₉	Jeewamrit 500 lit./ha + VC 5 t/ha + FYM 10 t/ha	2.20	39.16	39.04	61.08	39.01	72.53	13.75
F ₁₀	VC 5 t/ha + Panchagavya 15 lit./ha + FYM 10 t/ha	2.33	39.48	39.75	60.13	39.81	72.80	14.03
F ₁₁	Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10 t/ha	2.26	38.55	38.74	61.89	37.27	72.45	13.91
F ₁₂	VC 5 t/ha + Jeewamrit 500 lit./ha + Panchagavya 15 lit./ha + FYM 10t/ha	2.40	41.05	41.82	61.70	40.40	73.23	14.22
S.E.M±		0.06	0.09	0.12	0.14	0.09	0.19	0.10
CD (P=0.05)		0.17	0.26	0.35	0.41	0.27	0.56	0.28

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ESTIMATION OF TOTAL FUNGI COUNT (TFC) IN DAIRY PRODUCT SAMPLES BY STANDARD PLATE COUNT (SPC) TECHNIQUE

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Abstract

The preservation of foodstuffs has been essential for human survival. Without any knowledge of microbiology, the earliest food preservation techniques relied on the inactivation of bacteria and fungi responsible for food deterioration. Typically, food items were preserved from microorganisms through dehydrating, salting, heating, chemical preservatives, or fermentation. In order to prevent the proliferation of microorganisms that cause food to spoil, a variety of food preservation techniques and combinations thereof are employed. Microbial food degradation was characterized by the sensory alteration of food products that renders them unacceptable to the consumer. Food spoilage can be caused by physical damage, chemical changes (oxidation and color changes), or a change in flavors and odors induced by microbial growth and metabolism in the food products. The most prevalent cause of food spoilage is microbial growth in food substance, which manifests as visible growth (slime, colonies), textural changes (polymer degradation), or off-odours and off-flavors.

Key Words: Total Fungi Count (TFC), Dairy Product, Standard Plate Count (SPC) Technique, Microbial Spoilage, Butter, Cheese, Yoghurt, Curd, Raw Milk.

Introduction

Milk is the universal product with the highest commercial demand, regardless of its source, and it contributes essential protein (casein) to the diets of individuals of all ages. Consumers place a premium on the microbiological characteristics and quality of milk and milk products.

Most commonly, the growth and activity of microorganisms that cause food deterioration are described and studied as a function of substrate base and various parameters, such as temperature, pH, water activity, and atmospheric pressure. Food spoilage is one of the most significant issues that the food and dairy industries confronted. In fact, spore-forming and toxin-producing bacteria are primarily responsible for the majority of foodborne maladies, even in developing nations. The microbial proliferation in

a variety of dairy products is primarily responsible for the spoilage of dairy products (1). Many pathogenic microorganisms, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Candida albicans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Salmonella typhi* have been identified as the causing agents of the major food borne diseases, food borne illness and food spoilage (2, 3).

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella typhi* have been reported as some of the most prevalent pathogenic microorganisms in the dairy industry that are associated with food borne diseases, food borne diseases, and food borne

intoxification. This microorganism is a short filament that can grow under aerobic and microaerophilic conditions. The optimal temperature for growth of microorganisms that cause decomposition in dairy products is 35 to 37 °C, but some spoilage-causing organisms can grow at refrigerated temperatures, over a wide pH range of 4 to 9 with salt concentrations up to 10%. Although the dairy industry is most concerned about the presence of *Listeria monocytogenes* in cheese, yoghurt, raw milk, and curd (5,4,6,), *Listeria monocytogenes* has been found in other dairy products as well.

The use of chemical preservatives and organic acids currently employed to control the dairy product spoilage-causing microorganisms that are capable of growing in milk products, such as sulphites, sulphur dioxide, sodium chloride, phosphates, hydrogen peroxide, nitrates, nitrites, sodium diacetate, citric acid, acetic acid, lactic acid, benzoic acid, fumaric acid, and therapeutic antibiotics, is being increasingly questioned. These types of inquiries challenge the food processing and manufacturing industries to concentrate on natural methods of food preservation because food service customers demand high-quality products with a relatively long shelf life, but still prefer the appearance of minimally processed dairy food products (8, 9).

A number of microorganisms belonging to the *Lactobacillus* genus are able to produce biologically active antimicrobial peptides, also known as bacteriocins, which have broad-spectrum activity, a mode of action that achieves the target, the appropriate molecular weight, and the appropriate biochemical properties (10).

Bacteriocins are bacteriostatic or bactericidal to pathogenic bacteria, particularly those that are closely related taxonomically, as well as food spoilage-causing bacteria. Typically, the producer strain is resistant to the bacteriocin it produces (11, 12, 13, 2).

Milk is a well-balanced and nutrient-dense substance that is abundant in proteins, fats, carbohydrates, vitamins, minerals, essential amino acids, etc.

Milk and its derivatives contain an abundance of nutrients, making it an ideal growth medium for

all microorganisms (including bacteria, viruses, fungi, and protozoa).

Raw milk's microbial content is essential for the production of hygienic milk-based consumables.

Milk and its derivatives, including butter, cream, and cheese, are susceptible to microbial deterioration (14).

Materials and Methods

In Meerut district, Uttar Pradesh, India, five distinct varieties of dairy products, including raw milk, curd, cheese, yogurt, and butter, were collected for this study. Raw milk was collected from Daurala, whey from Pallavpuram, cheese from Shastrinagar, yogurt from Sivaya, and butter from Begumpul.

Estimation of Bacterial Population in Dairy Products Using the Standard Plate Count (SPC) Method

Using the Standard plate count (SPC) method, the total bacterial population in collected dairy products (Raw milk, Cure, Cheese, Yoghurt, and Butter) was determined. The gathered dairy products were serially diluted up to a concentration of 10⁻⁶. One milliliter of samples were pipetted into a sterile petri dish containing Nutrient agar. They were thoroughly combined and permitted to solidify. There were three replications, and the uninoculated platters served as a control. After 24 hours of incubation at 28±0.5°C, the colonies of bacteria that appeared on the plates were enumerated using a Qubec colony counter and expressed as 10⁻⁴ cfu/ml.

Evaluation of the Total Coliform Count (TCC)

Using the Total coliform count (TCC) method, the coliform population in collected dairy products (Raw milk, Cure, Cheese, Yoghurt, and Butter) was determined. The gathered dairy products were serially diluted up to a concentration of 10⁻⁶. One ml of samples was pipetted onto a sterile petridish with Coliform agar. They were thoroughly combined and permitted to solidify. There were

three replications, and the uninoculated platters served as a control. After 24 hours of incubation at 28^oC, the coliform colonies that appeared on the plates were enumerated using a Qubec colony counter and expressed as 10⁻³ cfu/ml.

Results and Discussion

Details of Dairy Product Sample Collection Location

In Meerut district, Uttar Pradesh, India, five distinct varieties of dairy products, including raw milk, curd, cheese, yoghurt, and butter, were collected for this study. Raw milk was collected from Daurala, whey from Pallavpuram, cheese from Shastrinagar, yogurt from Sivaya, and butter from Begumpul.

Tabulated in Table – 1 are the locations where samples of dairy products were taken.

Table – 1: Details of location of Dairy product sample collection

Name of the District	Name of the location	Dairy samples
Meerut District, Uttar Pradesh, India	Daurala	Raw milk
	Pallavpuram	Curd
	Shastrinagar	Cheese
	Sivaya	Yoghurt
	Begumpul	Butter

Estimation of Bacterial Population in Dairy Product Samples by Standard Plate Count (SPC) Technique

The bacterial population in five collected samples of dairy products, namely Raw milk, Curd, Cheese, Yoghurt, and Butter, was estimated using the Standard plate count technique (SPC), and the results are presented in Table – 2. Curd sample had the highest bacterial population among the five milk products with 9.4 10⁴ cfu/ml, followed by Cheese (8.6 10⁴ cfu/ml), Raw milk (7.1 10⁴ cfu/ml), and Yoghurt (6.5 10⁴ cfu/ml). Butter had the smallest population (5.3 10⁴ cfu/ml).

Begumpul samples contained the greatest number of microbes, followed by those from Pallavpuram, Daurala, and Sivaya. Shastrinagar has the lowest population density.

Table – 2: Estimation of bacterial population by Standard Plate Count (SPC) technique

S. No	Milk products	Bacterial population estimated by Standard Plate Count (SPC) technique (×10 ⁴ cfu/ml)				
		Chidambaram	Annamalai Nagar	Bhuvanagiri	Sethiyathope	Cuddalore
1	Raw milk	5.4	6.3	4.6	5.2	7.1
2	Curd	7.2	8.5	6.3	6.9	9.4
3	Cheese	6.5	7.8	5.5	6.2	8.6
4	Yoghurt	4.2	5.9	3.8	4.7	6.5
5	Butter	3.6	4.1	2.4	3.5	5.3
S.Ed		0.67	0.76	0.67	0.59	0.73
CD (P = 0.05)		1.35	1.54	1.34	1.19	1.47

Estimated Total Fungi Count (TFC)

The results of estimating the total fungal count (TFC) of dairy product samples (Raw milk, Curd, Cheese, Yoghurt, and Butter) are shown in Table – 4. Among the milk products, the highest total fungal count was found in cheese (5.3 10⁻³ cfu/ml) and yogurt (4.2 10⁻³ cfu/ml) from Annamalai nagar. Butter contained the lowest coliform count (3,6 10³ cfu/ml). Raw milk and curd contain no fungi, according to a study. Annamalai Nagar samples contained the highest number of fungi, followed by Cuddalore, Chidambaram, and Sethiyathope. Bhuvanagiri's coliform population was found to be the lowest.

Table – 4: Estimation of Total Fungal Count (TFC)

S. No	Milk products	Total Fungal Count (TFC) (10 ⁻³ cfu/ml)				
		Chidambaram	Annamalai Nagar	Bhuvanagiri	Sethiyathope	Cuddalore
1	Raw milk	-	-	-	-	-
2	Curd	-	-	-	-	-
3	Cheese	4.6	5.3	3.8	2.8	4.3
4	Yoghurt	3.5	4.2	2.2	1.9	3.7
5	Butter	2.5	3.6	1.7	1.2	2.8
S.Ed		0.60	0.49	0.63	0.46	0.43
CD (P = 0.05)		1.20	0.98	1.27	0.93	0.87

Conclusion

A number of microorganisms belonging to the *Lactobacillus* genus are able to produce biologically active antimicrobial peptides, also known as bacteriocins, which have broad-spectrum activity, a mode of action that achieves the target, the appropriate molecular weight, and the appropriate biochemical properties.

Bacteriocins are bacteriostatic or bactericidal to pathogenic bacteria, particularly those that are closely related taxonomically, as well as food spoilage-causing bacteria. Typically, the producer strain is resistant to the bacteriocin it produces (5, 9, 11, 4, 7).

Pasteurization, commercial sterilization, fermentation, dehydration, refrigeration, and chilling are involved in the dairy processing procedure. This chapter discusses contamination and deterioration of milk and milk products, as well as the interaction of microorganisms with dairy foods. Milk and milk products spoil due to the development of fermentative bacteria when storage temperatures are high enough to support psychrotrophs. Psychrotrophic bacteria's heat-resistant proteinases induce spoilage in processed milk due to enzyme-retaining activity after heat treatment. The most prevalent fermentative deterioration of fluid milk products is lactic acid bacteria (LAB)-caused acidification. There are three categories of concentrated milk products: evaporated milk, sweetened or unheated condensed milk, and concentrated milk. Since these bacteria produce highly active proteolytic and lipolytic enzymes, psychrotrophic bacteria play a pivotal role in cheese deterioration. Some milk microorganisms can be removed by centrifugation, clarification, or separation.

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ESTIMATION OF GENETIC DIVERGENCE IN TOMATO (*SOLANUM LYCOPERSICUM* L.) GENOTYPES

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Abstract

The present investigation conducted on 25 genotypes of tomato (*Solanum lycopersicum* L.) to assess the genetic divergence using Mahalanobis D² analysis during *rabi* season of year 2021-22 and 2022-23. The analysis of variance revealed significant differences among the genotypes for twelve characters under study. The genotypes were grouped into 5 clusters. It was showed that the cluster II comprises of highest 9 genotypes followed by cluster IV comprised 5 genotypes while, lowest genotypes were recorded in cluster I comprised 3 genotypes. The highest mean value was recorded in cluster V for days to 50% flowering, no. of primary branches per plant, fruit length, diameter of fruit, average fruit weight, fruit index, average yield per plant and fruit yield per hectare. Cluster II for plant height, plant spread and cluster I for no. of leaves per plant and number of fruit per plant respectively. The maximum intra-cluster distance was found in cluster V and minimum intra-cluster distance was recorded cluster III. The highest inter-cluster distance was recorded Cluster V with Cluster IV and the minimum inter-cluster D² distance was recorded cluster III with Cluster II. The highest contribution in the manifestation of genetic divergence was exhibited by days to 50% flowering followed by plant height and minimum was found in fruit index.

Keywords: Tomato, cluster, contribution, genetic divergence, yield.

Introduction

The tomato is a member of the Solanaceae family, popularly known as the nightshade family, the genus *Solanum* the Solanoideae subfamily and the Solaneae tribe ch. no. 2n=24. All closely related wild tomato species are indigenous to the Andean area, which includes parts of Chile, Colombia, Ecuador, Bolivia, and Peru (15). The cherry tomato, *L. esculentum* var. *cerasiforme*, which is native to tropical and subtropical America, is the most likely ancestor (14). Although the Peru-Ecuador region was where the tomato's

ancestors first appeared, Mexico appears to have been the first country to extensively domesticate the plant (15; 5).

Tomato is being cultivated an area of 843 thousand ha. with annual production of 20694 thousand Metric tons (2). Madhya Pradesh is the leading tomato producing state with production of around 2760.16 thousand Mt. from an area of 102.37 thousand ha. It is followed by Andhra Pradesh with production of around 2303.79 thousand Mt. from an area of 57.27 thousand ha, (2).

Tomato is a rich source of minerals, vitamins and organic acid, essential amino acids and dietary fibres. Tomato is known as a productive as well as protective food. It is a rich source of vitamins A and C; it also contains minerals like iron, phosphorus. Tomato contains lycopene and beta-carotene pigments. It plays a vital role in maintaining human health (8). A hundred grams of edible parts of tomato contains 0.9g protein, 0.1g fat, 0.7g fibre, 3.5g carbohydrates, 15–20 calorie energy, 500–1500 IU vitamin A, 0.1mg thiamine, 0.02mg riboflavin, 0.6mg niacin, 20–25mg vitamin C, 6–9mg calcium and 0.1–0.3mg iron (16). Tomato is used as a bioreactor in bio-pharming for the production and oral delivery of vaccines. It is available in almost all seasons of the year and in both tropical and subtropical regions (13). The D^2 statistic measure divergence at two levels, namely inter-cluster and intra-cluster levels and thus helps in the selection of genetically divergent parents for exploitation in hybridization programme (9). Therefore, the present study was undertaken to assess the genetic divergence in 25 genotypes of tomato to identify suitable genotypes.

Materials and Methods

The present investigation was conducted during *rabi* season of year 2021-22 and 2022-23 at Horticultural Research Centre (HRC). Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut. The experimental material comprised of 25 diverse genotypes of tomato. Total 25 genotypes were collected from different research institutes viz. IIVR Varanasi Uttar Pradesh, IARI New Delhi and IIHR Bengaluru **Table-1**. The diverse genotypes of tomato were sown in well prepared field with using standard experimental design in Randomized block design (RBD) with three replications. The schedules of various cultural operations were carried out during the course of investigation according to agronomically. All agricultural practices and packaging were adopted to raise a tomato crop healthy. Seed were sown in lines in raised nursery bed having 1 metre width and 3.0-meter length which were 10 cm apart from each other and the depth was maintained at 0.5 cm. Transplanting of young seedlings from nursery to the main field was done after 30 days of sowing with 60 x 75 cm

spacing. The observations were recorded from five randomly selected plants from each genotypes and each replication. The study was recorded twelve observations viz. days of 50% flowering, plant height, number of leaves per plant, number of primary branches per plant, plant spread, fruit length, diameter of fruit, average fruit weight, number of fruits per plant, fruit index, average yield per plant and fruit yield per hectare.

The data were evaluated with OPSTAT programme for assessed different parameters of genetic divergence analysis.

Table-1 List of genotypes

S. No.	Genotype	Source
1	VRst T-67	IIVR, Varanasi
2	ToLEV-32	IIVR, Varanasi
3	H-88-78-1	IIVR, Varanasi
4	Kashi Chayan	IIVR, Varanasi
5	VRT-51	IIVR, Varanasi
6	VRT-19	IIVR, Varanasi
7	Kashi Anupam	IIVR, Varanasi
8	H-88-78-5	IIVR, Varanasi
9	H-88-78-4	IIVR, Varanasi
10	ToLEV-16	IIVR, Varanasi
11	VRT-30	IIVR, Varanasi
12	VRT-16-1	IIVR, Varanasi
13	Kashi Amrit	IIVR, Varanasi
14	Kashi Aman	IIVR, Varanasi
15	ToLEV-28	IIVR, Varanasi
16	VRT-50	IIVR, Varanasi
17	VRT-34	IIVR, Varanasi
18	Pant T-3	GBPUAT, Pantnagar
19	Pusa Rohini	IARI, New Delhi
20	Punjab Ratha	PAU, Ludhiana
21	Punjab Gaurav	PAU, Ludhiana
22	Pusa Ruby	IARI, New Delhi
23	Arka Rakshak	IIHR, Bengaluru
24	Arka Samrat	IIHR, Bengaluru
25	Arka Abhed	IIHR, Bengaluru

Result

The cluster mean calculated for twelve characters under study is presented in **Table-2**. It shows that the plant height exerts highest mean for cluster II (72.32) followed by cluster V (61.50), cluster IV (61.26), cluster I (58.42) and lowest with cluster III (52.99). Days to 50 % flowering indications highest mean with cluster V (38.83) followed by cluster III (38.59), cluster IV (37.65), cluster II (35.11) and lowest for cluster I (33.30). Number of leaves per plant shows highest mean with cluster I (236.09) followed by cluster V (200.96), cluster II (195.16), cluster IV (141.13)

and lowest for cluster III (116.76). Number of primary branches per plant shows highest mean with cluster V (16.34) followed by cluster II (16.06), cluster III (15.36), cluster I (15.14) and lowest for cluster IV (14.51). Plant spread expressions highest mean with cluster II (70.12) followed by cluster V (68.14), cluster I (62.16), cluster III (59.64) and lowest for cluster VI (58.33). Fruit length shows highest mean with cluster V (5.58) followed by cluster I (5.24), cluster III (5.19), cluster II (4.96) and lowest for cluster IV (4.35). Diameter of fruit indications highest mean with cluster V (6.78) followed by cluster III (6.02), cluster II (5.70), cluster I (5.10) and lowest for cluster IV (4.47). Average fruit weight shows highest mean with cluster V (99.50) followed by cluster III (74.25), cluster II (68.69) cluster I (51.68) and lowest for cluster IV (41.63). Number of fruit per plant shows highest mean with cluster I (69.99), followed by cluster II (44.81), cluster V (40.99), cluster VI (40.06) and lowest for cluster III (39.96). Fruit index shows highest mean for cluster V (6.18) followed by cluster III (5.60), cluster II (5.33), cluster I (5.17) and lowest for cluster IV (4.41). Average yield per plant highest mean for cluster V (4.09) followed by cluster I (3.72), cluster II (3.07), cluster III (3.01) and lowest for cluster IV

(1.61). Fruit yield per hectare shows highest mean for cluster V (862.80) followed by cluster I (779.69), cluster II (628.67), cluster III (628.00) and lowest for cluster IV (328.18). Based on cluster analysis the selected genotypes expecting to bring the improvement in the germplasm and also serve as potential hybrids, as well as evaluation of genotypes for quality purpose. Similar result found by (6, 10, 3, 7).

A total of 25 genotypes were grouped into five different genetic clusters on the basis of genetic affinity or diversity by Tocher's methods presented on **Table-3**. Cluster II have maximum 9 genotypes namely VRT-67, ToLEV-32, VRT-51, H-88-78-5, ToLEV-28, VRT-50, VRT-34, Punjab Ratha and Arka Samrat followed by cluster IV have 5 genotypes namely H-88-78-4, ToLEV-16, Pusa Rohini, Punjab Gaurav and Pusa Ruby, Cluster III and V consist 4 genotypes. Cluster III namely VRT-19, Kashi Aman, Pant T-3, Arka Rakshak and Cluster V namely Kashi Chayan, Kashi Anupam, Kashi Amrit and Arka Abhed. Minimum 3 genotypes having Cluster I namely H-88-78-1, VRT-30, VRT-16-1. This demonstrates that a cluster's genotypes could be somewhat connected to one another. These results revealed that spatial variety and genetic.

Table-2. Cluster mean for 12 characters in Tomato

Cluster s		Plant height (cm)	Days to 50% floweri ng	No leaves/ plant	No primar y branch es/ plant	Plant spread (cm)	Fruit length (cm)	Diamet er of fruit (cm)	Averag e fruit weight (g)	Numb er of fruit per plant	Fruit index	Averag e yield (kg/pla nt)	Fruit yield (q/ha)
I	Mean	58.42	33.30	236.09	15.14	62.16	5.24	5.10	51.68	69.99	5.17	3.72	779.69
	SE±	3.52	2.86	71.76	0.55	5.88	0.12	0.25	5.87	14.30	0.18	0.88	199.20
II	Mean	72.32	35.11	195.16	16.06	70.12	4.96	5.70	68.69	44.81	5.33	3.07	628.67
	SE±	8.08	1.54	59.95	1.63	5.10	0.57	0.50	10.90	9.95	0.34	0.52	122.56
III	Mean	52.99	38.59	116.76	15.36	59.64	5.19	6.02	74.25	39.96	5.60	3.01	628.00
	SE±	5.05	0.55	13.11	0.75	3.83	0.45	0.42	14.55	6.19	0.27	0.85	175.11
IV	Mean	61.26	37.65	141.13	14.51	58.33	4.35	4.47	41.63	40.06	4.41	1.61	328.18
	SE±	12.78	2.62	53.97	2.64	5.70	0.55	0.19	8.85	7.63	0.34	0.23	40.78
V	Mean	61.50	38.83	200.96	16.34	68.14	5.58	6.78	99.50	40.99	6.18	4.09	862.80
	SE±	6.53	2.30	71.83	2.81	9.46	0.50	0.45	8.94	4.40	0.24	0.75	134.92

Table-3 Number of genotypes in each cluster in Tomato

Clusters	No of genotypes	Genotypes
I	3	H-88-78-1, VRT-30 and VRT-16-1
II	9	VRT-67, ToLEV-32, VRT-51, H-88-78-5, ToLEV-28, VRT-50, VRT-34, Punjab Ratha and Arka Samrat.
III	4	VRT-19, Kashi Aman, Pant T-3 and Arka Rakshak.
IV	5	H-88-78-4, ToLEV-16, Pusa Rohini, Punjab Gaurav and Pusa Ruby
V	4	Kashi Chayan, Kashi Anupam, Kashi Amrit and Arka Abhed.

Table-4 Inter and intra distances in Tomato

Clusters	I	II	III	IV	V
I	2.061				
II	3.219	2.302			
III	4.145	3.04	1.695		
IV	4.924	4.132	4.054	2.437	
V	4.842	3.484	3.131	6.596	2.519

Table-5. Contribution of various characters towards total genetic divergence

Genotypes	Contribution %
Plant height (cm)	12.50
Days to 50% flowering	13.67
Number of leaves/plant	9.06
No primary branches/plant	10.52
Plant spread (cm)	11.65
Fruit length (cm)	10.11
Diameter of fruit (cm)	8.84
Average fruit weight (g)	6.72
Number of fruit per plant	5.78
Fruit index	3.21
Average yield (kg/plant)	3.92
Fruit yield (g/ha)	4.03

diversity might not always be related. From the pattern of clustering, it could be inferred that sufficient divergence was present to enable the formation of individual character. The clustering pattern suggested that geographical diversity is not efficient index of genetic diversity. The view point has been supported by the work (10, 3, 17, 7, 12).

The average intra and inter cluster D^2 values and average intra and inter cluster distance values are presented in **Table-4**. The intra-cluster distance was observed maximum in cluster V (2.519) followed by cluster IV (2.437), cluster II (2.302), cluster I (2.061) and minimum in cluster III (1.695). The maximum inter cluster distance

was recorded for cluster V with cluster IV (6.596) followed by cluster IV with cluster I (4.924), cluster V with cluster I (4.842), cluster III with cluster I (4.145), cluster IV with cluster II (4.132), cluster IV with cluster III (4.054), cluster V with cluster II (3.484), cluster II with cluster I (3.219), cluster V with cluster III (3.131). However, the minimum inter-cluster distance for cluster III with cluster II (3.040). To select the best cluster pattern and crossed between the maximum inter and intra value are observed for developed high and best yield producing variety in the form of cluster. Similar findings were also earlier reported by (11, 6, 10, 3, 7, 12).

The contribution of various characters under study toward the expression of genetic divergence as presented in **Table-5**. The result showed that the highest contribution % was recorded for the characters viz., days to 50% flowering (13.67), plant height (12.50), plant spread (11.65), number of primary branches per plant (10.52), fruit length (10.11), number of leaves per plant (9.06), diameter of fruit (8.84), average fruit weight (g) (6.72), number of fruits per plant (5.78), fruit yield (g/ha) (4.03), average yield per plant (kg) (3.92) and lowest contribution was recorded for fruit index (3.21). The high contribution per cent of different characters for improving many breeding as well as genetic programme for development high yielding variety as well as cultivars. Similarly, research finding by (4, 1, 3 and 7).

Conclusion

It was concluded that the genotypes were grouped into five clusters. Cluster II comprised highest genotypes followed by Cluster IV, Cluster V, Cluster III and Cluster I. So, the cross combinations cluster I and Cluster II are indicating wide diversity for developing progeny with high vigour and desirable characters in tomato. The highest mean value was recorded in Cluster V, Cluster I, Cluster II, Cluster III and Cluster IV respectively. The maximum intra cluster distance was found in cluster V and minimum intra cluster distance was recorded cluster III. The highest inter-cluster distance was recorded cluster V with cluster IV and the minimum inter cluster D^2 distance was recorded cluster III with cluster II. The character days to

50% flowering contributed maximum divergence and fruit index exhibit minimum divergence. To develop high yielding tomato cultivars, selecting divergent genotypes and using them in cross-breeding programmes would increase the likelihood of obtaining high heterosis and high genetic variability for quantitative and other desirable traits.

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ANALYSIS OF LEGHAEMOGLOBIN, CHLOROPHYLL AND PROTEIN CONTENT IN THE SELECTED LEGUME PLANTS (*VIGNA RADIATA* L. AND *VIGNA MUNGO* L.)

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Abstract

The present study carried out to analysis of Leghaemoglobin, Chlorophyll and Protein content in the selected legume plants (*Vigna radiata* L. and *Vigna mungo* L.). The highest leghaemoglobin content (0.031 mg/g) was observed in (IARI PSB+IARI *Rhizobium*) while lowest was in Native *Rhizobium* (0.024 mg/g) and IARI *Rhizobium* (0.025mg/g) as compared to control in green gram experiment. The maximum protein content (15.66%), which was statistically significant in IARI PSB +IARI *Rhizobium* and higher than remaining treatments was found in Native *Rhizobium*+ Native PSB (14.93%). The protein content increased by 19.4%% in IARI PSB+ IARI *Rhizobium* as compared to control samples. In case of urd been the maximum leghaemoglobin content was recorded in IARI PSB+IARI *Rhizobium* (0.940 mg/g) while the minimum in Native *Rhizobium* (0.0596 mg/g) and IARI *Rhizobium* (0.835 mg/g) as compared to control. The maximum protein content (14.12 %) was observed in IARI PSB+IARI *Rhizobium* while minimum in Native PSB (10.61%) and Native *Rhizobium* (11.42%) respect to control. In green gram total chlorophyll content was observed to be more in IARI PSB +IARI *Rhizobium* (0.369 mg/g) as compare to control. In case of urd been the total chlorophyll content was observed highest (0.991 mg/g) in IARI PSB +IARI *Rhizobium* treated plants. Key Words: Microbial, Spoilage, Preservation, Milk and Milk Products.

Introduction

Legumes are unique among crop plants in their ability to fix nitrogen in symbiotic association with bacteria, mostly belonging to the genera *Rhizobium* and *Bradyrhizobium*. Nitrogen fixation by bacteroids (symbiotic form of bacteria) provides ammonia, which is incorporated by the plant into important biomolecules, such as proteins, nucleic acids, porphyrins, and alkaloids. In return, the plant furnishes bacteroids with sugars; these sugars are oxidized to organic acids and used as respiratory substrates by the bacteroids. The symbiosis takes place at the level of root nodules, tumor-like structures formed as a result of the infection of roots by the bacteria. Typical legume nodules possess three structurally

and physiologically distinct zones called external cortex, internal cortex, and central region. The external cortex is separated from the internal cortex by a layer of cells called endodermis which may act as a physical barrier to oxygen diffusion (Witty et al. 1986; King et al. 1988). The central region is formed mainly by infected cells containing the bacteroids. Bacteroids are not free in the cytoplasm of infected cells but are confined inside vesicles called symbiosomes. The membrane of these symbiosomes derives in part from the plant cell plasmalemma. Interspersed among the infected cells there are also uninfected cells whose functions, such as the synthesis of the nitrogenous compounds called ureides, are only now beginning to be understood (Aparicio-Tejo et

al., 1993 and M. Becana et al. 1995). The pigment of leghaemoglobin is similar to the haemoglobin of red blood cells. It has the ability to combine very rapidly with oxygen and thus acts as a very efficient oxygen scavenger. The enzyme nitrogenase is highly sensitive to molecular oxygen as it acts under anaerobic conditions. The plant interacts with various abiotic factors in their life cycle, among them light is also an important factor. The energy embedded in photon perceived by chlorophyll and converted into energy molecules ATP and NADPH. This energy consumed in fixation of CO₂ and synthesize photosynthetic assimilate. Apart from, the light also up-regulates various processes such as seed germination, seedling development, plant growth, biomass production, flowering, reproduction, and circadian rhythms.

Key words: Rhizobium, PSB, Native Rhizobium, Native PSB, Leghaemoglobin, Chlorophyll and Protein content

Materials and Methods:

Pot Based Experiment/Testing Firstly, collected forty-two pots, then filled with eight kg sterilized soil and leave for 24 hours. Media was prepared YEM for Rhizobium and KB for PSB or seed treatment. Take one native PSB isolate +one native Rhizobium isolate, one IARI PSB isolate +one IARI Rhizobium isolate (PSB+RB). Four bacterial strains were inoculated in particular medium and incubate at 37°C or 48 hrs. Take seven sterilized petri plates and add approximate 20 seeds per Petri plate after that add 20ml bacterial solution. After that sowing the seed into pots. Observed the data regarding leghaemoglobin, chlorophyll and protein content in the selected Legume Plants (*Vigna radiata* L. and *Vigna mungo* L.)

The experiments were conducted in the Department of Pathology & Microbiology, Sardar Vallabhai Patel University of Agriculture and Technology, Meerut, during kharif season 2017-18, 2018-19 and 2019-20. The experiment was laid out in Completely randomized research design with three replicates and seven treatments. The seed of *Vigna radiata* and *Vigna mungo* were obtained from seed certification office, Meerut. Healthy seeds of uniform size were shorted out and

washed with distilled water. The phosphate solubilizing bacteria and Rhizobium bacteria were procured from I. A. R. I., New Delhi. Collection and identification of native Rhizobium isolates was also done. Method for isolation of PSB and Rhizobium culture medium used colony morphology and microscopy biochemical characterization seed germination assay Pot experiment/testing- parameters used for pot assay.

Results and Discussion

Estimation of Leghaemoglobin mg/g and protein content % of *Vigna radiata* L.

Leghaemoglobin content (mg/g): The results obtained from average mean leghaemoglobin content of root nodule (mg g⁻¹) value increased in treated plants (0.031mg/g) as compared to control (0.024 mg/g). The highest leghaemoglobin content (0.031 mg/g) was observed in (IARI PSB+IARI *Rhizobium*) while lowest was in Native *Rhizobium* (0.024 mg/g) and IARI *Rhizobium* (0.025mg/g) as compared to control. The average mean leghaemoglobin content (mg/g)) value increased treated plants (0.025 to 0.031mg/g) as compared to control(0.024 mg/g). Similarly, as compared to control samples was observed (0.024 mg/g), while in treated plants was observed (0.026 mg/g) IARI PSB and (0.028mg/g) Native *Rhizobium* + Native PSB (0.028mg/g). The leghaemoglobin content increased in IARI *Rhizobium* (0.026 mg/g) and IARI PSB+IARI *Rhizobium*(0.031mg/g) (**Table 1(a)**). The highest leghaemoglobin was observed in case of IARI PSB+IARI *Rhizobium* (0.031mg/g). In comparison to control grain yield increased by 29.2% in T5 (IARI PSB+ IARI *Rhizobium*).

In the present study, co-inoculation of IARI PSB+ IARI *Rhizobium* performed better than *Rhizobium* and PSB alone with respect to leghaemoglobin content in green gram crop. In case of IARI PSB+IARI *Rhizobium*, higher leghaemoglobin content than lowest as comparison to control. The better nodulation in treated sample might be resulted in higher content of leghaemoglobin in nodular tissues. Similarly, higher leghaemoglobin content in IARI PSB+ IARI *Rhizobium* was mainly due to better root and nodules development.

Protein Content (%): The protein content of green gram in different treatment ranged from (13.96 to 15.66%). The maximum protein content (15.66%), which was statistically in IARI PSB + IARI *Rhizobium* and significantly higher than remaining treatments was found in Native *Rhizobium* + Native PSB (14.93%). The minimum protein content (13.11%) significantly lower than the rest of the treatments was found in control samples. Significantly higher protein was obtained with the *Rhizobium* and PSB inoculation of application of treatment in IARI PSB + IARI *Rhizobium*. The protein content increased by 19.4% in IARI PSB + IARI *Rhizobium* as compared to control samples (Table 1(a)).

Estimation of Leghaemoglobin mg/g and protein content % of *Vigna mungo* L.

Leghaemoglobin content (mg/g): The results given in (Table 1(b)) showed that the leghaemoglobin content in black gram root nodules increased with different treatments over the control samples. The maximum leghaemoglobin content was recorded in IARI PSB + IARI *Rhizobium* (0.940 mg/g) while the minimum in Native *Rhizobium* (0.0596 mg/g) and IARI *Rhizobium* (0.835 mg/g) as compared to control respectively. The leghaemoglobin content (mg/g) ranged from (0.835 to 0.940 mg/g) treated plant samples, while it found in 99% in IARI PSB + IARI *Rhizobium* as compared to control (0.034 mg/g). The results obtained from under treated plants shows higher nodule leghaemoglobin content as compared to control plants. The plant leghaemoglobin content values were compared to general mean value of treated plants as compared to control.

Protein content (%): The results obtained shows that average mean protein content (%) value varied from treated plants (14.11 to 14.12%) as compared to control (13.24%). The maximum protein content (14.12 %) was observed in IARI PSB + IARI *Rhizobium* while minimum in Native PSB (10.61%) and Native *Rhizobium* (11.42%) respect to control respectively. In comparison to control protein content increased by 6.65% in IARI PSB + IARI *Rhizobium* (Table 1(b)).

Estimation of leaf chlorophyll content mg/g of green gram (*Vigna radiata* L.)

Leaf chlorophyll content (mg/g): The mean values were recorded to measure the change in chlorophyll content. The results obtained shows that average mean chlorophyll value varied from treated plants (0.446 mg/g, 0.369 mg/g and 0.0815 mg/g) as compared to control (0.600 mg/g) chlorophyll 'a' 0.298 mg/g, chlorophyll 'b' 0.302 mg/g and total chlorophyll content (Table 2 (a)). The highest chlorophyll 'a' content was observed in IARI PSB + IARI *Rhizobium* (0.446 mg/g) and total chlorophyll (0.0815 mg/g). Similarly, the chlorophyll 'b' and total chlorophyll content was observed to be more in IARI PSB + IARI *Rhizobium* (0.369 mg/g) and total (0.0815 mg/g) as compared to control respectively. But, the chlorophyll 'b' content was higher in Native *Rhizobium* + Native PSB (0.351 mg/g). All the three chlorophyll parameters i.e., chlorophyll 'a', chlorophyll 'b' and total chlorophyll was found to be higher in plants treated with combination of IARI PSB + IARI *Rhizobium*. The average mean values were (0.446 mg/g) chlorophyll 'a', (0.369 mg/g) chlorophyll 'b' and (0.0815 mg/g) total chlorophyll. In comparison to control total chlorophyll content increased by 86.4% in IARI PSB + IARI *Rhizobium*.

The treated samples showed faster leaf chlorophyll increased in treatments. In controlled plants treated with tap water, there is no growth pigment was observed, although the chlorophyll the mean values among them varied due to inherent variations in chlorophyll content but in the treated plants. The highly enhanced chlorophyll pigment shows that the treated plants as compared to control.

Estimation of leaf chlorophyll content mg/g of *Vigna mungo* L.

chlorophyll parameters namely chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were maximum recorded in Native PSB (0.880 mg/g) i.e., plants treated with the combination of IARI PSB + IARI *Rhizobium* while, the lowest recorded in Native PSB (0.037 mg/g) chlorophyll 'a' and Native *Rhizobium* (0.596 mg/g) chlorophyll 'b' as compared to control (0.074 mg/g) respectively. The

chlorophyll 'a' and 'b' content varies ranged from (0.043 mg/g and 0.835mg/g) Native *Rhizobium*, IARI PSB+IARI *Rhizobium* (0.051 mg/g, 0.940 mg/g) and total chlorophyll (0.991mg/g) **Table 2(b)** respectively.

Chlorophyll is one of the important green pigments which is used as an index of plant production capacity. The pigment content is an indication of photosynthetic and metabolic activity. The chlorophyll is an integral part of plant pigments and play an important role in the process of photosynthesis. The highest chlorophyll content recorded in IARI PSB+IARI *Rhizobium* treated plant. The result obtained in the present study on black gram. The results of the study carried on the growth of *Vigna mungo* showed a significant improvement in the growth parameters. The total chlorophyll content was also found to be significantly higher in treated plants than the control plants.

Table 1(a): Leghaemoglobin and protein content of *Vigna radiata* variety with different treatments

Treatment	Leghaemoglobin (mg/g)	Protein %
Control (T _C)	0.024	13.11
IARI <i>Rhizobium</i> (T ₁)	0.025	13.96
Native <i>Rhizobium</i> (T ₂)	0.024	13.47
IARI PSB (T ₃)	0.026	14.26
Native PSB (T ₄)	0.025	13.33
IARI PSB + IARI <i>Rhizobium</i> (T ₅)	0.031	15.66
Native <i>Rhizobium</i> + Native PSB (T ₆)	0.028	14.93
CD 5%	0.004	1.394
SE(m)	0.001	0.455

Significant at P at 5% level of significant

Table 1(b): Leghaemoglobin and protein content of *Vigna mungo* variety with different treatments

Treatment	Leghaemoglobin (mg/g)	Protein %
Control (T _C)	0.034	13.24
IARI <i>Rhizobium</i> (T ₁)	0.835	14.12
Native <i>Rhizobium</i> (T ₂)	0.596	11.42
IARI PSB (T ₃)	0.909	12.01
Native PSB (T ₄)	0.843	10.61
IARI PSB + IARI <i>Rhizobium</i> (T ₅)	0.940	14.12
Native <i>Rhizobium</i> + Native PSB (T ₆)	0.909	12.99
CD 5%	0.346	1.842
SE (m)	0.113	0.601

Significant at P at 5% level of significant

Table 2(a): Chlorophyll content of *Vigna radiata* variety with different treatments

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll
Control (T _C)	0.298	0.302	0.600
IARI <i>Rhizobium</i> (T ₁)	0.364	0.347	0.711
Native <i>Rhizobium</i> (T ₂)	0.342	0.335	0.677
IARI PSB (T ₃)	0.348	0.342	0.690
Native PSB (T ₄)	0.334	0.329	0.663
IARI PSB + IARI <i>Rhizobium</i> (T ₅)	0.446	0.369	.0815
Native <i>Rhizobium</i> + Native PSB (T ₆)	0.429	0.351	0.781
CD 5%	0.056	0.034	0.090
SE(m)	0.018	0.011	0.029

Significant at P at 5% level of significant

Table 2(b): Chlorophyll content of *Vigna mungo* variety with different treatments

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll
Control (T _C)	0.040	0.034	0.074
IARI <i>Rhizobium</i> (T ₁)	0.043	0.835	0.878
Native <i>Rhizobium</i> (T ₂)	0.034	0.596	0.680
IARI PSB (T ₃)	0.041	0.909	0.960
Native PSB (T ₄)	0.037	0.843	0.880
IARI PSB + IARI <i>Rhizobium</i> (T ₅)	0.051	0.940	0.991
Native <i>Rhizobium</i> + Native PSB (T ₆)	0.046	0.909	0.955
CD 5%	0.009	0.346	0.355
SE(m)	0.003	0.113	0.116

Significant at P at 5% level of significant

Fig. 1(a): Leghaemoglobin and protein content of green gram variety with different treatments

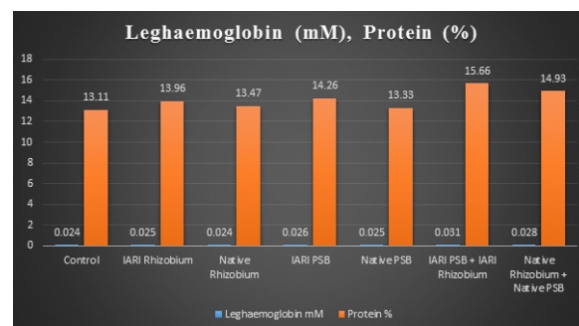


Fig. 1(b): Leghaemoglobin and protein content of black gram variety with different treatments

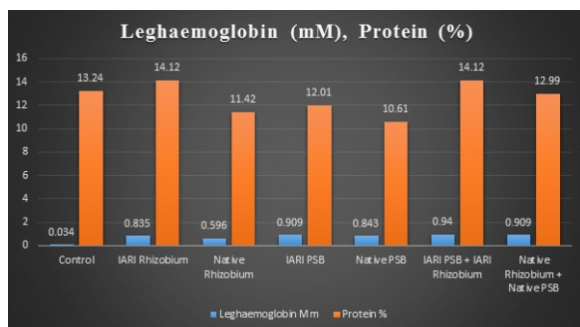


Fig. 2(a): Chlorophyll content of green gram variety with different treatments

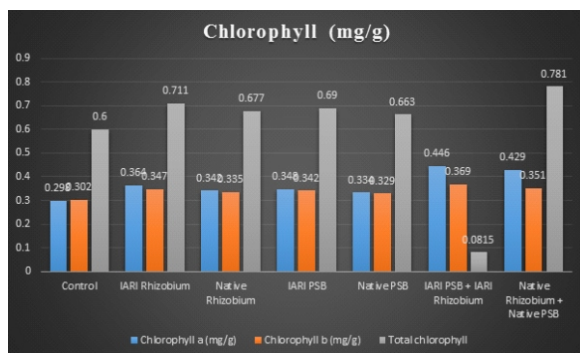
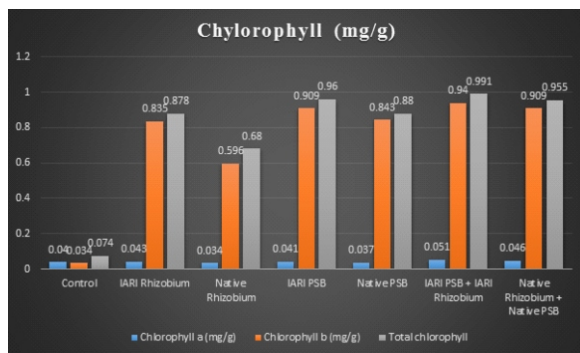


Fig. 2(b): Chlorophyll content of black gram variety with different treatments



Conclusion

It may be concluded that the better nodulation in treated sample might be resulted in higher content of leghaemoglobin in nodular tissues. Similarly, higher leghaemoglobin content in IARI PSB+ IARI *Rhizobium* was mainly due to better root and nodules development. The treatment combination effect of IARI PSB +IARI *Rhizobium* significantly increased in treated plants protein content as compared to control sample.

The highest chlorophyll content recorded in IARI PSB+IARI *Rhizobium* treated plant. The total chlorophyll content was also found to be significantly higher in treated plants than the control plants.

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PHYSIOLOGICAL CHARACTERS ASSOCIATED WITH WATER-STRESS TOLERANCE UNDER PRE ANTHESIS WATER-STRESS CONDITIONS IN WHEAT

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Abstract

This study was conducted to evaluate previously identified water-stress tolerance and water-stress susceptible varieties, including Falchetto and Chinese spring (CS) (water-stress tolerant) and Oxley and Flat (water-stress susceptible), for some physiological characters affecting the performance of varieties under water-stress conditions. The results indicated that water-stress tolerant varieties had higher Stomatal Resistance (SR), higher Leaf Relative Water Content (LRWC), higher Vegetative Water Use Efficiency (WUE_{veg}) and higher Vegetative Evapo-transpiration Efficiency (ETE_{veg}) than water-stress susceptible varieties. Varieties with higher LRWC generally had higher SR. This indicates that higher LRWC is, at least in part, due to higher SR. The variety Oxley (water-stress susceptible) showed no significant differences from Falchetto (water-stress tolerance) for stomatal area on both surfaces of the flag leaf but Falchetto had higher number of stomata per unit area and smaller stomata than Oxley. This implies that low frequency of stomata is compensated by high stomatal size. This also indicates that differences in SR between these two varieties are possibly due to differences in stomatal response to water-stress or due to differences in cuticle resistance.

Introduction

Assessment of wheat varieties for physiological components of water-stress tolerance has been used as a quick technique for screening water-stress tolerant varieties (1, 2, 7, 13, 14, 19, 22). Physiological characters of plants, particularly those related to plant water status, have a great deal of importance in growth and development of plants during water-stress conditions. Leaf relative water content (LRWC), stomatal resistance (SR) water use efficiency (WUE) and its components, stomatal characteristics and carbon isotope discrimination ($\delta^{13}C$) have been used as examples of the characters which determine water-stress tolerance of wheat genotypes.

LRWC is the relationship between fully turgid water content and actual water content of plant

tissues when they are subjected to water-stress. Therefore, LRWC indicates the ability of plants to keep their water status at a reasonable level when they experience water-stress. (7) pointed out that LRWC represents the water status of plants, while other parameters like water potential can be affected by soil, plant and atmospheric water status. Therefore, LRWC may be a more appropriate indicator of plant water status. LRWC is also closely related to cell volume and reflects the balance between water supply and transpiration.

Water use efficiency indicates the amount of assimilate produced for the amount of water used. (15) defined water use efficiency (WUE) as the ratio of grain yield to total water used (GY/TWU). Total water used includes both soil evaporation and plant transpiration. In some

papers, water use efficiency has been defined as the ratio of photosynthesis (net CO₂ assimilation) to total water transpired (18). Carbon isotope discrimination (Δ) is a character which indicates the amount of ¹³C which is depleted by photosynthesis mechanisms. This character is related to water-stress tolerance indicators such as stomatal resistance and water use efficiency (17, 21). Furthermore, this character has indicated negative association with grain yield under some water-stress conditions.

Material and Methods

Experiment 1

This experiment was carried out in a greenhouse cubicle at the Close House Field Station, UK. This experiment was started in the middle of July when natural light and day length was adequate for the plants. The temperature ranged from 17 to 31 & deg; C. The relative humidity fluctuated from 45-80%.

Four varieties Falchetto, Falat, CS and Oxley, were included in this experiment. Falchetto is an early flowering semi-winter variety bred in Italy and grown in the Sudan which possesses the Akakomugi semi-dwarf gene (Rht₈). This variety is tolerant to water-stress (24) and has hairy glumes and compact spikes. Falat is reported to be susceptible by (19). Seeds of the varieties were germinated and then vernalised for 4 weeks in a cold cabinet at 2-7 & deg;C and an 8 hour photoperiod. 20 seedlings of each variety were transplanted in 10-cm pots containing John Innes No. 2 compost. The pots were divided into two blocks so that plants in each block were similar in vigour and size. The pots were arranged randomly within each block.

At growth stage 37-39 of Zadok's scale (flag leaf emerging), plants in one of the blocks were subjected to water-stress and the plants in the other block remained under full irrigation conditions. In the full irrigation treatment, the compost was maintained at optimum water content. Several pots of each variety were weighed every three days and rewatered according to the amount of water lost. The water-stress treatment was initiated by bringing all the pots to optimum water content in the morning of the first day of treatment. The treated plants were subjected to

water-stress by withholding water for 6 days stomatal resistance in both full irrigation and water-stress treatments was measured at mid-day (11 am-2 pm) on the adaxial surface of flag leaf using a Delta-T Diffusion porometer model AP4. The leaf relative water content was determined by using the last fully expanded leaf (flag leaf) from each plant at the end of water-stress period. LRWC was calculated using the following equation:

$$\text{LRWC} = \frac{(\text{Fw} - \text{Dw})}{(\text{Tw} - \text{Dw})} \times 100$$

Where Fw = fresh weight, Dw = dry weight, Tw = turgid weight.

The fresh weights of leaf samples were obtained immediately after excision. Then the leaves were put into test tubes containing distilled water. After 24 hours, both adaxial and abaxial surfaces of leaves were dried with paper towel and turgid weights were obtained. Dry weights were obtained after oven drying the flag leaves at 80°C for 48 hours.

Vegetative Evapo-transpiration Efficiency (ETE_{veg}) and Vegetative Water Use Efficiency (WUE_{veg})

ETE_{veg} and WUE_{veg} were calculated based on vegetative dry matter production as follows:

ETE_{veg} = total dry matter/total water evaporated and transpired from the plant surface excluding the soil

WUE_{veg} = total dry matter/ total water evaporated and transpired from both the plant and soil surfaces (total water used).

At the end of the water-stress period, the plants were harvested at the surface of the soil, and weighed after oven drying at 80 & deg; C for 48 hours. Total water used was obtained by subtracting the weight of pots after harvesting the plants from the weight of pots at the transplant time and adding the amount of water added during growth period. Total water evaporated and transpired only from the plant surfaces was obtained by subtracting the total water evaporated from the unplanted pots from the total water used.

Table 1: Genotype of wheat used for drought tolerance assessment

No	Genotype	No	Genotype
01	Kohdasht	06	Dena
02	Aflak	07	Azar-2

03	Chonab	08	Behrang
04	Seymareh	09	Mahdavi
05	S-83-3	10	Sabalan

Table 2: Yield losses at reproductive growth stages under drought in wheat.

Reproductive stage	Yield loss (%)
Higher grain protein content, fewer days to physiological maturity,	
smaller kernel weight and diameter,	
less grain yield	Not applicable
Less grain yield (drought-tolerant variety)	43
Less grain yield (drought-sensitive variety)	26
1000-grain weight	18.29
ht 1000-grain weight (anthesis stage)	38.67
Biological yield	10
Maximum grain yield	22
Decreased seed number	64
Grain formation stage	101.23
Grain formation stage	65.5
Number of spikes	19.85
Number of spikes (anthesis stage)	15.79
Spike length (anthesis stage)	16.90
Number of spikelets per spike (anthesis stage)	26.20
Grains number (anthesis stage)	72.51
Grain yield (anthesis stage)	64.46

Carbon isotope discrimination ()

This character was measured in 5 plants of varieties Oxley and Falchetto under both control and water-stress conditions. To prepare plant samples, flag leaf fresh tissues were oven-dried at 80 & deg; C for 48 hours. Since water-stress was applied to the plants when flag leaves were half way emerged, the leaves were divided into two parts in order to identify whether there are differences between the tip and the base of leaves for $\delta^{13}C$. Leaves were finely ground to a powder to ensure homogeneity and to achieve greater accuracy in determination of carbon ratio (Boutton 1991). Only a small amount of plant material is required for the majority of combustion systems (Griffiths 1993), therefore samples were weighed in amounts of 1±0.05 mg.

Experiment 2

This experiment was conducted to study stomatal frequency and stomatal size in varieties Oxley and Falchetto. Plants from the two varieties were grown in completely randomized design with 5 replications in a greenhouse. The plants were irrigated evenly by putting them on a wet mat which was irrigated by an automatic water supply. When flag leaves of main stems were just visible

the plants were transferred into a growth chamber where day/night temperatures were 20-23/15-17 & deg; C, photoperiod was 16 hours and light intensity varied from 180 to 210 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the plant surface. In this experiment, when flag leaves were fully developed, stomatal frequency and size were measured on the adaxial and abaxial surfaces of flag leaves by the impression method (Wang and Clarke 1993a). Impressions were taken from the middle of both adaxial and abaxial surfaces of flag leaves.

Statistical methods

In Exp1, analysis of variance (ANOVA) was performed to determine whether there was any difference between varieties under water-stress conditions. Comparison of mean values between varieties was performed by Tukey-Test (Cochran and Cox 1992). Student t-test was performed to compare the treatments within each variety. In the case of carbon isotope discrimination and stomatal characteristics in which only two varieties were assessed, student t-test was used to compare varieties or treatments. LRWC was calculated as a percentage. Therefore, the values for this character were transformed into Arc Sin values before the analysis of variance.

Results and Discussion

Leaf Relative Water Content (LRWC) and Stomatal Resistance (SR)

For all varieties, the LRWC differences between water-stress and well water conditions were significant indicating the effect of water-stress on leaf water status. Falchetto and CS had higher LRWC than varieties Falat and Oxley under water-stress conditions. SR of all four varieties increased with water-stress. Falchetto showed the most and Falat showed the least amount of SR. Falchetto exhibited significant differences from Falat and Oxley for LRWC and SR but the differences between Oxley and CS were not significant for these characters. CS showed an intermediate response to water-stress and showed no significant differences from other varieties.

LRWC of plants after a period of water-stress represents the ability of plants to retain the water in their tissues. The leaf water retention, when subjected to water-stress, has been reported to

increase yield in wheat. Wheat varieties have shown variation for LRWC under both well water and water-stress treatments. A possible explanation of variation between varieties for LRWC under well water conditions is that this variation might be due to differences between varieties leaf thickness and stomatal conductance. Maintenance of LRWC, under water-stress conditions, can be due to osmotic adjustment or reduction in transpiration.

(26) reported that LRWC affects the ability of plants to recover from the stress and consequently influences yield and yield stability. Similar results were found in this experiment. Varieties CS and Falchetto with high LRWC showed a better recovery after water-stress than Oxley and Falat. Data from the previous experiment indicated that varieties Falat and Oxley lost their late tillers because of water-stress, while Falchetto kept their late tillers and eventually produced more fertile tillers. Another consequence of high LRWC is the delaying of leaf senescence. In this experiment, varieties with high LRWC postponed leaf senescence and leaf wilting even in old leaves. This character helps the plants to attain good recovery after stress and maintenance of assimilates transfer to the ear after water-stress.

WUE_{veg} and ETE_{veg}

WUE and ETE are generally affected by dry matter production and water consumption in a reverse direction. Sometimes reduction in dry matter production is compensated by reduction in the amount of water used during the growth season. In this situation, WUE and ETE remain insensitive to water-stress even when the plants are exposed to continuous water-stress in the field. This kind of insensitivity has been reported by (18) in the field experiments. This may seem surprising since it is well documented that water-stress causes stomatal closure and, in theory, temporary closure of stomata when evaporative demand is high should improve water use efficiency. (27) suggested that closure of stomata, caused by an increase in temperature, might reduce transpiration efficiency due to a

reduction in photosynthesis.

Carbon isotope discrimination ($\delta^{13}C$ & permil;)

Therefore measurement of $\delta^{13}C$ in samples prepared from the whole leaf can lead to a saving in time and expenditure and can sufficiently identify possible differences between varieties and treatments. In other words, from the present data, there was no firm evidence to support the hypothesis about the existence of a significant difference in $\delta^{13}C$ accumulation between the base and the tip of flag leaf. However, in both treatments and both varieties, $\delta^{13}C$ of the base was somewhat lower than the tip. Senescence and wilting is more severe in the leaf tip when water-stress is imposed on the plants. Nonetheless, this did not result in a significant reduction in carbon discrimination compared to the base. Since the increase in discrimination in the tip compared with the base is similar in the well watered and water-stressed treatment, the difference probably existed before the water-stress was imposed. The difference could result from a reduction in photosynthesis in the tip due to ageing effects.

Comparison between varieties based on pooled data (irrespective of leaf section) indicated significant differences between the treatments and between the two varieties. The overall reduction in $\delta^{13}C$ due to water-stress for Oxley was 0.84 & plus mn; 0.25 which was less than the reduction for Falchetto (1.06 & plus mn; 0.15). This indicates that discrimination against $\delta^{13}C$ decreases to a greater extent in the variety Falchetto as a result of water-stress. Since $\delta^{13}C$ is negatively correlated with WUE (21), the high WUE_{veg} of Falchetto in comparison with Oxley is probably partly due to the lower value for $\delta^{13}C$ in Falchetto. Despite wide use of $\delta^{13}C$ as an indicator of water use efficiency, (12) believe that measuring $\delta^{13}C$ as an indicator of water use efficiency has several shortcomings. For example, they have reported that measuring $\delta^{13}C$ provides no information on the magnitude of either assimilates or transpiration or whether variation in $\delta^{13}C$ is due to variation in photosynthesis capacity or stomatal conductance.

Relationship between Δ and other physiological characters under water-stress conditions

Carbon isotope data from the tip and base were pooled for 5 plants of Falchetto and Oxley and the relationship between the data and some physiological traits was investigated for these plants ($n=10$). As can be seen from significant relationships were found between Δ and $E_{TE\text{ veg}}$ ($R^2=0.53$, $df=8$, $P<0.01$), WUE_{veg} ($R^2=0.51$, $df=8$, $P<0.01$) and DM ($R^2=0.42$, $df=8$, $P<0.05$). On the other hand, no significant relationship was found between Δ and either LRWC or SR.

The absence of a significant relationship between Δ and SR under the conditions of this experiment allows us to postulate that variation in Δ is due to differences in photosynthetic capacity rather than differences in stomatal resistance. However, (33) did not find a significant correlation between specific leaf dry weight and Δ in 144 accessions of durum wheat grown under rain fed field conditions. They concluded that variation in Δ results from differences in stomatal resistance rather than from photosynthetic capacity. They also reported a positive significant correlation between LRWC and Δ . In the present study such a relationship was not observed. Environmental differences and duration of water-stress period are two possible reasons behind these inconsistent results. However differences between the varieties and temperature could also affect these relationships.

Stomatal characteristics

This indicates that stomatal length is more effective than stomatal width to compensate for the loss of stomatal area due to lower SF. Stomatal length has been used as an indicator of stomatal size by many investigators but no published work was found to use stomatal pore width as a trait which determines the capacity of stomata to reduce water loss. This is possibly due to difficulties which appear during the measurement of stomatal pores especially under unfavourable conditions. Venora and Calcagno (1991) measured and used stomatal width as an indicator of stomatal aperture. This application seems to explain differences between the varieties for water loss only under non-limiting conditions provided

varieties with larger size of stomata have larger stomatal pores. In unfavourable conditions, particularly under water-stress conditions, stomatal aperture is not the main determination of water lost because it is extremely variable as a result of the influence of atmospheric factors.

Higher number of stomata on the adaxial surface has also been reported by other workers (28). Teare et al. (1971) studied SF in different species of wheat. Their results indicated that the number of stomata was significantly greater on the adaxial than on the abaxial surface of the leaves of *Triticum*. This was the case for all species studied in the genus. The ratio of SF on abaxial to adaxial surface was 0.6902 over 4 varieties of common wheat. In the present study, this ratio was 0.6887 and 0.6909 for Oxley and Falchetto, respectively. These results are very close to those obtained by Teare et al. (1971). From these results, it can be suggested that the ratio of SF on the abaxial to that on the adaxial surfaces is possibly more stable than the absolute number of stomata in either surfaces of flag leaves in common wheat.

Conclusion

The results of other workers concerning the relationship between stomatal characteristics and plant water status are inconsistent. (30) found no consistent differences in SF between two durum genotypes with different water-retention capabilities. Wang and Clarke (1993b) reported that SF was not correlated with relative water loss and LRWC in field experiments. However, their results indicated that SF was positively correlated with the rate of water loss but not with LRWC under growth room experiments. The inconsistency of this relationship is possibly due to the influence of other characteristics of stomata rather than SF and due to negative relationships between stomatal size and frequency. In addition to SF and stomatal size, the stomatal responses to water-stress and cuticle resistance are other factors which influence water status of plants under water-stress conditions. Thus, the results presented in this section and those explained above indicate that stomatal characteristics are affecting water status of plants as a complex, and every component of this complex should be studied in relation to other components and with other factors which influence water status of plants.

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BIOTECHNOLOGICAL STRATAGEMS FOR THE PRODUCTION OF MICROBIAL BIOPLASTICS AND ITS COMMERCIAL APPLICATIONS

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Abstract

Different bacteria, fungi, and algae have been reported to synthesize the biopolymer PHA; nonetheless, it is still possible to isolate effective wild-type bacteria that produce PHA. The many harsh settings may serve as possible habitats for bacteria that synthesize PHA. PHA is used in many different sectors, although its production costs are often extremely high. Process parameter optimization has long been recognized as a means of increasing PHA yield and manufacturing in large quantities. The traditional optimization approach focuses primarily on studying one element at a time and obscures the interactions between two or more factors that have an impact on output. Optimizing the carbon and nitrogen sources is necessary to increase PHA synthesis because medium components, pH, temperature, and agitation speed all have an impact on PHA production. When several parameters are taken into consideration, Plackett-Burman statistical modeling offers a novel method of sorting through the components and determining which ones are relevant. The response surface methodology functions as a robust approach to investigate and explore the independent and interactive effects of variables on PHA production. It is a highly effective and dependable statistical tool for evaluating the factors influencing PHA production.

Key words: Synthetic plastic pollution, petro-plastics, marine ecology, production, natural bio-plastics, conventional plastics.

Introduction

Plastics

Because of their many qualities, conventional plastics are an essential component of daily life. These days, plastic pollution is the main issue because of its enormous buildup in the environment. There are only two ways to get rid of them: in landfills or by burning. Plastic waste that is not disposed of properly has a negative impact on the ecosystem, wildlife, and marine life. When marine organisms consume the plastics dumped into water bodies, it has been discovered that they can induce intestinal obstructions or even death. However, burning plastics could unleash harmful and greenhouse chemicals into the atmosphere,

including dioxins. Because of this, plastics made of synthetic materials or petroleum pose a major threat to the environment and need to be addressed right away (6).

Bio-plastics

Petroplastics can be suitably replaced with bio-plastics or plastics made from bio-based resources. Biodegradable plastics are favored mostly because they come from sustainable sources and don't harm the environment. While the majority of bio-plastics naturally degrade in response to environmental factors, certain man-made bio-plastics only partially break down. Starch-based bioplastics are the most widely sold type of bioplastic.

Natural Bio-plastics

Cellulose-based bio-plastics

The most common polysaccharide and organic polymer is cellulose, which has enormous applications. Microfibrils are made up of many cellulose molecules joined together, and cellulose fiber is made up of multiple layers of microfibrils.

Plants maintain their structural integrity because of the supramolecular structure of cellulose. The degree of hydrogen bonding in cellulose, whether crystalline, amorphous, or paracrystalline, is lower, making it less organized. The hydrophilic characteristic of cellulose explains why it is biodegradable (7). Biomaterials are made using cellulose derived from both plants and microbes. Recently, microbial cellulose-based sutures have been studied. Various modified cellulose forms, including sodium carboxymethyl cellulose, methyl cellulose, oxycellulose, and cellulose ether, are employed in various drug delivery systems.

Starch-based bio-plastics

The most common types of bio-plastics and those that hold a significant market share are those made from starch. Quotile starch has been altered to become thermoplastic starch. Compared to regular starch, thermoplastic starch (TPS) offers better material and thermal properties. One type of starch-based bioplastic that is commercially available is Mater-Bi, a composite. Food trays, food storage containers, and cling wrap for food packaging are a few of the popular consumer goods produced with starch-based bio-plastics. Thermoplastic starch is frequently combined with other bio-plastics, including polylactic acid (PLA), before being utilized in the industrial sector.

Synthetic Bio-plastics

Microbial or plant sources provide the raw materials for synthetic biopolymers. There are two types of polymers: biodegradable and non-biodegradable. Among the different synthetic biopolymers are polyanhydrides, PLA, PGA, and PHA.

Polylactic acid (PLA)

Polylactic acid, a fascinating polymer with a

wide range of uses, is frequently sourced from agricultural sources. Commercial interest has been piqued by its thermoplastic properties. Films and other packaging materials are made from PLA. To enhance PLA's mechanical qualities, a number of composites have been created. According to Pfister and Larock (2010), PLGA, a mixture of polylactic acid and polyglycolic acid, is one of the most popular PLA composites.

Synthetic plastic pollution: a global scenario

Because of their superior mechanical qualities and long lifespan, petroleum-based plastics are indispensable to our daily lives, but their growing use has had a detrimental effect on the environment. As of 2014, the world's plastic manufacturing had skyrocketed to above 311 million tons. The global urbanization and industrialization of the world have led to the widespread problem of plastic pollution. Discarding used plastic materials through open dumping has become a popular practice. The excessive buildup of plastics has had a detrimental impact on the ecology and health of the ecosystem. Municipal solid wastes make up the majority of the many kinds of garbage that are deposited in the ground. The ecosystem has suffered from plastic contamination. debris: water bottles, plastic bags, plastic covers, and plastic fibers are only a few of the various kinds of plastic debris that are dumped on the ground.



Fig. 1. The landfill contaminated by different types of plastics

Pollution of petro-plastics in the marine ecology

Plastic pollution has not only affected land areas but also marine habitats. Because plastics have a significant impact on marine life, plastic pollution has become a threat to this species. The main causes of marine pollution are the pollutants that various businesses discard into the ocean.

Plastic debris, microplastics, and nanoplastics from plastic waste in the oceans all have an impact on marine life. Microplastics are a major source of plastic pollution in the marine ecosystem. They are broken pieces of micro-sized plastic materials. Marine animals frequently eat these plastic wastes, which causes intestinal obstructions and bioaccumulation that ultimately results in the organism's mortality (Xanthos and Walker, 2017). Additional plastic waste that contaminates the marine environment are plastic fibers and microbeads.

Primary and secondary plastic abrasion results in the production of plastic fibers. Nanoplastics are created as microplastics continue to fragment. Nanoplastics are plastic particles smaller than one nanometer that are produced when microplastics break apart and pollute the environment. The most widely used plastic, polystyrene, is one of the main contaminating nanoplastics. In addition to polystyrene, polyethylene is a common plastic pollutant that ruins marine ecosystems. The marine ecology is negatively impacted by these microplastics, in addition to the marine life. The various plastic types that have contaminated the seas and oceans are depicted in the technique of extending plastic debris is essential. Plastic garbage is incinerated by first being dumped in huge landfills and then being burned. This approach to plastic management has a number of drawbacks, even though it can be used with a large quantity of plastic. The primary source of environmental contamination from incinerators is the release of many hazardous gases during the burning of plastics. In addition to carbon dioxide and other harmful gases like sulfur dioxide, the combustion process also releases a variety of deadly dioxins and furans. Land contamination results from the incineration process's burned plastic waste and ashes, which contaminate the soil.

The plastic waste is shredded, turned into a new product, and used during the recycling process. Recycling is a useful practice as well because it makes handling a lot of plastic garbage easier. Recycling reduces the amount of plastic produced, making it an energy-efficient solution.

Recycling reduces municipal solid waste and enhances the aesthetic value of the environment, making it an environmentally friendly activity. While recycling and incineration offer numerous benefits, there are also significant drawbacks to these waste management techniques. An alternative material with superior and improved material qualities is required to fight plastic pollution in both terrestrial and marine settings. Recently, there has been interest in bio-plastics as an alternative to plastics derived from fossil fuels.

Bio-plastics: A panoramic view

The widely used synthetic polymer may be adequately replaced by biopolymers or biodegradable polymers. Degradation is made possible by the ease with which biopolymers can split into smaller parts. The amount of plastic derived from petroleum might be significantly reduced by using biopolymers. Polyethylene plastics based on starch, cellulose, protein, and bio-based materials can be distinguished among the bio-plastics.

Thermoplastics with a biological origin are starch-based bioplastics. The cellulose-based plastics, as their name implies, are composed of cellulose that is obtained from cotton and tree plants. Sugarcane and recycled paper have recently been used to create cellulose esters (1).

Since ligno-cellulosic fibers make up the majority of plant fibers, matrix polysaccharides like hemicellulose and pectin are formed from the polymers derived from cell walls. The last ten years have seen the development of bio-composites, or mixtures of two or more materials, which have shown promise as high-quality bioplastics with improved mechanical strength and durability. Bio-based plastics have several minor drawbacks, despite being excellent substitutes for resistant synthetic polymers. Because of their high glass transition (T_g) and melting temperatures, starch-based bioplastics are less strong and are more challenging to convert into plastics (14). Therefore, this is the time to look for biologically derived polymers with improved material qualities.

Polyhydroxyalkanoates

One class of bacterially produced aliphatic structural biopolymers is called polyhydroxyalkanoates. One of the most prevalent PHAs is polyhydroxybutyrate (PHB). They are a good substitute for petroplastics because they have qualities that are comparable to those of ordinary plastics. In addition to its physical characteristics, PHA's biodegradability is another factor in its commercial production. Optimizing the conditions for microbe culture is necessary for the commercial production of PHA. Another crucial element that needs to be taken into account is the PHA's downstream processing. PHA has been utilized in packaging materials such as films.

There are two types of polyhydroxyalkanoates: co-polymers and homo-polymers. Co-polymers are composed of two distinct monomers, while homo-polymers are usually composed of repeating monomers of the same type. Monomers and polymer chain lengths are mostly influenced by the production substrates. The monomer's composition is mostly determined by the substrates that are employed. Gram-negative bacteria typically follow the beta-oxidation pathway for PHA production, but gram-positive bacteria follow a different mechanism. According to Anjum et al. (2016), gram-positive bacteria typically create short chain length (scl-PHA), and gram-negative bacteria produce medium chain length (mcl-PHA).

Genes associated with polyhydroxyalkanoate production

PhaA, PhaB, and PhaC are the three cardinal genes involved in the production of PHA. The phaA gene controls the enzyme 3-ketothiolase; the phaB gene, which modifies the enzyme acetoacetyl-CoA reductase, is the next important gene; and the phaC gene controls the enzyme PHB synthase. These key genes generally affect and regulate PHA production, even if various bacterial genera follow different paths to produce PHA.

Numerous bacteria have been found to have about 60 PHA synthases. Genetic engineering has the potential to be an effective method for increasing product production. The primary genes responsible for PHA production might be engineered to increase the yield, as it is currently

quite low. PHA synthesis from genetically modified *E. coli* has been extensively researched. *Alcaligenes eutrophus* is a microbe that is extensively explored in addition to *E. coli*.

One important factor affecting the polymer's mechanical and structural characteristics is its molecular weight. Cyanobacteria genera have been the subject of much research recently because of their economical PHA synthesis. It is possible to modify the chain length of the polymer (mcl-PHA) in *Pseudomonas* by genetic manipulation. Because they are photoautotrophic, certain cyanobacteria can create polyhydroxyalkanoates when their nutritional supply is limited. Modification and metabolic engineering were done to induce co-polymer synthesis in *Synechococcus* sp. PCC 7002. Genetic manipulation would boost the yield and differ the types of monomers and co-polymers.

Industrial production of PHA

Three main procedures are involved in the industrial manufacturing of PHA: PHA generation, PHA extraction, and PHA downstream processing. PHA is produced either continuously or under batch fermentation conditions. To make PHA, industries use huge fermenters, like 5L scales. The most commonly utilized bacterium for producing PHA on an industrial basis is *Alcaligenes latus*. When it comes to the production of PHA, *Bacillus megaterium* is the most researched model organism, aside from *Alcaligenes latus*. On a larger scale, single-culture PHA production is preferable to co-culture PHA production. Press filtration is the next step after the cell culture is centrifuged to extract the biomass. To extract the cell biomass and get rid of the supernatant, press filtration is used. Next, the cell biomass is ground into a fine powder and extracted. This is done using a rotating vacuum dryer to ensure that the biomass is totally dry. Large extraction tanks are used for the extraction process, and solvents that are sold commercially are used. Centrifugation was used to separate the solutions following extraction. After removing the cell debris through filtration, the PHA-containing solution was further purified using ultrafiltration. PHA was precipitated and subsequently centrifuged in the subsequent procedure. The PHA was spread-dried after being air-dried (11).

PHA is extracted and used for additional industrial and medical uses. A productivity of 0.5 g/l/h was noted during the large-scale production of PHBV in a 20,000-liter fermenter (11). Metabolix is a commercial PHA producer; Biomer, a German company that makes several PHB-based products, is another commercial PHA provider. Because PHB is more brittle, PHBV is recommended instead. The manufacturing of PHBV is aimed at by numerous businesses due to its superior material qualities.

Challenges in Polyhydroxalkanoates (PHA) Production

One intriguing class of biopolymers that has the benefit of biodegradability is polyhydroxyalkanoates. Despite being biopolymers produced by microbes, PHAs have extremely high production costs overall.

The three main factors affecting the production of biopolymers are the cost of carbon stocks, the labor-intensive solvent-based extraction procedure, and the PHA's subsequent processing. Even though a wide range of bacteria can manufacture PHA, bacterial systems only create a relatively small amount of PHA overall. The production of PHA by wild-type bacteria is significantly higher than that of non-wild-type strains. The production of PHA would be dramatically increased by screening and isolating wild-type bacterial strains from various polluted environments and severe settings. The carbon sources are the second important component influencing PHA formation. Because the metabolism of polyhydroxyalkanoates is heavily dependent on substrates, the main cost is thus dependent on carbon stores.

The utilization of renewable substrates, such as ligno-cellulosic biomass, can make the PHA synthesis process more affordable. Since waste from a variety of industries can be utilized to produce PHA, using multiple waste substrates would significantly lower costs. Utilizing waste substrates also serves a twofold purpose in terms of efficient waste management because the trash is converted into a valuable product.

An alternative for producing PHA is to use waste lipids produced by companies. One of the most commonly produced waste lipids is tallow. It

has been reported that PHA is produced from tallow waste by *Pseudomonas* species. Another type of waste that would be suitable as a carbon source is leftover frying oil. The production of co-polymers would arise from the use of different substrates.

Although pretreatment and detoxification of ligno-cellulosic biomass are necessary steps for the utilization of wastes, ligno-cellulosic biomass, such as wood hydrolysate, rice straw hydrolysate, and bagasses, are efficient resources that might be exploited in a sustainable manner. The production of PHA is made more affordable by the use of carbon sources such as waste water from various industries and excess whey from the dairy industry. These carbon sources are not sustainable in the traditional sense, but they are also less expensive than commercial sources (8).

Another major obstacle in the production of PHA is usually the extraction procedure, which calls for a lot of different organic solvents. Solvents are costly and sometimes dangerous when it comes to the recovery and purification of PHA. To get pure PHA, one of the main limiting steps is the separation of PHA from non-PHA cell biomass. PHA extraction without polymer degradation is a tedious process, which makes downstream processing of PHA a laborious task. PHA extraction requires comprehensive purification before it can be used in industrial and medical settings. Biological recovery techniques have also been suggested as alternatives to chemical extraction for the extraction of PHA. Enhanced product recovery necessitates process improvement overall, even though extraction is a significant step.

Conclusion

The overall productivity of PHA plays a major role in the economics of PHA production. The cost and type of fermentation method used determine the overall productivity. Reducing costs and increasing PHA production require cost-effective process recovery solutions. About half of the PHA produced commercially—roughly \$16/kg—is used for product purification. Waste material is transformed into goods with additional value using a new idea called waste valorization.

Costs can be significantly decreased by using mixed cultures, feeding plans, and fermentation techniques. Additionally, PHA and bio-hydrogen production could both benefit from the value-adding of substrates and waste water. The process of converting lingo-cellulosic wastes into products with added value has the potential to significantly lower expenses and facilitate waste management. Reusing and recycling trash offers the dual benefits of being an environmentally benign and energy-efficient technique, aiding in the sustainable production of PHA.

Application of PHA in packaging

Packaging that is biodegradable is essential because the amount of plastic in the world has significantly increased. Various types of plastic are used for packaging, depending on the type of food and its characteristics. Food packaging applications are the primary usage for cling films composed of various polymeric materials. Depending on the plastic grades used, other kinds of wrap films other than cling films are produced. These traditional plastics are not biodegradable while having strong chemical and heat resistance. Overuse of plastics leads to an accumulation of single-use plastics in the environment. Over time, packaging has progressively used more bioplastics. Because PHAs are biodegradable, they are a better option than petroplastics. Plastics' capacity to degrade is a crucial factor in the current pollution problems. Modifiable characteristics are PHA's next beneficial feature. By combining PHA with polymers or co-polymers, its diverse chemical and physical characteristics can be improved. PHA's functionality still needs to be enhanced before it can be applied to the food packaging industry. According to (8), research is still being done on the use of PHA in food packaging.

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CONSTRAINTS FACED BY HORTIPRENEURS IN KUMAON HILLS OF UTTARAKHAND

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Abstract

In Uttarakhand, the horticulture sector contributes to nutrition security and serves as a reliable source of income generation. It has almost all major climatic zones, making it amenable to a variety of commercial opportunities in horticulture, floriculture, and agriculture. Despite the vast potential and opportunities in the field, established industries remain limited. Within the horticulture sector, enterprises suffer from oversupply, and direct marketing channels are yet to be well established. The present study therefore attempted to analyse various technical, infrastructure related, marketing and economic constraints faced by hortipreneurs in undertaking various horticultural enterprises in hills. A descriptive research design was used to meet the objectives of the study. The study was carried out in purposively selected districts Nainital and Almora of Uttarakhand. The study was carried out on a sample of 70 hortipreneurs who were selected through snowball sampling from various villages and blocks and were interviewed using a pre-tested semi-structured schedule. Constraints were analysed using Friedman Analysis in SPSS. Lack of adequate information and technical support, High seasonality/ perishability of products, Inadequate facility of specialized transportation, Lack of sufficient integrated cold chain/storage facilities. High marketing margin by the middlemen and Prevalence of financial illiteracy among producers were the major constraints faced by the hortipreneurs in various horticultural enterprises.

Keywords: Constraints, hill horticulture, horticulture enterprises and Friedman test

Introduction

Horticulture crops have a significant contribution in the gross domestic production of the agricultural sector. Due to the increasing demand and important contribution in agricultural sector, this sector is becoming an area of priority. Although, it has been one of the fastest growing segments of agriculture, the sector also faces numerous emerging challenges. The most prominent ones are pressures emanating from climate change, postharvest losses, bio-security concerns, absence of market linkages and resultant price fluctuations, changing quality

consciousness and global competition. Uttarakhand enjoys almost all kinds of topography from subtropical plains to high altitude temperate hills hence all type of horticultural crops can be grown successfully, however, the productivity of most of the crops being low (1, 2 & 3). The region of Uttarakhand, holds a good potential for budding horticulture enterprises and acts as a basket of raw materials of export the quality for the country. Being a hill state, there lies the immense potential to excel in production of temperate fruits and off-season vegetation, as the state ranks first in the

production of temperate fruits like pear, plum, apricot, and peach in the country, second in walnut, and third in apple production in the country (4). It is blessed with a rare bio-diversity, inter-alia, and 175 rare species of aromatic and medicinal plants that are found in the State. The state has almost all major climatic zones, making it amenable to a variety of commercial opportunities in horticulture, floriculture, and agriculture (5). Despite having numerous policies and facilities for the development of hortipreneurship in the state, entrepreneurship fails to develop in the area. People are migrating to urban sectors for employment leaving their farm dwellings behind. The constraints on improving horticulture crops in the hills include poor orchard management practices, quality planting material, seeds, and other inputs, and little access to extension services and marketing. Across the Himalayan region, producers face problems in accessing market information, postharvest processing, and value-adding skills. Lack of regular markets and reliable marketing, hill farmers in many areas are finding it too risky to diversify into more lucrative high-value crops (6). The number of industries in horticulture is somewhat limited and emerging ones are facing several challenges and wide gaps in marketing, establishing, and running their enterprises. Technological interventions and market knowledge sometimes fail to reach the commoners and hence they are unable to exploit the opportunities to their fullest. The poor support infrastructure and lack of skilled training for budding entrepreneurs lead to underperformance and sometimes ultimate failures in the sector. These factors raise an issue of sustainability of hortipreneurs with their ventures despite the high demand for horticultural products (7).

Materials and Methods

Uttarakhand lies in the foothills of Himalayas where it serves an ideal environment for temperate and off-season horticultural crops. Uttarakhand State was carved out of the state of Uttar Pradesh on November 9, 2000. It is divided into two broad regions-Garhwal and Kumaon. The study was purposively carried out in Nainital and Almora district in Kumaon division of

Uttarakhand in the year 2022-23. Descriptive research has been used for the study of objectives. Total 70 respondents were selected by using snowball sampling method. The interview schedule was divided accordingly to the selected objectives. The questions asked were both open and close ended. Modifications were done afterwards accordingly to the requirements of the study and respondents. The data was arranged, classified and tabulated for further analysis in accordance with study objectives. A semi-structured interview schedule was used for the data collection. A semi-structured interview schedule was prepared based on the objectives of the study.

Constraint Analysis: The analysis of constraints was done using Friedman Test Analysis in SPSS. It is a non-parametric statistical test for testing whether samples originate from the same distribution. It is used for comparing more than two samples that are related. When the Friedman's test leads to significant results, then at least one of the samples is different from the other samples. The prime advantage of this technique over simple frequency distribution is that the constraints are arranged based on their severity from the point of view of respondents. Constraints were divided into four main sub heads viz., Technical, Infrastructure related, Economic and Marketing. The responses to these constraints were recorded on a three-point continuum of 'most severe, severe and not severe' with the respective weightage of 3, 2 and 1. Nonparametric test i.e., Friedman two-way ANOVA by ranks test, as described by (8) was also used to identify the most severe constraints among the four major constraints faced by hortipreneurs using the following formula:

$$F_r = \frac{12}{nk(k-1)} \sum_{j=1}^k R_j^2 - 3n(k-1)$$

Results and Discussion

A. Profile Characteristics of Hortipreneurs: The findings of the present study found that the most of the hortipreneurs were male (82.8%), belonged to general category (82.87%), were in middle age group (48.47%), and were graduate (44.28%). Maximum number of

respondents (81.43%) were married, belonged to nuclear family (74.29%) and had livestock/poultry occupation along with the enterprise (57.14%). Majority of the hortipreneurs had micro scale enterprises (97.14 %) and had small size land holding (85.71%). Maximum number of the hortipreneurs have their available markets locally i.e., 44.29 per cent and more than half of the respondents (55.71%) had low experience in their present enterprise. They had low institutional support (84.28%), low information seeking behaviour (42.86%) and had medium innovativeness (57.14%). Majority (70.00%) of the respondents had medium economic motivation along with medium level of achievement motivation (60%).

It could be inferred from the above results that a greater number of males turned out to be hortipreneurs compared to their counterparts. This due to the fact that male would have been given free choice, liberty and independence to become hortipreneurs to start their own business than female groups. Most of the class from where hortipreneurship was highest belonged to general category (9 & 10). Majority of the hortipreneurs were graduate and it showed that the educated persons with entrepreneurial thoughts showed less interest to work as employee, which strongly indicates a relationship between education and

entrepreneurship (11). The respondents were categorized into 5 categories based on their profession. Table 1 shows that majority (57.14 %) of the respondents had livestock/poultry occupation along with the enterprise followed by 31.44% of the respondents were engrossed with farming. None was found during the study who were having hortipreneurship as their sole medium of income which clearly states that for having risk bearing ability and sustaining their livelihood, the rural hortipreneurs prefer other occupation in which farming and livelihood rearing is a major preference. The enterprises were micro scale and no enterprise belonged to medium size category. The reason for majority of the units being micro followed by small might be the inadequate finance and knowledge in running the enterprise and lack of motivation and self-confidence to take up large scale enterprises. Hortipreneurs with high entrepreneurial experience were found to be less than twenty per cent (11.43%). This may be because there were only 7.14 per cent of the respondents who belonged to old age category. These results also indicate that hortipreneurship has recently started to develop in the area following the pandemic and gradually people have focused their interest towards self-employment.

Table 1. Distribution of respondents on the basis of their profile characteristics (n=70):

S. NO.	CATEGORIES	FREQUENCY	PERCENTAGE
AGE			
a.	Young (30-40)	31	44.28
b.	Middle (41-50)	34	48.57
c.	Old (51-60)	5	7.14
GENDER			
a.	Male	58	82.8
b.	Female	12	17.14
CASTE			
a.	General	58	82.87
b.	OBC	0	0
c.	ST	1	1.42
d.	SC	11	15.71
EDUCATION QUALIFICATION			
a.	Illiterate	0	0
b.	Can read and write only	0	0
c.	Primary School	1	1.42
d.	Middle School	3	4.28
e.	Higher School	5	7.14

f.	Intermediate	16	22.85
g.	Graduate	31	44.28
h.	Post Graduate and Above	14	20
FAMILY TYPE			
a.	Nuclear	52	74.29
b.	Joint	18	25.71
c.	Extended	0	0
OCCUPATION			
a.	Enterprise + Farming	22	31.45
b.	Enterprise + Livestock/ Poultry	40	57.14
c.	Enterprise + Business	4	5.71
d.	Enterprise + Govt. Job	2	2.85
e.	Enterprise + Others	2	2.85
LAND HOLDING			
a.	Small (2-28)	60	85.71
b.	Medium (29-55)	8	11.43
c.	Large (56-82)	2	2.86
SIZE of ENTERPRISE			
a.	Micro (15000-2500000)	68	97.14
b.	Small (2500001-50000000)	2	2.86
c.	Medium (5000001-10000000)	0	0
INCOME			
a.	Low Income (1,55,000-17,70000)	62	88.58
b.	Middle Income (1770001-3385000)	4	5.71
c.	High Income (3385001-5000000)	4	5.71
EXPERIENCE IN ENTERPRISE			
a.	Low (3-7)	39	55.71
b.	Medium (8-12)	23	32.86
c.	High (13-17)	8	11.43
INSTITUTIONAL SUPPORT			
a.	Low (0-1)	59	84.28
b.	Medium (2-3)	9	12.86
c.	High (3-4)	2	2.86
INFORMATION SEEKING BEHAVIOUR			
a.	Low (8-14)	30	42.86
b.	Medium (15-21)	20	28.57
c.	High (22-28)	10	14.29
INNOVATIVENESS			
a.	Low (32-28)	12	17.14
b.	Medium (39-45)	40	57.14
c.	High (46-51)	18	25.71
RISK TAKING ABILITY			
a.	Low (9-12)	10	14.29
b.	Medium (13-16)	40	57.14
c.	High (17-20)	20	28.57
ECONOMIC MOTIVATION			
a.	Low (21-24)	9	12.86
b.	Medium (25-28)	49	70
c.	High (29-32)	12	17.14

ACHIEVEMENT MOTIVATION

a.	Low (13-16)	7	10
b.	Medium (17-20)	42	60
c.	High (21-24)	21	30

The information seeking behaviour was found to be low among the hortipreneurs. Table 1 indicates that majority of the respondents had low information seeking behaviour, followed medium level of information seeking behaviour. The findings inferred that majority of the respondents prefer to use personal localite sources and mass media i.e., friends, local leaders, neighbours and progressive farmers in comparison to any other formal agency as their source for information. All the information sources in personal cosmopolite were negligible except VDO, SMS and Scientists. Among all the information sources of mass media excluding radio and mobile apps, other forms were used frequently with majority using the WhatsApp groups and YouTube. The reason of this might be limited contacts of the hortipreneurs from the personal cosmopolites as well as less use of extension education methods. Extension education methods of information were not that much popular among hortipreneurs and uses of these information sources of extension education methods were also little. It might be due to the cause that extension help fails to reach the people in an effective and adequate manner for which the respondents showed much interest in internet for catering their needs.

A. Constraints faced by Hortipreneurs: An attempt was made to identify the constraints faced by the hortipreneurs. The constraints reported by the respondents were grouped into several categories of constraints viz. technical constraints, infrastructure constraints, marketing constraints and economic and were ranked on the basis of Friedman Mean Score technique. For the Friedman test, the observations were arranged according to the statements in sets under these categories. Scores had been assigned for measurement of observations. The mean scores were calculated by using Friedman Mean formula in SPSS. The estimated mean scores were converted into mean ranks as presented in Table

Table 2. Constraints faced by Hortipreneurs.

S. No.	Constraints	Ranks				
	Technical constraints Friedman Mean Rank Value Score = 3.91	Most Severe	Severe	Least Severe	Mean Score	Overall Rank
1.	Inability to afford the cost of machineries	13	37	20	1.90	V
2.	Lack of year-round supply of raw material	10	41	19	1.87	VI
3.	High seasonality/ perishability of products	32	34	4	2.40	I
4.	Lack of suitable varieties for processing/cultivation	2	41	27	1.64	VII
5.	Poor support for diversification of enterprise	21	27	22	1.99	IV
6.	Lack of training programmes in their locality	22	31	17	2.07	III
7.	Lack of adequate information and technical support	18	40	12	2.09	II
Infrastructure related Constraints Friedman Mean Rank Value Score = 2						
1.	Inadequate facility of specialized transportation.	19	32	19	2.19	I
2.	Lack of sufficient integrated cold chain/storage facilities.	19	28	23	2.12	II
3.	Interrupted power supply with high cost.	3	32	35	1.69	III
Marketing Constraints Friedman Mean Rank Value Score = 4						
1.	Fluctuating price of raw material	18	30	22	1.94	V
2.	Inadequate market infrastructure	12	46	12	2.00	IV

3.	High marketing margin by the middlemen	28	24	18	2.14	II
4.	Difficulty in predicting demand and price of products	25	33	12	2.19	I
5.	Difficulty to meet the quality standards of products for export purpose	1	26	43	1.40	VII
6.	Distress sale of products	32	13	25	2.10	III
7.	Lack of skill to advertise and brand	6	32	32	1.63	VI
Economic Constraints		Friedman Mean Rank Value Score = 3.502				
1.	High operational & managerial cost	28	42	0	2.40	III
2.	No insurance coverage of produce	34	36	0	2.49	II
3.	Prevalence of financial illiteracy among producer	40	30	0	2.57	I
4.	Long payback period	31	15	24	2.10	V
5.	Lack of Initial Capital	12	40	18	1.91	VI
6.	Inadequate credit facilities in the area	38	14	18	2.29	IV

It is clear from Table 2 that the high seasonality/ perishability of products, lack of adequate information and technical support lack of training programmes in their locality were the key technical constraints faced by the hortipreneurs in their enterprises followed by poor support for diversification of enterprise. Horticultural commodities are highly perishable in nature thus there may be a glut of fruits and vegetables in the market during the peak harvest season. These crops undergo a rapid transformation between the harvest and consumption which results spoilage and reduces market value. The spoilage has been estimated to be nearly 30-40 per cent in most of the produce which account much economic losses. This is not only a loss to the growers but a net loss of huge human nutrition and wastage of inputs involved. These losses can be minimized to a considerable surplus with timely and safe management of post-harvest produce.

Furthermore, Table 2 clearly states that inadequate facility of specialized transportation, lack of sufficient integrated cold chain/storage facilities and interrupted power supply with high cost are the infrastructure related constraints faced by hortipreneurs while setting up and running their enterprises. Horticulture crops are mainly perishable in nature as they can contain up to 80-95% moisture, high rate of respiration, more surface area and delicate texture. Lack of harvesting equipments, collection centres in major

producing areas, suitable containers, commercial storage plants and lack of cold chain in complete post-harvest handling are the possible reasons for providing a suitable media for the rotting and senescence. The prevention measures include growing of varieties having better shelf-life, adoption of proper/ recommended cultural practices for the production quality produce free from insect pest, integrated approach to post harvest operations like harvesting at proper maturity, grading, sorting, removal of field heat, use of appropriate packing materials, appropriate packaging, efficient and effective transportation according to the domestic or export purpose and better storage and marketing. The data mentioned in Table 2 also depicts constraints in marketing viz. difficulty in predicting demand and price of products, high marketing margin by the middlemen, distress sale of products, inadequate market infrastructure, fluctuating price of raw material, lack of skill to advertise and brand and difficulty to meet the quality standards of products for export purpose affected hortipreneurs in their horticultural enterprises. It is evident from Table that, economic constraints viz. prevalence of financial illiteracy among producer, no insurance coverage of produce, high operational & managerial cost, inadequate credit facilities, long payback period, lack of initial capital in the area affected hortipreneurs in their horticultural enterprises.

The findings revealed that the marketing constraints had the highest mean rankings derived from using the Friedman test, making them the most severe of the four broad constraints. Technical constraints were the second-worst constraint. Third most severe constraint was economic constraints followed infrastructure related constraints, suggesting that it was the weakest overall constraint. Marketing in India is poor and producers are bound to sell their produce at a through away price through middle men (*adatiyas*) which are infamous for their high margins and exploitation behaviour. If a product is to sell, it must be made readily available to target segment such as fast-food chains, supermarkets, restaurants, hotels, institutional cafeterias and caterers. Hortipreneurs also are now adopting other means of market establishment among the hills by directly targeting their customer groups and establishing their own secure market by selling and producing according to the demand of customers.

Conclusion

The research findings underscore a significant gap in the availability of extension support for hortipreneurs, despite the presence of government policies and initiatives. This highlights the imperative for extension personnel to enhance their outreach efforts. Employing diverse extension teaching methods and optimizing communication networks are pivotal strategies to effectively disseminate technological information and provide necessary assistance to hortipreneurs. This proactive approach will better align with the needs of hortipreneurs and facilitate their entrepreneurial endeavours. Overall, the findings underscore the need for comprehensive support and interventions to address the multifaceted challenges faced by hortipreneurs in hills. Strategies such as enhancing post-harvest management, improving infrastructure, implementing effective marketing techniques, and providing financial literacy and access to credit could contribute to the success and resilience of

horticultural businesses. Additionally, the adoption of alternative marketing approaches, like targeting specific customer groups and establishing secure markets, can empower hortipreneurs to overcome some of these constraints and thrive in the competitive landscape of the horticultural industry.

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A REVIEW ON SPOILAGE AND PRESERVATION OF MILK AND MILK PRODUCTS

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Abstract

Milk and milk products that provide nutrition have been of fundamental importance since ancient times. These are aids for improving the economic status of cultivators and sellers, as well as the health of consumers. However, microbial contamination may cause these products to spoil, resulting in the potential loss of not only producers and vendors, but also consumers. As a consequence, there is an urgent need to investigate the causes of spoilage in addition to their preservation for long-term use. In this review, we examined the fundamental causes of deterioration of the examined products and highlighted their preservation techniques so that they can be used to meet the food demands of a growing global population. Microbiologist, engineer, and technologist are challenged by the variety of dairy foods to discover the most effective ways to prevent the entry of microorganisms, destroy those that do enter along with their enzymes, and prevent the growth and activities of those that survive processing. Aerobic psychrotrophic Gram-negative bacteria, yeasts, fungi, heterofermentative lactobacilli, and spore-forming bacteria are problematic spoilage microorganisms. The level of recontamination of pasteurized fluid milk products with psychrotrophic bacteria is a significant factor in determining their shelf life. In addition to visible changes in color or texture, the deterioration of dairy foods caused by fungi is characterized by the presence of a wide variety of metabolic by-products, which produce off-odors and flavors and cause visible color or textural changes.

Key Words: Microbial, Spoilage, Preservation, Milk and Milk Products.

Introduction

India is an agricultural nation that has undergone the white revolution and developed diverse dairy products to satisfy the needs of a growing population. Indian nationals consume a substantial quantity of dairy products such as lussy, shrikhand, basundi, ice cream, badam shake, etc. as desserts. Since the beginning of human civilization, however, these foods have always been subject to preservation errors over the long term. Due to improper preservation and storage facilities in India in cold environmental

settings, Indians are losing value-added food products, resulting in a loss for the manufacturer's economy and a worsening of malnutrition in the country, particularly in areas of poverty.

In this review, we focused on the most significant issues pertaining to the spoilage and preservation of milk and milk products, as well as the probable solutions that would support the economy and enhance the health of people, resulting in a superior economic status for producers.

Microbial Putrefaction

Microorganisms are the microscopic organisms responsible for the rapid deterioration of milk and milk products. Milk is lacteal secretion that is pure, fresh, and complete, and it is obtained from animals that are milked. According to (25), milk spoilage is the deterioration of its flavor, texture, and pigment that renders it unfit for human consumption. Milk is a potentially nutritive medium for the proliferation of microorganisms. Likewise, spoilage of milk and its derivatives may result in flavor and texture changes. Similarly, bitterness and moldiness can develop in milk products (19). Important dairy products become inedible when decomposition alters their flavor, nutritional value, and texture (6). (6) proposed a similar viewpoint and reported that deterioration is responsible for altering the texture, flavor, and nutritive value of dairy products, thereby rendering them unsafe for human ingestion.

Globally, contamination of milk and its products by microorganisms is a significant problem. It occurs during the processing of dairy products like khoa. Khoa on the market was contaminated with microorganisms due to a delay between processing and selling, primary bioburden on used implements, and improper handling and storage procedures (Ghodekar et al., 1974). Spoilage of food materials by microorganisms is caused by the degradation of fats, proteins, and carbohydrates by microorganisms or by enzymes produced by them (36, 4 and 4, 26).

There are numerous contamination sources for milk and milk products. The presence of feces can contaminate *pera*, *burfi*, and *khoa*. Numerous microflora are responsible for the degradation of milk and milk-based products. Pathogenic microflora, such as *Salmonella typhi*, *E. coli*, etc., can persist for extended periods in *khoa*, according to Bhat et al. Similarly, the presence of staphylococci and other food-spoiling organisms led to the loss of dairy products, exposing consumers to a substantial health risk. Milk with a higher water content is susceptible to microbial proliferation. Due to its high water and biochemical content, milk also serves as an optimal growth medium for numerous microbiota. In addition, it provides the essential nutrients

required for microbial proliferation.

Additionally, inadequate sanitation practices contribute to the decomposition of milk. According to Khan A.Q. (2006), low-quality cleaning in the production unit leads to microbial contamination, which reduces the shelf life of final products, and many products are introduced to the market with negligent packaging and exposure to environmental contamination. Similarly, milk and milk products become contaminated during marketing due to improper storage and handling. *Khoa* and delicacies derived from it become contaminated even when processed in sterile environments, and in India, molds contaminate dairy foods due to humid conditions.

In addition to coliforms, the preponderance of contaminants in it are psychrotrophs (5). In addition, the authors asserted that milk is a preferable source for microbial growth and spoilage microorganisms originating from the milkmaid, utensils, animal hair, water, and soil. Additionally, grass, feed, soil, and milking equipment are responsible for bacterial contamination of unprocessed milk. The milk is spoiled by both bacteria and fungi, resulting in economic loss for the dairy industry. Similarly, bacteria and fungi are responsible for the spoilage of milk products (6), and these products are susceptible to spoilage by a variety of organisms (22;).

Diseases are caused by the consumption of contaminated milk. This viewpoint was supported by (29), who asserted that contaminated milk is the cause of disease outbreaks. (11) reported that milk and milk derivatives are the healthiest options for Indians. Milk may be contaminated with microorganisms that cause deterioration, such as *Achromobacter*, *Alcaligenes*, etc., or pathogens that cause disease, such as *Staphylococcus aureus* (12). (11) Milk and dairy product contamination that occurs from the farm to the consumer is a worldwide issue. Nonetheless, the cleansing process safeguards consumer health (11).

Additionally, contaminated milk may contribute to the spread of infectious diseases. This notion was corroborated by (11), who reported that milk can serve as a medium for a variety of microorganisms that cause spoilage or disease.

Psychrotrophs have the greatest destructive potential in mesophilic conditions. Since numerous outbreaks of epidemics have been linked to the ingestion of raw milk, this practice should be discouraged (20). It is necessary to develop techniques that are both efficient and sensitive for monitoring the presence of psychrotrophs that degrade nutrients. In addition, milk is a medium in which microorganisms thrive (35) and may cause health issues.

Milk with fewer pathogens is of superior quality. Milk's microbial load is a major factor in determining its quality (33). Due to its chemical composition, milk is susceptible to microbial spoilage (35), and it is a potent growth medium for common spoilage organisms such as yeasts and molds (35). Pasteurized milk can be spoiled by heat-resistant streptococci that produce lactic acid and lower the pH. Moreover, milk is contaminated where it is collected (35).

In addition, dairy products should be inspected for their microbial burden, as microflora such as Gram-negative psychrotrophs, molds and yeasts, lactic acid bacteria, and coliforms contribute to their spoilage. *E. coli* is used as an indicator of the microbial quality of both milk and its derivatives. Molds and yeasts reduce the purity of milk, even in small quantities. Milk has nutritional value and is not only the optimal medium for microbial growth (7), but is also highly susceptible to microbial degradation.

Conservation techniques

To meet the requirements and demands of a growing global population, it is imperative that preservation strategies for milk and milk products be developed immediately. This view was supported by (6), who stated that preventing milk product deterioration is necessary to control economic loss in the dairy industry. According to (34), there would be practical benefits to microbial investigation of milk and its products. For milk to be secure for human consumption, it must be protected from external contamination during production, storage, and transportation (9). Depending on the facilities available in various nations, milk preservation strategies may vary. Indian rural residents preserve milk by producing *khoa* and *ghee* (34), and in India, *khoa* is regarded

as an excellent milk-preservation agent.

Pathogen activity reduction may reduce the risk of infection to some extent. By inactivating microbial burden responsible for spoilage, pathogens can be reduced and milk's shelf life can be extended (20). It is accomplished by means of high temperature treatment (Gedam et al. 2007), UV exposure (15), microwave treatment (31), membrane processing and microfiltration. In contrast, (20) argued that pasteurization does not sterilize milk, even though it is extensively used. To lengthen the expiration life of milk and milk products, sufficient preservation facilities should be made available.

Similarly, after milking, the milk can be stored for only 3 minutes and its shelf life can be extended to 24 hours at 5°C (Pal M et al. 2018), while it can be increased to 4 to 7 days via the pasteurization procedure (11). According to (19), pasteurization of milk at a temperature between 55°C and 70°C is capable of destroying bacteria, and according to Gunasekera (1993), it was implemented to eliminate human pathogens and reduce the development of spoilage-causing microorganisms.

Improve the milk's durability in order to preserve it for an extended period of time. Durability of pasteurized milk is influenced by duration of raw milk storage, heat exposure, availability of heat-resistant microflora, amount of contamination occurring after pasteurization, packaging platform employed, and storage conditions after pasteurization (Cromie 1991). Moreover, light (16) and raw milk quality (16) also affect pasteurized milk. The shelf life of dairy products can be extended through proper packaging and storage (11).

A significant quantity of pathogens are eliminated when milk is heated to high temperatures. Heat reduces the number of microorganisms in food. According to Jayamanne and Samarajeewa (2010), both High Temperature for a Short Time and Low Temperature for a Long Time were capable of killing *L. monocytogenes* in low concentrations. According to (17), bacteria that grow in cold environments and have the ability to produce spores have the potential to grow in pasteurized milk. According to Meer et al. (1991), this problem has become the primary obstacle in extending the shelf life of products beyond 14 days.

The shelf life of milk can be enhanced by chemical treatment diminishing pathogenic bioburden. Both chlorine and ozone are able to effectively inactivate microorganisms present in biofilm. (8) discovered a decrease in microbial load in pasteurized milk and suggested the creation of an automatic disinfection and cleansing system. CO₂ can be utilized to extend the shelf life of fresh milk (18). (2) supported this idea by stating that N₂ can be used for the same purpose. This is possible due to a decrease in the number of bacteria, as well as a decrease in the lysis of proteins and the degradation of lipids (18).

In contrast, heat-sensitive food products cannot be treated with heat, necessitating the use of alternative techniques to eliminate pathogens, including their particles. Similarly, milk-borne spores can be eliminated effectively through microfiltration (20). The authors also proposed that pasteurization can reduce the bioburden of milk, that improved hygienic practices during milking can prevent milk contamination, and that microfiltration can be used to completely remove milk spores to prevent contamination of pasteurized milk. Milk that is microbiologically safe can be produced by microfiltration's complete removal of microorganisms (20).

In order to extend the shelf life of milk, the implementation of recommended storage conditions becomes a necessity. Kumaresan G et al. (2007) supported this notion and argued that milk should be stored at 2°C for two days prior to being used to make its products, such as cheese, because the milk's shelf life is increased by two days at this temperature. Moreover, Sowmya Y (2017) asserted that new preservatives and technologies are necessary for preventing the development of microflora responsible for milk spoilage and extending the shelf life of dairy products. Pasteurization does not guarantee complete eradication of milk when it is significantly contaminated in its raw state and after pasteurization (27). By storing milk at the correct temperature, not only suppliers but also consumers can delay milk spoilage (7).

Consumption of contaminated milk and milk products can result in severe health issues and place the life of the consumer at risk. Due to the

fact that milk is susceptible to microbial proliferation and may contain pathogens, its consumption may be detrimental to health (20). The milk is protected by pasteurization (20). Milk that has been pasteurized can be made safe through proper pasteurization, milk storage at a low temperature, and the prevention of contamination after pasteurization (20). (11) proposed the use of contamination control to ensure the availability of safe, acceptable, and nutritious milk and milk products for food safety.

It is essential to establish methodologies for prevention of contamination of dairy products by microorganisms at all steps of the chain related with food supply (11). People with immune suppression issues favor milk and other dairy products susceptible to *L. monocytogenes* in India (30). (30) observed that in the Indian city of Tiruchirappalli, Tamilnadu, both infants and the elderly were at risk for listeriosis. (7) concluded that methylene blue reduction and phosphatase assays are recommended for determining the safety of milk for human consumption.

According to (29), the quality of raw milk in terms of its hygienic standards can be improved by disinfecting and cleaning milking machinery. In addition, (29) reported that a price incentive based on the microbial quality of raw milk may increase the microbial quality of raw milk. The presence of bacteria that wreak havoc on milk products can be reduced by proper sanitation (35).

Contamination source of milk

The mammals that provide milk include cows, buffalo, sheep, and goats. When obtained from animals, milk is not sterile. The sources of tainted milk are:

- contamination from animal dairy
- the interior and exterior of the udder, as well as
- the coat of lactating animals
- the atmosphere encompassing the farm and storage
- animal feed, the quality of water used, the quality of milk handling apparatus and storage tanks, and the formation of biofilms on dairy plant pipes.

Microbial Spoilage of milk and milk products

Milk is an outstanding growth medium for numerous microorganisms.

Therefore, various microorganisms proliferate and cause deterioration.

Fermentation of Milk and cream:

- Souring is indicated by an acidic taste and the coagulation of milk into a solid curd-like substance.
- Numerous lactic acid bacteria, coliform bacteria, and additional bacteria ferment the sugar in milk to produce acid. *Streptococcus lactis* is most likely to cause fermentation at 10-37°C, with possible proliferation of *Coliform*, *Enterococci*, *Lactobacillus*, and *Micrococcus*.
- *Streptococcus thermophilus* and *Streptococcus faecalis* may produce 1% acid at 37 to 50 degrees Celsius, followed by *Lactobacillus*, which produces more acid. At refrigeration temperatures, milk undergoes minimal souring.
- However, thermotolerant lactic acid bacteria such as *Enterococcus*, *Streptococcus thermophilus*, *Lactobacillus*, etc. are permitted to survive pasteurization.
- If conditions are adverse for lactic acid bacteria, acid is produced by bacteria other than lactic acid bacteria. coliforms, for example, produce acetic acid, formic acid, ethanol, CO₂, and hydrogen. *Clostridium* produce butyric acid similarly.

Gas production (Strong fermentation of milk): Organisms that ferment sugar also produce acid and gas

- Principal gas-forming organisms, such as *Coliform*, *Clostridium*, heterofermentative lactic, and *Propionibacterium*.
- *Coliform*, *Clostridium*, and *Bacillus* are the only microorganisms that produce both H₂ and CO₂, while all others produce only CO₂.
- Foam on the surface of liquid milk, confined gas bubbles in curd, and the formation of curd are indications of gas production in milk.

- Excessive gas production results in the shattering or disintegration of curd, causing what is known as stormy fermentation of milk.
- Typically, *Clostridium perfringens* induces turbulent fermentation.
- Proteolysis is aided by storage at a reduced temperature, which destroys lactic acid bacteria, or by the distribution of acid already produced by mold and yeast.

Changes brought about by a proteolytic organism include:

- Acid proteolysis characterized by concurrent acid production and proteolysis.
- Proteolysis that is neither acidic nor alkaline.
- Sweet curdling caused by a renin-like enzyme produced by microorganisms.
- After their autolysis, bacterial intracellular enzymes perform a sluggish proteolysis.
- Some heat-stable proteinase has residual proteolytic activity.
- Acid proteolysis is caused by *Micrococcus*, *Streptococcus faecalis* var *liquefaciens* var *liquefaciens*, and certain lactose-fermenting, proteolytic *Bacillus* species.
- *Bacillus cereus* produces sweet curdling.
- Ropiness/ sliminess: Ropiness of milk can be caused by both bacterial and non-bacterial factors. During cooling, non-bacterial ropiness occurs due to the thickness of the cream or a deposit of casein or Lactalbumin.
- Bacterial ropiness is caused by bacteria's viscous capsular material, which typically forms at low storage temperatures.
- *Alcaligenes viscolactis*, *Micrococcus freudenreichii*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, and *E. coli* are the bacteria responsible for ropiness in milk.
- Changes in milk fat: Bacteria, yeast, and molds hydrolyze milk fat, resulting in rancidity.
- Lipolytic bacteria include *Proteus*, *Pseudomonas fragi*, *Staphylococcus*, *Bacillus*, *Micrococcus*, *Clostridium*, etc.
- *Pseudomonas fragi* and *Staphylococcus aureus* manufacture lipases that are moderately heat resistant.

- Alkali production is accomplished by *Pseudomonas fluorescens* and *Alcaligenes viscolactis*.
- The production of alkali is caused by the formation of ammonia from urea and carbonate from organic acid.

Flavor flaw

- The acidic flavor may be aromatic or harsh. Coliform and *Clostridium* produce acetic acid, formic acid, butyric acid, and other sharp-tasting acids. It is unwelcome. When *Streptococcus lactic* and *Leuconostoc* thrive together, they produce an aroma. It is to be desired.
- *Streptococcus lactic* var. *maltigenes* is responsible for caramel or caramelized flavor.
- Bitter taste is the result of a proteolytic organism.
- Other flavors include earthiness from *Actinomycetes*, fruitiness from *Pseudomonas fragi*, and soapiness from *Pseudomonas sapolactis*, among others.
- The growth of pigmented microbes and other organisms results in an undesirable hue. Examples include the following:
 - It is caused by the bacteria *Pseudomonas synchyaneum*.
 - Yellow milk is the result of *Pseudomonas synchyanea* and *flavobacterium*.
 - The bacteria *Serratia marcescens* and *Micrococcus roseus* cause red milk.
 - Brown milk is the result of *Pseudomonas putrefaciens* and the enzymatic oxidation of tyrosin by *Pseudomonas fluorescens*.

Conclusion

The deterioration of milk and milk products is a significant health risk because it causes disease outbreaks. Current preservation methods for milk and milk products, such as pasteurization, are inadequate because they do not guarantee the microbiological safety of milk and milk products. Therefore, it is necessary to devise techniques and methods of the next generation for their preservation. In addition, standard training of

individuals, quality cleaning practices, recommended preservation and transportation facilities, etc., may aid in meeting the global demand for milk and milk products for an extended period of time by increasing their stabilities.

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