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Effect of Long Term Nutrients Management on Physical Properties of Soil During Pearl Millet-Fallow Cropping System Under Dry Land Conditions

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Abstract: The present investigation was carried out on the on-going long term experiment started in 2014 to study the yield sustainability and maintenance of soil fertility at Research Farm of Department of Agronomy, CCS, HAU, Hisar. The experiment was planned in a randomized block design (RBD) with four replications and five treatments *viz.* T_1-control, T_2- RDF(Recommended Dose of Fertilizer *i.e.* N 40 Kg ha^{-1} and $P_20_5$20 kg ha^{-1}), T_3- FYM (Farm Yard Manure) @ 6.7 t ha^{-1}, T_4-Vermi-compost @ 2.5 t ha^{-1}, T_5-Integrated (50 % N through FYM + 50 % N through Urea) which were applied during *kharif* season in pearl millet crop. The physical properties of the soil such as bulk density, infiltration rate, saturated hydraulic conductivity (Ks), mean weight diameter, water stable aggregate, soil moisture retention and penetration resistance of the soil improved with addition of organic matter either alone or in combination with fertilizer. The increase in infiltration rate was significant over control. Bulk density of the soil was negatively and linearly correlated with volumetric water content at field capacity and permanent wilting point while was found to be positively and linearly correlated with K_s.

Keywords: RDF, FYM, Vermin-compost, Pearl millet, Saturated hydraulic conductivity, Organic matter

Pearl millet crop is well known for its ability to produce consistent grain and forage yields despite crop production constraints such as low productive sandy soils, hot and dry climatic conditions (Jukanti et al 2016). Pearl millet excels all other cereals due to its unique features-C_4 plant with high photosynthetic efficiency, high dry matter production capacity and is grown under the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yields. Pearl millet [*Pennisetum glaucum* (L.)], the world's hardiest warm-season cereal crop (Reddy et al 2012), ranks sixth in terms of region after rice, wheat, maize, barley, and sorghum (Khairwal et al 2007) and contributes 42 % of total global production with an average productivity of 1156 kg ha^{-1}. India is the world's leading producer of pearl millet (10.05 MT) (Bhardwaj et al 2014). It is usually grown in climates with annual rainfall ranging from 150 to 700 millimeters (Khairwal and Yadav, 2005). It is a grain that is primarily grown in Eastern Uttar Pradesh and Haryana where rainfall is scarce.Pearl millet-wheat cropping system is the second most important after rice-wheat cropping system in the Indo-Gangetic plains of India. Pearl millet can extract a high yield in arid and semi-arid regions due to its ability to withstand high temperatures and dry climatic conditions, compared to other crops that would have performed poorly. Pearl millet is an exhaustive crop which needs heavy dose of fertilizer to meet its nutrient requirement. Inorganic fertilizers increase crop yield by providing immediate plant nutrients, but long-term usage can reduce macro and micro aggregate stability, moisture retention, and bulk density, lowering productivity (Sarkar et al 2003). Inorganic fertilizer consumption increased from 0.07 Mt in 1950-1951 to over 25.0 Mt in 2009-2010 as a result of haphazard application. Aside from that, there is a significant gap between inorganic fertilizer production and consumption in the region, forcing farmers to supplement plant nutrients with organic manures and composts. Organic manures provide nutrients to plants in limited amounts, and their release pattern is slower than chemical fertilizers, but the presence of growth hormones and enzymes helps to sustain soil quality, increasing development and efficiency, and therefore soil fertility. It is thought that long-term use of chemical fertilizers would degrade the physical, chemical, and biological properties of the soil (Singh et al 1999). Using an integrated nutrient strategy and balanced fertilization, such degraded soils can be brought back to life (Gudadhe et al 2015).Bhatt et al (2017) observed that applying fertilizers combined with FYM reduced bulk density for surface and sub-surface soil.Mubarak et al (2014) reported an increase in infiltration rate when organic manure and fertilizer were used together.Kumar et al (2012) reported the maximum saturated hydraulic conductivity at 0-10, 10-20, and 20-30 cm soil depth when comparing plots where FYM was applied continuously for 22 years to control plots. Higher values of soil saturated hydraulic conductivity due to increased organic carbon content and decreased soil bulk density, the long-term application of organic materials with fertilizers improved the soil structure.In order to sustain productivity, use of organic source of nutrients in combination with inorganic fertilizers is necessary not only to maintain soil fertility but also to improve the efficacy of chemical fertilizers (Bagla et al 2008). Hence, nutrient supply through organic source such as bio-fertilizers and vermicompost in conjunction with chemical fertilizers is necessary to meet the crop nutrient requirement and for sustaining agricultural productivity, without detracting from the fact that chemical fertilizer will continue to be main production input for quickening the pace for agricultural production (Kumar et al 2014). Therefore, use of both organic manure and chemical fertilizers in appropriate proportion assumes special significance as complementary and supplementary to each other in crop production and sustainability of soil health.

Keeping all these facts in consideration, the present study (initiated in 2014) was undertaken in the on-going long term experiment at DLA Research Farm, Department of Agronomy, CCS HAU, Hisar in 2020-21 to explore the long term use of fertilizer with or without organic manures on soil physical properties in pearl millet- fallow cropping system.

MATERIAL AND METHODS

The experiment was started during *Kharif* 2014 and pearl millet-fallow system was followed. The field experiment was conducted during *Kharif* season of 2020 at Dryland Agriculture Research Farm, CCS Haryana Agricultural University, Hisar. The average annual precipitation of the experimental site is estimated to 425.5 mm and most of which is received from South-Western monsoon during July to September. The experiment was planned in a randomized block design (RBD) with four replications and five treatments *viz.* T_1-control, T_2-RDF(Recommended Dose of Fertilizer *i.e.*N 40 Kg ha^{-1} and P_2O_5 20 kg ha^{-1}), T_3- FYM (Farm Yard Manure) @ 6.7 t ha^{-1}, T_4-Vermi-compost @ 2.5 t ha^{-1}, T_5-Integrated (50 % N through FYM + 50 % N through Urea) which were applied during *kharif* season in pearl millet crop.Pearl millet hybrid 'HHB 67 (Improved)' was used in row spacing at 45 cm. The other agronomic practices were followed as per package of practices during the crop growth period. The FYM and Vermicompost were applied one month prior to the sowing. Soil samples were taken at a depth of 0-15 cm before sowing and after harvest of crop and examined in the laboratory for physical characteristics using the standard techniques. Bulk density was determined using galvanised metal cores from 0–15 and 15–30 cm depth. Undisturbed soil samples were taken from all treatments before seeding of pearl millet 2020. To determine the dry weight of the soil, the soil cores were dried in an oven at 105° C for 24-48 hours. The soil's bulk density (Mg m^{-3}) was calculated as the ratio of the dry weight of the soil to the inner volume of the metallic core (Bodman 1942). At crop harvest, the Infiltration rate (cm hr^{-1}) of water from the surface of each plot was determined using double ring infiltrometer methods (Bertrand 1965). The soil cores used to calculate bulk density were also used to calculate saturated hydraulic conductivity using constant head method in the laboratory (Richards 1954). Soil moisture was determined using undisturbed soil samples obtained at 0-15 cm soil depth for bulk density. Soil moisture was determined in the laboratory by measuring the water content of soil samples at 0.3 and 15.0 bar pressures with a pressure plate apparatus (Richards 1954). The soil core samples were saturated overnight, placed in contact with a saturated pressure plate, and brought to equilibrium using the pressure plate apparatus at 0.3 and 15.0 bar. To determine the soil water content at 15 bar, saturated samples and the saturated pressure plate of the pressure plate equipment were retained. The soil samples were equilibrated at 15 bars. When the water flow through the outflow tube was stopped, the soil water content was calculated gravimetrically at each applied pressure. The volumetric water content was calculated by multiplying the gravimetrical water content by the bulk density of the sample. The wet sieving method was employed to analyse the aggregates (Yodder 1936). After harvest of pearl millet in 2020, soil penetration resistance was evaluated using a digital penetrometer (Davidson, 1965) by inserting it very softly and perpendicular to the soil surface.

RESULTS AND DISCUSSION

Soil bulk density: It as affected by various nutrient management strategies demonstrated a significant decrease at surface and subsurface soils in the treatments viz. vermicompost (T_4) and FYM (T_3) and integration of FYM with fertilizer (T_5) as compared to control and RDF (T_2) (Table 1). The bulk density decreased up to 5.4, 4.7 & 6 % in surface and 5.9, 6.5 and 4.6 % in sub-surface soil in T_3, T_4 and T_5 treatments, respectively over the control. Organic source of nutrient would have improved the structure of the soil by improving total aggregation which leads to improvement in the porosity of soil. The increase in aggregation and decrease in bulk density may also be attributed to increase in microbial activity that may release polysaccharide which holds the particles together to form stable soil structure. As a result, the soil bulk density reduced, resulting in increased pores space. Application of RDF also significantly decreased the bulk density of the soil as it may promotes more vigorous growth of plant root and may increases the biomass when compared to control. The highest bulk density in control is owing to a lack of fertilizer and organic manure treatment, as well as increased compaction. Furthermore, increase bulk density was observed in subsurface soil, which could be associated with low organic carbon content at 15-30 (Table 1.) cm soil. Furthermore, the weight of the top layer increased the subsurface soil bulk density. Moharana et al (2017) showed a reduction in bulk density of the soil after six years of constant organic matter addition as compared to control and fertilizer treatments at 0-15 cm and 15-30 cm soil depths. These findings showing a reduction in bulk density due to the inclusion or application of FYM are also consistent with the findings of Rudrappa et al(2006),Gong et al (2009) and Narwal et al (2010).Singh et al (2016) carried out to analyzed the effect of long-term FYM and vermicompost treatment on bulk density of sandy loam soil under pearl millet–wheat cropping system and found that bulk density reduced 8.4 and 6.9 % in *rabi* and*kharif* respectively at surface soil. Furthermore, bulk density of the soil was found to be negatively and linearly correlated with volumetric water content at field capacity and permanent wilting point in surface and sub-surface, as evidenced by R^2 values of 0.65 and 0.76 (Fig. 1) and 0.79 and 0.80, (Fig. 2) respectively. A similar correlation was observed for soil bulk density with infiltration rate (R^2 0.76 and 0.74) at the surface and subsurface (Fig. 4). The bulk density of the soil was positively and linearly correlated with saturated hydraulic conductivity (K_s) at 0-15 cm and 15-30 cm depth with R^2values of 0.75 and 0.69, respectively (Fig. 3). This significant correlation of bulk density implies an increase in the soil saturated hydraulic conductivity, field capacity, moisture content, and infiltration rate due to the addition of organic matter, which may have enhanced aggregation and resulted in more pore space (porosity).

Infiltration rate: The rate of infiltration varies according to soil type, organic matter concentration, water stable aggregates, and other factors. The increase in infiltration

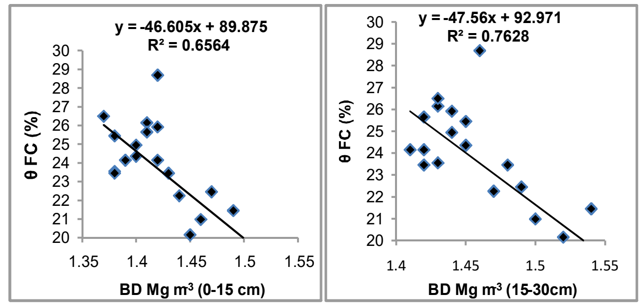

Fig. 1. Relationship of bulk density with volumetric water content at field capacity (Θ_{FC}) at 0-15 cm and 15-30 cm depth

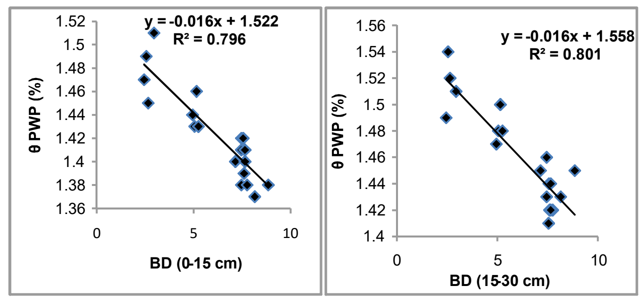

Fig. 2. Relationship of bulk density with volumetric water content at PWP (Θ_{PWP}) at 0-15 cm and 15-30 cm depth

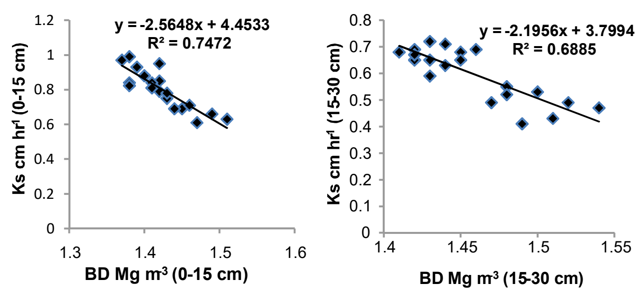

Fig. 3. Relationship of bulk density with saturated hydraulic conductivity (K_s) at 0-15 cm and 15-30 cm depth

Table 1. Effect of long term nutrients management on soils bulk density, saturated hydraulic conductivity andinfiltration rate during pearl millet-fallow cropping system under dryland condition

Treatments	Bulk density (Mg cm^{-1})		Saturated hydraulic conductivity (cm hr^{-1})		Infiltration rate (cm hr^{-1})
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	
T1	1.48	1.52	0.65	0.45	0.70
T2	1.44	1.48	0.73	0.52	0.94
T3	1.40	1.43	0.88	0.67	1.32
T4	1.41	1.42	0.82	0.65	1.27
T5	1.39	1.45	0.95	0.69	1.40
CD (p = 0.05)	0.032	0.02	0.06	0.06	0.05

rate was significant over control which recorded maximum for integrated treatment followed by, FYM and vermicompost sole treatment, RDF, and the lowest for control. The infiltration rate increased up to 88.5, 81.5 and 100 % in T_3, T_4 and T_5 treatments over control. The high infiltration rate was caused by higher organic matter, which was thought to promote pore size distribution and consequently soil structure and soil health. Singh et al (2007) observed that treatments with organic manures have higher infiltration rate than controls. Reddy et al (2017) and Chauhan et al (2018) demonstrated significant findings on infiltration rate due to long-term application of nutrient management.

Infiltration rate was positively significant and linearly connected with water stable aggregates and mean weight diameter of aggregates, with R^2 values of 0.96 and 0.92, respectively (Fig. 5). A rise in soil aggregation may results in an increase in pore size distribution, resulting in increased water infiltration over time.

Saturated hydraulic conductivity (K_s): Saturated hydraulic conductivity is affected by soil pore size distribution and total porosity soil, which are controlled by soil structure, texture, and organic carbon content. The Ks increased up to 35, 26 and 46 % in surface and 48.8, 44.4 and 53 % in sub-surface soil in T_3, T_4 and T_5 treatments respectively, over control. The higher saturated hydraulic conductivity in surface and subsurface soils with the addition of vermicompost, FYM individually or in combination with RDF treatments was attributable to improved soil aggregation, which enhanced porosity and hence hydraulic conductivity. The reduced hydraulic conductivity of subsurface soil compared to surface soil was owing to a lower amount of organic carbon at lower depths. Verma et al (2010) investigated the long-term use of various nutrient management strategies and observed higher hydraulic conductivity in *Typic Haplustept* in a maize-wheat cropping system. Singh et al (2016) also showed an increase in hydraulic conductivity of 15.7, 26 and 31 % and 12, 26 and 31 % in 15, 30 and 45 t FYM ha^{-1} in the *rabi* and *kharif* seasons, respectively. They also obtained the higher Ks in surface 0-5 cm, as opposed to 5-10 and 10-15 cm plots in FYM treatment. Katkar et al (2012) reported a similar pattern in hydraulic conductivity when organic matter was present. Earlier workers also observed similar findings(Bhattacharyya et al (2006), Mosaddeghi et al (2009) and Shwetha and Varija (2015).

Water stable aggregates (WSA) and mean weight diameter (MWD): Long-term nutrient management strategies had a significant impact on the water stable

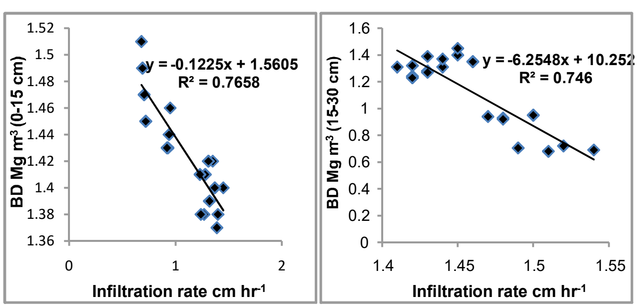

Fig. 4. Relationship of bulk density with infiltration rate at 0-15 cm and 15-30 cm

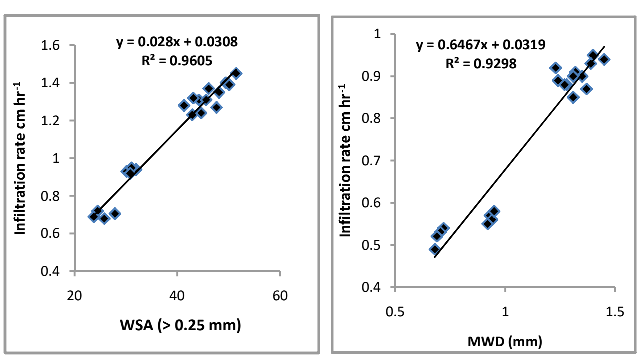

Fig. 5. Relationship of infiltration rate with water stable aggregates (WSA) and mean weight diameter (MWD)

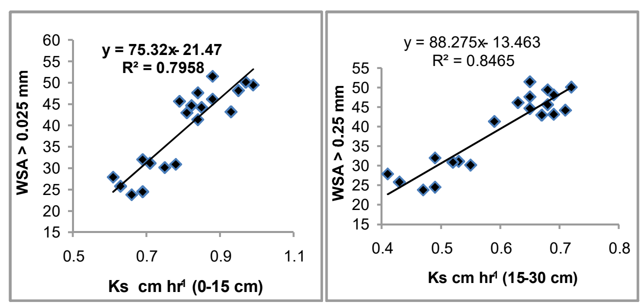

Fig. 6. Relationship of water stable aggregates with saturated hydraulic conductivity (Ks) at 0-15 cm and 15-30 cm soil depth

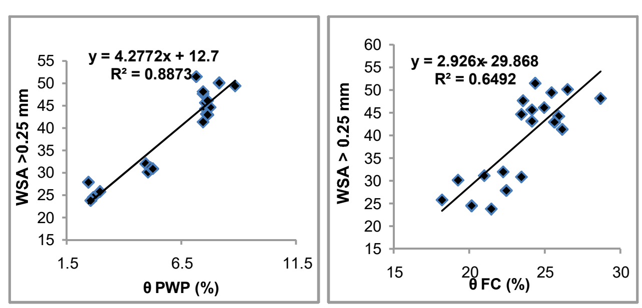

Fig. 7. Relationship of water stable aggregates with volumetric water content at field capacity and permanent wilting point at surface soil depth

aggregates (>0.25 mm) and mean weight diameter in the pearl millet crop. Mean weight diameter increased up to 9.6, 69, 73 & 77.3 % while water stable aggregates enhanced up to 21.7, 77.6, 71.6 and 95.3 % in T_2, T_3, T_4 and T_5 treatments over control, respectively. Addition of organic matter in the form of FYM or vermicompost either single or in integration with fertilizer significantly increased aggregate stability against water, due to more strong bonding of soil particles with each other or with organic matter, thus preventing its dispersion against disruptive action of water. Also FYM and vermicompost would increase the microorganism activity that might be responsible for formation of stable aggregates. Water stable aggregates has a significant and positive association with saturated hydraulic conductivity (Fig. 6), volumetric water content volumetric water content at field capacity and permanent wilting point (PWP) (Fig. 7), with R^2 values of 0.84 and 0.79 at 0-15 and 15-30 cm, respectively, while with FC and PWP having R^2 values of 0.64 and 0.88, respectively. The correlation coefficient was observed between WSA, θ_{FC} and θ_{PWP} which further showed the impact of organic matter in increasing the WSA & MWD of aggregates. Infiltration rate was shown to be highly significant and associated to WSA and mean weight diameter with $R^2$0.96 and 0.92, respectively (Fig. 8).

Chakraborty et al (2010) reported an improvement in water stable aggregates in integrated nutrient management of 100 % NPK + FYM. Furthermore, they observed a considerable positive association between aggregation indices and soil organic carbon in aggregates with sizes ranging from 8 to 4 mm. Similarly, increasing aggregate MWD significantly increased volumetric water content at field capacity and permanent wilting point (Fig. 9 and 10) with R^2 values of 0.65 and 0.87, respectively, and saturated hydraulic conductivity with R^2 values of 0.77 and 0.87 for 0-15 and 15-30 cm soil depth, respectively.

Bhatt et al (2017) evaluated the effect of vermicompost and fertilizers individually and in combination with FYM (Integrated) for 29 years experiment and found that the integrated treatment increased MWD and WSA by 31.74 and

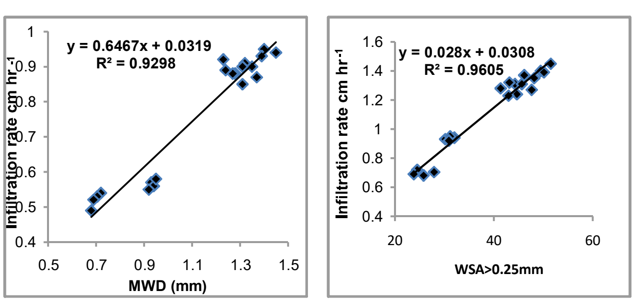

Fig. 8. Relationship of infiltration rate with mean weight diameter and water stable aggregates

53.35 and 21.45 & 27.16 percent in surface and sub-surface over the control.

Soil moisture retention: The study indicated that soil moisture retention at field capacity & PWP moisture content was found higher in treatment where FYM, vermicompost and FYM along with urea were applied with significant increase in soil retention as compared to control and RDF treatments (Table 1). Moisture retention at field capacity and permanent wilting point increased by the application of organic fertilizer up to 4, 19.8, 20 and 27 % and 92.4, 185.5, 186.8 and 198 % in T_2, T_3, T_4 and T_5 treatments as compared to control, respectively. Among treatments T_3, T_4& T_5 the

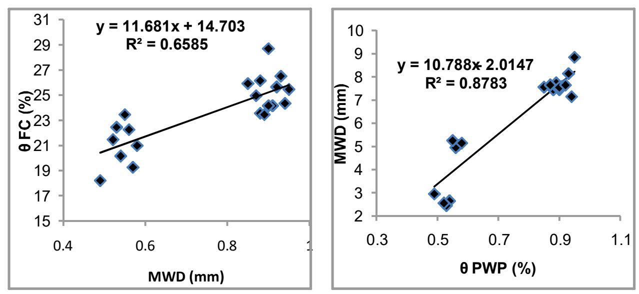

Fig. 9. Relationship of mean weight diameter with volumetric water content at field capacity and permanent wilting point

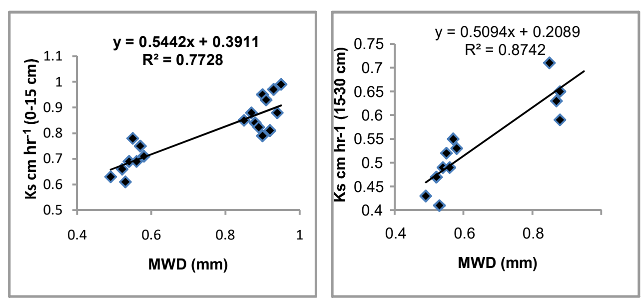

Fig. 10. Relationship of mean weight diameter (MWD) with saturated hydraulic conductivity (Ks) at 0-15 cm and 15-30 cm soil depth

Table 2. Effect of long term nutrient management on WSA (%), MWD (mm) and soil moisture (%) at different suctions of the soils during pearl millet-fallow cropping system under dry land condition

Treatments	Aggregate size analysis		Soil moisture (%)	
	MWD (mm)	WSA (%)	FC	PWP
T1	0.52	25.50	20.56	2.65
T2	0.57	31.05	21.48	5.10
T3	0.88	45.30	24.64	7.57
T4	0.90	43.65	24.85	7.60
T5	0.93	49.80	26.25	7.90
CD (p = 0.05)	0.03	2.55	2.55	0.55

Table 3. Effect of long term nutrient management on penetration resistance (MPa) of the soils at varying depths during pearl millet-fallow cropping system under dry land condition

Treatments	Penetration resistance (Mpa)						
	0 cm	5 cm	15 cm	5 cm	15 cm	30 cm	45 cm
T1	0.280	31.6	30.7	31.2	29.2	1.974	1.030
T2	0.260	32.2	31.3	31.6	30.4	1.959	1.015
T3	0.240	32.8	31.7	32.7	31.2	1.663	0.860
T4	0.240	32.6	31.1	31.6	30.8	1.735	0.805
T5	0.258	34.8	33.5	33.5	32.6	1.878	0.980
CD (P = 0.05)	0.020	1.5	1.3	0.9	1.2	0.02	0.019

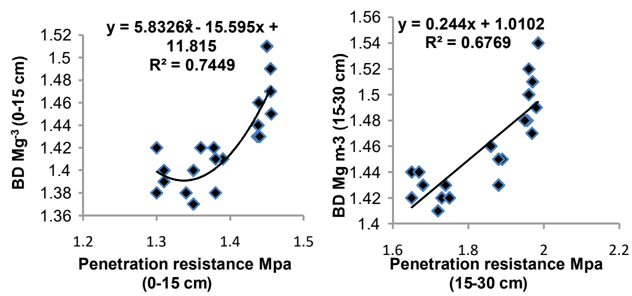

Fig. 11. Relationship of penetration resistance with bulk density at 0-15 cm and 15-30 cm soil depth

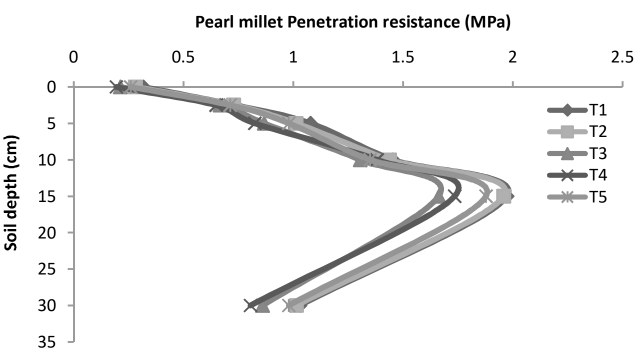

Fig. 12. Soil penetration resistance (MPa) at different depths of soil under long term nutrient management studies during pearl millet

effect was non-significant at both field capacity (-33 Kpa) and permanent wilting point (-1500 Kpa). High water retention due to organic matter was related to the creation of micro pores, which may retain the water more securely than the fertilizer-only treatment and control. Plant available water was high in treatments with Vermicompost and FYM alone or in conjunction with the urea fertilizer. The study findings were in consistent with those of Singh et al (2007), where high soil moisture holding at 0-5, 5-10, and 10-15 cm soil depths with the incorporation of organic matter in the rice crop. Gudadhe et al (2015) investigated the effect of FYM and fertilizers on moisture content at field capacity and permanent wilting point and observed that 10 t FYM ha^{-1} + RDF had the maximum field capacity, which could be attributed to the number of pores, their distribution, and the specific surface area.

Penetration resistance: The influence of different nutrient management strategies on penetration resistance at various depths in pearl millet was observed (Table 3 and Fig. 12). Significantly higher penetration resistance in control and RDF compared to integrated, vermicompost, and FYM alone may be attributable to reduced water retention due to the lower organic matter content in the earlier than to the latter. Greater organic matter content may have higher soil water holding capacity at field capacity and permanent wilting point. The increased moisture may help in reduction of the effect of crust in soil, allowing crop roots to penetrate the soil easily and promote crop growth. The study indicated that increasing the bulk density with increasing the penetration resistance in surface and subsurface soil was positively correlated, with R^2 values of 0.74 and 0.67 (Fig. 11) at 0-15 cm and 15-30 cm, respectively. Bassouny and Chen (2016) observed that the decline in penetration resistance with organic matter and fertilization was more prominent than the control and observed that use of organic and inorganic additives prevents soil compaction, which limits root growth, infiltration rate, and hydraulic conductivity. Celik et al (2010) reported that mycorrhizal anaesthetized compost had the least penetration resistance and the strongest in control and mineral fertilizer applications, which could be ascribed to the conditions imposed of glomalin naturally produced by mycorrhizae, which increased aggregation and thus porosity, lowering penetration resistance. Similarly, Chouhan et al (2018) and Borie et al (2018) reported a decline in penetration resistance due to organic and inorganic fertilization at the surface and subsurface.

CONCLUSION

The physical properties of the soil such as bulk density, infiltration rate, saturated hydraulic conductivity (Ks), mean weight diameter, water stable aggregate, soil moisture retention and penetration resistance of the soil improved with addition of organic matter either alone or in combination with fertilizer in surface soil. Bulk density of the soil was negatively and linearly correlated with volumetric water content at field capacity and permanent wilting point while was positively and linearly correlated with saturated hydraulic conductivity (K_s) and penetration resistance in surface and sub-surface soil. Significantly higher penetration resistance in control and RDF treatment was observed in comparison to left over treatments. Physical properties of the soil were improved by the continuous application of FYM and vermin-compost for 7 years on long term basis.

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Biological Activity and Distribution of Microbes as Influenced by Salinity in Coastal Rice Growing Soils of Guntur District, Andhra Pradesh

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Abstract: Research was conducted to study the biological activity and and isolate the dominant fungi and bacteria in coastal saline soils. Soil samples having different salinity levels *viz.* 0.60, 1.95, 3.59, 5.50, 7.50 and 9.35 dS m^{-1} were collected from coastal soils of Guntur district, AP. The soil enzymatic activity (dehydrogenase and phosphatase) and microbial population (fungi, bacteria, actinomycetes) were assayed by following standard procedures. The dominant fungal and bacterial isolates were identified using standard procedures. The data were analyzed statically. Biological assay of soil samples collected at different salinity levels revealed that the soil enzymatic activity and microbial population were significantly influenced by soil salinity. The soil biological activity was the highest at the low levels of salinity and vice-versa. A total of seven fungal and 18 bacterial isolates were observed in the coastal rice growing soils of Guntur district, AP. This study clearly indicated that the soil salinity significantly influenced the number and distribution of microbial population and enzyme activity.

Keywords: Enzyme activity, Isolates, Microbial populations, Bacteria, Fungi, Actinomycetes, Soil salinity, Biological activity

Andhra Pradesh has a vast stretch of 974 km coast line covering nine districts of the state. The major cropping systems of the coastal system are rice-rice, rice-pulse and rice-maize/jowar. Soil salinity is a major problem especially in the rice growing soils which are mostly subjected to irrigation, leading to secondary salinization affecting 20 per cent of irrigated land (Glick et al 2007). Soil salinization is a process of accumulation of excess salts comprising of chloride and sulphate ions. Soil salinity, a concern of the natural ecosystem in arid and semi-arid regions where, precipitation is insufficient to leach ions from the soil profile, is increasing day by day in agricultural soils (Shrivastava and Kumar 2015). Sometimes migration of salts upward in the soil from shallow groundwater or overuse of fertilizers can also be the contributors for soil salinity. In Andhra Pradesh, 7.48 per cent of coastal soils are affected by salinity of which 0.42 per cent is confined to Guntur district (Mandal et al 2018). Global soil salinization was estimated to continue spreading at a rate of up to 2 Mha yr^{-1} (Abbas et al 2013). Salinization is recognized as the main threat to environmental resources and human health in many countries which have resulted in serious consequences to global natural resources and diminished microbial activity/diversity (Patel et al 2011). Salinization effects in a long-term scenario, the biodiversity (Church et al 2013) and normal functioning of the soil ecosystems by affecting soil organisms, which participate in fundamental ecological processes like organic matter decomposition, nutrient cycling and maintenance of soil structure (Lavelle et al 2006). Soil microorganisms constitute less than 0.5 per cent (w/w) of the soil mass, but they play a key role in soil processes (Yan et al 2015), mediate numerous chemical reactions involved in soil nutrient cycling; transformation of plant and microbe debris; mineralization and transformation of organic matter within the carbon cycle, transformation and degradation of potentially hazardous pollutants etc., thus contributing to the restoration and remediation of polluted soils. Due to salinity, the existence of high osmotic pressure and ion toxicity in rice growing soils of coastal regions are serious constraints to many organisms (Rietz and Haynes, 2003).

Dehydrogenase activity is a good indicator of soil microbial activity, as this enzyme group occurs only within the living cells, unlike other enzymes which can occur in extracellular state (Kumar et al 2013) and play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters. Phosphorus (P) in soil is mainly in the organic form. Phosphatase enzymes (alkaline and acid) catalase the hydrolysis of both esters and anhydrides of phosphoric acid, increasing the availability of phosphorus in soils (Wang et al 2021). However, but as salinity increased, the metabolic quotient (respiration per unit biomass) is increased. The sensitivity of soil enzyme activities to salinity varies; Wang (2013) reported that the activities of dehydrogenase and phosphatase are strongly inhibited by salinity while, Rietz and Haynes (2003) stated that soil respiration is not significantly correlated with salinity. Isolation of different bacteria and fungi in saline environment provides us the knowledge of their dominance or diversity under high salt conditions. The isolates with high salt tolerance capacity are very much useful for biotechnological applications in terms of bio-remediation and bio-mineralization (Das et al 2011). Keeping this in view the study was carried out to find the soil enzymatic activity (dehydrogenase, phosphatase), microbial population (fungi, bacteria, actinomycetes) and to identify dominant micro-organisms (fungi and bacteria) in the coastal rice growing soils of Guntur district, Andhra Pradesh.

MATERIAL AND METHODS

Soil samples (60) up to a depth of 25 cm collected from rice growing areas representing the coastal region of Guntur district were processed and analyzed for soil electrical conductivity (1: 2.5 soil: water) (Jackson 1973). Based on the soil conductivity, a total of six salinity levels of approximately 0.60, 1.95, 3.59, 5.50, 7.50 and 9.35 were considered and for each level four samples were collected. Hence, a total of 24 fresh soil samples were collected and assayed for biological activity. The moisture content of the soils was estimated by gravimetric method (Gupta 2009) and the biological activity was expressed on dry weight basis**.** The method described by Casida et al (1964) was used to determine dehydrogenase activity whereas; phosphatase activity was estimated using the protocol given by Tabatabai and Bremner (1969). Enumeration of microbial population for bacteria, fungi and actinomycetes was carried out as per the procedures outlined by Paroda (2007) in fresh soil samples by following serial dilution plate count technique (Dhingra and Sinclair 1985) and spread plate method using nutrient agar for bacteria, Martins Rose Bengal Agar for fungi and Ken-Knight and Munaier's medium for actinomycetes and expressed on dry basis as colony forming units (CFU) g^{-1} soil. The data obtained were analysed statistically using completely randomized design. The fungal colonies obtained during enumeration were categorized based on colony morphology and further isolated, purified and preserved for studying their morphological features and to identify up to genus level. The fungal isolates were observed under microscope following wet mount method to distinguish characteristic shape and spore arrangement (Bartholomew and Mittwar 1950). Identification and characterization of bacterial isolates was done by recording morphological features microscopically (Claus 1992) and biochemical tests such as catalase test, Voges-Proskauer test, methyl red test, indole production test, citrate utilization test, starch hydrolysis, acid and gas production from glucose broth and comparing these tests with Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

Enzyme activity: Enzymatic activity was maximum at the lowest electrical conductivity of 0.60 dS m^{-1} and decreased with increasing salinity (Table 1). The decrease in dehydrogenase activity was 65.95 per cent with increase in salinity from 0.60 to 9.35 dS m^{-1}. The dehydrogenase activity at salinity level 7.50 and 9.35 dS m^{-1} were at par with each other while, other levels were significantly differing among one another. Similarly, phosphatase enzyme activity (acid and alkaline) vary significantly with different salinity levels. The maximum acid (161 µg PNP g^{-1} $soil^{-1}$ hr^{-1}) and alkaline (275 µg PNP g^{-1} $soil^{-1}$ hr^{-1}) phosphatase activities were at the lowest EC (0.60 dS m^{-1}). The decline in activity of acid and alkaline phosphatase enzymes was 59.00 and 71.63 per cent, respectively with increase in conductivity from 0.60 to 9.35 dS m^{-1}. The enzymes generally originate from microorganisms and the decrease in microbial population reduces enzymatic activity. Yan et al (2015) reported that increase in soil salinity reduced the soil respiration, which ultimately reduced the enzyme activity. The decline in enzyme activities might also be due to the change in osmotic potential of soil–water phase. The salting-out effect, which modifies the ionic conformation of the protein-enzyme active site and specific ionic toxicity cause a nutritional imbalance

Table 1. Soil enzymes activity at different levels of electrical conductivity in soils of Khajipalem revenue village

Electrical conductivity (dS m^{-1})	Enzyme Activity		
	Dehydrogenase (µg TPF g^{-1} soil day^{-1})	Acid Phosphatase (µg PNP g^{-1} soil hr^{-1})	Alkaline Phosphatase (µg PNP g^{-1} soil hr^{-1})
0.60	139.50	161	275
1.95	97.50	150	220
3.59	72.50	113	145
5.50	65.00	103	116
7.50	50.00	74	86
9.35	47.50	66	78
CD @ 0.05	4.40	5.10	6.91
CV (%)	3.77	3.09	3.04

for microbial growth and subsequent enzyme synthesis (Silva and Fay 2012). The osmotic desiccation of microbial cells and limitation of carbonaceous substrates also contribute to the reduction in enzyme activity (Siddikee et al 2011).

Generally, enzyme activity is low in summer due to lesser enzyme secretion by the surviving soil microorganisms as most of the microorganisms must have died as a result of rise in salt concentration in soil (Tripathi et al 2007). The higher amount of alkaline phosphatase than acid phosphatase activity might be due to predominance of neutral or alkaline soils in the study area. Similar observations were also reported by Kirankumar and Lakshmi (2015) in paddy growing areas of West Godavari district; Purvi et al (2016) in coastal ecosystems of Gujarat; Laxminayarana and Naik (2016) in the coastal saline soils of Orissa. Tripathi et al (2007) attributed the decrease in enzyme activity with soil salinity in coastal soils of West Bengal to the prevailing semi-arid conditions wherein, many of the enzymes are extracellular and form complexes with the organics and mineral colloids.

Microbial populations: The data pertaining to population of fungi, bacteria and actinomycetes of the coastal rice growing soils at different electrical conductivity levels indicated a significant effect of salinity on soil microbial population (Table 2). The population of all groups was maximum at the lowest EC level of 0.60 dS m^{-1} and decreased with increasing EC level up to the tested highest EC of 9.35 dS m^{-1}. The maximum fungi, bacteria and actinomycetes population of 18.00 x 10^3, 67.50 x 10^5 and 100.25 x 10^3 CFU g^{-1} soil, respectively, was at the lowest EC level (0.60 dS m^{-1}) whereas, minimum was observed at 9.35 dS m^{-1}. A significant reduction in microbial population was observed with increment in salinity level. The decrease in population with change in salinity from 0.60 to 9.35 dS m^{-1} was to a tune of 81.94 in fungi, while bacteria and actinomycetes declined up to 94.07 and 96.00 per cent, respectively. Bacterial population at salinity levels 1.95 and 3.59 were comparable with each other as there was no significant difference. The study indicated that salinity has a negative effect on soil organisms and this might be due to the low osmotic potential of the soil solution and ion toxicity or imbalanced ion uptake. The osmotic potential of soil water as a result of salinity removes water from microbial cells through plasmolysis, which leads to death of micro-organisms (Ibekwe et al 2010). Among different microbial populations, actinomycetes were more sensitive to salinity followed by bacteria and fungi. The reason for decline in actinomycetes population is the inability to release cell wall deficient (CWD) cells, which on prolonged exposure converted to L- forms, an adaptation strategy in actinomycetes (Ramijan et al 2018). The results were similar to Adilakshmi et al. (2018) where she reported biological activity reduced at high salinity (12 dS m^{-1}) when compared to low salinity level (1.5 dS m^{-1}). Presence of microbial population at higher salinity level indicates the adaption to low osmotic potential by accumulating osmolytes in the cell to counteract the increase in osmotic pressure and production of organic compounds which antagonize the concentration gradient between the soil solution and the cell cytoplasm (Hagemann 2011). Sensitive microbial cells are damaged by the low osmotic potential as soon as exposed to salinity while, some microorganisms get adapted by accumulating osmolytes that help to retain water. Synthesis of osmolytes requires large amounts of energy resulting in reduced proliferation of microorganisms (Yan et al 2015).

Table 2. Microbial populations at different levels of electrical conductivity in soils of Khajipalem revenue village

Electrical conductivity (dS m^{-1})	Microbial population (CFU g^{-1} soil)		
	Fungi (x 10^3)	Bacteria (x 10^5)	Actinomycetes (x 10^3)
0.60	18.00	67.50	100.25
1.95	14.50	54.50	57.00
3.59	10.75	52.75	41.75
5.50	8.50	22.50	36.50
7.50	7.50	14.00	15.75
9.35	3.25	4.00	4.00
CD @ 0.05	0.74	2.57	2.74
CV (%)	4.80	4.82	4.34

The high salt concentrations in saline soils have high bio-energetic taxation, since microorganisms need to maintain osmotic equilibrium between the cytoplasm and the surrounding medium, excluding sodium ions from inside the cell. However, the energy required for osmo-adaptation is lacking due to salinity stress (Silva and Fay 2012). Hence increased salinity becomes detrimental to the microbial community. Laxminarayan and Naik (2016) reported that increased concentration of soluble salts diminishes the multiplication of microbes in coastal saline soils of Eastern India and the same had been revealed in the present study where in the soil micro-fauna showed negative relationship with salinity. Similar reduction in soil population with increasing salinity was also observed by Jasmine et al (2020) in saline soils of Uppugunduru region of Andhra Pradesh.

Characterization and Distribution of the Microbial Isolates (Fungi and Bacteria)

Fungi: The colonies of isolates 1, 3, 4, 5 and 6 are circular while 2 and 7 were dendroid and irregular in shape, respectively (Table 3, Fig. 1). The colony margins of different isolates were entire (isolates 1, 3, 5 and 6), filiform (isolates 2 and 4) and undulate (isolate 7). All the isolates were flat in elevation except 2 and 6, which have raised and crateriform elevation, respectively. Colour varied among different isolates. Isolate 1 was black in colour, while isolate 2 was yellow coloured. White colour was observed in the isolates 3, 4 and 5 whereas, isolates 6 and 7 were orange and olivaceous green in colour, respectively. The distribution of different species varied with soil salinity (Table 4). The genera *Cladosporium, Aspergillus, Pencillium, Fusarium* and *Mucor* among fungi were dominant in the study area. There was a decrease in population abundance as the salinity increased from 0.60 to 9.35 dS m^{-1}. At the lower EC levels of 0.6 and 1.95 dSm^{-1}, fungal species of *Cladosporium, Aspergillus* and *Fusarium* were dominant, whereas *Pencillium* and *Mucor* along with *Fusarium* were dominant at 3.59 and 7.50 dS m^{-1}, respectively. At 5.50 dS m^{-1}, *Cladosporium* and *Fusarium* were observed whereas, at the highest salinity level (9.35 dS m^{-1}) only *Fusarium* prevailed. The study indicated that *Fusarium* is the only fungi that can survive and proliferate at all salinity levels due to certain salt tolerance mechanism. In fungi, low osmotic potential due to salt stress decreases spore germination, the growth of hyphae and also cause changes in the morphology and gene expression resulting in the formation of spores with thick walls (Mandeel 2006). The results are corroborating with those of Yang and Sun (2020) where Cladosporium fungal species in saline soils of Yellow river delta, China; Ashok et al. (2015) in coastal soils of Tamil Nadu with EC 2.95 dS m^{-1}; Rajpal et al. (2016) in the saline soils (0.4 to 15.0 dS m^{-1}) of Kutch Gujarat (dominance of Aspergilli, *Fusarium* and *Penicillium)*. Nayak et al (2019) isolated the fungal genera like *Fusarium sp., Penicillium sp. and Trichoderma* from coastal sandy soils of Orissa.

Bacteria: A total of eighteen bacterial isolates were identified on the basis of colony and cell morphology (Tables 5, 6). The colonies of the isolates 1, 2, 4, 8, 9, 10, 12, 14, 15, 16 and 17 were circular; colonies of 3, 6, 7 and 18 were irregular; colony 13 was rhizoid whereas the colonies of isolates 5 and 11 were filamentous in form. The isolates 3, 5, 7, 8, 10, 11 and 13 were white in colour whereas, isolates 6, 9 and 18 recorded light brown colour. Yellow colony colour was in isolate no 4, 15 and

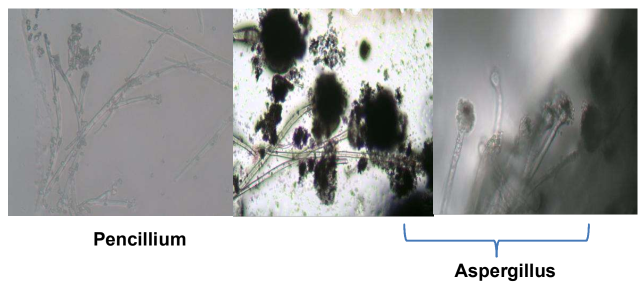

Fig. 1. Microscopic view of some of fungal isolates

Table 3. Characterization fungal isolates

Isolate No.	Form	Margin of the colony	Elevation	Colour	Tentative micro organism
1	Circular	Entire	Flat	Black	*Cladosporium sp.*
2	Dendroid	Filiform	Raised	Yellow	*Aspergillus-sp.*
3	Circular	Entire	Flat	White with pink	*Fusarium sp.*
4	Circular	Filiform	Flat	White with black	*Aspergillus sp.*
5	Circular	Entire	Flat	White	*Fusarium sp.*
6	Circular	Entire	Crateriform	Orange	*Mucor sp.*
7	Irregular	Undulate	Flat	Olivaceous greenish	*Pencillium sp.*

Table 4. Soil fungi at different EC levels

Isolate no.	Micro organism	Electrical conductivity (dS m^{-1})					
		0.60	1.95	3.59	5.50	7.50	9.35
1	*Cladosporium sp.*	✓			✓		
2	*Aspergillus-sp.*	✓	✓				
3	*Fusarium sp.*	✓	✓				✓
4	*Aspergillus sp.*	✓					
5	*Fusarium sp.*	✓	✓	✓	✓	✓	
6	*Mucor sp.*					✓	
7	*Pencillium sp.*			✓			

Table 5. Characterization of bacterial isolates

Isolate No.	Colony morphology				Cell morphology		Biochemical tests							Tentative micro organism
	Form	Colour	Margin of the colony	Elevation	Cell shape	Gram reaction	Catalase test	VP test	MR test	Indole test	Citrate test	*Starch hydrolysis*	Acid and Gas	
1	Circular	Orange	Entire	Raised	Cocci	+ve	+	+	+	-	+	+	-	*Micrococci sp.*
2	Circular	Light orange	Undulate	Flat	Cocci	+ve	+	+	-	-	-	-	+	*Staphylococci sp.*
3	Irregular	White	Undulate	Umbonate	Cocci	+ve	+	-	-	-	-	+	+	*Diplococci sp.*
4	Circular	Yellow	Entire	Flat	Cocci	+ve	+	-	-	-	-	+	+	*Diplococci sp.*
5	Filamentous	White	Filiform	Flat	Spiral	+ve	+	-	-	-	+	+	+	*Azospirillum sp.*
6	Irregular	Light brown	Undulate	Flat	Cocci	+ve	+	+	-	-	+	+	+	*Serratia sp.*
7	Irregular	White	Entire	Flat	Short Rods	+ve	+	-	-			-	+	*Diplococci sp.*
8	Circular	White	Entire	Convex	Spiral	-ve	+	-	-	-	+	+	+	*Azospirillum sp.*
9	Circular	Light brown	Entire	Raised	Short rods	-ve	-	-	-	-	+	-	+	*Pseudomonas sp.*
10	Circular	Dull White	Entire	Flat	Short rods	-ve	-	-	-	-	+	-	+	*Pseudomonas sp.*
11	Filamentous	White	Filiform	Flat	Short Rods	-ve	+	-	-	-	+	+	+	*Azospirillum sp.*
12	Circular	Pink	Entire	Raised	Rods	+ve	+	-	-	-	+	+	-	*Bacillus sp.*
13	Rhizoid	White	Filiform	Flat	Short Rods	+ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
14	Circular	Light orange	Entire	Raised	Cocci	-ve	+	-	-	-	+	+	+	*Xanthomonas sp.*
15	Circular	Yellow	Entire	Flat	Cocci	+ve	+	+	+	-	+	+	-	*Micrococci sp.*
16	Circular	Light pink	Entire	Raised	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
17	Circular	Light yellow	Entire	Flat	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
18	Irregular	Light brown	Undulate	Spiral	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*

VP - Voges-Proskauer, MR - Methyl red test, IND - Indole production light yellow was in isolate 17. An orange shade was observed in isolates 1, 2 and 14 while, pink shades were found in isolates 12 and 16. The isolates 1, 4, 7, 8, 9, 10, 12, 14, 15, 16 and 17 were having entire margin. Undulate margin was observed in isolates 2, 3, 6, 18 and filiform margin was observed in the isolates and 5, 11, 13. Isolates 2, 4, 5, 6, 7, 10, 11, 13, 15 and 17 were having flat elevation while raised elevation was recorded in isolates 1, 9, 12, 14 and 16. Likewise umbonate, convex and spiral elevations were reported in isolates 3, 8 and 18, respectively. Cell morphology includes cell shape and gram reaction of bacterial colonies where observing under microscope with the help of MICAPS-Micro View. The bacterial isolates 1, 2, 3, 4, 6, 14 and 15 were cocci while, isolates 7, 9, 10, 11, 12, 13, 16, 17 and 18 were short rods in shape. Isolates 5 and 8 were spiral in shape when observed under microscope. Among eighteen bacterial isolates, only 10 isolates *viz.* 1, 2, 3, 5, 4, 6, 7, 12, 13 and 15 were gram positive bacteria and remaining isolates were gram negative.

Among all salinity levels, dominance of *Micrococci, Staphylococci, Diplococci, Azospirillum, Serratia, Pseudomonas and Xanthomonas* bacterial species were observed, whereas at the higher salinity levels of 7.50 and 9.35 dS m^{-1} only *Azospirillum, Diplococci* and *Pseudomonas sp.* survived (Table 6). Different species of *Micrococci, Diplococci, Azospirillum* and *Pseudomonas* were dominant at 1.95 dS m^{-1} salinity level. *Diplococci, Azospirillum and Pseudomonas* bacterial species were dominant at 3.59 and 5.50 dS m^{-1} salinity levels. Among all, *Pseudomonas sp.* survived at all soil salinity levels.

Presence of bacteria under high saline conditions indicates the salt tolerance mechanism which require both energy and carbon for the synthesis of compatible osmolytes (Kakumanu and Williams 2014). Decreased growth of bacterial isolates with high salt concentration may be due to the detrimental effect of salts on bacterial populations through direct toxicity as well as osmotic stress. Corroborative findings were reported in the rice soils of the coastal region of the Gangetic delta of West Bengal (Barua et al 2011) and Orissa (Dangar et al 2017) where dominance of Bacillus bacterial species was observed. Bhatt et al (2015) reported the prevalence of *Pseudomonas, Serratia, Bacillus* species in the coastal soils of Jamnagar, Gujarat. Azmi and Chatterjee (2016) reported that, the microbial population of coastal belt of West Bengal comprised of aerobic heterotrophic, gram negative and spore forming bacteria with dominant *Pseudomonas* species. A total of 47 bacterial strains of *Psuedomonas sp.* were isolated from coastal sandy soils of Chennai as reported by Nayak et al (2019).

Table 6. Soil bacteria at different EC levels

Isolate no.	Tentative micro organism	Electrical conductivity (dS m^{-1})					
		0.60	1.95	3.59	5.50	7.50	9.35
1	*Micrococci sp.*	✓					
2	*Staphylococci sp.*	✓					
3	*Diplococci sp.*			✓	✓	✓	
4	*Diplococci sp.*		✓			✓	
5	*Azospirillum sp.*						✓
6	*Serratia sp.*	✓					
7	*Diplococci sp.*	✓				✓	
8	*Azospirillum sp.*	✓	✓	✓	✓		✓
9	*Pseudomonas sp.*	✓	✓				
10	*Pseudomonas sp.*	✓	✓				
11	*Azospirillum sp.*			✓			
12	*Bacillus sp.*	✓					
13	*Pseudomonas sp.*						✓
14	*Xanthomonas sp.*	✓					
15	*Micrococci sp.*	✓	✓				
16	*Pseudomonas sp.*	✓					
17	*Pseudomonas sp.*	✓	✓	✓	✓		
18	*Pseudomonas sp.*		✓				

CONCLUSION

The study elucidated the variable effect of salt concentration in soil on proliferation and distribution of different microbial species and soil enzyme activity. At high salinity level only, few organisms *viz*. *Fusarium sp.* among fungi and *Azospirillum sp., Pseudomonas sp.* among bacteria survived. The salt tolerance capacity of the organisms surviving at high salinity levels need to be further explored and these microorganisms are very useful for biotechnological applications in terms of bioremediation and bio mineralization.

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Optimization of Irrigation and Nitrogen Levels in Chickpea (*Cicer arietinum* L.) under Loamy Sand Soil of North Gujarat

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Abstract: Shrinking of water resources and poor fertility status of soil in semi-arid region, call for diversification from wheat to low inputs demanding crop chickpea in North Gujarat. A field experiment was conducted during the *rabi* seasons of 2021-22 at Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat to optimize irrigation and nitrogen fertilizer for chickpea under loamy sand soil. All growth traits, yield components and seed and straw yield of chickpea increased significantly with at each higher level of irrigation scheduling up to 0.8 IW/CPE ratio with water application of 450 mm along with 100% RDN. The highest field water use efficiency of chickpea with 0.6 IW/CPE ratio. Seed yield with applied water and field water use efficiency followed quadratic function with regression coefficient (R^2) of 0.99. The predicted maximum seed yield of chickpea was 2306 and 2217 kg/ha with optimum irrigation and nitrogen level of 549.6 mm and 24.03 kg N/ha beyond which yield seed yield shows decreased in trends.

Keywords: Chickpea, Economics, Irrigation, Nitrogen, WUE, Yield

Demand for agricultural products will further increase to satisfy the needs of an increasing population. However, the availability of water for agriculture has been declining due to an increasing demand of water for non-agricultural sectors (Srivastava et al 2018, Jafarikouhini et al 2020). Shortage of irrigation water is seriously affecting agricultural production particularly in arid and semi-arid regions because irrigated agriculture is required for agricultural production in these regions. In Northwestern semi-arid region of India, particularly in Gujarat, the wheat based cropping system has dominated other cropping systems because of the introduction of high yielding varieties, availability of favorable soil and climatic factors and better remunerative support prices. With the large scale adoption of wheat based cropping system even on coarse textured and light soils, Gujarat state has led to overexploitation of water resources caused a decline in the water table in several districts leads to sever water scarcity. As frequent drought in every 2-3 years in North Gujarat areas were very common. Farmers and planners in this area are seeking alternative suitable crops with low water and energy requirement for diversification and long term sustainable agricultural productivity point of view. Chickpea was identified as an alternative crop to North Gujarat area, because of its lower water demand (evapotranspiration) and irrigation requirement has been identified as a suitable alternate crop to wheat.

Among the pulses, chickpea (*Cicer arietinum* L.) is one of the major crop of tropics and sub-tropics and top most important pulse crop of India cultivated over an area of 9.99 million hectare with a production of 11.91 million tonnes and productivity of 1092 kg/ha (DES 2020-21). Earlier chickpea was cultivated in Maharashtra, Punjab, Haryana, Rajasthan as rainfed *rabi* crop only for subsistence and not for commercial in heavy black soil, resulting in low yield. In Gujarat, chickpea was cultivated as rainfed crop only in Saurashtra region under black heavy soil. Commercial cultivation of crop in loamy sand soil with limited irrigation facility was started in water scarcity area of North Gujarat with yield potential of 2500 kg/ha. Though, chickpea crop doesn't require much amount of nitrogen fertilizer due to legume-rhizobia association fix nitrogen in root nodules. However, in light textured soil which was deficit in organic carbon and nitrogen, there may be good response of irrigation along with nitrogen fertilization. As these two factors which are very important for proper growth and development of plant, rather than by crop genetics (Sinclair and Rufty 2012). Although, number of experiment in chickpea were conducted under heavy black soil. However, research on light textured soil where there is potential to get higher yield due to proper cultivar selection and inputs management is very meagre. Considering the above fact the present study was conducted to optimize the irrigation and nitrogen levels to chickpea under loamy sand soil of North Gujarat.

MATERIAL AND METHODS

A field experiment was carried out during winter (*Rabi)* season of 2021-22 at S. D. Agricultural University, Sardarkrushinagar. The site is geographically situated at 24° 19' N Latitude and 72° 19' E Longitude with an elevation of 154.52 m above the mean sea level. The region is characterised by tropical and semi-arid with dry winter (November-February)and soil is loamy sand in texture having low in organic carbon (0.21%) and available nitrogen (168.4 kg/ha) and medium in available phosphorus (35.22 kg/ha) and available potassium (264.10 kg/ha). Chickpea variety "Gujarat Gram 5" was sown manually on 22nd November 2021 with recommended dose of fertilizer was 20:40:00 NPK kg/ha. The experiment was laid out in a split plot design with four replications, consisting of nine treatment combinations comprising three irrigation levels in main plot *viz*., 0.6 IW/CPE (I_1), 0.8 IW/CPE (I_2) and 1.0 IW/CPE ratio (I_3) with 50 mm depth of irrigation water at each irrigation and three nitrogen levels in sub plot *viz.*,125% RDN (N_1), 100% RDN (N_2) and 75% RDN (N_3).One common pre sowing irrigation was applied for crop establishment in all treatments thereafter; 50 mm depth of irrigation water was given through flood method as per the treatment wise. Full dose of phosphorus was applied as basal dose through SSP and nitrogen was given in two splits at half in basal and remaining half at 30 DAS as per the treatment wise. Crop yield (dependent variable) was assumed as a function of various growth traits, yield components and nutrient uptake (independent variables) and the following straight line model was established by least square technique (Gomez and Gomez 1984):

$$Y = a + b\ (x)$$

where, Y = Grain yield of chickpea(kg/ha), a = Y–axis intercept, b = Regression coefficient, x = Growth and yield components

The functional relationship between crop yield with irrigation water applied (W) defined as water production function and crop yield with applied nitrogen (N) is fertilizer production function. The production function equations evaluated in this study are as follows.

Linear production functions:

$$Y = a + b\ (W)$$

$$Y = a + b\ (N)$$

Quadratic production functions:

$$Y = a + b\ (W) + c\ (W^2)$$

$$Y = a + b\ (N)\ c\ (N^2)$$

where in: Y = Crop yield (kg/ha), W = Applied irrigation water, N = Applied nitrogen (kg/ha), a = Y – axis intercept, b and c = Regression coefficients reflecting the yield variation per unit change in irrigation/nitrogen.

The data obtained on the different growth and yield components and yield were analyzed statistically by the method of analysis of variance as per the procedure outlined for split plot design given by Gomez and Gomez (1984) in Microsoft excel sheet. Statistical significance was tested by P–value at 0.05 level of probability and critical difference was worked out wherever the effects were significant.

RESULTS AND DISCUSSION

Growth and yield attributes: The higher level of irrigation from 0.6 to 1.0IW/CPE ratio produced significantly higher growth parameters and yield attributes of chickpea (Table 1). Maximum growth parameters and yield attributesof chickpea were with irrigation scheduled at 1.0 IW/CPE ratio which was at par with 0.8 IW/CPE and significantly superior over 0.6 IW/CPE ratio.Adequate moisture supply promoted the elongation, division and expansion of cell components and ultimately, increased plant growth causes higher yield attributing characters. Similar result reported by Pawar et al (2013) and Srinivasulu et al (2016). Chickpea fertilized with

Table 1. Effect of irrigation scheduling and nitrogen levels on growth and yield attributes of chickpea

Treatment	Plant height (cm) at harvest	Dry matter accumulation at harvest (g/plant)	Days to 50% flowering	Number of branches per plant	Number of pods per plant	Number of seeds per pod	Seed index (g)
Irrigation scheduling: (I)							
I_1: 0.6 IW/CPE ratio	44.06	24.62	53.77	7.56	40.80	1.36	19.47
I_2: 0.8 IW/CPE ratio	49.69	27.63	57.38	9.53	49.45	1.41	20.86
I_3: 1.0 IW/CPE ratio	52.24	28.95	58.37	9.82	52.39	1.42	21.49
C.D. at 5%	6.16	2.37	3.64	0.68	8.93	NS	1.57
Nitrogen levels: (N)							
N_1: 125% RDN	50.82	28.37	57.60	9.48	51.21	1.42	21.05
N_2: 100% RDN	49.00	27.47	56.75	9.37	49.12	1.40	20.90
N_3: 75% RDN	46.18	25.37	55.16	8.06	42.31	1.37	19.87
C.D. at 5%	3.65	2.34	NS	1.20	3.70	NS	NS

125% RDN increased growth and yield attributes which was at par with 100% RDN and significantly superior over 75% RDN. The overall improvement in growth and yield attributes with an application of nitrogen might have stimulated increased activity of nucleotide, protein, chlorophyll formation, cell division, cell elongation and enzymes involve in various metabolic process which have direct impact on vegetative phase. Similar results were also reported by Rani and Krishna (2016), Bhadoria (2018), Verma et al (2019).

Seed and straw yield: Irrigation scheduled at 1.0 IW/CPE ratio recorded significantly the highest seed yield (2306 kg/ha) and straw yield (3123 kg/ha) of chickpea which was at par with 0.8 IW/CPE ratio and significantly superior over 06 IW/CPE ratio (Table 2). Increase in irrigation frequency from 0.6 to 1.0 IW/CPE ratio tented to increase consumptive use of water, which provided congenial condition throughout the growth period of the crop. Besides adequate soil moisture in the rhizosphere of chickpea crop which results in higher photosynthesis and translocation of photosynthesis towards reproductive structures. Several researchers reported improved seed yield with irrigation scheduled at 0.8 and 1.0 IW/CPE (Pawar et al 2013, Kumbhar et al 2015, Srinivasulu et al 2016, Khot et al 2021). Application of 125% RDN recorded significantly the highest seed yield (2213 kg/ha) and straw yield (3068 kg/ha) which was at par with 100% RDN. The improvement in yield components might have resulted from favorable influence of nitrogen on growth attributes and efficient and greater partitioning of metabolites and adequate translocation of nutrients to developing reproductive structure. The results were in agreement with the findings of Bhadoria (2018), Dwivedi et al (2019) and Verma et al (2019).

Interaction effect between irrigation scheduling and nitrogen levels: The irrigation scheduled at 1.0 IW/CPE ratio along with application of 125% RDN recorded significantly higher seed yield (2521 kg/ha) which was at par with irrigation scheduled at 1.00IW/CPE along with 100% RDN, 0.8 IW/CPE along with 125% RDN and 0.8 IW/CPE with 100% RDN and significantly superior over rest of treatments (Table 3). The results are in agreement with the Gadade et al (2021). The crop fertilized with nitrogen along with adequate amount of irrigation can increased in nitrogen uptake by plant. Nitrogen accumulation during vegetative growth period, which was conducive to transfer to seed at maturity stage improved dry matter accumulation at harvest and ultimately seed yield.

Regression of growth traits and yield components on seed yield of chickpea: All the independent variables showed a significant positive and linear relationship with seed yield suggesting an increment in seed yield of chickpea with increase in given growth trait and yield component (Table 4). However, the magnitude of this reinforcement varied with the independent variable, *viz.,* growth trait and yield component and their units. The explained total variation as indicated by coefficient of determination (R^2) in grain yield by various growth traits and yield components (number of pods per plant, number of seeds per pod, seed index) chosen as independent variables individually ranged from 88.51 to

Table 3. Seed yield of chickpea as influenced by interaction between irrigation scheduling and nitrogen levels

Irrigation scheduling (I)	Nitrogen levels (N)		
	N_1: 125% RDN	N_2: 100% RDN	N_3: 75% RDN
I_1: 0.6 IW/CPE ratio	1734	1711	1702
I_2: 0.8 IW/CPE ratio	2384	2280	1854
I_3: 1.0 IW/CPE ratio	2521	2436	1961
C.D. at 5%	277.8		

Table 2. Effect of irrigation scheduling and nitrogen levels on yield, FWUE and economics of chickpea

Treatment	Seed yield (kg/ ha)	Straw yield (kg/ha)	Harvest index (%)	FWUE (kg/ha/mm)	Gross realization (₹/ha)	Total cost of cultivation (₹/ha)	Net realization (₹/ha)	BCR
Irrigation scheduling: (I)								
I_1: 0.6 IW/CPE ratio	1715	2660	39.20	4.90	96390	48109	48281	2.00
I_2: 0.8 IW/CPE ratio	2173	2953	42.39	4.83	120462	50470	69992	2.39
I_3: 1.0 IW/CPE ratio	2306	3123	42.48	4.19	127792	52832	74960	2.42
C.D. at 5%	357.6	265.9	NS	-	-	-	-	-
Nitrogen levels: (N)								
N_1: 125% RDN	2213	3068	41.90	4.92	122922	40189	82733	3.06
N_2: 100% RDN	2142	2927	42.26	4.76	118808	40120	78688	2.96
N_3: 75% RDN	1839	2742	40.14	4.09	102918	40251	62667	2.56
C.D. at 5%	160.4	248.9	NS	-	-	-	-	-

94.08% and 81.24 to 98.58%. The variance ratio for testing R^2 was highly significant in all the relationships. This suggests that the seed yield of chickpea can be adequately predicted using the tested independent variables, *viz.,* growth traits and yield components.

Irrigation water and yield relation: The relation between seed yield (Y) of chickpea under each level of applied water (water production function) was developed which explained 99% variation (Fig. 1). It showed quadratic response. The resultant water production function and test statistics are as follows.

$$Y = -2447 + 17.58W - 0.016W^2 \quad (R^2 = 0.99)$$

Quadratic: The test statistic (R^2 and F – value) of quadratic production function were highly significant. The water production functions developed for chickpea under different irrigation scheduling were used to determine economic irrigation water level that a farmer can use.The predicted maximum seed yield was 2306kg/ha with applied water of 549.4 mm beyond which the yield decreased (Fig. 2). However, the maximum grain yield (Y_{max}) during experimental period was bracketed within the tested range of applied water levels (350 to 550 mm). Similar results were in tune with the finding of Malve et al (2017).

Nitrogen levels and yield relation: The relation between chickpea seed yield (Y) under each nitrogen levels (N) was established following quadratic production function (Fig. 2). The resultant fertilizer production function and test statistics are as follows.

$$Y = -462 + 223\,N - 4.64\,N^2 \qquad (R^2= 0.99)$$

Quadratic: The test statistic (R^2 and F – value) of fitted quadratic production function were highly significant. The yield – nitrogen production function did not emerge through the origin and the value of regression constant (intercept 'a') was positive indicating that some minimum grain yield of 462kg/ha can be realized based on the native fertility of the experimental soil. The positive linear coefficient for N term denoted that grain yield increased linearly from the addition of initial N levels. On the other hand, the negative second power (quadratic) regression coefficient (N^2) suggested that the increase in grain yield from each increment of N diminished at higher levels. The predicted maximum grain yield was 2217kg/ha with an input level of 24.03 kg N/ha, beyond which the yield decreased (Fig. 3). However, the maximum grain yield (Y_{max}) during experimental period was bracketed within the tested range of nitrogen levels (20 to 30 kg N/ha).

Yield water use efficiency (Y-WUE): Y-WUE was significantly affected by irrigation water applied during

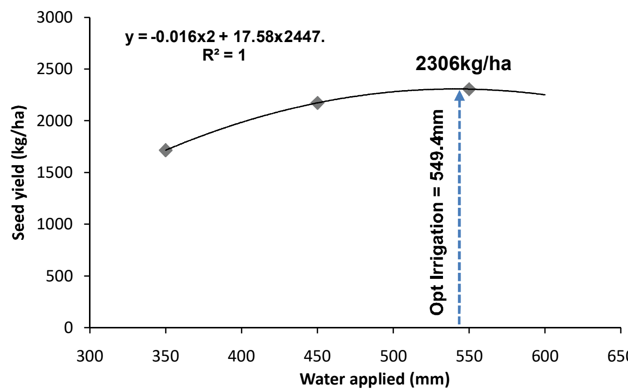

Fig. 1. Predicted yield response of chickpea to applied water applied

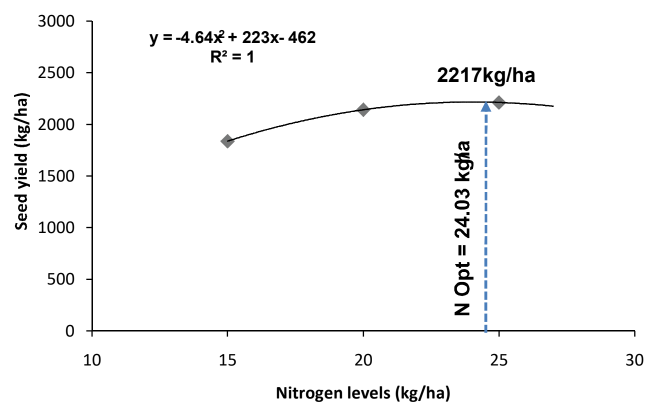

Fig. 2. Predicted yield response of chickpea to nitrogen levels

Table 4. Empirical estimates for the regression of growth traits and yield components on seed yield of chickpea

Relationship	Regression constant		Coefficient (R^2)	Test statistic F value for testing R^2
	a	b		
Yield – Plant height (cm)	-1611	75.53	0.885**	53.94
Yield – Dry matter production (g/plant)	-1625.8	136.33	0.897**	60.98
Yield – Days to 50% flowering	-5670.2	136.89	0.940**	111.14
Yield – No. of branches per plant	-231.12	255.95	0.922**	83.03
Yield – No. of pods per plant	-185.92	47.33	0.985**	484.64
Yield – No. of seeds per pod	-10455.2	8974.7	0.812**	30.31
Yield –Seed index (g)	-4203.22	304.15	0.964**	189.34

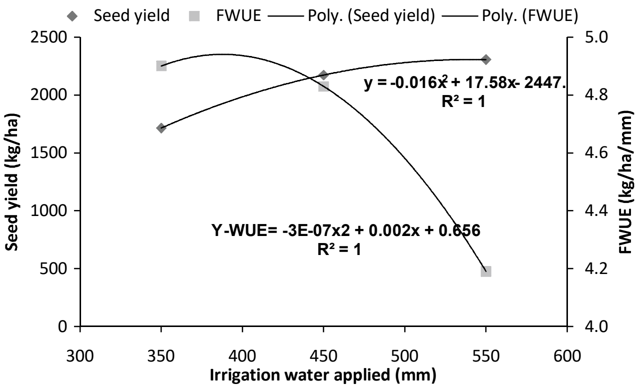

Fig. 3. Relationship of irrigation water applied to seed yield and water use efficiency

experimental period. Each higher level of irrigation from 0.6 to 1.0 IW/CPE ratio decreased water use efficiency of chickpea. Numerically, highest water use efficiency (4.90 kg/ha/mm) was recorded at 0.6 IW/CPE ratio. The relationship between Y-WUE was curvilinear (polynomial of second order) graphically depicted in Fig 3. This relationship could be expressed as follows:

$$Y - WUE = -3E - 07x^2 + 0.002x + 0.656 \ (R^2 = 1)$$

Y-WUE increased with increasing water shortage in the root zone, indicating that yield losses was proportionally smaller than the amount of water used by crops. The different effects of water deficit on Y-WUE observed in various studies can be attributed to the level of water stress experienced by the crop. Chickpea crop develop deep root system which potentially increases the water availability for the plants and attenuates negative effects of water deficit. This may bring up the crop more resistant to water stress and a greater Y-WUE. In the shallow soils, as in our experiment, the development of the rooting system was very limited; it resulted in severe water stress with a very negative impact on the yield and Y-WUE. The application of each higher level of nitrogen improved Y-WUE, as well as yield, in agreement with results reported by Gadade et al (2021).

Economics: The application of irrigation at 1.0 IW/CPE ratio gave the highest net return (74960 ₹/ha) with maximum BCR value 2.42 followed by 0.8 and 0.6 IW/CPE ratio. In case of 125% RDN application recorded the highest net monetary returns (82733 ₹/ha) with maximum B:C ratio of 3.06 followed by 100% and 75% RDN (Table 2).

CONCLUSIONS

The remunerative higher yield of chickpea can be achieved by irrigation scheduled at 0.8 IW/CPE ratio along with application of 100% recommended dose of nitrogen (20 kg/ha). The predicted maximum seed yield of chickpea was 2306 and 2217 kg/ha with optimum irrigation and nitrogen level of 549.6 mm and 24.03 kg N/ha beyond which yield seed yield shows decreased in trends. However, the maximum seed yield was bracketed within the tested ranged of irrigation scheduling and nitrogen levels which confirmed optimum inputs levels for getting yield of chickpea on loamy sand soil.

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Assessment of Soil quality in Rural and Peri-urban Areas of Southern Transect of Bengaluru by using Principal Component Analysis

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***Abstract*:** Environmental degradation is caused by urbanization in developing countries affects soil and water quality year by year in the surrounding areas with this our objective was to assess the soil quality of rural and peri-urban areas of southern transect of Bengaluru. Among the different regions, soils of rural areas had recorded significantly higher available N (323.01 kg ha^{-1}), P (28.98 kg ha^{-1}), K (246.16 kg ha^{-1}), exchangeable Ca [9.67 c mol (p^{+}) kg^{-1} soil], Mg [7.22 c mol (p+) kg^{-1} soil], available S (19.32 kg ha^{-1}), Zn (0.73 ppm), B (0.84 ppm) and dehydrogenase activity (DHA) (14.59 µg TPF g^{-1} soil 24 h^{-1}) compared peri-urban. Soils of peri-urban areas were recorded higher Cd (0.041 ppm), Cr (0.049 ppm), Pb (0.033 ppm) and Ni (0.043 ppm). The principal component analysis was used to evaluate the soil quality index of rural and peri-urban areas. The most influenced indicators were: OC, soil pH, S, P and clay. The soils of rural areas had SQI 0.61 and peri-urban 0.54. Conclusively, soil quality of rural and peri-urban areas falls under the medium category of soil quality *viz*., 0.50-0.75.

Keywords: Soil quality, Principal component analysis, Southern transect of Bengaluru

Soil is the most vital and precious natural resource that sustains life on the earth (Kumar Naik et al 2020). Soils degraded year by year due to the pressure exerted by various sectors of society including urbanization and industrialization. Urbanization is an unavoidable trend in human development (Yan et al 2018) that affects soil physico-chemical properties, shifts in input application and the incorporation of imported materials in soil (Stephen et al 2018). Intensification of agriculture by the addition of excess fertilizers, irrigation with contaminated water from industries and application of urban compost would have an adverse effect on the physico- chemical and biological properties of soils and also their potential to synchronise nutrient supply and demand in cropping systems. The major driving forces of soil degradation are deforestation, soil erosion, waste disposal, change in land use, uncontrolled grazing and unscientific land management practices (Vasu et al 2020). Sustainable management of soil and water resource is extremely critical in urban cities, particularly in developing countries and one more challenge in urban cities is the handling and management of municipal wastes and sewage water (Abdel-shafy 2018). Most urban residents in developing counties lack access to adequate management of solid waste and sewerage systems resulting in significant environmental pollution (Navarro and Vincenzo 2019). The assessment of soil quality is necessary to evaluate the degradation status and trends followed in different land use and management techniques (Spandana et al 2013). Soil quality is defined as the continued capacity of soil to function as a vital living system, as it contains biological elements which are key to the ecosystem, within land-use boundaries. The SQI combines both physical and chemical characteristics of soil into a single parameter that may be used as a general measure of soil quality for agricultural purposes (Andrews et al 2002). Bengaluru is an example of many other Indian megacities where urbanization encroaches on traditional land-use systems and their ecosystem services such as soil and water. In this context, the present study was approached to investigate the effect of urbanization on soil quality and to identify the most appropriate indicators that influence soil quality in rural and peri-urban areas of the southern transect of Bengaluru.

MATERIAL AND METHODS

Bengaluru district is divided into two transects one is towards north *i.e.,* the Northern transect (N-transect) and another one *i.e*., the Southern transect (S-transect). The S-transect is a polygon covering a total area of 300 km^2 and it was further divided into three sub-regions *viz*., rural, peri-urban and urban areas based on the survey stratification index (SSI) by Ellen et al (2015) where distance to the city center (Vidhana Soudha) and percentage of built-up area in that village were considered to calculate SSI. If the value comes 1 and 2 then those villages were considered as urban, likewise 3 and 4 for peri-urban, 5 and 6 for rural. The present study is confined only to rural and peri-urban areas. Twenty villages were selected from each rural and peri-urban areas, four representative soil samples were collected from each village then processed and analysed for various physical, chemical and biological properties of soil by adopting standard analytical procedures.

Mechanical analysis of soil was carried out by international pipet method (Piper 1966). Soil pH (1: 2.5) and EC (dS m^{-1}) were analyzed as outlined by Jackson (1973). The method followed for the estimation of SOC by Walkley and Black (1934). The available N by Subbiah and Asija (1956), available P, available K, exchangeable Ca and Mg as outlined by Jackson (1973). The method followed for the analysis of micronutrients and heavy metals by Lindsay and Norwell (1978). In case of biological properties, dehydrogenase activity was analyzed by Casida et al (1964).

Statistical analysis: All data were checked for normality of distribution, and the one-way analysis of variance was performed using SPSS (version 16) to assess the effects of different sub-regions of southern transect on soil properties. Pearson's correlation coefficients were used to determine the strength of relationships among soil attributes. In the present study PCA was performed for the determination of SQI where four steps were followed (Andrews et al 2002). In the first step correlation analysis was carried out to determine whether the soil indicators were redundant, only the significant variables ($p < 0.05$) were included for PCA. In the second step selection of most critical soil quality indicators, for this step: The PCs with eigenvalues >1 (Brejda et al 2000) were selected and subjected to varimax rotation (Shukla et al 2006). The selection of indicators from the PC was done according to Masto et al 2008. In the third step scoring of indicators (S_i). The scoring was done by the linear scoring functions (LS). Each indicator is categorized as "more is better", "less is better", or "optimum is better" according to Masto et al 2008. In the last step scores of individual attributes were added to get final SQI. Once score is assigned to each indicator, weight is computed for them by using the PCA results. The percentage of variation divided by the total per cent variation provide the weighted factor (W_i) for each selected indicator from the PCA (Singh et al 2013). The SQI is computed by integrating score and weight factor of each indicator. This can be explained by the following equation:

$$SQI = \sum_{i=1}^{n} W_i S_i$$

Where S_i is the score for variable *i* and W_i is the weighting factor derived from the PCA.

RESULTS AND DISCUSSION

Soil physical attributes: The sand (%) and silt content (%) were statistically non-significant among two regions of southern transect of Bengaluru. The highest mean values of sand (55.03 %), silt (16.31 %) were in the peri-urban soils, whereas clay content (30.47 %) was significantly higher in the rural soils (Table 1). The soil texture varies from sandy loam to clay in both rural and peri-urban soils. Pradeep et al 2018 reported that the clay content was high in the rural soils than that of suburban and urban soils. Soils with higher clay contents tend to have greater organic matter which is crucial in determining the microbial biomass, microbial activity, and composition of the microbial community (Mcculley and Burke 2004).

Soil chemical attributes: The soil pH and organic carbon showed significant difference among the two regions of southern transect of Bengaluru. The soil pH ranges from 5.10 to 8.56 in soils rural areas and 4.23 to 8.50 in soils of peri-urban areas (Table 1). The soils of peri-urban were acidic (6.02) and this might be due to excess application nitrogenous fertilizer has resulted in the contribution of H^+ ions and led to soil acidity. Similar results were reported by Sumita et al (2019). The OC content was recorded at 18.64 per cent higher in soils of rural areas compared to peri-urban. The regular application of manures to crops might have added organic matter to soil thereby rural soils recorded higher OC content. The rural soil possessed higher OC (0.44 %) than sub-urban (0.42 %) and urban (0.36 %) soils (Pradeep et al 2018).

Soil available nutrients: Soils of rural areas recorded significantly higher per cent available N (13.30 %), available P (13.95 %) and available K (23.28 %) over the soils of peri-urban areas. This might be due to the continuous application of the judicious level of fertilizers and increased level of organic inputs had led to the increased availability of these nutrients in soils of rural. Pradeep et al (2018) reported that the soils of rural areas had higher levels of macronutrients (N, P, K) than sub-urban and urban soils. The exchangeable Ca and available sulphur varied with different areas of southern transect of Bengaluru. Exchangeable Ca, Mg and available sulphur recorded higher in soils of rural areas (9.67 c mol (p^+) kg^{-1}, 7.22 c mol (p^+) $kg^{-1}$and 19.32 kg ha^{-1}) compared to soils of peri-urban areas (Table 2). This might be due to the process of urbanization which affected the availability of Ca, Mg and S in peri-urban. Exchangeable calcium and magnesium contents are highly pH dependent and dynamic in their reaction. Since soil reaction is acidic in peri-urban areas of southern transect, exchangeable Ca and Mg content found less in these areas. Higher availability of sulphur in rural areas is mainly attributed to higher soil organic matter content than peri -urban.

The content of DTPA extractable micronutrients (Fe, Zn and B) were significantly varied among the different areas of southern transect of Bengaluru. The Fe was high in soils of peri-urban areas (13.20 ppm) and this might be due to urbanization causes the acidification of soil which resulted in increase of Fe. Generally, the micronutrient content of the soil depends on other soil parameters such as pH, OM, and soil moisture content (Peraza et al 2017). Zn was recorded significantly higher in rural areas (0.73 ppm) than peri-urban (0.63 ppm). Similarly, B content found significantly higher in rural areas (0.84 ppm) compared to peri-urban. Higher availability of Zn and B in rural compared to peri-urban areas is mainly due to the use of crop residues and bulky organic manures in rural areas which is not possible in the vicinity of peri-urban areas. Cu and Mn availability did not vary among the different areas of southern transect of Bengaluru.

Heavy metals: The contents of heavy metals were significantly influenced by the different areas of southern transect of Bengaluru. Soils of peri-urban areas recorded higher Cd (0.041 ppm), Cr (0.049 ppm), Pb (0.033 ppm) and Ni (0.043 ppm). The higher level of heavy metals in peri-urban soils might be due to industrialization and urbanization activities resulted in the accumulation of heavy metals.

Dehydrogenase: The DHA was found significantly different among areas of southern transect of Bengaluru. The dehydrogenase content recorded higher in soils of the rural areas (14.59 µg TPF g^{-1} soil 24 h^{-1}). The high DHA in soils of rural areas might be due to regular application organic manure and agricultural practices. The low DHA is due to strong anthropogenic influences, such as mechanical disturbance, soil sealing, and contamination. These factors have an impact on soil microbial properties (Dobrovolsky and Nikitin 2012).

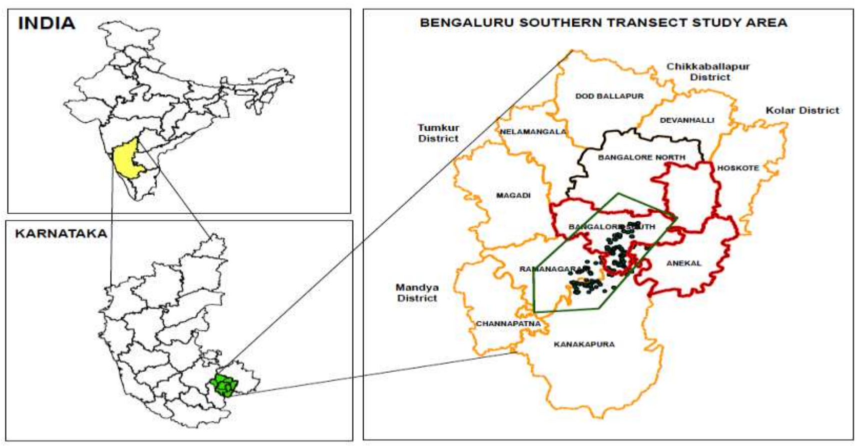

Fig. 1. Base map showing present study area

Table 1. Soil physico-chemical and biological properties of different regions of southern transect of Bengaluru

Treatments	Sand (%)	Silt (%)	Clay (%)	pH	EC (dSm^{-1})	OC (%)	DHS (µg TPF g^{-1} soil 24 h^{-1})
Rural	53.77	15.27	30.47	6.87	0.65	0.70	14.59
Peri -Urban	55.03	16.31	28.19	6.02	0.59	0.59	12.36
CD (p=0.05)	NS	NS	1.64	0.32	NS	0.08	1.96

Table 2. Soil available nutrients of different regions of southern transect of Bengaluru

Treatments	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Ca (c mol (p^+) kg^{-1})	Mg (c mol (p^+) kg^{-1})	S (ppm)
Rural	323.02	28.98	246.16	9.67	7.22	19.32
Peri -urban	285.08	25.43	199.66	8.83	6.84	17.52
CD (p= 0.05)	22.83	2.59	16.74	0.76	NS	1.78

Table 3. Micronutrients and heavy metals in soils of different regions of southern transect of Bengaluru (ppm)

Treatments	Fe	Mn	Cu	Zn	B	Cd	Cr	Pb	Ni
Rural	9.89	7.58	0.60	0.73	0.84	0.031	0.037	0.020	0.030
Peri -urban	13.20	8.24	0.58	0.63	0.63	0.041	0.049	0.033	0.043
CD (p= 0.05)	1.10	1.36	NS	0.10	0.07	0.153	0.153	0.197	0.176

Selection of soil quality indicators: The PCA and per *cent* contribution of each soil attributes towards soil quality indicated by communalities and sowed that among the different soil attributes the SOC (0.911) has contributed high percentage to soil quality followed by N (0.900), dehydrogenase (0.881), soil pH (0.815) and so on (Table 4). The four PC with eigenvalues >1 and explaining at least 5 per cent of the variance in the dataset and which together explains around 67.15 per cent variation in the total data set (Fig. 2). The first PC, second PC, third PC and fourth PC explains around 30.20, 17.76, 10.04 and 9.13 per cent variation, respectively in the data set. Based on rotated factor loadings of soil attributes, selected indicators were: Under PC-1, SOC, available N, and DHA were the three highest weighted variables with the rotated factor loadings of 0.951, 0.949, and 0.937, respectively. Since these three attributes were highly correlated, to avoid redundancy in indicator selection, only SOC was chosen based on its highest correlation sum. Under PC-2, soil pH and Fe were within 10 per cent of the maximum value, so they were considered as highly weighted variables. There was a strong correlation between soil pH and Fe (r = -0.79), therefore the variable with high rotated factor loading was selected *i.e.,* soil pH (0.900). Under PC-3, only sulphur (0.782) was within 10 per cent of the maximum value, so it as considered as a highly weighted variable. Under PC-4, available P (0.681) and clay content (0.649) were within 10 per cent of the maximum value but there was no strong correlation coefficient between these two soil variables (Table 5), so each soil variable as its importance in determination of soil quality that's why both were selected under MDS.

SOC is the most important attribute of soil quality as it improves soil physical, chemical, and biological properties of soil. In the present study, there was a wide gap in the application of organic manure in rural and peri-urban soils. So, the selection of OC as a key indicator of soil quality is advisable (Masto et al 2008, Singh et al 2013, Basak et al

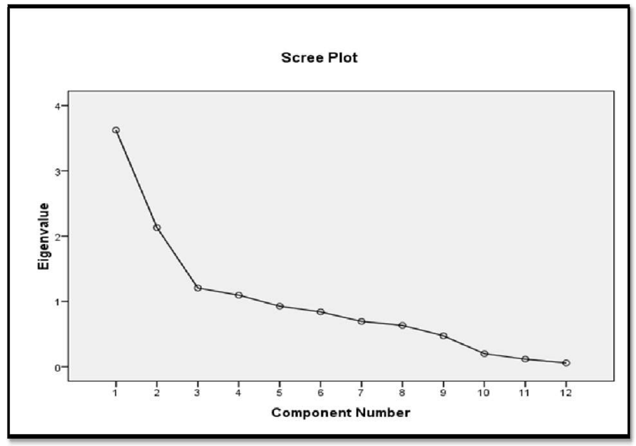

Fig. 2. Relationship between eigenvalue and principal components

Table 4. Principal component analysis and communalities of soil quality parameters to evaluate soil quality index

Soil attributes	PC_1	PC_2	PC_3	PC_4	Communalities
Clay	0.004	0.035	0.465	0.649	0.638
pH	0.051	0.900	-0.034	-0.032	0.815
OC	0.951	0.016	0.065	-0.042	0.911
N	0.949	0.058	0.043	-0.044	0.907
P_2O_5	0.238	0.206	0.146	-0.681	0.585
K_2O	0.659	0.224	-0.115	-0.033	0.499
Ca	-0.011	0.658	0.280	-0.134	0.530
S	0.085	0.045	0.782	0.000	0.621
Fe	-0.074	-0.867	0.059	0.001	0.761
Zn	0.371	0.185	-0.367	0.455	0.513
B	0.240	0.474	-0.331	0.074	0.397
Dehydrogenase	0.937	-0.021	0.030	-0.038	0.881
Maximum value	0.951	0.900	0.782	0.681	
10 per cent of the maximum value	0.0951	0.0900	0.0782	0.0681	
Highly weighted variable	0.856	0.810	0.704	0.613	
Eigenvalues	3.625	2.132	1.206	1.096	
Variance explained (%)	30.20	17.76	10.04	9.13	
Cumulative variance explained (%)	30.20	47.97	58.02	67.15	

Table 5. Correlation coefficients among highly weighted variable

	Clay	pH	OC	N	P_2O_5	Fe	DHA
Clay	1.00						
pH	-0.01	1.00					
OC	0.00	0.09	1.00				
N	0.01	0.13	0.93**	1.00			
P_2O_5	-0.11	0.18*	0.20**	0.23**	1.00		
Fe	0.03	-0.79	-0.10	-0.14	-0.16	1.00	
DHA	-0.03	0.06	0.92**	0.88**	0.18*	-0.07	1.00

* and ** correlations are significant at the 0.05 and 0.01 levels

2016). The measure of soil pH is an important parameter that helps in the identification of the chemical nature of the soil (Shalini et al 2003). P is the second most important macronutrient available in biological systems. S being soil a conditioner helps to reduce the sodium content of soils. The soil texture (clay) was considered another important indicator and it interacts with soil organic matter to form aggregates that protect the organic matter from decomposition. By considering the importance of these indicators in improving soil quality, these indicators such as SOC, soil pH, S, P and clay content were selected as the most appropriable key indicators of soil quality as MDS.

Scoring of indicators: "More is better" approach followed for SOC, available P and available S, while "optimum is better" approach followed for soil pH (6.5-7.5) and clay content (20-35 %). The weight of each PC on the basis of per cent variance to total variance ranges from 0.136 to 0.450 (Table 4). The weighted factor for each PC was: 0.450 (PC-1),0.267 (PC-2), 0.150 (PC-3) and 0.136 (PC-4).

Soil quality index: The per cent contribution of selected soil quality indicators to SQI of rural and peri-urban areas are depicted in Figure 3. Among different indicators, SOC (41.23 %), pH (29.65 %), S (16.49 %), P_2O_5(6.49 %) and clay content (6.49 %) were contributed towards SQI of rural and peri-urban areas of southern transect of Bengaluru. The rural SQI 0.61 and peri-urban 0.54. The high soil quality in rural areas might be due to the high SOC (0.70 %), nearly neutral soil pH (6.8), high S (19.32 kg ha^{-1}), high P (28.98 kg ha^{-1}) and clay content (30.47 %). The low soil quality in peri-urban might be due to the industrialization and urbanization activities resulted in accumulation of heavy metals suppress the availability of other nutrients in soil which leads to deficiency of nutrients in soil. The excess application of nitrogenous fertilisers leads to soil acidity which in turn affects the availability of nutrients. The quality of the soil is altered by imbalance in fertilizer use, acidification, intensive farming activities and most undesirably, soil erosion (Masto et al 2008, Bilgili et al 2017).

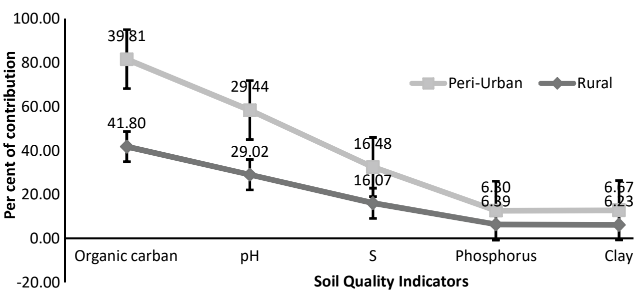

Fig. 3. Per cent contribution of soil key indicators towards soil quality index of rural and peri-urban areas of southern transect of Bengaluru

CONCLUSIONS

Soil organic carbon and soil pH are the most important key indicators of soil quality as they govern the nutrient supplying power of soil and availability of nutrients in soil**.** Thus, if the soil quality and its productivity is to be improved in the region, appropriate measures need to be taken to improve soil organic carbon, ameliorate soil acidity and improve P and Zn supply. Conservation agriculture, judicious use of chemical fertiliser, soil erosion control measures, organic residues incorporation, *etc*., should be promoted to increase the organic carbon status of the soil. The process of urbanization leads to shift in land use system, farmers instead of growing agriculture crops they started growing commercial crops with injudicious use of fertilizers or dumping of fertilisers to get higher returns, in long run which lead to soil acidity and imbalance of nutrients and further lead to drastic reduction in the productivity of crops and soils become unsuitable for crop production.

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Effect of Tree Species and Organic Manures on Physio-Chemical Properties of Coal-Mine Soil and Seed Germination

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Abstract: The present study was attempted to document the influences of three tree species viz., *Dalbergia sissoo* (shisham), *Gmelina arborea* (ghamari) *and Acacia nilotica* (babul) and organic manures such as vermicompost, farm yard manure and poultry manure on physico-chemical properties of coal mine soil. Coal mine soil amendment mix was prepared and used as top layer in all treatments. The organic amendments viz. vermicompost (VC), farm yard manure (FYM) and poultry manure (PM) were applied uniformly @ 0, 20, 40 and 60 kg as a top layer. Top soil amendment was also used as one of treatments along with species and organic manures. Treatments were studied as individual and different combinations of treatments. Ninety viable seeds of each test species were sown in each treatment and each treatment replicated three times. The tree species, top soil and organic manures had significant positive effect on physico-chemical properties of coal soil. Highest seed germination of all test species was in control and *D. sissoo* had highest germination percent. Organic amendments and tree species had significant effect in coal mine soil amelioration and re-vegetation on it.

Keywords: Coal-mine, Vermicompost, Reclamation, Soil, Revegetation

The coal industry alone converts about 500 ha of land as biologically unproductive in 1994-95, which rose to 1400ha by 2000AD in India (Chari et al 1989). During opencast mining, the entire existing vegetation is uprooted (Kundu and Ghose 1997). Mining causes several changes in physical, chemical and microbiological properties of mine soils and are unsuited for plant and microbial growth because of low organic matter content, unfavorable pH, and drought arising from coarse texture of oxygen deficiency due to compaction (Dutta and Agarwal 2000). It is universally known that the plants produce many beneficial interactions with the surroundings in which they grow and vice-versa. The mine soil is influenced greatly by plant species in many ways. Addition of organic manures help plants to bio-rejuvenate by themselves. It also promotes microbial activity and release organic acids through litter decomposition which improve physical properties such as porosity, field capacity, bulk density and aeration of coal mine soil. Organic matter is the most capable and potent substance that greatly influences mine soil composition due to humus deposition in a man-made forest. The tree species may speed up reclamation and bio rejuvenate process in a relatively short period of time. Therefore, self-sustainability and regenerative capacities of abandoned mining areas should be developed for the proper functioning of the ecosystems through re-vegetation. Addition of organic manure plays vital role in increasing the organic C content, CEC and t soil fertility in mine soil and also macro nutrient fertilizer (N, P, K) is required at high doses reclamation and enhancing productivity (Pranayama et al 2019). During open cast mining, topsoil stripping and stockpiling are important practices since topsoil is critical element for the successful restoration of mine soil (Ghose 2001). Topsoil cannot always be placed directly onto mined out land. Therefore, it may be necessary to stockpile the resource for future use (CMSACRA, 2007). Poor management of topsoil and stockpiles will lower the rehabilitation potential of the soils and increase rehabilitation costs. Restoring the soil productivity and re-vegetation are primary objectives of mine soil reclamation (Mushia et al 2016). The present study was aimed to study the combined effect of tree species and organic manures on physical and chemical properties of coal mine soils and to find out the promising tree species for revegetation in coal mine dumps.

MATERIAL AND METHODS

Study area: A study was conducted during in the month of March – June, 2011 at Forest Research Centre, Mandar, Jharkhand (23^0 27' 41.3" N and 085^0 05' 57.0"N) at an altitude of 703m above mean sea level, having an annual average rainfall of 1400mm; humid to sub humid tropical type of climate. Annual temperature ranges from maximum 42 to 20^{0}C during summer and 25 to 4^{0}C during winter season. Soil of study site is lateritic in nature.

Experiment details: On the cemented platform (1.0 m height from ground level), eight rectangular shape nursery beds with size 3.40 X 1.36 m were made with bricks lining. The beds were filled uniformly with coal mine soil @ 150 kg per bed. Then each bed was divided into 4 equal sub- plots with dimensions of 1.36 X 0.75 X 0.17 m. Stone chips (2mm jelly) was spreaded uniformly at base of bed up to about 30 cm thickness prior to filling of amended coal-mined soil treatment. Above this layer, another layer was formed with coal- mine-soil - amendment mix which includes 50,10 and 10 % municipal waste, saw dust and bio-char of coal mine soil (w/w). Then, three organic amendments viz. vermicompost (VC), farm yard manure (FYM) and poultry manure (PM) were applied uniformly @ 0 (T1), 20 (T2), 40 (T3) and 60 kg (T4) as a top layer. Each dosage represents as a treatment and each treatment replicated thrice. Influences of three tree species viz., *Dalbergia sissoo* (Shisham)*, Gmelina arborea* (Ghamari) *and Acacia nilotica* (Babul) and organic amendments on physico-chemical properties of coal mined soil at end of 3- month period. Representative soil samples were collected randomly two times from each the treatment, i.e., at time of seed sowing and at three-month-old end of experiment. The collected samples were processed and subjected to analysis of physical and chemical properties (bulk density, soil pH, EC, organic carbon, avail N, P, K were determined with standard methods (Black 1963).

RESULTS AND DISCUSSION

Physical properties of coal mine soil: The soil physical properties viz. bulk density, particle density, porosity, maximum water holding capacity and volume expansion were changed significantly with species, top- soils and addition of organic manures such as VC, FYM and PM (Table 1). The physical properties of coal mine soils were significantly changed with influence of species planted, organic manures and combination of both. The bulk density of treated coal mine soil under all tree species was lower than that of control. The highest bulk density was in *D. sissoo* and lowest in *A. nilotica*. The particle density was high (2.03) in *G. arborea* and low in *A. nilotica*. The porosity of all species plots was significantly higher than that of control. The porosity, water holding capacity and volume were higher in *G. arborea*. The tree species and top-soil had combined influences the soil physical properties of coal mine soil significantly. The bulk density of all top-soil incorporated plots had higher value than that of control. The highest bulk density was in top-soil added *D. sissoo* plot whereas top-soil added *G. arborea* plot had lowest bulk density. Similarly, the particle density of top-soil added plots had shown higher values than that of control. On other hand, the porosity, water holding capacity and volume were found low in all top-soil added plots.

Similarly, both organic manures and species had shown significant effect on physical properties of coal mine soils. The bulk density and particle density of organic manures added coal mine soils were found lower than that of control under *D. sissoo*. The bulk density of organic manure- added coal mine plots followed the order viz. vermicompost plot > FYM plots > Poultry manure. The lowest bulk density was 0.50 found in PMD40 whereas highest was 0.92 in control followed by FYMD20 and VCD20. Both particle density and porosity of manured plots was FYM plots > Vermicompost > Poultry manures. The particle density was maximum in control and minimum in PMD20. The lowest porosity too was noted in PMD20. The highest porosity and maximum water holding capacity was in PMD60, whereas lowest volume expansion was in PMD60. As stray dogs were damaged some manured plots under *A. nilotica and G.arborea*, the results were interpreted with undisturbed treatments only. The data revealed that the bulk density was slightly increased with organic manures under *A. nilotica* when compare with control. The highest bulk density was observed in PMA60 and lowest was in PMA40. The particle density was lower than control in all manured plots except FYM60 and PMA60. Further, the porosity was decreased with increasing poultry manure. Both water holding capacity and volume expansion were high in PMA40. The lowest water holding capacity and volume expansion was in PMA60 and FYMA60.The bulk density of manured plots under *G. arborea* was ranged from 0.72 (PMG20) to 0.91 (control). The bulk density of manured plots was lower than that of control. The particle density too was shown similar trend as bulk density. The maximum porosity and water holding capacity was recorded in PMG20 and minimum of the same was in FYM20 and control, respectively. Besides, the highest volume expansion was in PMG40 and lowest in FYMG20 (Table 1). Thus, both plant species and organic manures had caused favorable effect on bulk density and water holding of coal-mine soil.

Chemical properties of coal mine soil: The plant species and organic manures caused significant changes in chemical properties of coal- mine soils. In general, plants raised pH level from acidity to near-alkalinity. The high pH was 7.10 in TSMD and low (5.7) in control. The EC changed with species. The EC of control and *A. nilotica* plots were same. The highest EC was 0.23 dS/m in TSMD. The organic content increased in all plots when compare with control. The OC content was high d in TSMA and low in TSMD. The available N content ranged from 106.8 to 422.55 ppm. The available N and K content had shown same trend. The high available N and K was noted in TSMG and low in control. The available P content had shown reverse trend of N and K.All top-soil plots were acidic in nature and pH ranged from 5.6 to 6.3. The EC

Table 1. Effect of species and organic manures on physical properties of coal-mine soil

Treatment	Treatment details	Bulk density	Particle density	Pore space	Maximum water holding capacity/100 g of soil	Volume expansion per 100 g of soil
TC00	Coal mine-amendment mixonly	1.2	1.96	42.13	34.78	2.01
Species effect						
TSMG	Coal mine-amendment + *G. arborea*	0.91	2.03	61.53	64.54	2.98
TSMA	Coal mine-amendment + *A .nilotica*	0.9	1.69	50.52	52.16	0.79
TSMD	Coal mine-amendment + *D. sissoo*	0.94	2.16	61.19	61.56	1.64
	Mean	0.9	2.0	57.7	59.4	1.8
Top soil effect						
TTSA	Coal mine-amendment + top soil + *A. nilotica*	1.22	2.03	42.61	34.43	1.54
TTSD	Coal mine-amendment + top soil + *D. sissoo*	1.26	2.1	41.66	32.09	0.35
TTSG	Coal mine-amendment + top soil + *G. arborea*	1.19	2.07	44.3	36.22	0.91
	Mean	1.22	2.07	42.86	34.25	0.93
Combined effect of species and organic manures(kg/1.1m^2)						
D. sissoo	VC -2O	0.9	1.4	50.4	60.9	6.1
	VC -4O Kg/1.1m2	0.8	1.1	48.5	68.5	13.0
	VC -6O Kg/1.1m2	0.9	1.5	53.8	69.6	5.6
	Mean	0.8	1.4	50.9	66.3	8.3
	FYM -2O Kg/1.1m2	0.9	1.8	57.1	61.1	1.7
	FYM -4O Kg/1.1m2	0.8	1.4	53.4	65.6	2.2
	FYM -6O Kg/1.1m2	0.8	1.2	52.9	80.1	11.8
	Mean	0.8	1.5	54.5	68.9	5.2
	PM -2O Kg/1.1m2	0.5	0.6	42.4	93.9	17.8
	PM -4O Kg/1.1m2	0.5	0.7	47.8	98.6	8.1
	PM -6O Kg/1.1m2	0.6	1.2	63.4	99.3	4.7
	Mean	0.5	0.8	51.2	97.3	10.2
A. nilotica	FYM 20kg /1.1m2	1.0	2.3	61.0	59.4	1.0
	FYM 60kg /1.1m2	0.9	1.7	55.9	59.6	3.7
	Mean	0.9	2.0	58.4	59.5	2.3
	PM 20kg /1.1m2	0.8	1.8	58.2	64.1	2.2
	PM40kg /1.1m2	0.8	1.5	57.1	71.9	6.6
	PM 60kg /1.1m2	1.1	2.2	54.5	47.9	1.5
	Mean	0.9	1.8	56.6	61.3	3.4
G. arborea						
	VC -4O Kg/1.1m^2	0.8	1.3	53.2	78.4	9.9
	FYM -2O Kg/1.1m^2	0.8	1.4	47.3	61.3	0.3
	FYM -4O Kg/1.1m2	0.9	1.5	52.9	62.5	2.4
	Mean	0.8	1.5	50.1	61.9	1.4
	PM -2O Kg/1.1m2	0.7	1.6	63.7	86.9	2.2
	PM -4O Kg/1.1m2	0.8	1.0	47.4	69.4	18.6
	PM -6O Kg/1.1m2	0.7	1.1	54.7	79.9	10.8
	Mean	0.8	1.3	55.3	78.7	10.5

of the top – soil added – plots were lower than that of control. The lowest EC was 0.06 dS/m in TTSD and highest in TTSG. The organic content of top-soil plots was higher than that of control and rest of the treatments. Available N content in top-soiled plots was influenced by top soil. The highest N content among top-soil added plots was 405.1ppm in TTSG and lowest in TTSD. The available P content was comparatively lower than that of control. The maximum P was inTTSA among top-soiled plots. The available K content was much lower than that of rest of treatments in top soil added plots. The high K was 26.4ppm in top-soil added TTSA and low K in TTSG.

Both vermicompost and tree species positively influenced the chemical properties of coal–mine soil equally. Soil pH was increased towards neutral pH from the acidic conditions under VC treated plots under *D. sissoo*. The range of pH under *D. sissoo* was 6.7-7.5. The highest pH in TFYMD2 and lowest in control followed by TVCD1. The pH of organic treated plots under *D. sissoo* exhibited the following order: VC< FYM < Poultry manure. EC of the all manured plots under *D. sissoo* was much higher than that of control. It was constantly increased with addition of organic manures. The highest EC was 1.07 dS/m in PMD and lowest was 0.2 dS/m in TFYMD1. The OC recorded decreasing trend with VC and FYM under *D. sissoo* whereas the OC of PM had increasing trend. The maximum OC was 19.82% in TPMD3 and lowest (4.48%) in TVCD2. The available N, P and K of the treated plots too were changed with organic manures and *D. sissoo*. Both N and K had shown increasing with addition of FYM while others did not any trend. The available nutrient content was higher in all treated plots when compare with control. The N, P and K ranged between 267.39 – 1572.8 ppm, 1.0 – 14.1ppm and 342.8ppm, respectively. The highest value of N, P and K was in TPMD2, TVCD1 and TPMD1, respectively. The TVCD1, TPMD2 and TPMD3 plots had shown low content of N, P and K, respectively (Table 2).

The pH of manured treated plots under *A. nilotica* was neutral when compare with control. The pH range of treated plots was between 6.7 in TVCA1and 7.2 in TPMA2. EC had significantly increased in all treated plots. The highest increase was in poultry manured plots (TPMA3) and low in TPMA1. The OC content ranged from 5.44 to 13.4%. The OC was high in FYM treated plots and low in TPMA1. The available N, P and K was between 100 – 305.5, 0.61 – 10.82 and 142.5 - 783.7ppm, respectively. The maximum N, P and K was in TPMA1, TVCA1 and TPMA3, respectively. The pH of all treated plots under *G. arborea* ranged from 6.57 to 7.4. Among treated plots, poultry manured had high pH values than in others. The highest and lowest pH was in TPMG1 and TVCG1, respectively. EC of organic manured plots were ranged from 0.17 to 0.69 dS/m. The highest EC was in TPMG3 and lowest in TVCG1. The OC also changed with addition of manures and was between 4.26-12.4%. The TFYMG2 had shown highest OC among the treated plots. In the *G. arborea* planted plots, the available N, P and K content varied from 277.48-586.81, 1.53-13.27 and 135.4-1033.5ppm, respectively. The highest P and K content were recorded in TPMG3, whereas available N was maximum in TPMG1 (Table 2).

The physical and chemical properties of mine soils tend to inhibit soil-forming processes and plant growth due to a lack of nutrients associated with SOM, nitrogen and phosphorus (Ussiri and Lal 2005). Soil amendments had significant effect on improvement on soil properties. Soil amendments had showed high-rate plant growth (Mohapatra and Goswami 2012).Organic manures contain humic substances with many functional groups viz., carboxyl and phenolic groups that are able to consume protons at their natural pH values (Stevenson and Vance 1989). Crop yield is maximum in poultry manure amended soil as it supplies more balanced nutrition especially Ca and Mg and adds large quantity of cations to the soil (Adediran et al 2005, Adediran and Ojeniyi 2006). Moreover, compost may increase the cation exchange capacity of soils, and also allow crops to utilize nutrients more effectively while reducing nutrient loss through leaching.

Germination: The germination percentage differ significantly among treatments and between tree species. The highest germination per cent of *D. sissoo* was in control and top-soiled coal mine. Among the vermi-composted coal mine soils, maximum germination was 30.4% in control whereas in FYM incorporated coal mine soil treatments, the germination was 43.3% in control. Among all treatments, top soiled coal mine soil had highest germination (44.4%). The mean germination per cent of *D. sissoo* followed the order: top – soil treated mine soil > FYM treated mine soils > poultry manures (PM) treated mine soils > VC treated soils. The highest germination of *Gmelina arborea* was 68.5% in TPM0 and lowest was 4.8% in TVC3. Maximum germination was in TVC0 and TPM0 under VC and PM treated mine-soils. The germination per cent of *Gmelina arborea* shown an increasing trend with addition of organic manures (FYM and PM) in coal mine soils. The germination of *Gmelina arborea* had shown the typical trend PM mine soils > FYM mine soils > VC mine soils. The germination of *Acacia nilotica* in amended coal mine that germination percent was decreasing trend in VC treated mine soils and increasing trend in PM treated mine soils. Among the three test tree species, the rate of germination of both *D. sissoo* and *G. arborea* was higher than that of *A. nilotica*. Over all, the highest germination per cent

Table 2 Chemical properties of organic manure treated coal mine under *Dalbergia sissoo*, *Gmelina arborea* and *Acacia nilotica*

Species	Treatment	Treatment details	pH (1:2.5) @ 30°C	EC (1:5) mS/cm	OC%	N (ppm)	P (ppm)	Avail K (ppm)
-	TC00	Soil mixture with no manure/top soil/spp.	5.7	0.2	3	107	12	44
Species effect								
G. arborea	TSMG	Soil mixture with no organic manure	6.9	0.2	6	423	8	221
A. nilotica	TSMA	Soil mixture with no organic manure	6.8	0.2	7	136	9	142
D. sissoo	TSMD	Soil mixture with no organic manure	7.1	0.2	3	149	2	330
	Mean		6.9	0.2	5	236	6	231
Top soil effect								
A.nilotica	TTSA	Soil mixture with top soil	5.6	0.1	3	152	8	26
D. sissoo	TTSD	Soil mixture with top soil	6.3	0.1	4	110	8	24
G. arborea	TTSG	Soil mixture with top soil	5.6	0.2	4	405	8	19
	Mean		5.8	0.1	4	222	8	23
Combined effect of species and organic manures (kg/1.1m^2)								
D. sissoo	TVCD1	VC @ 2O	7	0	6	267	14	414
	TVCD2	VC @ 4O	7	1	4	456	8	1122
	TVCD3	VC @ 6O	7	1	5	322	10	654
	Mean		7	0	5	348	11	730
	TFYMD1	FYM @ 2O	7	0	6	100	8	343
	TFYMD2	FYM @ 4O	7	0	6	224	8	587
	TFYMD3	FYM @ 6O	7	0	5	328	1	750
	Mean		7	0	6	217	6	560
	TPMD1	PM @ 2O	7	1	18	1400	1	1537
	TPMD2	PM @ 4O	8	1	19	1573	1	988
	TPMD3	PM @ 6O	7	1	18	1034	9	139
	Mean		7	1	18	1335	4	888
Acacia nilotica	TFYMA1	FYM @ 20kg	7	1	6	572	1	729
	TVCA3	FYM @ 60kg	7	0	13	180	0	440
	Mean		7	0	10	376	1	584
	TPMA1	PM @ 20kg	7	0	5	305	7	196
	TPMA2	PM@ 40kg	7	0	6	257	1	547
	TPMA3	PM @ 60kg	7	1	6	100	7	784
	Mean		7	0	6	221	5	509
G.arborea	TVCG1	VC @ 4O	7	0	10	277	8	871
	TFYMG1	FYM @ 2O	7	0	12	166	13	392
	TFYMG2	FYM @ 4O	7	0	12	252	2	454
	Mean		7	0	12	209	7	423
	TPM1	PM @ 2O	7	0	3	587	8	491
	TPM2	PM @ 4O	7	0	10	411	3	768
	TPM3	PM @ 6O	7	1	4	537	8	1034
	Mean		7	0	6	512	6	764

Table 3. Germination percent of different tree species in organic manure treated soil

Treatment code	Particulars	Germination (%)		
		Dalbergia sissoo	*Gmelina arborea*	*Acacia nilotica*
T00	Control	40.6	28.4	5.2
TVC1	T1: Vermicompost @ 20 kg/ 1.13 m^2	15	5	6
TVC2	Vermicompost @ 40 kg/ 1.13 m2	4	7	5
TVC3	T1: Vermicompost @ 60 kg/ 1.13 m2	8	5	1
	Mean	9	6	4
TFYM1	T1: FYM @ 20 kg /1.13 m^2	21	9	4
TFYM2	T1: FYM @ 40 kg /1.13 m2	22	10	7
TFYM3	T1: FYM @ 60 kg /1.13 m2	21	11	6
	Mean	22	10	6
TPM1	Poultry manure @ 20 kg/ 1.13 m^2	16	18	4
TPM2	Poultry manure @ 40 kg/ 1.13 m2	16	34	5
TPM3	Poultry manure @ 60 kg/ 1.13 m2	17	40	7
	Mean	16	31	6

was in control (Table 3) which may due to seeds were sown before beginning of reclamation process.

CONCLUSION

Addition of organic amendments in coal mine soil not only increased pH towards neutral from acidic conditions, but also enhanced available nutrients content to plants. Among amendments, poultry manure had quicker response in reducing soil acidity in coal mine soil. Seed germination percent varies with type of species and amendments. Among three tested species, *D. sissoo* was performed well in amended coal mine. Addition of organic amendments in coal mine help to reduce physiological nutrient stress by ameliorating physicochemical properties of soil and enhance re-vegetation capacity of coal mine soil. However, more detailed studies are required for development of site-specific reclamation methods.

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Effect of Varying Irrigation and Fertigation Levels on Soil Water Dynamics and Water Use Efficiency of Potato in Wet Temperate Zone of Himachal Pradesh

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Abstract: The present study was conducted at the experimental farm of Department of Soil Science, CSK HPKV, Palampur to study the effect of varying irrigation and fertigation levels on soil moisture content, relative leaf water content and water use efficiency of Potato. The experiment comprised of ten treatments, each replicated three times, including three drip irrigation levels viz., 0.4 PE, 0.6 PE and 0.8 PE, three fertigation levels viz., 50% RDF, 75% RDF and 100% RDF and Recommended Practice. Soil moisture content and relative leaf water content (RLWC) were significantly higher at irrigation level of 0.8 PE as compared to Recommended Practice. The WUE and Irrigation water use efficiency (IWUE) decreased with increase in the irrigation levels whereas fertigation levels increased it. Both WUE and IWUE were significantly highest at irrigation level of 0.4 PE followed by 0.6 PE and minimum at irrigation level of 0.8 PE over recommended practice.

Keywords: Drip irrigation and fertigation, RLWC, WUE, Soil moisture content, Potato

Potato (*Solanum tuberosum* L.) is an herbaceous plant with sparse and shallow rooting system and requires light and frequent irrigations throughout period of crop growth. It is very sensitive to the changes in the soil moisture content and the decrease in water content, leading to a significant reduction in tubers quantity as well as quality. Many factors affected potato production, including cultivars, weather conditions, planting date, nutrition and irrigation. The water requirement of potato vary under different growth stages. Tubers initiation and tubers bulking stages are the most sensitive growth stages of potato to water deficit. During water deficit conditions, many physiological and biochemical processes are disturbed. Continuous water supply is generally recommended from tuber initiation to maturity stage (Khalel 2015). Improved irrigation methods like drip method can save water without compromising potato yield or quality. As conventional method of irrigation results in losses of nutrients through leaching, surface runoff and also create adverse condition for plant growth like water logging to some extent. Efficient irrigation techniques are therefore needed for maximizing the efficiency of water and applied nutrients. Drip irrigation is the most efficient method of water utilization for the crop growth which helps to achieve considerable amount of water saving with high WUE compared to surface irrigation method, where irrigation efficiency is low due to losses in water distribution on the field. So, scheduling water application is very critical to make the most efficient use of drip irrigation system, as excessive irrigation reduces yield, while inadequate irrigation causes water stress and reduces production. Drip irrigation method has ability to apply small but frequent irrigation, and has found to be superior over flood method in respect of water saving, yield, quality of produce and WUE (Kassu et al 2017).

Moreover, the drip fertigation systems offer opportunity to apply appropriate amount of nutrients and chemicals along with water, which reduces leaching losses and enhances water content in soil as well as IWUE. Drip irrigation can save water use by 30 to 70 per cent and raises crop yields by 20 to 90 per cent depending on soil, climatic and crop characteristics. It mainly controls the vegetative growth and improving the IWUE (Ayas, 2021). The relative leaf water content (RLWC) is an important indicator of water status in plants as it reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan and Ciulca, 2011). Research on drip fertigation conducted so far in India and abroad has shown that this method leads not only the appreciable saving of water as well as fertilizer but also returns in achieving higher crop yields as compared to flood irrigation method. For this, advanced irrigation and fertilization techniques are required. Therefore, the present investigation is executed to study the effect of different irrigation and fertigation levels on soil water content, RLWC and WUE of potato in wet temperate zone of Himachal Pradesh.

MATERIAL AND METHODS

The present study was conducted at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The experimental farm is situated at 32° 6' N latitude and 76° 3' E longitude at an altitude of about 1290 m above mean sea level. The site lies in the Palam valley of Kangra district representing mid hill wet temperate zone (Zone 2.2) of Himachal Pradesh. Taxonomically, the soils of study area fall under order Alfisol and sub-group Typic Hapludalf. These soils have originated from rocks like slates, phyllites, quartzites, schists and gneisses. The field experiment on potato (cv. Kufri Jyoti) was laid out in randomized block design with ten treatments, each replicated three times. Ten treatments comprised of three drip irrigation levels viz., 0.4 PE, 0.6 PE and 0.8 PE corresponding to 40, 60 and 80 per cent of cumulative pan evaporation, respectively, three fertigation levels viz., 50% RDF, 75% RDF and 100% RDF equivalent to 50, 75 and 100 per cent of recommended dose of NPK, respectively and RP i.e. recommended practice (recommended dose of fertilizers was applied through conventional methods and 6 flood irrigations of 50 mm each). The recommended dose of fertilizers (RDF) for potato was N, P_2O_5 and K_2O were 120, 80 and 60 kg/ha, respectively. The irrigations were applied through online drip system on alternate days for each treatment. Fertigation was done as per treatments using urea and water soluble fertilizers 17:44:00 and 00:00:50 in calculated proportions. Fertigation was started after complete emergence of the crop. Fertigation with 17:44:00 and 00:00:50 was completed in 5 splits whereas, urea was applied in 10 splits. In the last treatment i.e. RP, recommended dose of fertilizers was applied through urea, single super phosphate and muriate of potash. Half of the nitrogen dose (60 kg/ha N) and full dose of phosphorus and potassium was applied in the RP treatment at the time of sowing. The remaining dose of nitrogen in this treatment was applied at the time of earthing up. Six irrigations @ 50 mm water per irrigation were applied during the crop period. Soil samples were collected periodically at an interval of 15 days after the start of drip irrigation from each plot from a depth of 0-0.15 m and 0.15-0.30 m for determination of moisture content in soil.

Relative leaf water content: The relative leaf water content (RLWC) was determined at tuber initiation and bulking stages at 7:00 am and 12:00 noon. It was computed from the fresh weight, turgid weight and oven dry weight according to the method given by Weatherly (1950).

$$RLWC = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Fully turgid weight} - \text{Oven dry weight}} \times 100$$

Water use efficiency: The tuber yield obtained for each treatment was divided by the quantity of water used (irrigation water + rainfall) for the respective treatments by this method. Water use efficiency was worked out and expressed in kg ha-mm^{-1} of water used.

$$WUE = \frac{\text{Yield (kg ha}^{-1}\text{)}}{\text{Total water use (ha-mm)}}$$

Likewise, irrigation water use efficiency was worked out as:

$$IWUE = \frac{\text{Yield (kg ha}^{-1}\text{)}}{\text{Irrigation water use (ha-mm)}}$$

RESULTS AND DISCUSSION

Soil moisture studies during crop growth: The soil moisture contents determined at 15 days interval throughout the growth period (Fig. 1 and 2). The soil moisture contents determined on dry weight basis at first sampling after initiation of the crop on February 17, 2016 were 22.6 and 23.9 per cent in 0.4 PE, 22.7 and 23.5 per cent in 0.6 PE and 22.5 and 23.8 per cent in 0.8 PE at 0-0.15 and 0.15-0.30 m depths, respectively. The soil water contents were almost similar under all the irrigation levels. The contents in the sub surface layer were found to be higher in comparison to the top layer. The moisture contents determined after 15 days of first sampling that is on 3-3-2016 were higher in comparison to previous sampling owing to the effect of drip irrigations. The highest moisture content of 25.6 per cent was with irrigation level of 0.8 PE whereas, minimum (23.8 %) was in 0.4 PE followed by 0.6 PE (24.8 %) in 0-0.15 m depth. Like the previous sampling, the soil moisture contents in 0.15-0.30 m depth were higher in comparison to the top soil and varied between 26.3 per cent (0.4 PE) to 27.2 per cent (0.8 PE). The moisture content recorded on March 19, 2016 did not show much difference among various levels as it varied from 28.5 per cent at 0.4 PE to 28.9 per cent at 0.8 PE. Almost similar contents were recorded in the sub surface layer of 0.15-0.30 m depth also as these varied from 28.0 per cent in 0.4 PE to 29.1 per cent in 0.8 PE.

This was probably due to the significant amount of water received during the preceding week through rainfall which might have neutralized the effect of irrigation levels. The moisture contents in samples collected on 2-4-2016 varied from 21.6 per cent at 0.4 PE to 24.6 per cent at 0.8 PE in surface soil whereas, in the 0.15-0.30 m soil depth these varied from 24.5 per cent in 0.4 PE to 25.3 per cent in 0.8 PE. The moisture content at the next sampling on 17-4-2016 varied from 22.5 per cent at 0.4 PE to 23.8 per cent at 0.8 PE. Little higher moisture contents were recorded in the sub surface layer of 0.15-0.3 m depth also as these varied from 23.5 per cent in 0.4 PE to 24.7 per cent in 0.8 PE.

The moisture contents in top soil (0-0.15 m) recorded on May 2, 2016 varied from 21.7 per cent at 0.4 PE to 23.6 per cent at 0.8 PE and from 22.3 per cent at 0.4 PE to 24.1 per cent at 0.8 PE in samples collected on 17-5-2016. Likewise, the moisture contents in 0.15-0.30 m soil depth on 2-5-2016 and 17-5-2016 varied between 24.6 and 23.8 per cent, respectively at 0.4 PE to 25.4 and 24.7 per cent, respectively at 0.8 PE. The moisture contents at the last sampling on June 1, 2016 were comparatively higher in comparison to the previous sampling and similar trend was observed with respect to the drip irrigation levels. The moisture contents in the lower layer were higher in comparison to the surface layer. Soil moisture contents under recommended practice were almost equal or higher than recorded under 0.8 PE at all the sampling stages except the last where these were lower in both the soil depths. In general, higher moisture contents were observed at irrigation level of 0.8 PE in comparison to the other levels due to higher amount of water applied in this treatment. The soil water content changed more under the influence of irrigation levels in the upper soil layer than in the lower layer. Similar results have been reported by Mokh et al (2014), Kapoor (2016) and Abedin et al (2017).

Relative Leaf Water Content (RLWC)

Tuber initiation stage: Application of different levels of irrigation water influenced the relative leaf water contents significantly at this stage (Table 1). Application of water @ 0.8 PE recorded significantly higher RLWC at tuber initiation

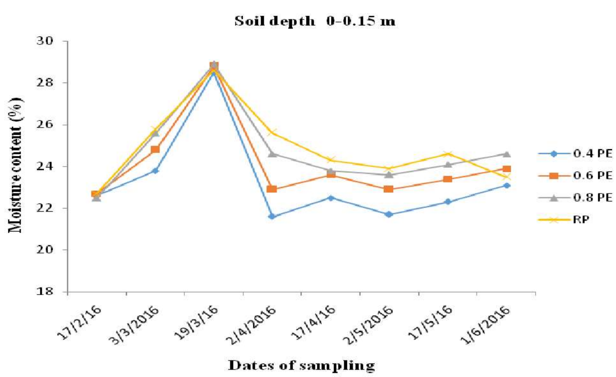

Fig. 1. Effect of irrigation levels on soil water content during crop growth at 15 days interval in 0-0.15 m soil depth

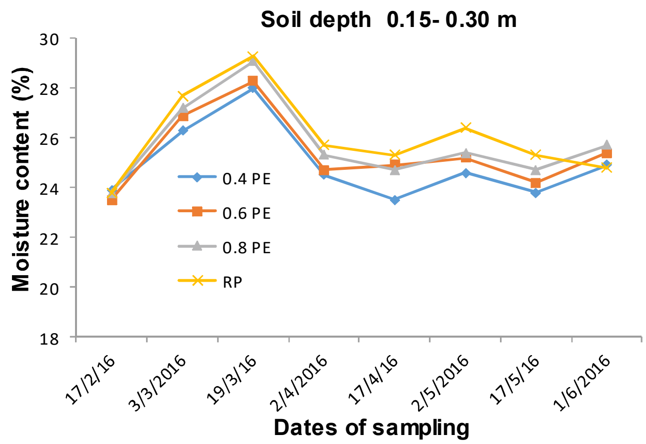

Fig. 2. Effect of irrigation levels on soil water content during crop growth at 15 days interval in 0.15-0.30 m soil depth

Table 1. Effect of drip irrigation and fertigation levels on relative leaf water content (RLWC) at tuber initiation and bulking stage

Treatment	RLWC (%)			
	Tuber initiation stage		Bulking stage	
	7 am	12 pm	7 am	12 pm
Irrigation level				
0.4 PE	80.2	70.5	80.6	71.5
0.6 PE	81.2	72.4	81.5	72.7
0.8 PE	81.7	74.4	82.0	74.7
CD (P=0.05)	1.2	2.7	1.0	2.4
Fertigation level				
50 % RDF	80.8	71.8	81.2	72.5
75 % RDF	81.1	72.2	81.4	72.5
100 % RDF	81.2	73.3	81.5	73.9
CD (P=0.05)	NS	NS	NS	NS
Recommended practices (RP) vs others				
RP	82.6	76.1	82.5	76.8
Others	81.0	72.4	81.4	73.0
CD (P=0.05)	1.5	3.5	NS	3.1

stage (81.7 % and 74.4 %, at 7 am and 12 pm, respectively) in comparison to 0.4 PE (80.2 % and 70.5 %, at 7 am and 12 pm, respectively). However, no significant differences were observed in RLWC between irrigation levels of 0.4 PE and 0.6 PE. Likewise, RLWC at 0.6 PE was statistically at par with that observed at 0.8 PE. The higher relative leaf water content under irrigation level of 0.8 PE in comparison to 0.4 PE might be due to considerably higher amount of water applied under this level in comparison to later. Almost similar findings in different crops have been reported by Thakur (2015), Dorota et al (2016) and Kapoor (2016). RLWC under recommended practice was significantly higher (82.6 per cent and 76.1 per cent, at 7 am and 12 pm, respectively) in comparison to overall mean of others (81.0 per cent and 72.4 per cent, at 7 am and 12 pm, respectively) probably due to higher amount of water applied under recommended practice. Fertigation levels did not influence the relative leaf water content of potato plants significantly. The interaction between irrigation and fertigation was not significant.

Bulking stage: Application of different levels of irrigation water influenced the relative leaf water contents significantly at bulking stage also (Table 1). Similar trends with respect to the effect of irrigation and fertigation levels were observed in the RLWC at this stage as were observed during the tuber initiation stage. The RLWC contents of plants under drip irrigation levels of 0.4, 0.6 and 0.8 PE were 80.6, 81.5 and 82 per cent, respectively at 7.00 am and 71.5, 72.7 and 74.7 per cent, respectively at 12.00 pm. The higher RLWC values at 0.8 PE during bulking stage too might be ascribed to more availability of water to plants due to higher amount of irrigation water applied. The RLWC under recommended practice was statistically at par with overall mean of others at 7.00 am, though at 12 PM on same day, recommended practice recorded significantly higher RLWC (76.8 %) in comparison to overall mean of others (73 %). Fertigation levels did not influence the relative leaf water content of potato plants significantly. Also the interaction between irrigation and fertigation was not significant. Irrespective of irrigation or fertigation levels, during both the growth stages, RLWC contents recorded at 7.00 am were higher than recorded at 12.00 pm. However, no water stress was observed at any level of irrigation even at 12.00 pm as RLWC was more than 70 percent even at lower level of irrigation.

Water use efficiency (WUE): Water use efficiency at irrigation level of 0.4 PE was highest (38.4 kg/ha-mm) followed by 0.6 PE and minimum at irrigation level of 0.8 PE (33.5 kg/ha-mm) (Table 2). However, the difference in WUE was significant only between 0.4 PE and 0.8 PE. As regards the effect of fertigation levels, data revealed that WUE was significantly higher in 75 per cent recommended dose of fertilizers (39.3 kg/ha-mm) in comparison to fertigation level of 50 per cent recommended dose of NPK (32.2 kg/ha-mm) by about 22 per cent. However, 75 per cent recommended dose of fertilizer was statistically at par with 100 per cent recommended dose of fertilizers which recorded WUE of 37.6 kg ha-mm^{-1}. Similar results were reported by Ati et al (2012), Mokh et al (2014), Ghiyal et al (2016) and Kassu et al (2017). In recommended practice vs. others', WUE under others (36.4 kg/ha-mm) was significantly higher by about 37 per cent in comparison to the recommended practice (26.5 kg/ha-mm).

Table 2. Effect of drip irrigation and fertigation levels on WUE and IWUE

Treatments	WUE (kg/ha-mm)	IWUE (kg/ha-mm)
Irrigation level		
0.4 PE	38.4	137.6
0.6 PE	37.2	101.4
0.8 PE	33.5	76.8
CD (p=0.05)	4.0	12.9
Fertigation level		
50 % RDF	32.2	93.1
75 % RDF	39.3	113.5
100 % RDF	37.6	109.3
CD (p=0.05)	4.0	12.9
Recommended practices (RP) vs others		
RP	26.5	57.9
Others	36.4	105.3
CD (p=0.05)	5.2	16.7

Irrigation water use efficiency (IWUE): IWUE was significantly higher in 0.4 PE (137.6 kg/ha-mm) in comparison to 0.6 PE (101.4 kg/ha-mm) and 0.8 PE (76.8 kg/ha-mm) (Table 2). Among different fertigation levels, the IWUE was highest in 75 per cent recommended dose of fertilizers (113.5 kg/ha-mm) which was about 21.9 per cent higher than that obtained with 50 per cent recommended dose of fertilizers (93.1 kg/ha-mm). Increase in the fertigation level to 100 per cent of recommended fertilizers recorded IWUE of 109.2 kg/ha-mm, however, it was statistically at par with 75 per cent recommended dose of fertilizers. Similar results were reported by Badr et al (2012), Mokh et al (2014) and Elhania et al (2019). As regards the comparison of 'Recommended practice (RP)' vs. 'others', IWUE under overall mean of others (105.2 kg/ha-mm) had significantly higher irrigation water use efficiency in comparison to recommended practice (57.9 kg/ha-mm), the magnitude of difference being 47.3 kg/ha-mm (an increase of about 81.7 % over RP).

CONCLUSION

The study concluded that gravimetric moisture content varied in soil as per irrigation levels and comparatively higher moisture contents were observed in the sub-surface samples. The soil moisture contents under all the irrigation levels were close to the field capacity during the crop season. The RLWC at tuber initiation and bulking stages were significantly higher at irrigation level of 0.8 PE in comparison to 0.4 PE. However, there was no significant difference between 0.6 PE and 0.8 PE irrigation levels. RLWC under various fertigation levels were statistically at par. The WUE and IWUE decreased with increase in the irrigation levels. Both were highest at irrigation level of 0.4 PE and minimum at irrigation level of 0.8 PE. WUE and IWUE under drip irrigation were recorded an increase of about 37 and 81.7 per cent over recommended practice, respectively.

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Assessment of Soil Properties in Krishna Western Delta Ecosystem of Guntur district Andhra Pradesh

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Abstract: A study was conducted to assess the soil properties in Krishna western delta eco region in Guntur district of Andhra Pradesh. A total of 231 soil samples from 77 locations in three depths *viz.,* 0-15, 15-30 and 30-60 cm were collected for assessment. Soil properties *viz.,* texture, pH, EC_2, ECe, CEC, ESP, organic carbon, water soluble, exchangeable ion composition and available N, P and K were analyzed. Half of the soils of the study area are with clay loam texture. Soil reaction is mostly moderately alkaline, non-saline, none to slightly alkali hazard at all three depths of the soil. The organic carbon is low (<0.5%) with 42, 91 and 100 per cent samples at 0-15, 15-30 and 30-60 cm depth of soil respectively. Available nitrogen is low and available phosphorus is high at all the three depths of the soil. High available potassium was with the half of the soils at 0-15 and 15-30 cm depths of the study area. The study area consists of 87.01 per cent good soil, 9.09 per cent saline soil, 3.90 per cent saline alkali soil at 0-15 cm depth and 72.7 per cent good soil, 18.2 per cent saline soil, 9.09 per cent saline alkali soil at 15-30 and 30-60 cm depth of the soil for cultivation.

Keywords: Soil properties, Krishna western delta ecosystem, CEC, ECe, ESP

Krishna western delta ecosystem falls under eastern coastal plain, hot sub-humid to semi-arid eco region in Guntur district of Andhra Pradesh. The soils of the study area are covered with river borne alluvium and coastal sands in the area bordering to the coast. The thickness of alluvium varies from few meters to over 100m. The eco region is known for intensive cultivation of crops with the availability of good quality canal water. The soil resources are under severe stress due to lack of suitable management practices, indiscriminate use of input resources and also the salt laden winds and rains along the sea coasts carry oceanic salts in quantities sufficient to cause salinization in coastal areas. The coastal regions are also exposed to the risk of progressive salinization of land due to process like storms, cyclones, tidal surges, flooding (Rao et al 2014). In coastal areas of Andhra Pradesh, many farmers are practicing aquaculture by storing brackish water, drawn from the sea through creeks and drains, in big tanks for raising high value prawns this may leads soil degradation in adjacent agricultural soils. Hence, a study was conducted to assess the soil properties and their extent of degradation interms of salinity and alkalinity in Krishna western delta eco region.

MATERIAL AND METHODS

A total of 231 Soil samples were collected from 0-15 cm, 15-30 cm and 30-60 cm with geo-reference at 77 locations by taking location co-ordinates at Ponnur, Amarthalur, Tenali, Kolluru, Karlapalem, Duggirala, Nizampatnam and Bapatla mandals between 15.8577 and 16.2579 of Northern latitudes and 80.4727 and 80.8310 Eastern longitudes occupies northeastern part of Guntur district in Krishna western delta ecosystem in Andhra Pradesh. The collected samples were air dried under shade in room temperature. Roots and other debris present in soil samples were removed before grinding the soil samples using wooden pestle and mortar to pass through 2 mm sieve. Processed soil samples were analyzed for various physico-chemical and ionic compositions for characterization of salt affected soils. Saturation paste extract (1:1) was obtained by following standard procedure given by Gupta et al (2019) and same was used for analysis of water soluble ionic composition, pH in saturated paste extract and in 1: 2 soil water suspension was determined potentiometrically by using pH meter (Jackson 1973). Electrical conductivity was determined by using Conductivity Bridge (Willard et al 1974). Chlorides (Mohr's method), carbonates and bicarbonates (double indicator method) and calcium and magnesium (versenate method) were determined by adopting the procedures given by Richards (1954). Similarly the sodium and potassium were determined by using flame photometer (Richards 1954). Particle size analysis has been carried out through international pipette method (Piper 1966) using sodium hexametaphosphate as a dispersing agent. The textural class was determined by using USDA textural triangle. The cation exchange capacity (CEC) was determined by saturating a known weight of the soil with 1.0 N sodium acetate (pH 8.2), the excess sodium acetate was leached with 95% ethanol. The adsorbed sodium was displaced with 1.0 N neutral ammonium acetate. The concentration of the sodium in the lechate was determined by aspirating directly into the flame photometer. The CEC was calculated and was expressed in cmol (p^+) kg^{-1} soil (Bower et al 1952). The Sodium Adsorption Ratio (SAR) and Residual Sodium Carbonate (RSC) were calculated by using the formulas given by Richards (1954) such as SAR = Na/ $((Ca^{2+}+Mg^{2+})/2)^{0.5}$ and RSC = $(CO_3^{2-} + HCO_3^-) - (Ca^{2+} + Mg^{2+})$. The Na^+, Ca^{2+} and Mg^{2+} are in meq L^{-1}. RSC, CO_3^{2-}, HCO_3^-, Ca^{2+} and Mg^{2+} are in meq L^{-1}. The exchangeable sodium percentage (ESP) of soils was computed by the following equation (CSSRI 2004).

$$ESP = \frac{\text{Exchangeable sodium (cmol(p}^+\text{) Kg}^{-1}\text{ soil)}}{\text{Cation exchange capacity (cmol(p}^+\text{) Kg}^{-1}\text{ soil)}} \times 100$$

The soils are classified based on physicochemical properties into various salt affected soils (Richards 1954).

Statistical analysis: Research data were analyzed in SPSS 20.0 using Pearson correlation coefficient matrix to know significant variations between the soil physicochemical properties. Descriptive statistics were calculated using Microsoft Excel (Microsoft, WA, USA) spread sheet.

RESULTS AND DISCUSSION

Characteristics of Soils

Grouping of soils based on texture: The texture of the soil varied with depth and space in the study area (Table 1). The soils at 0-15 cm depth are mostly clay loam (50.0%), followed by loamy sand (22.7%), sandy clay loam (11.0%), sand (11.0%). At 15-30 cm depth the soils are mostly clay loam (50.6%), followed by loamy sand (23.4%), sand (14.3%) sandy clay loam (9.1.0%). At 30-60 cm depth 7 textural classes were identified; the dominant soils are clay loam (45.5%), followed by sand (14.3%), loamy sand (13.0%), sandy clay loam (9.1%), sandy loam (9.1%), sandy clay (5.2%) and loam (3.9%). The variation in soil texture with space and depth might be due to translocation and transportation of clay and sand materials (Purandar and Naidu 2020)

Grouping of soils based onsoil reaction: Based on soil reaction (pH) the soils at 0-15, 15-30 and 30-60 cm depth were mostly slightly to strongly alkaline in reaction, with increase in depth the alkalinity of soil was increased (Table 2). This might be due to variation in soil solution ionic composition (Gupta et al 2019).

Grouping of soils based on electrical Conductivity (ECe): Based on 1:1 Saturated soil water extract, the electrical conductivity (ECe) of the soils of the study area are grouped into 4 classes *viz.,* Non saline, Slightly saline, Moderately saline and Very strongly saline (Table 3) at all the three depths. The dominance of soluble ions *viz.,* Ca^{+2}, Mg^{+2}, Na^+ and Cl^- in soil may contribute higher salinity of soil at different depths (Mandal et al 2018).

Grouping of soils based on Exchangeable Sodium per cent (ESP): Based on exchangeable sodium percentage

Table 1. Textural class of soil in Krishna western delta region

Textural class	No. of samples	Per cent of samples	No. of samples	Per cent of samples	No. of samples	Per cent of samples
	0-15 cm		15-30 cm		30-60 cm	
Sandy clay loam	11	13.6	7	9.1	7	9.1
Sandy clay	0	0.0	0	0.0	4	5.2
Clay loam	39	50.0	39	50.6	35	45.5
Sand	11	13.6	11	14.3	11	14.3
Loamy sand	18	22.7	18	23.4	10	13.0
Sandy loam	0	0.0	0	0.0	7	9.1
Loam	0	0.0	2	2.6	3	3.9

Table 2. pH and the respective reaction of soils

Reaction class	pH range	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
		0-15 cm		15-30 cm		30-60 cm	
Neutral	6.6-7.3	18	22.73	11	13.64	4	4.55
Slightly alkaline	7.4-7.8	11	13.64	11	13.64	14	18.18
Moderately alkaline	7.9-8.4	35	45.45	39	50.00	39	50.00
Strongly alkaline	8.5-9.0	14	18.18	14	18.18	18	22.73
Very strongly alkaline	9.1-10.6	0	0.0	4	4.55	4	4.55

(ESP) the soils (Table 4) of the study area are classified into none to slightly alkali hazard (ESP<15) and slight to moderate alkali hazard (ESP 15-30) at 0-15, 15-30 and 30-60 cm depth, no evidence of moderate, high and extremely high alkali hazard was noticed. Sodic soils are developed due to the presence of sodium on exchangeable complex of soil at very few parts of the study area. The higher sodicity may cause ill drainage of soil. The lesser sodicity of soil might be due to dominance of Ca^{+2}, Mg^{+2}, Na^{+} and Cl^{-} ions rather than HCO_3^{-} and CO_3^{-2} ions in coastal areas (Shahid et al 2018).

Physical, Physico-Chemical, Chemical Properties of Soils

Soil texture: Sand was dominant in all the depths of soil followed by clay and silt. The clay content of soil increased with the depth Table 5.

Soil reaction: The soil reaction (pH in 1: 2 soil water suspension) ranged from 6.6-8.8, 6.6-9.8 and 6.7-9.8 at 0-15, 15-30 and 30-60 cm respectively (Table 5). With increase in depth of the soil the pH of soil increased, this might be due to the presence of soil organic carbon at surface may lower the pH of the soil due to release of some organic acids during decomposition (Dutta et al 2021).

Electrical conductivity: Electrical conductivity (1:2 soil water suspension) ranged from 0.1-6.9, 0.1-6.1 and 0.09-5.8 dS m^{-1} and electrical conductivity (1:1 saturated soil water extract) ranged from 0.4-29.6, 0.3-40, and 0.5-31.0 dS m^{-1},at 0-15, 15-30 and 30-60 cm depths respectively (Table 5). The relationship between EC_2 and ECe of soil at 0-15, 15-30 and 30-60 cm presented in Figure 1-3, respectively indicated that ECe of soil was 4 times higher than EC_2 at 0-15, 15-30 cm depth of soil indicated that maximum conductivity is with the surface layers. At 30-60 cm depth the soil ECe is 3.72 times

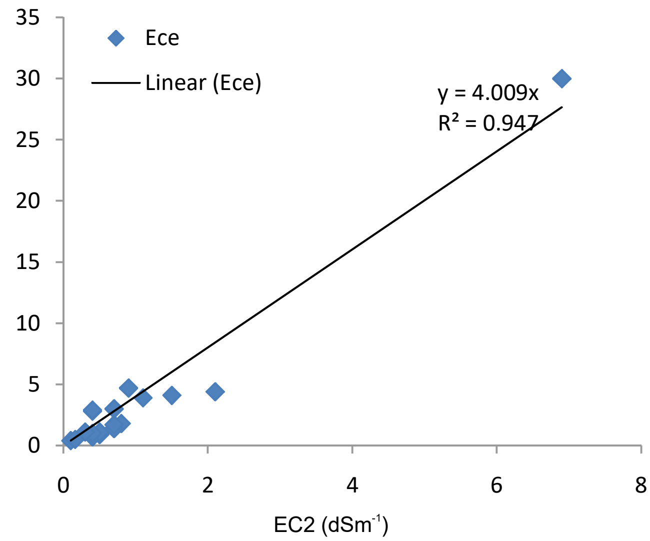

Fig. 1. Relationship between EC2 and ECe (0-15 cm)

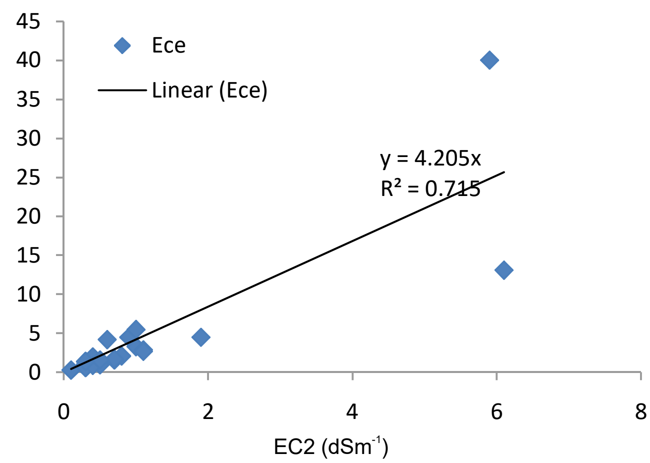

Fig. 2. Relationship between EC2 and ECe (15-30 cm)

Table 3. ECe (dSm^{-1}) and degree of salinity hazard in soils

Soil salinity class	Ece (dSm^{-1})	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
		0-15 cm		15-30 cm		30-60 cm	
Non-saline	0-2	49	63.6	42	54.6	46	59.0
Slightly saline	2-4	14	18.2	14	18.2	11	13.6
Moderately saline	4-8	11	13.6	14	18.2	11	13.6
Strongly saline	8-16	0	0.0	4	4.6	7	9.0
Very strongly saline	>16	4	4.5	3	4.0	4	4.5

Table 4. ESP and degree of alkali hazard

Soil salinity class	Alkali hazard	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
		0-15 cm		15-30 cm		30-60 cm	
Up to 15	None to slight	77	100	73	94.8	73	94.8
15-30	Slight to moderate	0	0.0	4	5.2	4	5.2

No evidence of moderate, high and extremely high alkali hazard at all the three depths of the study area higher than EC_2 indicate the lesser conductivity at lower depths. The salinity was decreased with the depth of the soils of the study area, this might be due to the capillary rise of salts at surface increased the conductivity of the soil at surface (Sharma and Chaudhari 2012).

Cation exchange capacity, ESP and organic carbon: Cation exchange capacity (CEC) ranged from 3.76-53.4, 3.28-58.3 and 2.13-60.66 cmol (p+) kg^{-1} soil. Exchangeable sodium percentage (ESP) ranged from 1.0-12.8, 1.12-16.87 and 0.95-16.95 and organic carbon ranged from 2.3 to 14.2, 2.2-5.8 and 1.9-4.8 g Kg^{-1} at 0-15, 15-30 and 30-60 cm depth of soil respectively (Table 5). The increased per cent of clay content might influence the cation exchange capacity of soil with the increased depth of the soil. ESP was increased with the depth. Soil organic carbon content was decreased with the depth of the soil.

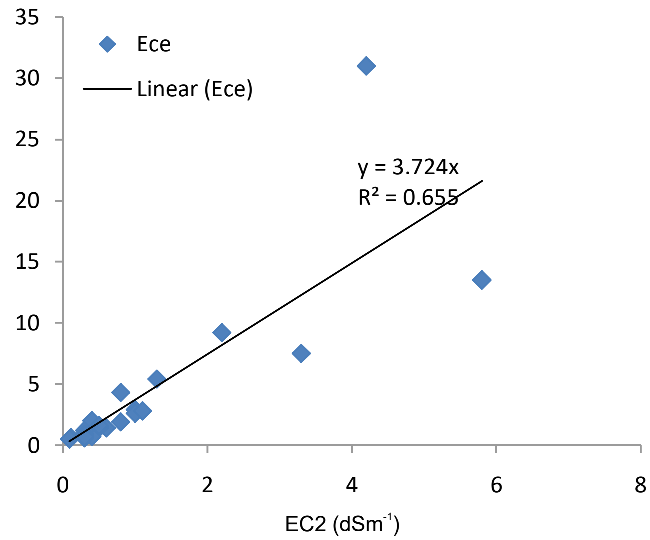

Fig. 3. Relationship between EC2 and ECe (30-60 cm)

Table 5. Physical, physico-chemical and chemical properties of soil

Parameter (me l^{-1})	0-15 cm		Standard deviation	Standard error	15-30 cm		Standard deviation	Standard error	30-60 cm		Standard deviation	Standard error
	Range	Mean			Range	Mean			Range	Mean		
Sand (%)	34.64-90.64	56.8	21.5	2.45	36.64-90.64	56.5	22.11	2.52	36.64-90.64	56.74	21.7	2.47
Silt (%)	0-30.0	12.36	8.36	0.95	0.0-33.0	12.06	9.19	1.04	0.0-36.0	11.98	9.81	1.11
Clay (%)	9.36-59.4	30.82	16.79	1.91	9.36-58.36	31.34	17.33	1.97	9.36-59.36	31.2	17.15	1.95
pH_2	6.6-8.8	7.82	0.78	0.09	6.6-9.8	8.06	0.79	0.09	6.7-9.8	8.12	0.67	0.07
EC_2(dS m^{-1})	0.1-6.9	0.91	1.29	0.14	0.1-6.1	1.17	1.59	0.18	0.09-5.8	1.19	1.46	0.16
ECe (dS m^{-1})	0.4-29.6	3.08	5.62	0.64	0.3-40	4.27	7.75	0.88	0.5-31.0	4.16	6.35	0.72
CEC (cmol(p+) kg^{-1})	3.76-53.4	38.35	24.2	2.75	3.28-58.3	38.38	24.24	2.76	2.13-60.66	38.18	24.5	2.79
ESP	1.0-12.8	4.3	2.67	0.30	1.12-16.87	4.93	3.37	0.38	0.95-16.95	5.41	3.43	0.39
Organic carbon (%)	0.23-1.42	0.62	0.43	0.04	0.22-0.58	0.13	0.29	0.03	0.19-0.48	0.11	0.21	0.02
Water soluble ionic Composition (me L^{-1})												
CO_3^{2-}	0-0.6	0.05	0.17	0.01	0.0-0.4	0.01	0.07	0.01	0.0-0.0	0.0	0.0	0.0
HCO_3^-	0.4-6.6	2.56	1.59	0.18	0.0-4.4	1.78	1.03	0.11	0.0-6.0	1.74	1.22	0.13
Cl^-	1.6-301	22.75	57.65	6.57	0.8-298	31.13	62.19	7.08	1.2-334	31.9	65.2	7.43
Ca^{2+}	1.6-20.0	6.84	5.38	0.61	2.0-34.0	8.10	8.07	0.91	2.0-26.0	7.51	6.60	0.75
Mg^{2+}	0.8-24.4	4.32	4.85	0.55	1.2-46.0	7.07	11.03	1.25	0.4-33.2	6.66	8.58	0.97
Na^+	0.62-358	25.69	68.3	7.78	0.47-533	37.4	102	11.71	0.83-374	32.52	72.62	8.27
K^+	0.08-0.92	0.21	0.16	0.01	0.07-1.20	0.22	0.23	0.02	0.06-0.94	0.21	0.21	0.02
Exchangeable ionic composition (me/100 g soil)												
Ca^{2+}	1.6-21.6	11.4	7.0	0.79	0.8-24.4	11.8	7.55	0.86	1.2-25.6	11.76	7.68	0.87
Mg^{2+}	0.4-8.8	4.9	2.72	0.31	0.0-9.2	4.7	3.15	0.35	0.4-10.0	5.05	3.23	0.36
Na^+	0.2-4.02	1.41	1.21	0.13	0.17-4.30	1.61	1.35	0.15	0.15-5.97	1.79	1.54	0.17
K^+	0.03-1.26	0.46	0.36	0.04	0.03-1.01	0.35	0.27	0.03	0.02-0.95	0.33	0.27	0.03
Available major nutrients (kg ha^{-1})												
N	37.62-250	147	58.4	6.65	25.08-225	119	53.8	6.13	12.54-200	97.06	51.75	5.89
P	39.0-179	78.85	29.9	3.41	29.0-156	58.0	26.34	3.00	28.4-99.4	47.29	18.4	2.10
K	48.38-600	361	263	30.0	34.0-597	291	218	24.8	22.0-560	270	219	25.05

Table 6. Nutrient status of soil

Availability status	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
	0-15 cm		15-30 cm		30-60 cm	
Organic carbon (g kg^{-1})						
Low	32	42	70	91	77	100
Medium	14	18	7	9	0	0
High	31	40	0	0	0	0
Available N						
Low	77	100	77	100	77	100
Available P						
High	77	100	77	100	77	100
Available K						
Low	13	17	23	30	20	26
Medium	23	30	14	18	25	32
High	41	53	40	52	32	42

Table 7. Soil types in salt affected areas of Krishna western delta region of Guntur district

Soil type	No. of samples	Per cent	No. of samples	Per cent	No. of samples	Per cent
	0-15 cm		15-30 cm		30-60 cm	
Good	67	87.01	56	72.7	56	72.7
Saline	7	9.09	14	18.2	14	18.2
Saline alkali	3	3.90	7	9.09	7	9.09

Water soluble ions: The dominance of Na^{+} in water soluble ionic composition ranged 0.62-358, 0.47-533 and 0.83-374 meq L^{-1}, Cl^{-} ranged from 1.6-301, 0.8-298 and 1.2-334 meq L^{-1}, Mg^{+2} ranged from 0.8-24.4, 1.2-46.0, and 0.4-33.2 meq L^{-1}, Ca^{+2} ranged from 1.6-20.0, 2.0-34.0 and 2.0-26.0 meq L^{-1}, K+ ranged from 0.08-0.92, 0.07-1.20 and 0.06-0.94 meq L^{-1}, HCO_3^{-} ranged from 0.4-6.6, 0.0-4.4 and 0.0-6.0 meq L^{-1} and CO_3^{-2} ranged from 0.0-0.6, 0.0-0.4meq L^{-1} at 0-15, 15-30 and 30-60 cm depth of soil. Presence of Na^{+}, Mg^{+2} Ca^{+2}, Cl^{-} and HCO_3^{-} ions indicates the development of saline soils in the study area (Table 5). The salt laden winds and rains (sea sprays) along sea coasts carry oceanic salts along with them in quantities sufficient to cause salinization in coastal areas (Kumar and Sharma 2020).

Exchangeable ions: The exchangeable ions *viz.,* Ca^{+2} ranged from 1.6-21.6, 0.8-24.4 and 1.2-25.6 cmol (p+) kg^{-1}, Mg^{+2} ranged from 0.4-8.8, 0.0-9.2 and 0.4-10.0 cmol (p+) kg^{-1}, Na^{+} ranged from 0.2-4.02 , 0.17-4.30 and 0.15- 5.97 cmol (p+) kg^{-1} and K^{+} ranged from 0.03-1.26, 0.03-1.01 and 0.02-0.95 cmol (p+) kg^{-1} at 0-15, 15-30 and 30-60 cm depth of soil . The dominance of calcium and magnesium minimized the impact of sodium on exchangeable complex of soil (Table 5). Hence, very lesser area is occupied by sodic soils.

Available N, P and K: The available nitrogen ranged from 37.62-250, 25.08-225 and 12.54-200 kg ha^{-1}, Available P ranged from 39-179, 29-156 and 28.4-99.4 kg ha^{-1} and available K ranged from 43.38-600, 34.0-597 and 22.0-560 kg ha^{-1}. The lowest availability might be due to presence of sandy soils and highest might be due to clay loam soils of the study area (Table 5 & 6).

Soils of the study area: Based on the physico-chemical properties the soils are classified in to 3 categories (Table 6) *viz.,* good soil (87.01 %), saline (9.09 %) and saline alkali (3.90%) at 0-15 cm depth, good soil (72.7 %), saline (18.2 %) and saline alkali (9.09 %) at 15-30 and 30-60 cm depth (Table 7).

CONCLUSIONS

The Krishna western delta eco-region soils are under greater stress with lack of sufficient organic matter resulted low available nitrogen of soils. Around 40 per cent of the soils reporting slightly saline to very strongly saline in nature in all three depths of the soil. The alkali hazard is slight to moderate with 5.2 per cent at subsurface depth of soil, these soils are grouped in to saline and saline alkali. Hence, good agronomic management practices *viz.,* balanced fertilization, addition of organic and green manures, suitable reclamation, cereal-pulse crop rotation and selection suitable crops and varieties can sustain the long-term production of agri-ecosystem in the study area.

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Maize Grain Quality as Influenced by Chemical, Organic and Natural Farming Systems in an Acid Hill Soil of North-Western Himalayas

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Abstract: An understanding of nutritional quality response to different nutrient management practices is important to counter the widespread deficiencies of nutrients among humans. This study aimed to evaluate the effect of chemical fertilizers, lime, organic farming and natural farming practices on the yield and quality of maize (*Zea mays* L.) The experiment consisted of 11 treatments *viz.,* 100% NPK , 100% NPK+FYM (Farmyard manure) , 100% NPK + lime , organic farming practices , NFS (Natural Farming System)-*Desi* cow ; NFS-Crossbred cow , NFS-buffalo , organic farming practices + 25% NPK , NFS-*Desi* cow + 25% NPK , NFS-Crossbred cow + 25% NPK , NFS-buffalo + 25% NPK) The 100% NPK+ FYM @ 10 t ha^{-1} recorded highest maize grain equivalent yield (42.25 q ha^{-1}), reducing sugars (1.01%), non-reducing sugars (0.64%), crude protein (9.88%) and ash content (1.34%). Organic farming recorded highest total carbohydrate (72.65%) as well as starch content (68.68%). The calcium content in maize grains was highest in 100% NPK+ lime, whereas, phosphorus, magnesium, zinc and iron content were highest in 100% NPK+ FYM. The study concluded that higher crop yield and better nutritional quality can be achieved with a balanced application of NPK fertilizers along with FYM and lime, and organic manures plays a significant role in enhancing carbohydrate and starch content of maize grains.

Keywords: Grain quality, Maize, Chemical fertilizers, Lime, Organic farming, Natural farming

Maize (*Zea Mays*) is the third leading cereal crop across the globe after rice and wheat. It is a staple food for a huge section of the world's population, accounting for more than one-third of total calorie and protein intake in various countries. However, maize is inherently poor in protein and mineral content; particularly zinc. It is a high nutrient intensive crop which is sensitive to micronutrient deficiency. It is cultivated on an average of 9.5 million hectares area, with a total production of 28.7 million tons and a productivity of 3.0 tons per hectare (Agricultural Statistics at a Glance 2018). Intensive, high-input agriculture reliant on chemical fertilizers, insecticides, irrigation and other chemicals has resulted in deterioration of soil health leading to poor grain production and quality. According to the Food and Agriculture Organization of the United Nations (FAO 2017), about 190.7 million people in India are malnourished, with zinc (Zn) and iron (Fe) being the most often deficient micronutrients. To meet the nutritional needs of such a large population, the country needs to boost the production as well as nutritional quality of food grains. Proper nutrient management practices is one of the key factors for maintaining soil health and ultimately obtaining higher yields with increased nutrient content in grains. The integrated use of organic manures and balanced fertilizers has been reported to improve yield and nutritional quality of maize. Soil acidity limits agricultural production by affecting plant growth and restricting the absorption of water and nutrients (mainly micronutrients) by plant roots. Reclamation of soil acidity by use of lime is very effective in enhancing crop yield particularly in acidic regions. In addition to this, it is equally important to grow food grains of superior nutritional quality to address the wide-spread nutritional deficiencies among people. However, little is known about the effects of lime application on the nutritional quality of maize.

Recently, with the increasing concern of environment and human health, the scientists across the world are focusing on the adoption of alternate nutrient management practices which can improve crop yield and nutritional quality of food grains sustainably. In India, about 2.78 million hectare of farmland is under organic cultivation which accounts only 2 per cent of the net sown area in the country (Anonymous 2020). In organic farming, soil fertility is maintained through the use of organic amendments *viz.,* compost, manures and cover crops.. The soil has a critical role in organic farming and therefore, soil quality has a direct influence on the quality of food grown in it. When compared to conventional farming, organic farming brings minimal or no changes in starch quality, protein content, antioxidant activities and secondary metabolites of grains (Kesarwani et al 2021). Therefore, the effect of organic farming on nutritional quality of grains is still a matter of debate.

Natural farming is another alternative farming system to raise food crops that counters commercial expenditure and farmers' dependency on market for inputs. It involves the use of formulations (Jeevamrit, Beejamrit etc.) generally prepared from the dung and urine of indigenous cows (*Desi*) and are used as a replacement for the chemical fertilizers. These formulations are the quality natural sources which can supply the essential nutrients for the plant growth, and economical in preparation as all the ingredients are naturally available on farm. Government of India as well as the state government has laid emphasis on evaluation and popularization of natural farming system. The Himachal Pradesh government hopes to bring 9.61 lakh farmers under natural farming by the end of 2022. However, very little is known about the effect of natural farming practices using products of indigenous cow, and crossbred cow and buffalo in particular on yield and grain quality of maize. Keeping this in view, the present study was carried out with the aim to evolve a more efficient nutrient management practices in terms of yield and grain quality of maize.

MATERIAL AND METHODS

The present study was carried out on maize during 2020 at CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India (32°6´N latitude and 76°3´E longitude, 1290 m above the mean sea level). The research site falls under the mid-hills sub-humid agro-climatic zone and its climate is characterized as wet temperate. Nearly 80% of annual rainfall is received during July–September months. During the study period, total rainfall of 1449.0 mm was received and weekly relative humidity ranged between 57.95 to 92.05%. The maximum and minimum weekly temperatures varied from 26.0 to 30.5 °C and13.0 to 20.1°C, respectively. The soil of the study site has been classified as Typic Hapludalf and the texture is silty loam. The soil properties at the initiation of the experiment before sowing of maize have been depicted in Table 1.

Experimental details: The experiment comprised of eleven treatments in three replications which were laid out in a randomized block design. These treatments were 100% NPK (T_1), 100% NPK+FYM (Farmyard manure) (T_2), 100% NPK + lime (T_3), organic farming practices (T_4), NFS (Natural Farming System)-*Desi* cow (T_5); NFS-Crossbred cow (T_6), NFS-buffalo (T_7), organic farming practices + 25% NPK (T_8), NFS-*Desi* cow + 25% NPK (T_9), NFS-Crossbred cow + 25% NPK (T_{10}), NFS-buffalo + 25% NPK (T_{11}). 100% NPK corresponding to 120 kg nitrogen (N), 60 kg phosphorus (P), and 40 kg potassium (K) ha^{-1} for maize. Urea, single super phosphate, and muriate of potash were used to supply N, P, and K, respectively. One pre-sowing irrigation was given and thereafter, water requirement of crop was met through rainfall. The whole quantity of FYM, containing 0.98, 0.47 and 0.85 % N, P and K, respectively on a dry weight basis, was added at the time of sowing in 100% NPK + FYM @ 10 ha^{-1} treatment. Lime @ 3.2 t ha^{-1} was applied about four weeks prior to sowing of the maize in specified plots comprising 100% NPK + lime treatment. The full doses of P and K and half dose of N were applied at the time of sowing and the remaining half N was top-dressed in two equal splits at knee high and pre-tasseling stages. In organic farming plots, 60 kg N ha^{-1} was supplied through FYM and another 60 kg N ha^{-1} was supplemented through vermicompost containing 29.4% moisture with average nutrient content of 1.83, 0.97 and 0.73% of N, P and K, respectively on dry weight basis. In NFS plots, before sowing, the seeds were treated with beejamrit for a period of 30 minutes. At sowing, *Ghan-jeevamrit* (@ 250 kg ha^{-1}) was applied along with sieved FYM (@ 250 kg ha^{-1}), followed by application of *Jeevamrit* (@ 500 l ha^{-1}), and sprays of *Jeevamrit* @ 10% were given five times at 21days interval during crop growth. Soybean was intercropped in between the rows of maize plants in the ratio of 2:1. Mulching

Table 1. Initial physico-chemical characteristics of the surface soil

Soil property	Value
Mechanical analysis	
Mechanical separates (%)	
Sand	18.80
Silt	41.40
Clay	34.90
Chemical analysis	
Bulk density (g cm^{-3})	1.36
Water holding capacity (%)	44.42
Mean weight diameter (mm)	2.12
Soil pH (1:2.5)	5.56
Organic carbon (g kg^{-1})	8.31
Available nutrients (kg ha^{-1})	
N	257.4
P	33.17
K	211.36
Exchangeable cations (c mol (p$^+$) kg^{-1})	
Ca	2.51
Mg	1.32

with locally available organic residues was also done. The crop was harvested manually on 9th October, 2020 and yield was recorded at the time of harvesting. The NPK composition of beejamrit and jeevamrit prepared using dung of different cattle is given in Table 2.

Sampling and analysis: After the harvesting, the maize grain samples were collected and dried in an electric oven at 60°C to a constant weight. The dried grain samples were finely ground in a mixer grinder in stainless steel jar, sieved through 1 mm sieve and stored under moisture-free conditions in plastic bags. The reducing sugar content was estimated by the Dinitrosalicylic acid reagent method as proposed by Miller (1959). Non-reducing sugar content was determined as per the standard procedure (Malhotra and Sarkar 1979). To estimate crude protein, ash content, and total carbohydrate content, the standard procedure for proximate analysis given by AOAC (2005) was followed. The starch content was estimated using the Anthrone method given by Hedge and Hofreiter (1962).

Statistical analysis: The data recorded was analysed using MS-Excel, OPSTAT and SPSS 16.0 package as per design of the experiment.

RESULTS AND DISCUSSION

Maize grain equivalent yield: The highest maize grain equivalent yield (42.25 q ha^{-1}) was in 100% NPK + FYM (T_2) which was statistically at par (40.51 q ha^{-1}) with 100% NPK + lime (T_3). Addition of FYM, and lime along with 100% NPK fertilizers recorded 15.9% and 11.1% higher maize grain equivalent yield than sole application of 100% NPK, respectively. The lowest maize grain equivalent yield (23.07 q ha^{-1}) was in NFS-Buffalo (T_7) (Table 3). Among organic treatments, organic farming treatment resulted in higher maize grain equivalent yield (29.13 q ha^{-1}) which was statistically at par (26.54 q ha^{-1}) with NFS-*Desi* cow (T_5). Among integrated organic treatments, higher maize grain equivalent yield (33.99 q ha^{-1}) was in NFS-*Desi* cow + 25% NPK (T_9) with non-significant differences with NFS-Crossbred cow + 25% NPK , NFS-Buffalo + 25% NPK and organic farming + 25% NPK. NFS-*Desi* cow recorded 15% higher grain equivalent yield than NFS-Buffalo treatment, whereas NFS-*Desi* cow + 25% NPK 8.9% higher maize grain equivalent yield over NFS-Buffalo + 25% NPK treatments. The significant increase in maize grain equivalent yield with integrated use of FYM and inorganic fertilizers could be due

Table 2. NPK content (%) of *Beejamrit* and *Jeevamrit* prepared using dung of different cattle

Dung used	*Beejamrit*			*Jeevamrit*		
	N	P	K	N	P	K
Desi cow	0.457	0.115	0.273	0.203	0.048	0.078
Cross-bred cow	0.485	0.119	0.297	0.220	0.049	0.085
Buffalo	0.476	0.118	0.270	0.216	0.046	0.069

Table 3. Effect of conventional, organic and natural farming treatments on maize grain equivalent yield and quality parameters in maize grains

Treatments	Maize grain equivalent yield (q ha^{-1})	Reducing sugars (%)	Non-reducing sugars (%)	Crude protein (%)	Total carbohydrates (%)	Starch (%)	Ash (%)
100%NPK (T_1)	36.46	0.96	0.59	9.77	69.21	64.84	1.23
100%NPK+FYM (T_2)	42.25	1.01	0.64	9.88	70.92	65.12	1.34
100%NPK+Lime (T_3)	40.51	0.99	0.62	9.83	70.12	65.04	1.28
Organic farming (T_4)	29.13	0.90	0.51	9.52	72.65	68.68	1.05
NFS-*Desi* cow (T_5)	26.54	0.88	0.49	9.48	72.12	68.50	1.04
NFS-Crossbred cow (T_6)	24.15	0.88	0.46	9.45	72.09	68.23	1.02
NFS-Buffalo (T_7)	23.07	0.87	0.44	9.43	71.82	68.15	1.01
Organic farming +25% NPK (T_8)	31.64	0.93	0.57	9.70	70.82	66.94	1.17
NFS-*Desi* cow +25% NPK (T_9)	33.99	0.92	0.55	9.67	70.64	66.70	1.15
NFS-Crossbred cow +25% NPK (T_{10})	33.06	0.92	0.53	9.65	70.29	66.42	1.12
NFS-Buffalo +25% NPK (T_{11})	31.21	0.91	0.52	9.63	70.14	66.21	1.11
LSD (P=0.05)	4.32	0.03	0.02	0.08	1.60	1.07	0.04

to the improved physical, biological and chemical properties of soil as well as supply of higher quantity of nutrients with FYM and fertilizer which consequently resulted in better growth and higher crop yields (Ghosh et al 2022).

Reducing sugars: The reducing sugar content varied from 0.87 to 1.01% (Table 3). The highest reducing sugar content was in 100% NPK+ FYM @ 10 t ha^{-1} (1.01%) which was statistically at par with 100% NPK + lime treatment (0.99%). Addition of FYM, and lime along with 100% NPK fertilizers resulted in 5.2 and 3.1% higher reducing sugar content than sole application of 100% NPK, respectively. NFS-buffalo recorded the lowest reducing sugar content (0.87%) in maize grains among all the treatments. Among organic treatments, organic farming recorded higher reducing sugar content (0.90%) which was statistically at par with NFS-*Desi* cow (0.88%), NFS-Crossbred cow (0.88%) and NFS-Buffalo treatments (0.87%). Among integrated organic treatments, organic farming + 25% NPK recorded higher reducing sugar content (0.93%) which produced non-significant differences with NFS-*Desi* cow + 25% NPK (0.92%), NFS-Crossbred cow + 25% NPK (0.92%) and NFS-Buffalo + 25% NPK treatments (0.91%). The significant increase in reducing sugar content of maize grain with conjunctive use of FYM and inorganic fertilizers could be attributed to good nutrient status, improved plant conditions, more efficient functioning of leaf area and increased photosynthetic activity (Sharma et al 2018). .

Non-reducing sugars : The 100% NPK + FYM (T_2) recorded the highest non-reducing sugar content (0.64%) which was statistically at par with 100% NPK + lime (0.62 %), while the lowest non-reducing sugar content (0.44%) was in NFS-Buffalo (T_7) (Table 3). Integration of FYM, and lime with NPK fertilizers recorded 8.5% and 5.1% higher non-reducing sugar content than application of 100% NPK (T_1) alone, respectively. Integration of 25% NPK with organic farming recorded 11.8% higher non-reducing sugar content when compared to organic farming treatment only. Among organic treatments, organic farming recorded higher non-reducing sugar content (0.51%) which was statistically at par with NFS-*Desi* cow treatment (0.49%). The significant increase in non-reducing sugar content with combined application of FYM and inorganic fertilizers could be due to balanced supply of essential nutrients, growth promoting substances such as hormones, enzymes, etc. and effective regulation of metabolic functions which might have led to better synthesis of sugars (Safiullah et al 2018).

Crude protein: Integrated use of fertilizers with organic manure or lime influenced the crude protein content of maize grain significantly over sole use of fertilizers (Table 3). The highest crude protein content (9.88%) was in 100% NPK + FYM (T_2), followed by 9.83% under 100% NPK+ lime (T_3), and the lowest (9.43%) in NFS-Buffalo (T_7). Addition of FYM, and lime along with inorganic fertilizers recorded 1.1 and 0.6% higher crude protein content than sole use of 100% NPK, respectively. The significant higher crude protein content was in integration of FYM with inorganic fertilizers which might be due to the increased nitrogen availability, uptake as well as its storage in maize grains (Liu et al 2016). The positive effect of lime use on the crude protein content of maize grain could be due to the enhanced activity of nitrifying bacteria resulting in higher availability of N for uptake (Castro and Crusciol 2015). Among organic treatments, organic farming recorded higher crude protein content (9.52%) which produced non-significant differences with NFS-*Desi* cow (9.48%) and NFS-Crossbred cow treatments (9.45%). Among integrated organic treatments, organic farming + 25% NPK recorded higher crude protein content (9.70%) which was statistically at par with NFS-*Desi* cow + 25% NPK (9.67%), NFS-Crossbred cow + 25% NPK (9.65%) and NFS-Buffalo + 25% NPK treatment (9.63%). Organic farming resulted in higher crude protein content which could be due to better supply of macro and micro nutrients by vermicompost along with FYM and thereby, better nitrogen absorption and transformation in crops (Manjhi et al 2016).

Total carbohydrates: The total carbohydrate content of maize grains ranged from 69.21 to 72.65% (Table 3). The organic farming (T_4)recorded the highest carbohydrate content which was statistically at par with NFS-*Desi* cow , NFS-Crossbred cow and NFS-Buffalo treatments (Organic farming recorded 4.97% higher carbohydrate content than sole application of inorganic fertilizers. The lowest total carbohydrate content was in 100% NPK (T_1) (69.21%). The significantly higher total carbohydrate content with organic farming practices could be due to the slow and continuous supply of both micro and macro nutrients from manures (vermicompost), which might have helped in improved root growth, higher dry matter accumulation with adequate supply of nutrients to the plants leading to increase in the accumulation of carbohydrates. Addition of FYM, and lime along with NPK fertilizers showed 2.5% and 1.3% higher total carbohydrate content than sole application of 100% NPK, respectively. Integration of FYM, and lime with chemical fertilizers provided the adequate and balanced supply of nutrients required for several metabolic activities within the plants, resulting in high carbohydrate content over sole application of inorganic fertilizers (Chauhan et al 2020).

Among integrated organic treatments, organic farming + 25% NPK (T_8) recorded higher total carbohydrate content (70.82%) which was statistically at par with NFS-*Desi* cow + 25% NPK (70.64%), NFS-Crossbred cow + 25% NPK

(70.29%) and NFS-Buffalo + 25% NPK (70.14%) treatments which might be due to balanced and continuous supply of nutrients provided by both organic and inorganic sources of nutrients.

Starch content: The significant difference in starch content in maize grains due to different nutrient management practices was recorded (Table 3). The highest starch content in maize grains (68.68%) was recorded under organic farming treatment (T_4), while the lowest (64.84%) was in 100% NPK treatment (T_1). The treatment, 100% NPK + FYM (T_2) as well as 100% NPK + lime (T_3) also recorded higher starch content than sole application of 100% NPK which was 65.12% and 65.04%, respectively. Among integrated organic treatments, organic farming + 25% NPK (T_8) recorded higher starch content (66.94%) which produced non-significant differences with NFS-*Desi* cow + 25% NPK (66.70%), NFS-Crossbred cow + 25% NPK (66.42%) and NFS-Buffalo + 25% NPK treatments (66.21%). The beneficial effect of organic farming practices on starch content could be attributed to the organic manures that contain significant amounts of macro and micronutrients which help in improving the grain chemical constituents, as well as the effect of organic acids produced during soil mineral decomposition in enhancing grain quality (Anjum et al 2020). Combined application of NPK fertilizers and FYM resulted in significant increase in starch content over sole application of inorganic fertilizers which could be due to release of hormones, nutrients and other growth promoting substances from organic manures. Liming also resulted in significant improvement in starch content over 100% NPK which could be attributed to the increased nutrient availability due to the neutralization of soil acidity.

Ash content: There was a significant effect of application of fertilizers and amendments on ash content of maize grains. Highest ash content of 1.34% was in 100% NPK + FYM (T_2), followed by 100% NPK + lime (1.28 %), while the lowest ash content (1.01%) was in NFS-buffalo treatment (Table 3). Integration of FYM, and lime along with NPK fertilizers recorded 8.9% and 4.1% higher ash content than sole application of 100% NPK fertilizers (T_1), respectively. Among integrated organic treatments, organic farming + 25% NPK (T_8) recorded higher ash content (1.17%) which was statistically at par (1.15%) with NFS-*Desi* cow + 25% NPK treatment (T_9). Integration of FYM with inorganic fertilizers resulted in significant increase in ash content which might be due to improved availability of mineral nutrients from the soil and enhancement in the microbial activity as well, which in turn, would have helped in better absorption of macro and micro nutrients from the soil thereby resulting in higher mineral content in maize grains. Chauhan et al (2020) also reported higher ash content in FYM amended plots as compared to other treatments due to the presence of higher amount of bran portion in it.

Nutrient content: The significant difference in nutrient content of maize grains was recorded under different nutrient management practices (Table 4). Highest P, Mg, Zn and Fe content of maize grain were recorded under organic farming (T_4). However, 100% NPK + lime (T_3) recorded the highest Ca content. Addition of FYM, and lime with 100% NPK increased the P content by 39.3% and 21.1%, Mg content by 40 and 30% , Zn content of maize grains by 18.9% and 4.5% when compared to 100% NPK (T_1), respectively. The Ca content of maize grain recorded a significant increase with lime over 100% NPK, the increase being 21.9%. The significant increase in phosphorus, magnesium and zinc content of maize grains with organic farming might be due to positive

Table 4. Effect of conventional, organic and natural farming treatments on P, Ca, Mg, Zn and Fe content in maize grains

Treatments	P (%)	Ca (%)	Mg (%)	Zn (mg kg^{-1})	Fe (mg kg^{-1})
100% NPK (T_1)	0.48	0.32	0.10	24.09	54.01
100% NPK+FYM (T_2)	0.52	0.37	0.14	28.64	56.99
100% NPK+Lime (T_3)	0.49	0.39	0.13	25.17	53.97
Organic farming (T_4)	0.55	0.38	0.15	31.36	58.32
NFS-*Desi* cow (T_5)	0.53	0.37	0.14	29.03	56.99
NFS-Crossbred cow (T_6)	0.52	0.36	0.14	28.36	56.38
NFS-Buffalo (T_7)	0.51	0.36	0.13	27.03	55.89
Organic farming + 25% NPK (T_8)	0.48	0.34	0.13	24.69	55.66
NFS-*Desi* cow + 25% NPK (T_9)	0.47	0.35	0.13	24.36	55.01
NFS-Crossbred cow + 25% NPK (T_{10})	0.45	0.33	0.12	24.69	55.23
NFS-Buffalo + 25% NPK (T_{11})	0.44	0.33	0.12	24.02	54.10
LSD (p=0.05)	0.03	0.02	0.01	2.77	1.62

effect of vermicompost on soil physical-chemical properties, root growth, its ability to release plant growth regulators, phytohormones, mineral nutrients and also humic substances that prolonged the bioavailability of micro nutrients and thereby improved the uptake of nutrients. Addition of organic manures also resulted in significant increase in iron content of maize grains which could be due to decrease in leaching losses and increase in the availability of nutrients with the use of organic manures (Joshi et al 2019). The significant improvement in calcium content of maize grains due to application of lime might be attributed to increase in soil pH and improved availability of calcium in the soil.

CONCLUSION

The present study demonstrated that balanced use of fertilizers in conjunction with FYM or lime significantly improved the maize grain quality in terms of reducing and non-reducing sugars, crude protein, ash and nutrient content. Application of organic manures significantly enhanced the total carbohydrates and starch content of maize grains. Thus, it can be concluded from the study that combined use of inorganic fertilizers and FYM, and organic manures and amelioration of soil acidity are essential to improve yield and nutritional quality of maize grains.

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Survey and Occurrence of Major Biotic Stresses of Rice in Sangrur District of Punjab

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Abstract: Field survey was conducted to investigate the prevalence of major biotic stresses of rice in different blocks of district Sangrur in Punjab. The Leaf folder, brown plant hopper, sheath blight, false smut and kernel smut were observed the major biotic stresses of rice in this region. The more damage of leaf folder and brown plant hopper populations observed in rice with combination of favourable meteorological factors such as reduced rainfall coupled with high humidity, high temperature and diminished sunshine hours. The damage of leaf folder and brown plant hopper population varied with weather conditions and significantly positive correlated with maximum temperature and negatively with occurrence of rainfall. The disease sheath blight might be due to the prevalence of highly favorable factors of high relative humidity, less temperature and occurrence of more rainfall convenient for disease development. The false smut and kernel smut diseases observed high incidence due to prevailing of favourable weather for long time coinciding with rainy days at the time of flowering stage of the crop, ultimately increasing the relative humidity which is a crucial factor for development of disease.

Keywords: Biotic stresses, Weather parameters, Rice, Standard week, Survey

Rice (*Oryza sativa* L.) is an important cereal crop, which provides food and nutritional security for half of the human race. About 90 per cent of the world rice is grown and consumed in Asia. India is next only to China with respect to area under rice cultivation. In India, it is grown over an area of 45.07 mha with a production and productivity of 122.27 m tonnes and 2712 kg ha, respectively during 2020-21. In Punjab, it occupies an area of 31.49 lakh ha with a production and productivity of 208.83 lack tonnes and 6631 kg ha, respectively during 2020-21.

The yield is affected by many factors, out of which, climatic conditions and pest epidemics are most important. Insect-pests damage started from nursery sowing to transplanting and continues upto maturity of the crop. Although crop is subjected to attack by a number of pests under field condition, only a few of them are responsible for triggering severe economic damage (Heinrichs et al 2017). Yield losses due to rice pests in tropical Asia are expected to be about 25-43% (Savary et al 2012). In India, yield losses on account of insect-pests in rice occur to the extent about 25% (Dhaliwal et al 2010) and diseases have been assessed as 15.6% (Mondal et al 2017). Singh et al (2012) observed the maximum number (percent) of insect-pest infestation by plant hopper (44%) followed by leaf folder (30%) and stem borer (29%) during years of 2000-2009 in Punjab. The sheath blight of rice caused by *Rhizoctonia solani* Kuhn is one of the major fungal diseases of rice in tropical Asia causing upto 50 percent loss in grain yield (Roy 1993). It is a devastating disease in all rice growing regions of the world. In tropical and sub-tropical Asia, the disease is reported to cause an average loss of 5-10 percent (Willocquet et al 2011). Studies carried out at IRRI, Philippines reported 24 percent yield loss in susceptible cultivars under highest level of disease intensity and nitrogen application. However, in Arkansas, USA 5-15 percent yield losses have been attributed to sheath blight (Annou et al 2005). In India, losses due to this disease has been reported to vary from 5-13.5 per cent in the Punjab state (Thind et al 2001). False smut is an ascomycete fungal pathogen *Villosiclava virens* (anamorph: *Ustilaginoidea virens* [Cooke] Takahashi) (Tanaka et al 2008). The emergence of this disease is believed to be partially due to grown of hybrid rice varieties, which are mostly susceptible to the false smut. The smut ball density varied, on an average, between 0.6 and 4.8 (per infected panicle) depending upon locations in Kashmir region of India (Sanghera et al 2012) and average 2.0-12.2 smutted ball intensity (per infected panicle) recorded in Uttar Pradesh (Singh et al 2014). Quintana et al (2016) observed at least 2-3 balls per infected panicle when investigated 120 panicles in Paraguay of which 40 percent were symptomatic. The climatic factors favouring the disease are high humidity, low temperature and rainy days at the time of flowering. Infection with *U. virens* is promoted by high relative humidity (>90%), temperatures between 25 and 30°C, late planting and high soil fertility and high amount of nitrogen fertilization (Ahonsi et al 2000, Rani et al 2015). The temperature, rainfall and sunshine hours are the most important environmental factors that are decisive for sporulation and formation of false smut balls by U. virens (Yashoda et al 2000). The incidence of false smut has increased after introduction of high yielding varieties and hybrids receiving high inputs, particularly excessive use of nitrogen fertilizers (Mohiddin et al 2012, Rani et al 2015). About 10-20 percent incidence has been reported with popular inbred rice varieties such as PR 116 and PAU 201 (Ladhalakshmi et al 2012). In India, crop loss has been recorded up to 44 percent (Pannu et al 2010). Kernel smut (*Neovossia horrida* Takah.) is considered a minor disease worldwide, but it has reached epidemic levels at rare intervals (Carris et al 2006, Brooks et al 2009). The disease was reported for the first time by Butler in 1913, occasionally the disease prevalent in most of paddy growing states as destructive form in Punjab, Uttar Pradesh, Haryana and Bihar states of North India.

The insect-pest scenario of rice has considerable changes due to the inclement climatic conditions in Punjab. The incidence and population buildup of a pest is highly dependent on prevailing weather conditions, excessive use of nitrogenous fertilizers and irrigation with growth stage of the crop. The present study has been carried out to find the prevalence of major biotic stresses in rice crop in this region, therefore, to formulate the tools required for issuing agro-advisory in speculation period for biotic stress in rice crop.

MATERIAL AND METHODS

A random field survey was conducted to investigate the prevalence of major biotic stresses of rice in different blocks of Sangrur district in Punjab during rice crop season 2017 and 2018 (Table 1). The regular observations on insect-pests and disease were recorded at stipulated period from farmers' fields. The data of leaf folder damage and brown plant hopper populations were recorded after appearance in field and an interval of 15 days to calculate the percent incidence as per leaf damage and number of brown plant hopper population per plant. Incidence of sheath blight was recorded on appearance of disease by following the standard evaluation system (IRRI 2002). Incidence of false smut and kernel smut diseases was recorded at the time of maturity randomly in per square meter area for the number of smut balls per infected plant and further the disease incidence was calculated as per infected grains. The incidence of leaf folder, false smut and kernel smut were calculated by using the formula as given below:

$$\text{Incidence of leaf folder damage (\%)} = \frac{\text{Number of damage leaf observed}}{\text{Total number of leaf examined}} \times 100$$

$$\text{Incidence of false and kernel smut (\%)} = \frac{\text{Number of smutted balls per plant}}{\text{Total number of grains in plant}} \times 100$$

Standard Evaluation System (0-9) based on relative lesion height of sheath blight (IRRI 2002).

0 : No infection observed,
1 : Lesions limited to lower 20% of the plant height
3 : 20-30%
5 : 31-45%
7 : 46-65%
9 : More than 65%

$$\text{Disease severity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of observations x Maximum disease ration of scale}} \times 100$$

RESULTS AND DISCUSSION

The weather parameters like rainfall, temperature and relative humidity (Fig. 1) played important role for emerging and spread of insect-pests and diseases in rice crop. The incidence leaf folder and brown plant hopper observed more in *kharif* 2017 due to prevailing of high temperature. The damage of leaf folder recorded during 31th to 43th standard week and maximum damage incidence during 35th to 41th standard week due to high temperature and low rainfall. The damage of leaf folder was the maximum *i.e*. 41.24 percent in block Moonak and 41.12 percent in block Lehra during 39th standard week in season 2017 (Fig. 2). However, the damage was observed low due to continuous occurrence of rainfall in season 2018 (Fig. 3). The highest damage of recorded in 39th standard week during season 2017 and 35th standard week during season 2018 in all blocks due to maintain the high maximum temperature. Singh et al., 2012 noticed that the active phase of leaf folder from July to September with peak activity during August to September in Punjab. Shyamrao and Raghuraman (2019) noticed the infestation of leaf folder in the field from 31th standard week to 41th standard week. Singh et al (2012) also reported higher incidence of leaf folder positively correlated with the maximum temperature during the growing season. However,

Table 1. Survey of major insect-pest and disease of rice in different block of Sangrur district

Blocks	Location as per GPS Coordinates	
	Latitude	Longitude
Sangrur	30°14'40.4"N	75°50'28.0"E
Sunam	30°07'47.1"N	75°48'16.8"E
Dhuri	30°22'20.5"N	75°51'40.2"E
Malerkotla	30°31'39.9"N	75°52'47.0"E
Lehra	29°56'10.8"N	75°48'22.1"E
Moonak	29°49'29.0"N	75°53'24.0"E

Baskaran et al (2017) observed that the relative humidity and rainfall were positively correlated with trap catches, temperatures were positively correlated to the susceptibility to leaf folder in rice. Supawan and Chongrattanameteekul (2017) recorded with the increasing temperature within the upper threshold of the species generally promoted insect population growth. Although statistically non-significant, relative humidity and rainfall indicated negative correlation

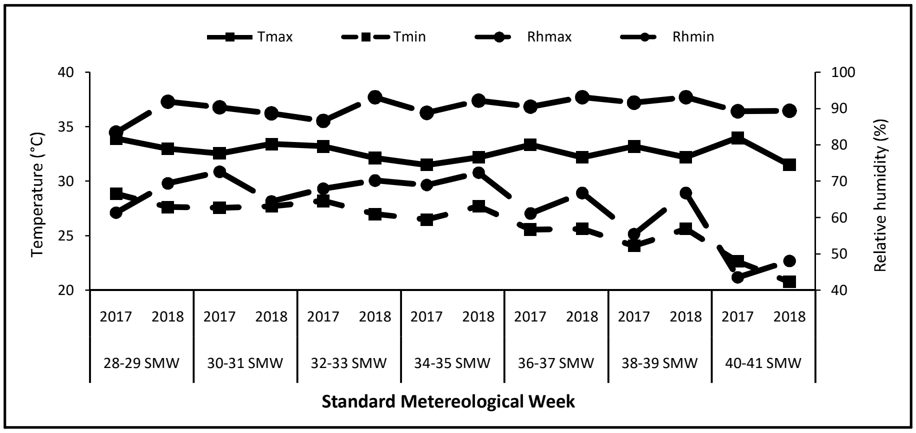

Fig. 1. Temperature and relative humidity of Sangrur district during 2017 and 2018

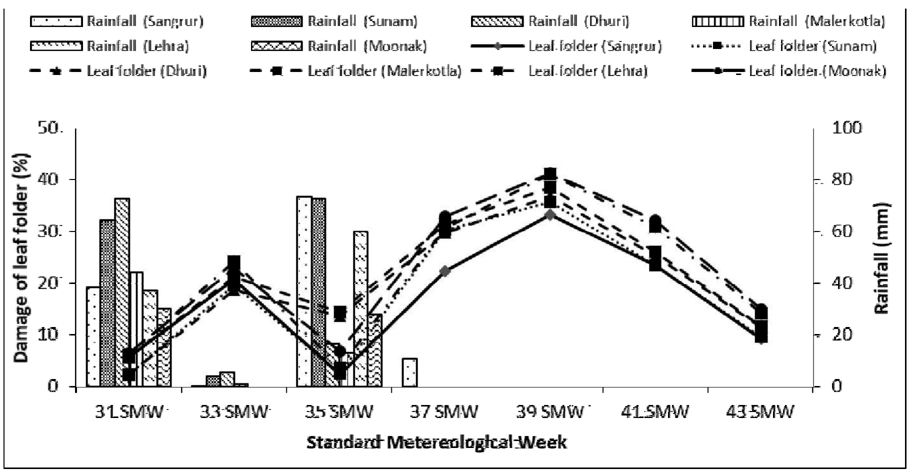

Fig. 2. Effect of rainfall on leaf folder damage of rice in different blocks of Sangrur district during 2017

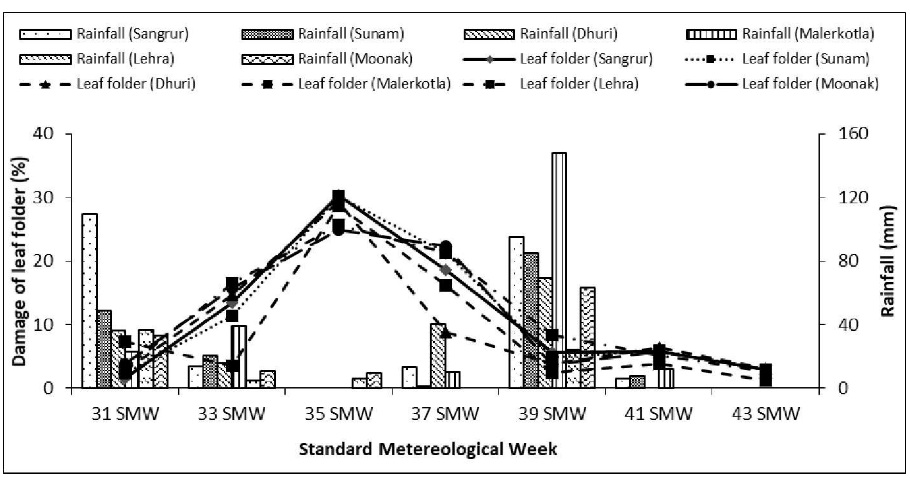

Fig. 3. Effect of rainfall on leaf folder damage of rice in different blocks of Sangrur district during 2018

with rice leaf folder population.

In season 2017, the populations of BPH recorded from 33th to 43th standard week in all blocks due to occurrence of very less rainfall and continuous high temperature upto 34°C. The maximum population of BPH was recorded as 41.37 BPH per hill in block Lahra and 40.47 BPH per hill in Malerkotla on 39th standard week (Fig. 4). The population recorded low as pervious season but highest observed in 37th-39th standard week (Fig. 5). Similarly, the maximum population reached 38th week and afterward started declining from 39th week and negligible by the end of October *i.e.* 42th and 43th weeks during 2011 and 2012 in Rice Research Station, Kaul, Haryana (Kumar et al 2017). The results from this study also support the view that peak level of population occurred during the 38th standard week in third week of September in BHU Varanasi, Uttar Pradesh (Chaudhary et al 2014), in Meerut, Uttar Pradesh (Kumar et al 2019, Verma et al 2021), in Srikakulam district of Andhra Pradesh (Chitti Babu et al 2020). BPH population appeared during 30th standard week and reached to its peak during 40th standard week in Varanasi, Uttar Pradesh (Sharma et al 2018). In present study, as maximum temperature of 33-34°C, minimum temperature 22-25°C and occurrence of very less rainfall were favorable factors for buildup of peak damage of leaf folder and brown plant hopper and such conditions were prevalent during crop season 2017. The damage of leaf folder and brown plant hopper populations correlated positive with maximum temperature and negatively with rainfall.

Sheath blight disease might be due to the prevalence of highly favorable factors like high relative humidity, less temperature and more rainfall for disease development. The

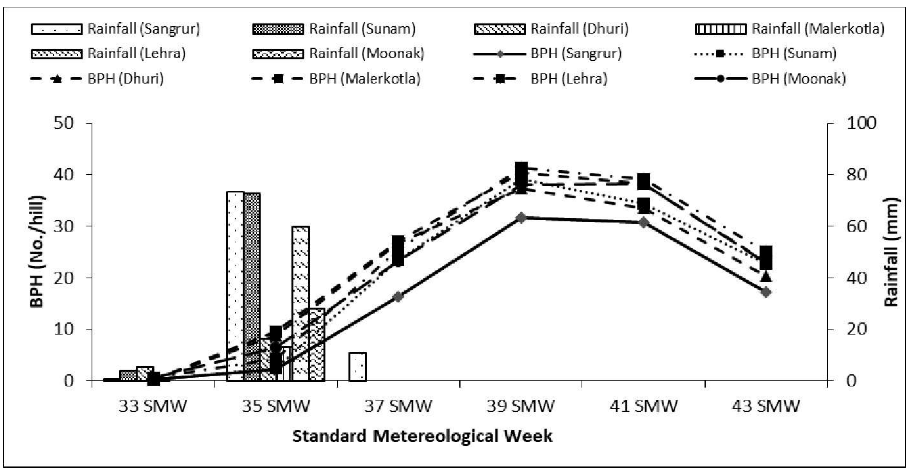

Fig. 4. Effect of rainfall on BPH population of rice in different blocks of Sangrur district during 2017

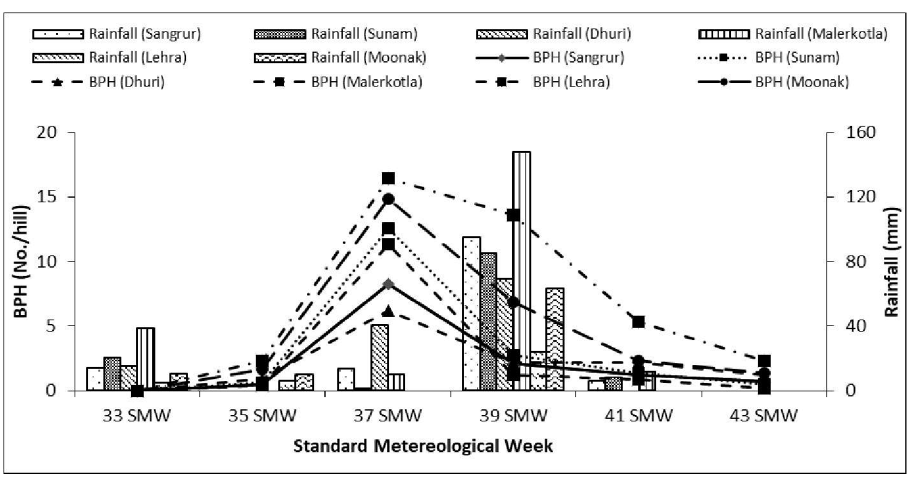

Fig. 5. Effect of rainfall on BPH population of rice in different blocks of Sangrur district during 2018

disease severity of sheath blight was recorded from 31^{th} to 43^{th} standard weeks in both season of crop in all blocks. In 2017, the disease severity was found highest in Sangrur (28.64%), Sunam (26.71%) and Lehra (22.15%) in 35^{th} standard week followed by decreasing the disease severity (Fig. 6). However, in crop season 2018 the disease severity was more remain throughout the season in field from 31^{th} to 43^{th} standard week due to continuous occurrence of rainfall. The highest disease severity was found 39th standard week in Malerkotla (46.65%) and Sangrur (38.64%) (Fig. 7). The disease incidence of sheath blight recorded 10.5 to 36.5 percent incidence in Cuddalore district of Tamil Nadu (Neha et al 2016), 30-76 percent incidence in Chhattisgarh, (Parshuram et al 2017), 15 to 42 percent incidence in areas of Allahabad (Yaduman et al 2018) and 20 to 80 percent incidence in five districts of eastern Uttar Pradesh (Thera et al 2021).

The incidence of false smut and kernel smut recorded per meter square area from infected field. In season 2017, false smut (0.115%) and kernel smut (0.048%) infected smut balls were highest in Sangrur block (Fig. 8). However, the incidence of false smut and kernel smut was more in 2018 which might be attributed to the favourable weather conditions coinciding with the susceptible stages of the crop. The prevailing weather remain favourable for long time coinciding with rainy days at the time of flowering stage of the crop, ultimately increasing the relative humidity which is a crucial factor for development of disease like false smut and kernel smut. The highest incidence of false smut (0.242%) and kernel smut (0.146%) recorded in Malerkotla during season 2018 (Fig. 9). The severe density of smut balls (per infected panicle) in the range of 1.6-7.1 in the seven surveyed locations of Egypt (Atia 2004), average between 0.6 and 4.8

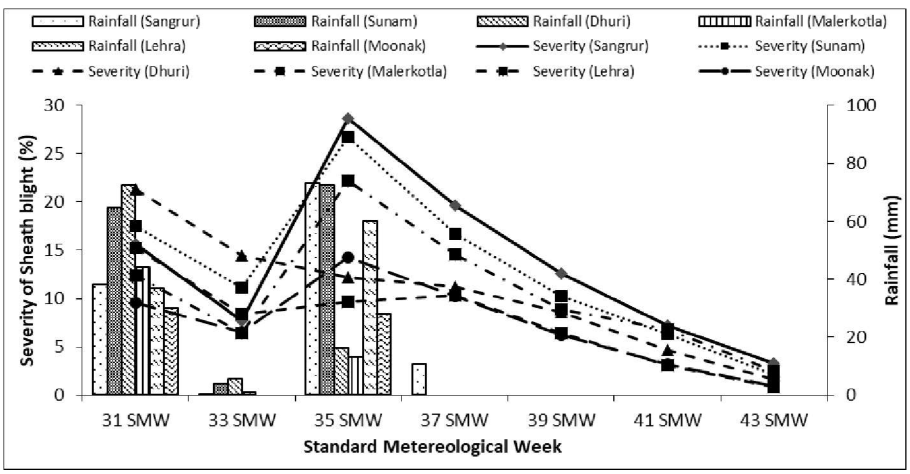

Fig. 6. Effect of rainfall on sheath blight disease severity of rice in different blocks of Sangrur district during 2017

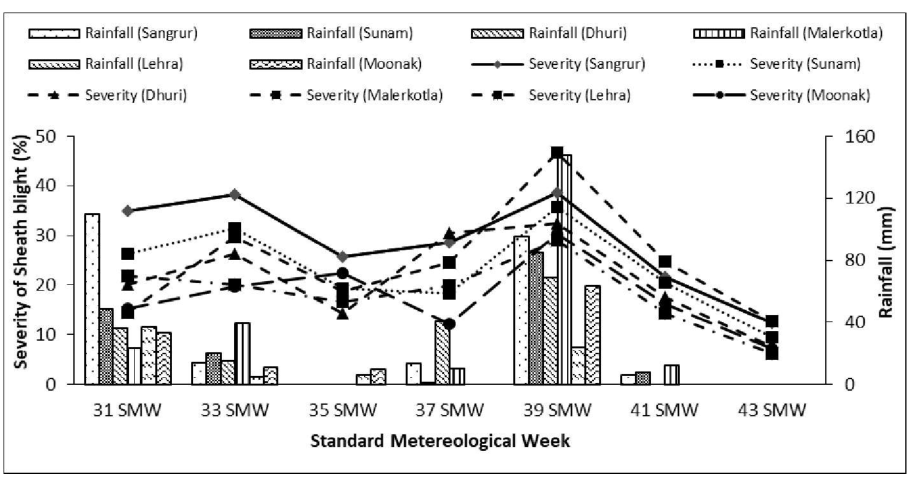

Fig. 7. Effect of rainfall on sheath blight disease severity of rice in different blocks of Sangrur district during 2018

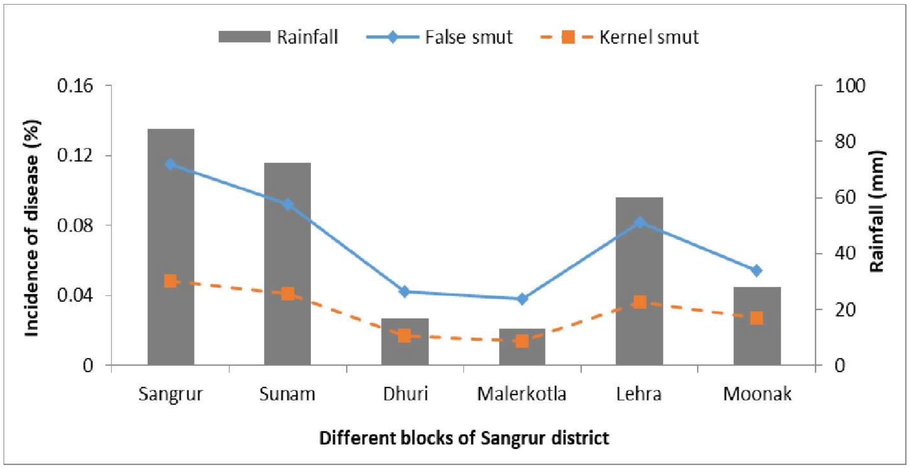

Fig. 8. Effect of rainfall on disease incidence of rice in different blocks of Sangrur district during 2017

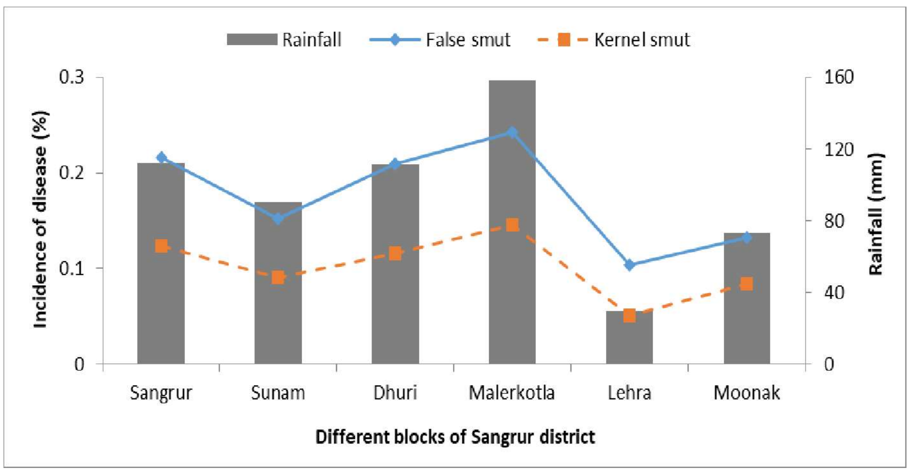

Fig. 9. Effect of rainfall on disease incidence of rice in different blocks of Sangrur district during 2018

smutted balls (per infected panicle) depending on locations and years (2010 and 2011) in Kashmir region of India (Sanghera et al 2012) and range of 2.0-8.0 average smut ball density (per infected panicle) during an extensive survey in the six districts of Uttar Pradesh of India (Singh et al 2014). In Punjab, 10 to 20 per cent infected tillers were recorded in popular inbred of rice like PR 114, PR 116 and PAU 201 (Ladhalakshmi et al 2012). Duhan and Jakhar (2000) reported incidence of kernel smut across different rice cultivars ranged from 0.05 to 1.20 percent. Mandhare et al (2008) collected the 630 seed samples of different varieties of rice from Nasik, Pune, Satara, Sangli, Kolhapur districts of state Maharashtra and observed that the incidence of kernel smut was low (0.00-0.4%).

CONCLUSIONS

It has observed that the high temperature and low rainfall favour to spread and build-up of insect population and in contrast occurrence of more rainfall and maintained the high humidity helpful for developing of disease in rice crop. It is very important study to know the preference weather conditions for occurrence and peak period of activity to spread and development of major biotic stresses. Therefore, proper monitoring and management strategies will be planned against these biotic stresses to avoid economic loss of the crop.

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Effective Seed Placement and Weed Management in Dry Direct Seeded Rice (DDSR) using Zero Till Seed Drill cum Herbicide Applicator

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***Abstract*:** Rice is typically grown in Asia by transplanting seedlings into puddled soil (land preparation with wet tillage). With accelerated economic growth and COVID-19 outbreak with subsequent lockdown has led to the shortage of labour. There was concern among the farmers on timely completion of transplanting due to shortage of labour. Dry Direct seeded rice (DDSR) is one such technique, probably the oldest method of crop establishment, is gaining popularity because of its low-input requirement. To overcome the effect of rain on the seeds sown by DDSR technique there is a need for creating a provision for settling the seeds in place. The energy spent in the field for individual sowing and herbicide application practice can be reduced by combining both operations. In view of these, the following study was undertaken to perform simultaneous sowing and herbicide application in DDSR technique using a zero till drill and herbicide applicator. A tractor operated zero-till drill cum herbicide applicator serves as a multi-purpose implement that helps in carrying out two or more operations simultaneously. The sole purpose of the implement was to settle the seed in the furrow by using levellers followed by herbicide application which acts as a pre-emergence herbicide preventing the immediate emergence of weeds. By adopting this practice, about 300 MJ ha^{-1} energy and Rs. 3000 spent for weed control can be saved. The emergence of the seed was not affected by the herbicide application as they are separated using a protective hood.

Keywords: Dry direct seeded rice, Herbicide, Nozzles, Zero till drill, Weeding

Rice is the world's most cultured crop, and more than half of the world's population devours it as a staple food. With the continuous rising in world's population, there is a need to increase rice productivity to meet global rice demand. The chances of increasing the rice-growing area in the near future are beyond our control. So the additional rice production must come from increased productivity. The main difficulty is to make this improvement while using less water, labour and chemicals as well as assuring long-term viability. Rice is typically grown in Asia by transplanting seedlings into puddled soil (land preparation with wet tillage). Puddling benefits rice because it reduces water loss through percolation, controls weeds, makes seedling establishment easier and creates anaerobic conditions to improve nutrient availability. On the other hand, puddling repeatedly has a negative impact on soil physical qualities by disintegrating soil aggregates, limiting permeability in subsurface layers and producing hard pans at shallow depths (Sharma et al 2004). It's a conventional practise that requires not only a lot of water but also a lot of effort. Various issues such as dwindling water tables, labour shortages during peak seasons and declining soil health, demand an alternate establishment strategy to ensure rice yield and natural resource sustainability. With accelerated economic growth and COVID-19 outbreak with subsequent lockdown has led to the shortage of labour. There was concern among the farmers on timely completion of transplanting due to shortage of labour. Direct seeded rice (DSR) is one such technique, probably the oldest method of crop establishment, is gaining popularity because of its low-input requirement. DDSR is direct seeding of rice in which seeds are directly sown in the field rather than using transplanting seedlings from the nursery. It aims to sow short duration and high yield varieties. It is done through DSR machines (Zero till drill, Happy seeder etc.,) which are used for planting seeds directly into combine harvested paddy fields.

Farmers who practiced DDSR technique, complained about rodent attacks. Usually in normal transplanting method, the crops are not vulnerable to rodent attacks. This is because, they cannot survive in flooded fields. Rice is commonly farmed using this DSR approach in Bangladesh and India, but it has been discovered that poor weed management procedures resulted in ineffective and poor weed control in DSR, resulting in yield reductions of 50-91 percent (Gupta et al 2006, Hussain et al 2008). In farmers' field trials in Tevai, Uttaranchal, India, herbicide spraying within two days of sowing resulted in good weed control and higher grain yield in DSR (Singh et al 2005), indicating that adopting appropriate weed management practises at the right time is critical to harvesting good paddy yields of direct seeded rice (Akhtar et al 2010). The farmers complained about DDSR technique getting effected by overnight rains that transport the seeds and reducing the yield. And since it is a new technique, farmers are having misconceptions and fears related to yield.

Hence to overcome the effect of rain on the seeds sown there is a need for creating a provision for settling the seeds in place. Also the energy spent in the field for individual sowing and herbicide application practice can be reduced by combining both operations. In view of these, the following study was undertaken. The objective of the study was to perform simultaneous sowing and herbicide application in DDSR technique using a zero till drill and herbicide applicator.

MATERIAL AND METHODS

The experiments are conducted at the Department of Farm Machinery and Power Engineering, Dr. NTR College of Agricultural Engineering, ANGRAU, Bapatla. A herbicide spraying technology/sprayer was designed and attached behind the existing tractor operated zero till seed drill for simultaneous sowing and herbicide application. The major components of the machine are a simple leveller, hood to separate seed from herbicide, triplex plunger pump and a spray boom fitted with nozzles.

Existing zero-till seed-cum-fertilizer drill: Zero-till-drill consists of frame, seed box, fertilizer box, seed metering mechanism, fertilizer metering mechanism, seed tubes, furrow opener, seed/fertiliser adjusting mechanism and transport cum power transmitting wheel. The frame is built of mild steel box section of dimensions 185 x 60 cm. There are 9 to 13 inverted T-type furrow openers on it. When affixed to a tine, these T-type furrow openers open a tiny slit 3-5 cm wide. The tynes are clamped in place to achieve infinite row spacing, as required by various crops. Furrow openers are positioned 17.5 cm apart in a zero-till seed-cum-fertilizer drill. The fundamental difference between a zero-till drill and a standard drill is that instead of reversible shovels, it features inverted T-type furrow openers placed on the tynes. The main benefit of inverted t-type furrow openers is that they do not generate clods, uses less draught, and penetrate the soil more easily.

Design of leveller: Inverted T-type furrow opener provided on the tynes creates a small opening less than 3 cm depth to drop the seed. Now the purpose is to design a leveller capable enough to close this opening without creating much disturbance on the soil. An annealed mild steel material of 5 mm thickness will be used to develop supporting flanges and end collector's plates. Supporting flanges will be spaced at 20 cm apart as shown in fig. 2 so that it matches the tyne spacings. End collector plates help in rolling the soil in the direction of travel covering the opened furrows.

Immediately after closing the furrow pre emergence herbicide will be applied to prevent weeds. There is a chance of herbicide coming in contact with seeds due to drift. So in order to prevent this, a protective hood will be installed on the supporting flanges as shown in Figure 2. A light weight 1 mm thick GI sheet will be used for developing protective hood.

Static structural analysis of leveller: Leveller is 1.6 m long and has to withstand soil resistance and undulations in the

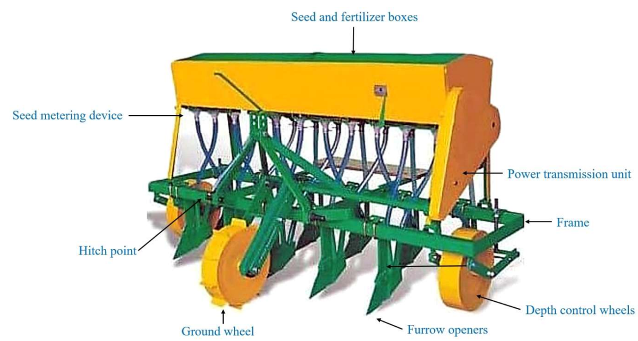

Fig. 1. Zero-till seed-cum-fertilizer drill

Table 1. Specifications of the existing zero-till seed-cum-fertilizer drill

Particulars	Zero-till drill
Working width, cm	180-200
Row spacing, cm	18-20 (Adjustable)
Furrow opener	Inverted "T" and narrow shoe
No of furrow openers	9/11
Drive wheel	Lug-front mounted
Weight, KN	2100
Unit Price, (Rs)	75,000

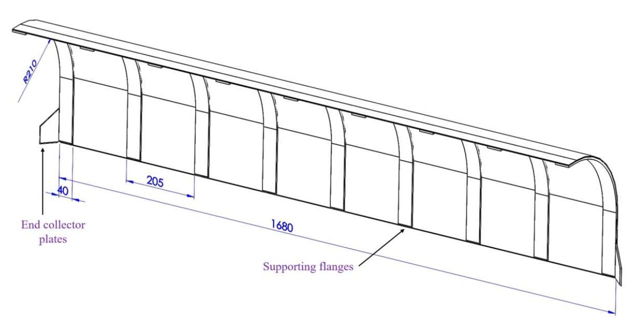

Fig. 2. Isometric view of leveller

field. So a static structural analysis has been carried out in solid-works simulation software with assumed load of 1000 N (including weight of frame, soil resistance and undulations). Mild steel AISI 1018 was applied to the body. A soil resistance of 1000 N has been applied on the face of leveller to determine the strength of the design. The stress, displacement and factor of safety plots of leveller were shown in Figure 3 to 5. Upon application of 1000 N, a maximum stress of 8.07×10^{7} N m^{-2} was experienced by the body which is in safe limit since yield stress of selected material was much higher i.e., 4.7×10^{8} N m^{-2}. In case of static displacement, upon 1000 N load a very less displacement has been noted. At the point where flange is welded with frame, a negligible displacement of 1×10^{-30} mm was noted. At the face of leveller a displacement i.e., backward thrust of 5.3 mm was noted but no failure in the design was observed. Factor of safety was about 5.8 i.e., it can withstand 5.8 times more load than the applied load.

Design of herbicide applicator: In direct-seeded rice systems, herbicides are one of the most essential instruments for weed control. Because of the scarcity of labour at important weeding times and high labour costs, herbicide use in these systems is projected to rise in the near future. Improper and ineffective herbicide application can result in nontargeted plant damage, a large waste of chemicals that pollutes the environment, and harmful impacts on human health. Herbicides can be applied with a knapsack, foot sprayer or pedal pump, as well as tractor-mounted and aerial sprayers; however, in Asia, knapsack sprayers are the most common (Chauhan 2012). Flat fan, hollow cone nozzle and solid cone nozzles are among the spray nozzles utilized. In multiple-nozzle booms, flat fan nozzles are employed because the spray pattern is tapered from the centre to the edges. By overlapping nearby nozzles, such patterns aid in the creation of consistent coverage. Hence, a plunger type pump compatible with tractor p.t.o. as shown in Figure 4 was selected to integrate with tractor operated zero till drill. Power to the sprayer will be tapped from ground wheel using a simple four bar mechanism.

Plunger pump: The pump had to supply four nozzles at 2.25 L min^{-1} discharge each at high pressure. The HTP (Horizontal

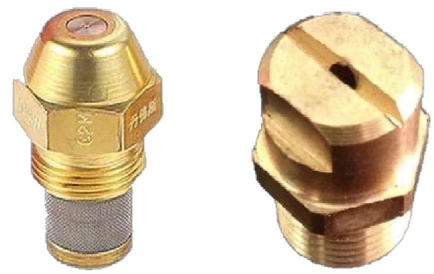

Fig. 4. Solid cone and flat fan nozzles

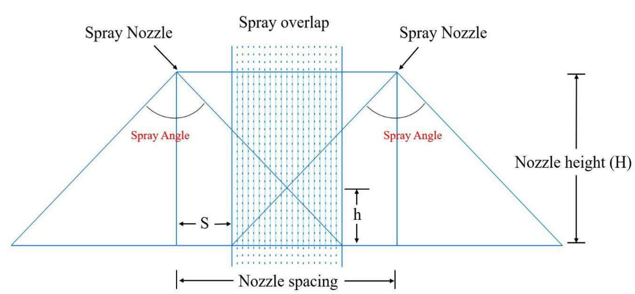

Fig. 5. Effect of nozzle height on spray pattern

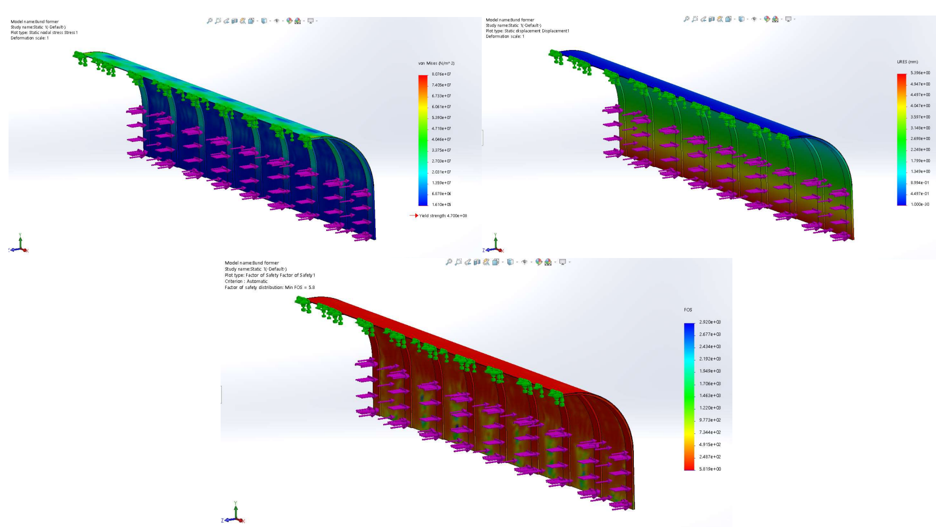

Fig. 3. Stress distribution, displacement curve and factor of safety curve of the designed leveller

Triplex Pump) was chosen, which has a free discharge capacity of 40-45 litres per minute. The pump's installation and driving arrangement from the tractor's PTO (Power Take Off) was at the rear. For operation, the shut off trigger valve of the lance is closed and the engine/ electric motor is started to actuate the pump. The pump draws the spray liquid from the tank, imparts pressure energy and sends it to the delivery line/lines (Mohan et al 2021).

Patternator: Effectiveness of a sprayer nozzle depends on the ability to provide good deposition on target area. Calibration of sprayer nozzles was usually carried out on a spray patternator. It is the most simple method of evaluating the spray distribution by a nozzle. A simple channel roof cover can also be used as a patternator. Grooves are maintained at equal spacing and mounted on an inclined stand. Directly below the grooves a provision was created to hold the corresponding spray collecting jars. To mount the spray nozzle at certain height, a height regulator or adjustable frame provided alongside the patternator. Markings were made on the mounting frame to adjust the height of nozzle from the surface.

Solid cone nozzle: Solid cone nozzle consists of an additional internal jet which strikes the rotating liquid within the orifice. Due to this impact the breaking of liquid droplets takes place causing fine distribution of spray particles inside the cone.

Flat fan nozzle: In a flat fan nozzle the liquid droplets are sprayed in the shape of a thin sheet. It is widely used for spraying pesticides over and between the rows. A flat fan nozzle attached with spray lance was operated at low pressure to maintain the spray pattern. Size of the liquid droplets will be larger than that in case of other nozzles.

Design considerations: Nozzle mounting height can be decided directly by measuring the swath length and spray angle. Tan of spray angle is equal to the division of nozzle mounting height and half of swath length. 2

$$\mathrm{Tan}\,\frac{\theta}{2}=\frac{S}{h}$$

$$\frac{H}{h}=\frac{\mathrm{Overlap}+S}{S}$$

Where,

θ = Spray angle, H = Nozzle mounting height, S = Half of swath length, h = Height of overlap

Uniformity and density of spray deposition varies from centre to side. Density of the spray will be thick in the middle and further becomes thin over the edges. So overlap of the spray is recommended in a multi nozzle or boom type sprayer for uniform distribution.

The spacing between the nozzles was calculated by given formula:

$$S_d = 2h\ \mathrm{Tan}(\frac{\theta s}{2})(1-\frac{S}{100})$$

Where,

S_d = Spacing between nozzles, mm, h = boom height, mm, θ_s = Nozzle spray angle

s = Overlap, %

An overlap of 40% for fan nozzles, 25% for cone nozzles and 100% for flood jet has been recommended (Varshney et al 2004))

The length of the boom was calculated by using the following formula.

$$L = n \times S_d$$

Where,

L = Length of the boom, S_d = Spacing between the nozzles, mm, n = Number of nozzles

From above formula's and design considerations, the following have been calculate. Let the possible nozzle spacing be 45 cm with a spray angle of 60° and an overlap of 40 %. The required height of mounting will be,

$$\mathrm{Tan}\,\frac{\theta}{2}=\frac{S}{h}\quad \text{(Since, overlap = 18 cm, } \theta = 60^0\text{, s= 13.5 cm)}$$

Height of overlap, h = 23 cm

$$\frac{H}{h}=\frac{\mathrm{Overlap}+S}{S}$$

Height of mounting, H = 50 cm

Discharge rate: Discharge rate is the amount of herbicide discharged from the nozzles. It is calculated as $l\,h^{-1}$.

Discharge/Flow rate is determined by

$$Q=\frac{1}{600}\times M\times A\times V$$

Where, Q = Flow rate/discharge, m^3/s, M = Application rate, $l\ ha^{-1}$ A = Area of cross section of pipe, m^2

V = Velocity of sprayer, kmph

From above formula's and design considerations, the following have been calculated **(Section 2.3)**

Application rate (M) = 300 $l\ ha^{-1}$

(Since, active ingredients in concentrated herbicide solution are high, dilution with prescribed amount of water is needed. 3 g for every 1000 ml of water is usually preferred. Herbicide application rate was limited to 1000 g a.i per ha (Singh et al 2016) (Singh et al 2015))

Area of cross section of pipe (A) = 1.8 m^2

Velocity of sprayer (V) = 2.5 kmph

$$\text{Flow rate/discharge from nozzle} = \frac{1}{600}\times M\times A\times V = 2.25\ l\ min^{-1}$$

Total discharge rate from boom = flow rate of nozzle × No. of nozzles = 2.25 × 4 = 9 $l\ min^{-1}$

The available tank capacity is 75 litres (2 No's), the time required to apply 150 litres of chemical will be

$$= \frac{150 \times 10 - 3}{9 \times 10 - 3} = 16.6 \min$$

Thus, total area covered in 16.6 min will be=

$$16.6 \min \times \frac{2.5 \times 1000}{60} \times 18$$

= 1250 m^2 or 0.125 ha

Hence 8 fillings will be needed to cover over a hectare of land.

Suggestions

- Use clean water and a plastic container to make spray solution as herbicides bind with suspended soil particles and metal surfaces (e.g., iron bucket).
- Use a multiple-nozzle boom fitted with flat-fan nozzles for full coverage

Tractor operated zero-till drill cum herbicide applicator: A tractor operated zero-till drill cum herbicide applicator serves as a multi-purpose implement that helps in carrying out two or more operations simultaneously. The sole purpose of the implement was to settle the seed in the furrow by using levellers followed by herbicide application which prevents the emergence of weeds. The leveller and spray nozzles are spaced apart to prevent contact with seed. Spray nozzles frame was placed at a distance of 52 cm from the frame which holds two 75 litre sprayer tanks as shown in Figure 7. From the design calculations, spray boom with 4 nozzles at a spacing of 45 cm will be fixed at 45-50 cm height from ground as shown in Figure 6.

Field capacity: It is the actual area covered by the machine based on its total time consumed and actual working width under field condition. It is expressed in terms of area covered per unit time of operation. It is calculated by

$$\text{Field capacity (ha h}^{-1}\text{)} = \frac{\text{Actual area covered}}{\text{Total time consumed}}$$

(Mohan and Jayan 2021)

Field efficiency: Field efficiency is the actual average rate of coverage by the machine, based upon the total operation set time. It is a function of the rated width of the machine, speed of operation and the amount of time lost during the operations. Effective field capacity is usually expressed as hectare per hour (Kepner et al 2005).

$$\text{Field efficiency (\%)} = \frac{\text{Actual field capacity}}{\text{Theoritical field capacity}}$$

(Mohan and Jayan 2021)

Application rate: It is the ratio of volume of herbicide sprayed in the test plot and the area of test plot.

$$\text{Application rate, 1 ha}^{-1} = \frac{\text{Volume of herbicide sprayed}}{\text{Area of test plot}}$$

RESULTS AND DISCUSSION

Laboratory evaluation of triaxial plunger pump was carried out with two different nozzles at three operating pressures and three mounting heights. Variation in discharge with change in operating pressure and nozzle height, change in swath width with pressure were analysed by using a patternator (Farooq et al 2001).

Variation in discharge rate w.r.to operating pressure and mounting height in a solid cone nozzle: Solid cone nozzle emits thick sprays throughout the diameter with equal density of spray particles. Variation in discharge along the grooves was comparatively less than the other nozzles. Figure 8 shows the parabolic increase and decrease in discharge rate from 4^{th} to 12^{th} groove at 50 cm mounting height. The discharge rate of solid cone nozzle ranged from 5-130, 5-135 and 5-150 ml min^{-1} with change in operating pressure from 2-3 kg cm^{-2} where the nozzle was fixed at a height of 30, 40 and 50 cm respectively.

Variation in discharge rate w.r.to operating pressure and mounting height in a flat fan nozzle: A flat fan nozzle achieves more spread of spray over the grooves compared to solid cone and hollow cone nozzles. Figure 9 clearly shows the uniform spray distribution throughout the grooves with change in operating pressure. The flat fan nozzle discharge rate varied from 15-150, 5-140 and 5-155 ml min^{-1} with change in operating pressure from 2-3 kg cm^{-2} where the nozzle is fixed at a height of 30, 40 and 50 cm respectively.

Variation in swath width w.r.to operating pressure and mounting height: Swath width is a major operational parameter which is effected by operational pressure, mounting height and type of nozzle during laboratory and

Table 2. Levels of variables

Description of variable	Levels	No. of levels
Type of nozzle	Solid cone Flat fan	3
Operating pressure	2 kg cm^{-2} 2.5 kg cm^{-2} 3 kg cm^{-2}	3
Mounting height	30 cm 40 cm 50 cm	3

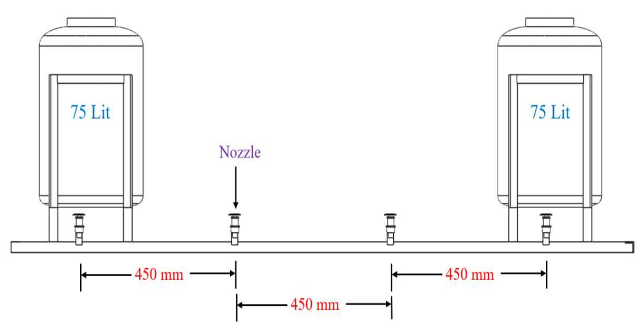

Fig. 6. Supporting frame and nozzle's arrangement

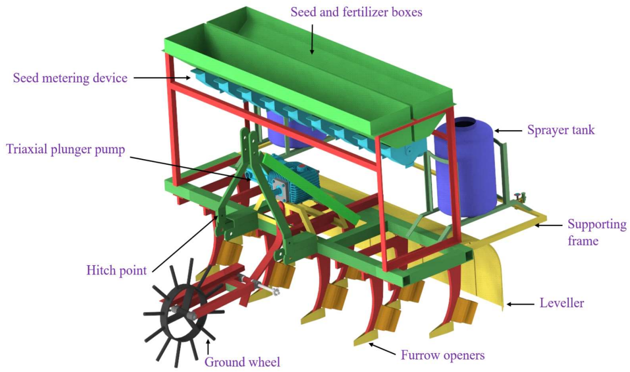

Fig. 7. Isometric view of zero till drill with herbicide applicator

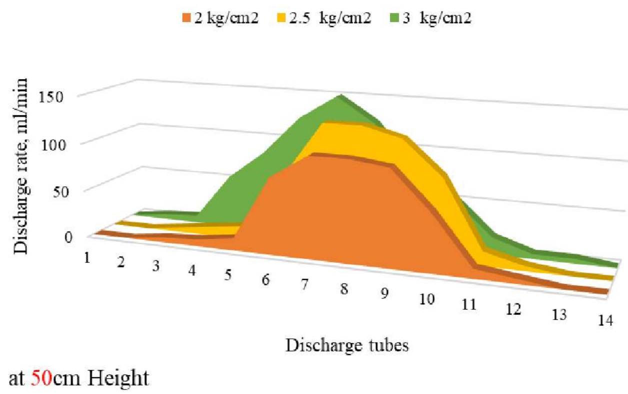

Fig. 8. Variation in discharge rate at 50 cm height

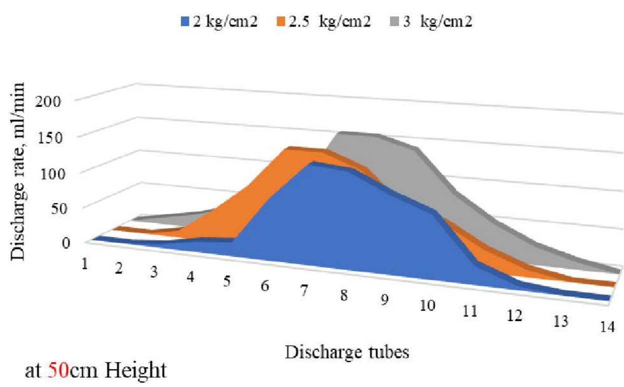

Fig. 9. Variation in discharge rate at 50 cm height

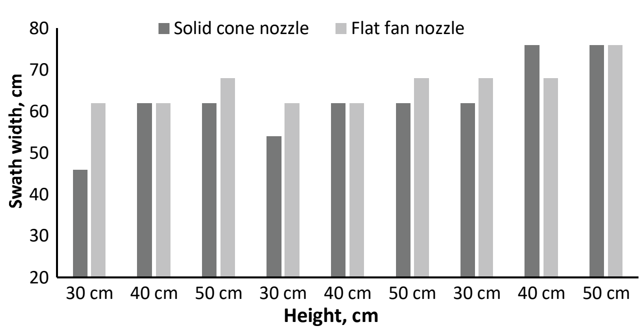

Fig. 10. Variation in swath width with mounting height and operating pressure

field evaluation of a sprayer. A flat fan nozzle covers more swath width compared to solid cone nozzles as shown in Figure 10. At 50 cm mounting height and 3 kg cm^{-2} pressure, equal swath width was achieved for both the nozzles.

Solid cone nozzle swath width ranged in between 46-76 cm at a height of 30-50 cm with operating pressure of 2-3 kg cm^{-2} respectively. Flat fan nozzle swath width varies from 62-76 cm at a height of 30-50 cm with a pressure of 2-3 kg cm^{-2} respectively. Width of the swath was increased with increasing the height of the nozzle. The actual field capacity of the machine was found to be 0.18 ha h^{-1} at a forward speed of 1.5 kmph, 0.20 ha h^{-1} at 1.7 kmph and 0.23 ha h^{-1} at 2.0 kmph. Maximum field capacity was noted at a forward speed of 2.0 kmph but the application rate of herbicide was relatively less. Leveller attachment immediately behind the furrow openers helped in settling the seeds followed by herbicide application.

CONCLUSION

Rice is the world's most cultured crop, and more than half of the world's population devours it as a staple food. Puddling is a conventional practise that requires not only a lot of water but also a lot of effort. It benefits rice because it reduces water loss through percolation, but puddling repeatedly has a negative impact on soil physical qualities like limiting permeability in subsurface layers and producing hard pans at shallow depths. DDSR is direct seeding of rice in which seeds are directly sown in the field rather than using transplanting seedlings from the nursery. Due to sudden rains, seeds sown with DDSR technique are transported. Also due to direct drilling of seeds without any prior tillage, emergence rate of weeds is high. Hence a tractor operated zero-till drill cum herbicide applicator must be adopted since it serves as a multi-purpose implement that helps in carrying out two or more operations simultaneously. The sole purpose of the implement was to settle the seed in the furrow by using levellers followed by herbicide application which prevents the emergence of weeds. Maximum field capacity of the machine was noted at a forward speed of 2.0 kmph i.e., 0.23 ha h^{-1}. By adopting this practice, about 300 MJ ha^{-1} energy and Rs. 3000 spent for weed control can be saved. Herbicide application did not affect the emergence rate of seed and it will economically benefit the farmers in overcoming the drudgery.

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Nutrients Content, Uptake and Soil Biological Properties as Influenced by Various Nutrient Management Practices under Fodder Pearl Millet Cultivation

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Abstract: The experiment was conducted during *kharif* season of 2019-20 at Agronomy research farm, ICAR-NDRI, Karnal (Haryana) with eight treatments including recommended dose of fertiliser (80:30:30 kg/ha, N: P_2O_5: K_2O, respectively) in combinations with plant growth promoting Rhizobacteria (PGPR) as seed treatment and cow urine foliar spray (10%). The nutrients content and uptake by fodder pearl millet significantly affected with different nutrient management practices and maximum nitrogen (1.57%), phosphorus (0.27%) and potassium (1.66%) contents as well as uptake of nitrogen (177.40 kg ha^{-1}), phosphorus (30.62 kg ha^{-1}) and potassium (188.56 kg ha^{-1}) was recorded with 100% RDF + PGPR + Cow urine foliar spray, which was statistically at par with 100% RDF + PGPR. Maximum microbial biomass carbon (163.47 and 192.44 µg/g dry soil), dehydrogenase activities (13.25 and 23.83 µg TPF/g soil/day), alkaline phosphatase activities (84.55 and 106.96 µg PNP/g soil/hr.), bacterial counts (14.46×10^6 and 22.09×10^6 CFU/g soil), actinomycetes counts (26.40×10^5 and 37.15×10^5 CFU/g soil) and fungal counts (14.07×10^4 and 18.85×10^4 CFU/g soil) at 40 DAS and harvest in 100% RDF + PGPR + cow urine foliar spray, which was statistically at par with 100% RDF + PGPR followed by 75% RDF + PGPR + cow urine foliar spray and 75% RDF + PGPR at 40 DAS, while at harvest it was found at par with 100% RDF + PGPR and both were found significantly higher over rest of the treatments.

Keywords: Cow urine, Fodder, Pearl millet, Quality, Yield

India has the highest number of livestock animals (535.78 million) in the world's, according to 20^{th} livestock census 2019, the population of major livestock animals *viz*., buffalo, cattle, sheep and goat in India is 109.85, 192.49, 74.26 and 148.88 million, respectively (Anonymous 2020). Fodder demand increase for ever increasing population of livestock and it is essential component for livestock production as it can curtail the cost of feeding because livestock feeding contributes about 65 to 70% of total cost of livestock farming (Kumar et al 2022). Nutrient management is an important aspect to achieve sustainable crop production. Scenario from green revolution era, shows productivity of cereals increased largely with the use of high yielding variety, intensive agronomic practices and indiscriminate use of higher rate of chemical fertilizers with little or without use of organic source of nutrients to plant, that create adverse effect on soil *viz*., inadequacy in one or more nutrients and deterioration of soil fertility which leads to stagnating or even declining of crop productivity and quality (Shormy et al 2013). Deficiency of nutrients in soils leads to produce mineral deficient fodder (Shukla et al 2015). However, animal production level and health adversely affected due to nutrients deficient or low-quality fodder offered in feeding, which ultimately influence returns from livestock sector (Surve et al 2011). Judicious use of organic and inorganic sources of nutrients may sustain and enhance fodder quality and soil health. Among the different fodder crops pearl millet (*Pennisetum glaucum*) is the gifted crops of the tropical and sub-tropical regions that provide fodder and stover (dry straw) to millions of livestock animal of poor resources farmer. Among different organic source of nutrients for plant, cow urine and plant growth promoting rhizobacteria (PGPR) are excellent and important for agriculture uses. Cow urine (CU) contains; nitrogen, phosphorus, potassium, sulphur, sodium, manganese, iron, carbolic acid, silicon, chlorine, enzyme and hormones (Saunders 1982). PGPR, is a consortium of microorganism that actively colonize around plant roots and enhances plant growth and yield (Wu et al 2005). PGPR strains belongs to a wide range of genera *viz*., *Pseudomonas, Azospirillum, Bacillus, Serratia* and *Azotobacter* (Bashan et al 2004). The beneficial effects of PGPR due to their ability to produce various organic compounds *viz*., auxins, gibberellins, cytokinin, ethylene, organic acids, siderophores, nitrogen fixation, solubilization of insoluble inorganic soil phosphate to available form, sulphur oxidation, extra cellular production of antibiotics, increases in root permeability, enhancement of essential plant nutrients uptake (Enebak and Carey 2000). Considering above facts in view the present study was proposed to finds out a suitable combination of nutrient source to enhance the fodder quality and soil health.

MATERIAL AND METHODS

Experimental site: The experiment was conducted during *kharif* season, 2019-20 at Agronomy Research Farm, ICAR-NDRI, Karnal (Haryana) located at 29º45' North latitude and 76º58' East longitude and at an altitude of 245m above mean sea level. The climate of the area is semi-arid with a mean annual rainfall is 70.7 cm, and about 70-80% of which is received during the months of July-September and rest during winter and spring season. The mean minimum and maximum temperature during this study was 20.49°C and 34.54°C, respectively. The soil of experimental site was clay loam in texture (Piper 1942) with pH 7.35, Electrical conductivity (EC) 0.37 dS/m (Jackson 1967), organic carbon 0.49 percent (Walkley and Black's 1934), available nitrogen 215 kg/ha (Subbiah and Asija 1956), available phosphorus 24.70 kg/ha (Olsen et al 1954), available potassium 285 kg/ha (Jackson 1967), microbial biomass carbon 120.30 µg/g dry soil (Nunan et al 1998), dehydrogenase activities 8.10 µg TPF/g soil/day (Casida et al 1964), alkaline phosphatase activities 63.55 µg PNP/g soil/hour (Tabatabai and Bermner 1969), bacterial counts 8.2×10^6 CFU/g soil (Gordon et al 1973), actinomycetes counts 17.56×10^5 CFU/g soil (Kenknight and Muncie 1939) and fungal counts 8.86×10^4 CFU/g soil (Martin 1950).

Treatments details and input applications: The experiment was laid out in randomized complete block design with eight treatments *viz*., T_1 : Absolute control; T_2 : 100% RDF; T_3: 100% RDF + Cow urine foliar spray; T_4: 100% RDF + PGPR; T_5 : 100% RDF + PGPR + cow urine foliar spray; T_6: 75% RDF + cow urine foliar spray; T_7: 75% RDF + PGPR; T_8 : 75% RDF + PGPR + Cow urine foliar spray with three replications. The land preparation was done by one deep ploughing with disc plough followed by two cross harrowing with disc harrow and planking. Application of recommended dose of fertilizers (80:30:30 kg/ha, N: P_2O_5: K_2O, respectively) were applied as per treatment. The N, P_2O_5 and K_2O were applied through urea, DAP, and muriate of potash, respectively. The half of N and full doses of P and K were applied before final harrowing and remaining half dose of nitrogen was top-dressed in two split doses as per the treatment. The PGPR liquid culture (100 ml/10 kg seeds) contains 10^9 CFU/ml was diluted in one litres of water, and applied on seeds. Thereafter, inoculated seeds were dried in shade for 60 minutes, after drying seeds was manually sown. Nutrifeed variety of fodder pearl millet was sown using 10 kg seed per hectare with maintaining spacing 30cm × 10cm row to row and plant to plant, respectively. Other package of practices was followed as per standard for cultivation of fodder pearl millet. The 10% cow urine was applied as foliar spray in early morning hours, when the dew has been evaporated at 30 and 45 DAS as per treatments.

Analysis of nutrients content in plant sample: The crop was harvested manually at 50% flowering stage. The harvested chopped plant samples from net plot area were dried in hot air oven at 70º C for at least 72 hours until a constant weight was reached. Further, these oven-dried plant samples were ground to pass through two mm sieve in a Wiley mill. The sieved samples were used for chemical analysis, *viz*., nitrogen (Jackson 1967), phosphorus (Olsen and Sommers 1982) and potassium (Richards 1954). Thereafter, the uptake of nutrients by plant was calculated through following equation:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%)} \times \text{Dry matter yield (kg)}}{100}$$

Analysis of soil biological properties: At 40 DAS and harvest of crop, soil samples randomly collected from five spot at 0-15 cm depth in each net plot area and make it a composite sample. Taken 500g soil from composite sample after quartering for further soil analysis. Microbial biomass carbon, dehydrogenase activities, alkaline phosphatase activities, bacteria count, actinomycetes count and fungal count in fresh soil was estimated by using standard procedure given by Nunan et al (1998), Casida et al (1964), Tabatabai and Bermner (1969), Gordon et al (1973), Kenknight and Muncie (1939) and Martin (1950), respectively.

Statistical analysis: All the data recorded were processed in Microsoft excel 2010 and analysed with the help of analysis of variance at 5% level of significance. Simple Pearson's correlation coefficient was computed by using mean values of different nutrient contents in plant sample with the help of SPSS 23.0 Version.

RESULTS AND DISCUSSION

Dry matter yield: The dry matter yield of pearl millet was significantly influenced with different nutrient management practices and recorded significantly higher dry matter yield (11.33 t/ha) at harvest with application of 100% RDF+PGPR+CU, which was found statistically at par with 100% RDF+PGPR and both were significantly higher than other treatments (Fig. 1). Balanced and regular supply of essential plant nutrients, PGPR produce phytohormones (Enebak and Carey 2000) and cow urine supply enzyme and hormones that attributed to stimulate plant physiological

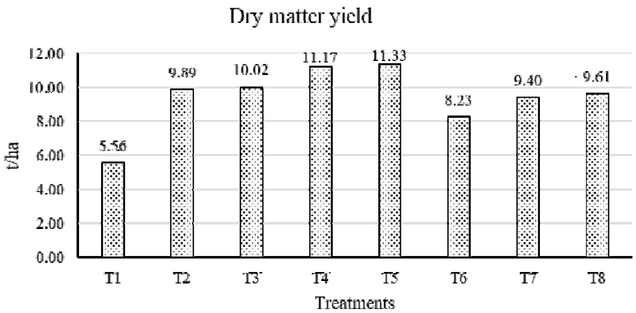

Fig. 1. Effect of nutrient management practices on dry matter yield of fodder pearl millet

processes leads to increase leaf area index that responsible for higher interception of solar radiation and produce more photosynthates and nutrients acquired, resulted in to increase dry matter assimilation in different part of plant leads to increase dry matter yield. Further, higher biomass production and dry matter content attributed to increase dry matter yield. The similar results also reported by Chattha et al (2017).

Nutrient content and uptake: The chemical analysis of fodder pearl millet showed that nutrient content and their uptake significantly influenced with different nutrient management practices and recorded maximum nitrogen (1.565%), phosphorus (0.273%) and potassium (1.663%) contents in pearl millet fodder on dry matter basis with application of 100% RDF+PGPR+CU, which was statistically at par with 100% RDF+PGPR (Table 1). Nutrients supplied with 100% RDF+PGPR+CU improved nitrogen by 3.27, 6.65, 8.78 and 21.33%; phosphorus by 1.23, 14.85, 15.49 and 41.38% and potassium by 1.42, 8.24, 8.89 and 38.96% over 100% RDF+PGPR, 100% RDF+CU, 100% RDF and absolute control, respectively. The pearl millet supplied with 100% RDF+PGPR+CU approached maximum nutrients contents in fodder on dry matter basis due to nutrients concentration in soil solution increase with application of fertiliser, PGPR supplement N by biological fixation of atmospheric nitrogen, P by solubilizing of fixed inorganic soil phosphate to available form and K by secretion organic acid that increase availability to plant (Cakmakci et al 2007). The cow urine additionally supplies N, P and K to plant foliage leads to quickly supply of nutrients to plant. Optimum and continues availability of nutrients to plant increase uptake as well as assimilation in plant tissue, leads to increase nutrients content. Likewise, contents the uptake of nutrients reported to follow the similar trend (Table 1) and recorded maximum nitrogen (177.40 kg/ha), phosphorus (30.62 kg/ha) and potassium (188.56 kg/ha) uptake with application of 100% RDF + PGPR + CU, which was statistically at par with 100% RDF + PGPR and both were significantly higher than other treatments. Higher assimilation of nutrients in plant tissue as well as biomass production attributed to increase nutrients uptake.

Soil biological properties: The analysis of soil showed that soil biological properties significantly influenced with different nutrient management practices and recorded maximum microbial biomass carbon (163.47 and 192.44 µg/g dry soil), dehydrogenase activities (13.25 and 23.83 µg TPF/g soil/day), alkaline phosphatase activities (84.55 and 106.96 µg PNP/g soil/hr.), bacterial counts (14.46×10^6 and 22.09×10^6 CFU/g soil), actinomycetes counts (26.40×10^5 and 37.15×10^5 CFU/g soil) and fungal counts (14.07×10^4 and 18.85×10^4 CFU/g soil) at 40 DAS and harvest respectively, with application of 100% RDF+PGPR+CU, which was statistically at par with 100% RDF+PGPR followed by 75% RDF+PGPR+CU and 75% RDF+PGPR at 40 DAS, while, at harvest it was found at par with 100% RDF+PGPR and both were found significantly higher over rest of the treatments. In addition to fertiliser, PGPR increase availability of phosphorus through solubilization and

Table 1. Effect of nutrient management practices on nutrient content and uptake by fodder pearl millet

Treatments	Nutrient content (%)			Nutrient uptake (kg/ha)		
	N	P	K	N	P	K
T_1: Absolute control	1.290	0.193	1.197	71.82	10.76	66.59
T_2: 100% RDF	1.439	0.237	1.528	142.28	23.40	150.92
T_3: 100% RDF+CU	1.468	0.238	1.537	147.19	23.75	154.24
T_4: 100% RDF+PGPR	1.516	0.270	1.640	169.33	30.55	183.24
T_5: 100% RDF+PGPR+CU	1.565	0.273	1.663	177.40	30.62	188.56
T_6: 75% RDF+CU	1.356	0.220	1.403	111.61	18.11	115.53
T_7: 75% RDF+PGPR	1.395	0.237	1.463	131.23	22.26	137.57
T_8: 75% RDF+PGPR+CU	1.418	0.240	1.477	136.37	23.10	142.08
CD (P=0.05)	0.057	0.013	0.097	10.14	2.16	13.59

nitrogen by biological nitrogen fixation that increase root growth, surface area and biomass leads to produce more exudates resulted increase SOC. However, SOC considered as food for microbes leads to increase microbial counts in soil attributed to increase microbial biomass carbon. Similar result also reported by Lestari and Andrian (2017) and Cakmakci et al (2007). Soil enzymatic activities are important indicator of soil health and play vital role in organic matter decomposition and nutrient cycling of carbon, nitrogen, phosphorus and sulphur, which indicate higher metabolic activity of microbes (Dinesh et al 2013). Soil dehydrogenase enzymes indicate oxidation activity of soil microorganisms. Higher microbial population in soil attributed to increase dehydrogenase activities. Alkaline phosphatase responsible for organic phosphorus transformation in soil. PGPR attributed to increase native phosphorus availability by solubilization leads to increase alkaline phosphatase activities. Similar result also reported by Rana et al (2012).

Correlation studies: The correlation between N, P and K uptake and dry matter yield of fodder pearl millet (Table 4) revealed that N uptake (r = 0.992), P uptake (r = 0.985) and K uptake (r = 0.994) showed significant highly positive correlation with dry matter yield of fodder pearl millet. However, dry matter yield increase with nutrients uptake.

Regression studies: The regression analysis exhibited (Fig. 2) a significant polynomial correlation between nutrients uptake and dry matter yield of fodder pearl millet at harvest. The R^2 value between dry matter yield and N, P and K uptake was 0.99, 0.99 and 0.99, respectively. This indicates that N, P and K at harvest accounted 99% of total variation in dry matter yield.

Table 2. Effect of nutrient management practices on soil microbial biomass carbon and enzymatic activities under fodder pearl millet cultivation

Treatments	MBC (µg/g dry soil)		DHA (µg TPF/g soil/day)		APA (µg PNP/g soil/hr.)	
	40 DAS	Harvest	40 DAS	Harvest	40 DAS	Harvest
T_1: Absolute control	130.00	149.07	10.15	15.63	72.86	82.84
T_2: 100% RDF	149.10	174.11	12.02	19.36	79.37	93.29
T_3: 100% RDF+CU	149.10	174.13	12.03	19.43	79.85	93.37
T_4: 100% RDF+PGPR	163.46	192.41	13.12	23.68	84.08	106.24
T_5: 100% RDF+PGPR+CU	163.47	192.44	13.25	23.83	84.55	106.96
T_6: 75% RDF+CU	140.59	166.30	11.33	18.68	77.79	88.62
T_7: 75% RDF+PGPR	156.30	181.53	12.63	20.54	81.54	98.03
T_8: 75% RDF+PGPR+CU	156.31	181.73	12.70	20.70	82.03	98.19
CD (P=0.05)	9.81	10.43	0.93	1.77	3.60	4.95

MBC: Microbial biomass carbon, DHA: Dehydrogenase activities, APA: Alkaline phosphatase activities

Table 3. Effect of nutrient management practices on soil microbial population under fodder pearl millet cultivation

Treatments	BC (10^6 CFU/g soil)		AC (10^5 CFU/g soil)		FC (10^4 CFU/g soil)	
	40 DAS	Harvest	40 DAS	Harvest	40 DAS	Harvest
T_1: Absolute control	10.07	14.36	19.80	26.88	9.71	13.07
T_2: 100% RDF	12.98	18.34	23.78	32.15	12.17	16.04
T_3: 100% RDF+CU	13.03	18.35	23.95	32.20	12.23	16.07
T_4: 100% RDF+PGPR	14.34	21.97	26.13	37.10	13.98	18.80
T_5: 100% RDF+PGPR+CU	14.46	22.09	26.40	37.15	14.07	18.85
T_6: 75% RDF+CU	12.07	17.25	22.47	30.78	11.32	14.90
T_7: 75% RDF+PGPR	13.41	19.46	24.90	34.00	13.07	17.00
T_8: 75% RDF+PGPR+CU	13.63	19.48	25.02	34.17	13.10	17.27
CD (P=0.05)	1.14	1.34	1.67	2.29	1.09	1.34

BC: Bacterial count, AC: Actinomycetes count and FC: Fungal count

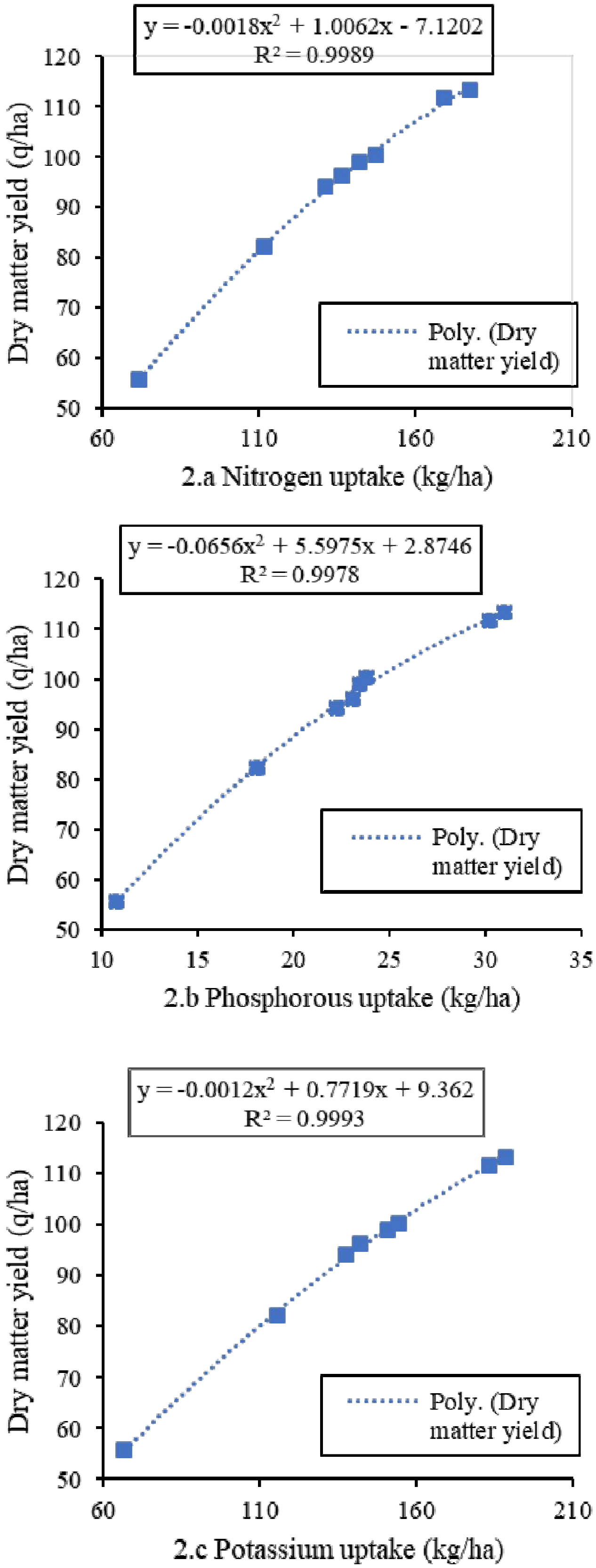

Fig. 2. Relationship between nutrient uptake and dry matter yield of fodder pearl millet

Table 4. Correlation coefficient (r) between dry matter yield and nutrients uptake by fodder pearl millet

Pearson	Correlations			
	DMY	N	P	K
DMY	1			
N	0.992**	1		
P	0.985**	0.995**	1	
K	0.994**	0.999**	0.995**	1

Note: DMY - Dry matter yield, ** - Correlation is significant at the 0.01 level (2-tailed)

CONCLUSION

Nutrient management is an important aspect, where intensive cropping system are dominates. Judicious use of organic and inorganic sources of nutrients sustains fodder quality and soil health. Maximum nitrogen, phosphorus and potassium content and uptake, as well as soil biological properties recorded with T_5 treatment under fodder pearl millet cultivation. For future line of work, as like pearl millet, different cereal fodder crops can be explored location wise along with proper dose and sources (inorganic and organic) of nutrients for better fodder quality.

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Impact off INM on Yield and Economics of Turmeric in Gangetic Alluvial Zone of West Bengal

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Abstract: As productivity of turmeric and soil health are reducing day by day due to improper application of chemical fertilizer, so present study was conducted to examine the effect of biofertilizers along with organic and different grades of inorganic nutrients on yield and economics of turmeric var. Suguna. Main objectives of this study were to use multiple sources of nutrients to increase productivity of turmeric rhizome and increase the B:C ratio. 13 treatments combination of nutrient sources like, 100%, 75% & 50% of NPK, 4 organic manures (compost, vermicompost, mustard cake and neem cake) and 3 bio-fertilizers (Nitrogen fixing - *Azotobacter chroococcum*, PSB - *Bacillus polymixa* and Potash mobilizer - *Fraturia aurantia)*. Turmeric rhizomes were harvested from the farm and then yield, and economic parameters were calculated. Treatment combination Vermicompost + NPK (100%) + Biofertilizer significantly superior in clump, primary & secondary rhizome characters, projected yield (38.55 t ha^{-1}), cost of production, gross return, net return (350688 Rs ha^{-1}) and B:C ratio (1.54). 25% of the full NPK can be replaced by organic sources of nutrients, which will improve soil health and productivity.

Keywords: B:C ratio, Economics, INM, Turmeric, Yield

The Zingiberaceae family includes turmeric (*Curcuma Longa* L), a significant ancient spice and medicinal plant with origins in South East Asia. It is a perennial herbaceous crop that is primarily farmed in tropical and subtropical areas of the world (Ronya et al 2020). As a long-term (8-9 months), demanding, and heavy feeder crop, turmeric needs a lot of nutrition to generate huge yield and higher-quality crop. It was found that using chemical fertilisers at high doses continuously has negative impacts on the ecology and the health of the soil (Chanchan et al 2018). Poor soil fertility and ineffective farming techniques result in a turmeric crop with low yield and productivity (Das et al 2020). The rising cost of chemical fertilisers and their negative effects on the environment, human health, and soil quality compelled the farmer to switch to an alternate source of nutrients for the production of spices. There are multiple sources of plant nutrients besides fertilisers, such as organic manures, bio-fertilizers, etc. The role of bio-fertilizers is perceived as growth regulators besides biological nitrogen fixation collectively leading to much higher response on various growth and yield attributing characters (Tinna et al 2020). The gap between potential yield and realized yield in a location can be markedly reduced with an appropriate nutrient management technique. Besides, enhanced rhizome yield, a suitable nutrient management technique can enhance rhizome quality and can also reduce the deleterious impact of fertilization by eliminating nutrient over use. Organic manures and biofertilizers offer an alternative to chemical fertilizers and increasingly used in spice crop production including turmeric (Datta et al 2017, Sahoo et al 2020). Organic source of nutrients is recommended for retaining productivity of soil, reducing usage of chemical fertilizers, improving soil health and minimize environmental pollution. Application of organic manures also quickly increases soil microbial biomass and their activity. Soil microorganisms and their activities play an important role in transformation of plant nutrients from unavailable to available forms and helpful for improvement of soil fertility. The combined use of organic and inorganic fertilizers known as Integrated Nutrient Management, not only increases the yield but also improves the physical, chemical, and biological property of soil which further improves fertility, productivity and water holding capacity of soil. The organic source will help to maintain nutrient equilibrium in soils. Application of different combinations of organic manures like FYM, neemcake, vermicompost also influence the growth and yield of turmeric (Sarma et al 2015). In addition to strengthening productivity and soil health, efficient management of chemical, organic, and biological forms of plant foods have demonstrated encouraging results in fulfilling some of the crop's chemical fertilizer needs (Prasanth et al 2018, Mohanty et al 2022). Integrated plant nutrient system involving a combination of fertilizers, manures and bio fertilizers are essential to sustain crop production, preserve soil health and biodiversity. This is especially important for developing countries where farming will continue to be in the hands of small farmers (Bhupenchandra et al 2022). Though a lot of trials on varietal, fertilizer, spacing, date of planting, size of planting material, mulching material and irrigation schedule etc. have been conducted to increase the production of turmeric, but till now very little work has so far been undertaken to increase the production. With such a premise, the current research was conducted to determine the effects of bio-fertilizers, manures, and grades of inorganic fertilizer on the yield characteristics and economics of turmeric in the Gangetic alluvial plains of West Bengal.

MATERIAL AND METHODS

The present investigation was conducted at Horticultural Research Station, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Nadia in 2019. Variety of turmeric is Suguna, number of treatments is 13 with 3 replications and followed RBD design (Table 1). Turmeric was fertilized with NPK @ 150:60:150 kg ha^{-1} and four types of organic manure namely Compost @ 25 t/ha, Vermicompost @ 5 t/ha, Neem Cake @ 3 t/ha and Mustard Cake @ 3 t/ha were applied during final land preparation. Three bio-fertilizers namely *Azotobacter chroococcum*, phosphate solubilizing bacteria (*Bacillus polymixa*) and potassic mobilizer (*Fraturia aurantia*) each @ 20 kg/ha was applied with organic manure during final land preparation and thoroughly mix up with soil. The fertilizers were applied in three splits but started 15 days after the application of bio-fertilizer and interval between the splits was same. Urea, SSP, and MOP were used as inorganic source of N, P and K respectively. Fresh turmeric rhizomes were harvested 9 months after planting with the help of spade. Then the roots and dirt material present on the rhizomes were removed. After that weight, length, breadth of the clump, primary and secondary rhizomes were collected. Then yield per plot and projected yield were written. According to the yield parameters, B:C ratio of 13 treatments were calculated.

Table 1. Treatment details

Treatments	Nutrient combinations
T1	Compost + NPK (100%) + Biofertilizer
T2	Compost + NPK (75%) + Biofertilizer
T3	Compost + NPK (50%) + Biofertilizer
T4	Vermicompost + NPK (100%) + Biofertilizer
T5	Vermicompost + NPK (75%) + Biofertilizer
T6	Vermicompost + NPK (50%) + Biofertilizer
T7	Mustard Cake + NPK (100%) + Biofertilizer
T8	Mustard Cake + NPK (75%) + Biofertilizer
T9	Mustard Cake + NPK (50%) + Biofertilizer
T10	Neem Cake + NPK (100%) + Biofertilizer
T11	Neem Cake + NPK (75%) + Biofertilizer
T12	Neem Cake + NPK (50%) + Biofertilizer
T13	NPK 100%

N-Nitrogen, P-Phosphorus, K-Potassium

Benefit: cost ratio: The economic assessment for the different treatment combinations were done based on cost of cultivation, gross return, and net return, considering the cost of inputs and market price of the produce during the period of experimentation (Sahoo et al 2020).

Statistical analysis: To do combined analysis, the mean data were analyzed using MSTAT-C. Various graphical representations have been created using the combined data of the relevant characters. The tables of findings provide the critical difference (C.D.) value to compare the variance between means.

RESULTS AND DISCUSSION

Yield Attributes

Clump: The highest weight of clump was documented under treatment combination of T4 (327.54 g) as compared to lowest clump weight of 205.16 g was associated with treatment of T9 (Table 2). The longest clump (16.31 cm) was in T10 followed by T4 as compared to minimum length (12.28 cm) under T9. The plants raised under T11 exhibited the widest (13.62 cm) clump as compared to minimum breadth (10.87 cm) of clump in T3 combination.

Primary finger: The T7 exhibited maximum (7.63) number of primary fingers as compared to minimum number of finger (4.96) in T3 (Table 3). Maximum weight (176.36 g) of primary finger was T1 as compared to minimum weight (108.24 g) under T11. The maximum length (9.24 cm) of primary finger was in plants grown under T1 as compared to minimum length (6.45 cm) under T9. The plants raised under T1 exhibited the widest (2.54 cm) primary finger as compared to minimum breadth (1.62 cm) of primary finger in T9 combination.

Secondary fingers: The plants raised under T11 exhibited maximum (10.34) number of secondary fingers as compared to minimum number of fingers (3.62) in T3 (Table 4). Maximum weight (135.38 g) of secondary finger was recorded in T4 as compared to minimum weight (38.16 g) under T3. The maximum length (5.83 cm) of secondary finger in T10 as compared to minimum length (4.34 cm) under T9. The maximum breadth of secondary finger (1.92 cm) was associated with T11 and minimum breadth of 1.45 cm under T9. Application of compost along with biofertilizers and NPK improves the soil tilth and aeration, rises the soil's capacity to hold water, and encourages the activity of microorganisms that makes the plant food elements in the soil readily available to the crop as compared to only NPK (100%) without any organic input. Which in return increase the photosynthesis, then accumulated carbohydrate increase the number of clump, primary & secondary fingers and finally increase total yield. As a result, integrated nutrient management sustain the soil fertility and productivity (Ambala et al 2019, Srinivasan et al 2016).

Yield per plot: The maximum (15.42 kg 3m^{-2}) plot yield was recorded in T4 (9.84 kg 3m^{-2}) as compared to minimum yield in T3 (Table 4). The plot yield under only inorganic fertilizer NPK 100% recorded yield of 11.52 kg 3m^{-2}. C:N ratio narrowed down during vermicomposting, which ensure the release of nitrogen to the crop when applied to the soil (Amala et al 2018).

Projected yield: The maximum projected yield (38.55 t/ha) was observed with T4 as compared to minimum yield (24.60 t/ha) in T3 combination (Table 4). The plot yield under only inorganic fertilizer NPK 100% recorded projected yields of 28.80 t/ha. Bhoomika et al (2018), Anuradha et al (2018) and Tripathi et al (2018) reported identical results. By enhancing the soil aggregates with organic manures, favourable pore

Table 2. Influence of different levels of organic, inorganic and biofertilizers on clump characters of turmeric

Treatment	Weight of clump (g)	Length of clump (cm)	Breadth of clump (cm)
Compost + NPK (100%) + Biofertilizer	285.24	16.14	12.94
Compost + NPK (75%) + Biofertilizer	262.36	15.65	12.46
Compost + NPK (50%) + Biofertilizer	214.18	12.32	10.87
Vermicompost + NPK (100%) + Biofertilizer	327.54	16.24	12.64
Vermicompost + NPK (75%) + Biofertilizer	278.39	15.56	12.18
Vermicompost + NPK (50%) + Biofertilizer	246.52	13.82	11.46
Mustard Cake + NPK (100%) + Biofertilizer	272.84	15.16	13.28
Mustard Cake + NPK (75%) + Biofertilizer	249.32	15.76	12.38
Mustard Cake + NPK (50%) + Biofertilizer	205.16	12.28	10.96
Neem Cake + NPK (100%) + Biofertilizer	304.85	16.31	13.12
Neem Cake + NPK (75%) + Biofertilizer	258.56	15.72	13.62
Neem Cake + NPK (50%) + Biofertilizer	228.38	14.62	11.36
NPK 100%	242.12	15.25	12.92
C.D. (P=0.05)	0.767	0.637	0.531

Table 3. Influence of different levels of organic, inorganic and biofertilizers on primary finger of turmeric

Treatment	Number	Weight (g)	Length (cm)	Breadth (cm)
Compost + NPK (100%) + Biofertilizer	6.51	176.36	9.24	2.54
Compost + NPK (75%) + Biofertilizer	6.32	139.28	8.68	1.92
Compost + NPK (50%) + Biofertilizer	4.96	148.05	6.86	1.67
Vermicompost + NPK (100%) + Biofertilizer	7.12	156.38	8.96	2.34
Vermicompost + NPK (75%) + Biofertilizer	6.24	132.12	8.74	2.14
Vermicompost + NPK (50%) + Biofertilizer	5.36	115.06	7.38	1.89
Mustard Cake + NPK (100%) + Biofertilizer	7.63	136.24	8.76	1.86
Mustard Cake + NPK (75%) + Biofertilizer	7.24	142.18	7.48	1.94
Mustard Cake + NPK (50%) + Biofertilizer	6.33	116.34	6.45	1.62
Neem Cake + NPK (100%) + Biofertilizer	6.82	142.43	8.62	2.08
Neem Cake + NPK (75%) + Biofertilizer	6.34	108.24	7.84	2.32
Neem Cake + NPK (50%) + Biofertilizer	5.46	123.17	6.86	1.82
NPK 100%.	6.24	136.45	7.32	1.84
C.D. (P=0.05)	0.288	5.706	0.336	0.082

geometry was created, increasing soil porosity, and enabling healthy rhizome development beneath the soil. The fact that organic manures after decomposition supply readily available nutrients directly to the plants and had a stabilizing effect on fixed forms of nutrients in soil, in addition to the nutrients supplying capacity, could be attributed to the combination's beneficial effects on yield and yield attributes (Babu et al 2014).

Economics: The maximum cost of production was Rs. 237,562/- recorded in T7 followed by T8 (Rs. 235,717/-) as compared to lowest cost of production Rs. 192,562/- in 100% NPK (Table 5). The highest gross return (Rs. 578,250/-) was observed in T4 as compared to lowest gross return (Rs. 369,075/-) in T3 combination. In case of net return, the maximum value of Rs. 350,688/- was registered under T4 as compared to lowest net return in T9 (Rs. 137,378/-). Highest B:C ratio was noticed in T4 (1.54) as compared to lowest B:C ratio (0.58) in T9 combination. Similar results were presented by Tripathi et al (2021).

Considering gross return, net return, and B:C ratio, the combination of T4 proved best for higher production of turmeric through integrated nutrient management. Though the next best net return (Rs. 298013/-) realized from T10 but for identifying the second-best treatment we should compare

Table 4. Influence of different levels of organic, inorganic and biofertilizers on secondary finger and yield of turmeric

Treatments	Number	Weight (g)	Length (cm)	Yield per plot (kg 3m^{-2})	Projected yield (t ha^{-1})
Compost + NPK (100%) + Biofertilizer	6.64	78.62	4.62	13.68	34.20
Compost + NPK (75%) + Biofertilizer	7.32	94.92	4.84	12.30	30.75
Compost + NPK (50%) + Biofertilizer	3.62	38.16	4.56	9.84	24.60
Vermicompost + NPK (100%) + Biofertilizer	9.23	135.38	5.62	15.42	38.55
Vermicompost + NPK (75%) + Biofertilizer	8.64	117.64	5.72	13.38	33.45
Vermicompost + NPK (50%) + Biofertilizer	4.75	94.32	4.76	11.76	29.40
Mustard Cake + NPK (100%) + Biofertilizer	7.92	106.38	5.58	12.96	32.40
Mustard Cake + NPK (75%) + Biofertilizer	9.46	75.62	5.12	11.34	28.35
Mustard Cake + NPK (50%) + Biofertilizer	7.34	54.36	4.34	9.90	24.75
Neem Cake + NPK (100%) + Biofertilizer	6.20	125.44	5.83	14.04	35.10
Neem Cake + NPK (75%) + Biofertilizer	10.34	119.38	5.46	12.72	31.80
Neem Cake + NPK (50%) + Biofertilizer	8.25	72.48	4.82	10.68	26.70
NPK 100%.	6.18	76.38	4.96	11.52	28.80
C.D. (P=0.05)	0.34	3.81	0.21	0.49	1.23

Table 5. Influence of different levels of organic, inorganic and biofertilizers on economics of turmeric

Treatment	Cost of production (Rs. ha^{-1})	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B : C ratio
Compost + NPK (100%) + Biofertilizer	217562	513000	295438	1.35
Compost + NPK (75%) + Biofertilizer	215717	461250	245533	1.13
Compost + NPK (50%) + Biofertilizer	213872	369075	155203	0.72
Vermicompost + NPK (100%) + Biofertilizer	227562	578250	350688	1.54
Vermicompost + NPK (75%) + Biofertilizer	225717	501750	276033	1.22
Vermicompost + NPK (50%) + Biofertilizer	223877	441000	217123	0.96
Mustard Cake + NPK (100%) + Biofertilizer	237562	486075	248513	1.04
Mustard Cake + NPK (75%) + Biofertilizer	235717	425250	189533	0.79
Mustard Cake + NPK (50%) + Biofertilizer	233872	371250	137378	0.58
Neem Cake + NPK (100%) + Biofertilizer	228562	526575	298013	1.30
Neem Cake + NPK (75%) + Biofertilizer	226717	477075	250358	1.10
Neem Cake + NPK (50%) + Biofertilizer	224872	399000	174128	0.77
NPK 100%	192562	432075	239513	1.24

the two-treatment combination of T10 and T1 in respect of cost of production, net return, and B:C ratio. As compared to T1, through incurring the extra expenditure of Rs. 11,000/- in T10 towards cost of production only Rs. 2575/- was realized as net return. Though net return is lower in case of T1, but through consideration of cost of production and B:C ratio, the second-best combination is T1. These organic manures create a reserve of humic substances, which improves the soil's ability to store water and its porosity and structural stability. Therefore, a progress in the physical and chemical conditions of the soil must have aided in the growth of the valuable soil microbial inhabitants, enhanced enzymatic activity, and stimulated the development of new roots, all of which contributed the soil acquire more nutrients and moisture from a larger area. This must be exactly triggered the rhizome production (Singh et al 2012).

CONCLUSION

When manure, biofertilizer, and fertilizer are applied together, the soil's water-holding capacity, photosynthesis, enzyme activity, and friability all increase, which improves yield and productivity and preserves the health of the soil and ecosystem. It also reduces cost of production with increasing net return. Through consideration of yield, cost of production, gross return, and B:C ratio, the best combination was Vermicompost + NPK (100%) + Biofertilizer. Moreover, through comparison of projected yield at 75% NPK level with four organic manures and 100% NPK, there was a chance of substitution of 25% inorganic fertilizer by the application of organic manure and bio- fertilizer. By reducing chemical fertilizer utilization and INM, farmers can reduce the cost of cultivation and environmental pollution as well as ensure sustainable soil fertility and productivity.

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Productivity, Profitability and Energy Efficiency Analysis of Rice-Wheat Cropping System in Mid Hills of Himachal Pradesh

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***Abstract*:** A field experiment was conducted to evaluate the productivity, profitability and energy efficiency analysis of rice-wheat cropping system on a silty clay loam soil of Himachal Pradesh. Tillage options had no significant effect on the grain yield of wheat while significantly higher grain yield of rice was recorded in conventional tillage as compared to zero tillage. Zero tillage resulted in higher net returns per rupee invested as compared to conventional tillage whereas among nutrient management practices, N-rich plot recorded maximum net returns per rupee invested as compared to other treatments in rice-wheat cropping system. Maximum energy input and output was obtained in conventional tillage followed by zero tillage. However, energy use efficiency and energy productivity was highest in zero tillage as compared to conventional tillage. In case of nutrient management practices, energy use efficiency and energy productivity was maximum in SSNM + Green Seeker with top dressing of nitrogen before irrigation followed by other treatments.

***Keywords*:** Rice, Wheat, Conventional tillage, Zero tillage and nutrient management

The rice–wheat is the predominant cropping system of Indo-Gangetic plains of India, covering about 10.5 million ha area and contributing about 38% to the national food basket (Gangwar 2009). The system is considered as the backbone for food grain security. Farmers realize much of their food security from this cropping system. This is also an important cropping system in Himachal Pradesh. The productivity of both rice and wheat is low in this country which may be due to poor soil fertility and inadequate, imbalanced and inefficient use of fertilizers (Yadav et al 2000). The tillage practices play an important role in influencing crop growth, yield and crop's micro-environment. It is an integral part of cropping system aimed at optimizing crop production by solving specific soil related ecological constraints. Soil tillage systems such as zero and conventional tillage are considered important soil management practices. These practices alter the soil physical environment and affect the plant and root growth, thereby, water and nutrient uptake and crop yields. Energy is one of the most valuable inputs in agriculture for crop production. Agriculture itself is an energy consumer and energy supplier in the form of bio-energy (Alam et al 2005). Sufficient availability of the right energy and its effective and efficient use are prerequisites for improved agricultural production. Agricultural intensification requires more energy and energy input pattern for crop production depends on economic, technological and social constraints. Commercial and noncommercial energy are available in agricultural operations. Commercial energy inputs arrive on farm in many different forms, e.g. fuel, irrigation water, chemical fertilizer, machinery and pesticides (Khan and Hussain 2007). Energy analysis, therefore, is necessary for efficient management of scarce resources for improved agricultural production. Hence, the present study was carried out with the objective to analyze the profitability, input, output and net return energy of different tillage methods with nutrient management treatments.

MATERIAL AND METHODS

The field experiment was conducted for two years from *Rabi* (wheat) season of 2015-16 through *Kharif* (rice) at CSK Himachal Pradesh Krishi Vishvavidyalaya at Rice and Wheat Research Centre, Malan situated at 32°07' N latitude, 76°23'E longitude and at an altitude of 950 m above mean sea level. The area receives a high rainfall that ranges between 1500-2500 mm per annum, of which 80 per cent is received during monsoon months from June to September. The soil of the experimental site was silty clay loam in texture, acidic in reaction, high in organic carbon, medium in available nitrogen, high in available phosphorus and medium in available potassium. The experiment was laid out in strip plot design with tillage in horizontal plot and nutrient management in vertical plot with three replications. The experiment consisted of 10 treatments combinations comprising five nutrient management practices in wheat *i.e.* recommended fertilizer dose (120:60:30 kg ha^{-1} NPK) with top dressing of nitrogen after irrigation; recommended fertilizer dose with top dressing of nitrogen before irrigation; fertilizer dose as recommended by software Nutrient Expert – Wheat (125:45:78 kg ha^{-1} NPK) with top dressing of nitrogen before irrigation; Nutrient Expert – Wheat guided fertilizer dose (70% nitrogen recommended by software and rest with green seeker technology) with top dressing of nitrogen before irrigation and N-rich plot which received 150% of recommended nitrogen with top dressing of nitrogen before irrigation with two tillage options *i.e.* conventional tillage and zero tillage. In rice only tillage practices were studied as trial was laid out in fixed plots. Rice was uniformly fertilized. Wheat crop variety HPW 349 was sown at a spacing of 20 cm using a seed rate of 100 kg ha^{-1}. HPR 2795 (Him Palam Lal Dhan 1) variety of rice was used for sowing. Nutrient management in wheat was as per the details given in Table 1. Rice was fertilized with uniform application of 60 kg N, 30 kg P_2O_5 and 30 kg K_2O per hectare in the form of urea (46%), single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. Wheat received five irrigations, first irrigation was given at CRI stage (21 days after sowing) and subsequent irrigations were applied at tillering stage (40–45 days after sowing), late jointing stage (70–75 days after sowing), flowering stage (90–95 days after sowing) and dough stage (110–115 days after sowing) and in each irrigation 5±0.5 cm water was applied. Rice was irrigated as and when needed. In zero tillage, glyphosate 3 l ha^{-1} was applied prior to wheat and rice to tackle weed menace. Net returns per rupee invested were worked out by dividing net returns (Rs ha^{-1}) with cost of cultivation (Rs ha^{-1}).To study the energy input and output of crop, a complete inventory of all crop inputs (fertilizers, seeds, plant protection chemicals, fuels, human labour and machinery power) and outputs of both main and by-products was taken into account which are given in Table 5 (Singh and Mittal 1992). Inputs and outputs were converted from physical to energy unit measures through published conversion coefficients (Devasenapathy et al 2009 and Tuti et al 2013). The energy use efficiency and net energy was worked out as per Dazhong and Pimental (1984). Since data followed the homogeneity test, pooling was done over the seasons.

$$\text{Energy use efficiency} = \frac{\text{Total energy return (Output)}}{\text{Total input involved in term of energy (Input)}}$$

$$\text{Net energy} = \text{Energy Output (MJ ha}^{-1}\text{)} - \text{Energy Input (MJ ha}^{-1}\text{)}$$

$$\text{Energy productivity} = \frac{\text{Wheat equivalent yield (kg ha}^{-1}\text{)}}{\text{Energy input (MJ ha}^{-1}\text{)}}$$

RESULTS AND DISCUSSION

Yield of wheat and rice: Tillage options failed to produce significant variation on grain yield and straw yield of wheat. This showed that wheat sown either in conventional or zero tillage gave similar wheat yield. Similar results were reported across the country in different wheat producing zones (Anonymous 2016). Among nutrient management practices, N-rich plot while remaining at par with the application of recommended NPK where nitrogen was top dressed both after and before irrigation gave significantly higher grain yield and straw yield than the treatments which received fertilizer doses as recommended by Nutrient Expert – Wheat. The higher grain yield and straw yield recorded in nitrogen rich plot as well as with recommended dose may be due to the higher nitrogen application in these treatments (180 and 120 kg ha^{-1}) as compared to the nitrogen added on the basis of Nutrient Expert – Wheat and SSNM + Green Seeker which resulted in higher photosynthesis, which ultimately resulted in better growth and higher yield. Increase in grain yield of wheat with increasing nitrogen application has also been reported by Jat et al (2013).

Conventional tillage significantly increased the grain yield and straw yield of rice over zero tillage. The yield was higher with conventional tillage might be due to profuse root system and higher yield attributes under better soil condition as compared to zero tillage. In zero tillage higher immobilization of the nitrogen applied to wheat and high C: N ratio may be the reason of lower yield. Similar result was also reported by Singh et al (2006). Nutrient management practices adopted in wheat had no significant influence on grain yield of succeeding rice.

Economics of wheat and rice: In wheat, cost of cultivation

Table 1. Nutrient management in wheat for 2015-16 and 2016-17

Nutrient management	Tillage (2015-16)		Tillage (2016-17)	
	Conventional	Zero	Conventional	Zero
RFD – AI	120:60:30	120:60:30	120:60:30	120:60:30
RFD – BI	120:60:30	120:60:30	120:60:30	120:60:30
SSNM Nutrient Expert – BI	125:45:78	125:45:78	125:45:78	125:45:78
SSNM + Green Seeker – BI	101.4:45:78	100.3:45:78	94.1:45:78	95.9:45:78
N-rich plot – BI	180:60:30	180:60:30	180:60:30	180:60:30

*RFD: Recommended fertilizers dose; AI: Top dressing of nitrogen after irrigation; BI: Top dressing of nitrogen before irrigation

Table 2. Effect of treatments on yield of wheat and rice

Treatment	Wheat		Rice	
	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Tillage				
Conventional	4062	6450	4366	6354
Zero	3926	6255	4050	5928
CD (p=0.05)	NS	NS	200	271
Nutrient Management				
RFD – AI	4111	6517	4156	6011
RFD – BI	3966	6300	4153	6034
SSNM Nutrient Expert – BI	3893	6213	4240	6213
SSNM + Green Seeker – BI	3858	6142	4189	6106
150% RFD – BI	4145	6590	4301	6342
CD (p=0.05)	212	323	NS	NS

Table 3. Effect of treatments on economics of wheat and rice

Treatments	Wheat				Rice			
	Cost of cultivation (Rs ha^{-1})	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Net returns per rupee invested	Cost of cultivation (Rs ha^{-1})	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Net returns per rupee invested
Tillage								
Conventional	35553	109173	73621	2.07	32957	82677	49720	1.51
Zero	31399	105643	74245	2.37	28802	76777	47975	1.67
CD (p=0.05)		NS	NS	0.13		3298	NS	0.12
Nutrient management								
RFD – AI	33088	110420	77332	2.34	30880	78607	47727	1.55
RFD – BI	33088	106608	73520	2.23	30880	78626	47746	1.55
SSNM Nutrient Expert – BI	33670	104810	71141	2.11	30880	80397	49517	1.60
SSNM + Green Seeker – BI	33689	103777	70088	2.08	30880	79348	48468	1.57
150% RFD – BI	33844	111456	77613	2.29	30880	81659	50779	1.64
CD (p=0.05)		5083	5083	0.16		NS	NS	NS

Table 4. Effect of treatments on economics of rice-wheat cropping system

Treatments	Cost of cultivation (Rs ha^{-1})	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Net returns per rupee invested
Tillage				
Conventional	68509	191850	123340	1.80
Zero	60200	182419	122219	2.03
Nutrient management				
RFD – AI	63968	189026	125058	1.95
RFD – BI	63968	185233	121265	1.89
SSNM Nutrient Expert – BI	64550	185207	120657	1.87
SSNM + Green Seeker – BI	64569	183124	118555	1.84
150% RFD – BI	64724	193115	128391	1.98

and gross returns was highest in conventional tillage than zero tillage while net returns and net returns per rupee invested was incurred maximum under zero tillage as compared to conventional tillage, respectively which may be due to the lower cost of cultivation than conventional tillage. The higher cost of production under conventional tillage was due to more number of tillage operations, which diminished the net returns per rupee invested. Lower cost of cultivation in zero tillage resulted in higher net returns though the differences were not significant. Among nutrient management, significantly highest gross returns and net returns were observed in N-rich plot. However, statistically at par with application of recommended NPK where split dose of nitrogen was applied both after and before irrigation. The higher gross returns in these treatments were due to the higher grain yield and straw yield recorded in these treatments. Recommended NPK where split dose of nitrogen applied after irrigation recorded highest net returns per rupee invested and was statistically at par with recommended NPK where split dose of nitrogen applied before irrigation and N-rich plot. These results obtained in the present investigation are in accordance with those reported by Suryawanshi et al (2013) and Pandey et al (2018). Lowest gross returns and net returns per rupee invested were recorded under SSNM + Green Seeker with top dressing of nitrogen before irrigation. This was due to the lowest yield recorded in this treatment. In rice, maximum cost of cultivation, gross returns and net returns was recorded with conventional tillage (Rs. 32957 ha^{-1}, Rs 82677 ha^{-1}and Rs 49720 ha^{-1}) over zero tillage. However, zero tillage significantly increased the net returns per rupee invested (1.67) as compared to zero tillage (1.51). More number of tillage operations contributed greatly to cost of cultivation in any crop production system resulting to lower net returns per rupee invested. Among nutrient management, cost of cultivation is similar in all nutrient management practices as only tillage practices were studied in rice whereas N-rich plot recorded maximum gross returns, net returns and net returns per rupee invested.

Table 5. Equivalents for various sources of energy

Particulars	Units	Equivalent energy (MJ)
Inputs		
Human labour	Man-hour	1.96
Diesel	litre	56.31
Chemical fertilizers		
Nitrogen	kg	60.60
Phosphorus	kg	11.10
Potassium	kg	6.70
Chemicals (I) Superior chemicals (chemicals requiring dilution at the time of application)	kg	120
(ii) Inferior chemicals (chemicals not requiring dilution at the time of application)	kg	10
Output		
Grain yield (rice/wheat)	kg	14.7
Straw yield (rice/wheat)	kg	12.5

Source: Singh and Mittal (1992)

System economics: The conventional tillage resulted into higher cost of cultivation, gross returns and net returns (Table 4). Higher net returns per rupee invested were recorded with zero tillage than with conventional tillage. Among nutrient management, N-rich plot recorded maximum cost of cultivation but also resulted in higher gross returns, net returns and net returns per rupee invested followed by other treatments. Lowest values were recorded under SSNM + Green Seeker with top dressing of nitrogen before irrigation.

Energy indices of rice-wheat cropping system: The energy input for different tillage and nutrient management practices in rice-wheat system has been computed on the basis of two years (Table 6). Highest total input energy requirement was with conventional tillage (27.1 x 10^3 MJ ha^{-1}) followed by zero tillage (24.3 x 10^3 MJ ha^{-1}). Higher energy

Table 6. Effect of treatments on energy indices of rice-wheat system

Treatments	Total energy input (X 10^{3}MJ ha^{-1})	Total energy output (X 10^{3}MJ ha^{-1})	Net energy return (X 10^{3}MJ ha^{-1})	Energy use efficiency	Energy productivity (kg MJ^{-1})
Tillage					
Conventional	27.1	283.9	256.8	10.5	0.29
Zero	24.3	269.5	245.2	11.1	0.31
Nutrient management					
RFD – AI	25.0	277.7	252.7	11.1	0.31
RFD – BI	25.0	273.8	248.7	10.9	0.30
SSNM Nutrient Expert – BI	25.5	273.6	248.0	10.7	0.30
SSNM + Green Seeker – BI	23.8	272.9	249.1	11.5	0.32
150% RFD – BI	29.0	285.4	256.4	9.8	0.27

input requirement might be due to higher requirement of labour and field preparation. Among nutrient management, total energy input was in range from 23.8 to 29.0 x10^3 MJ ha^{-1}. Energy input was observed maximum in N-rich plot with top dressing of nitrogen before irrigation followed by SSNM Nutrient Expert with top dressing of nitrogen before irrigation, recommended NPK where top dressing of nitrogen was given both after and before irrigation. The lowest energy input was recorded in SSNM + Green Seeker with top dressing of nitrogen before irrigation. Total energy output was computed from main product and by-product of crop. Conventional tillage resulted in highest energy output (283.9 x 10^3 MJ ha^{-1}) as compared to zero tillage (269.5 x 10^3 MJ ha^{-1}). Among nutrient management, N-rich plot recorded maximum energy output followed by other treatments. The maximum energy productivity was observed with zero tillage (0.31 kg MJ^{-1}) followed by conventional tillage (0.29 kg MJ^{-1}). Among nutrient management practices, highest energy productivity was recorded in SSNM + Green Seeker with top dressing of nitrogen before irrigation followed by other treatments.

CONCLUSION

Wheat yield recorded with the application of fertilizer dose recommended by software Nutrient Expert – Wheat for a target of 5.5 Mg ha^{-1} was considerably lower than the targeted yield. Therefore, for higher productivity and profitability from rice-wheat system in mid hill region of Himachal Pradesh there is needed to improve the software.

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Ecological Relationship of Earthworms with Soil Physicochemical Properties and Crop Yields in Conservation Agriculture

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Abstract: Earthworms are important macro-organisms in the soil as they play a vital role in improving soil properties. This paper aims to estimate the population and their relationship with crop yields and soil physicochemical properties (soil bulk density, soil organic carbon, and soil penetration resistance) in fields where long-term conservation agriculture has been practised since 2012. The study found a higher population in August (24 individuals). Earthworm populations increased in zero tillage with residue plots than without residues. ZTWR 50% BN+GS plot had a higher earthworm population, and it recorded 24, 17 and 17 individuals in August, September and October months, respectively. From the Pearson correlation analysis, it was observed that earthworm population was positively correlated with soil organic carbon and maize, wheat and mung bean crop yields; earthworm population was negatively correlated with bulk density and penetration resistance. PCA and cluster analysis revealed ZTWR 50% BN+GS as the best treatment. Hence, crop residues retention with appropriate balanced fertiliser is considered as a sustainable practice that improves the soil earthworms and increases crop yields in the North West Indo Gangetic plains.

Keywords: Soil fertility, Bulk density, Penetration resistance, Carbon, earthworm

Earthworms are called ecosystem engineers because they can change the soil environment. Because earthworms influence, many chemical and physical soil qualities and is vital to understand how soil management affects their populations (Na et al 2022). Earthworms are essential macro soil organisms crucial in soil formation (Schon and Dominati 2020). Earthworms have the ability to move 40 cm of soil every century (Sharma et al 2017). Charles Darwin calculated that around 1140 kg ha^{-1} $year^{-1}$ of earthworm cast material was eliminated from the environment. Since grazed pasture in New Zealand has been seen to produce 1120 kg ha^{-1} $year^{-1}$, (Blouin et al 2013). Biomass, such as agricultural waste, is required for soil earthworms to thrive (Joseph and Kathireswari 2020). Conservation agriculture is a method of resource-efficient practice which effectively utilises crop residues and recycles agricultural waste, which is the nutrient hub. Conservation agriculture is growing in popularity in IGP because it uses agricultural waste as mulch right in the field (Dinesh et al 2019, Dinesh et al 2021). Continuous minimal tillage, residue retention, crop diversification, and appropriate fertiliser usage are the four main principles of a no-tillage farming technique known as conservation agriculture (Vanlauwe et al 2014). Both earthworms effectively utilise organic matter produced by the crops. Primarily, it transforms complex organic materials into a very microbiologically active nutrient-rich organic matter (Andrews et al 2021). Heavy metals and inorganic fertilizers together suppress soil microbial activity, and secondary metabolites created by the breakdown of herbicides have more detrimental effects than direct ones (Sinduja et al 2022). The ploughing frequency influences the earthworm population because of the mechanical damage and habitat loss (Peigné et al 2018). The investigating earthworm populations is critical in tillage and agricultural residues studies. The earlier research has shown that earthworms offer ecosystem services in various habitats, the advantages of earthworms in conservation agriculture have remained unexplored until recently (Sharma et al 2017). Hence this study in long-term conservation agriculture provides an opportunity to study more the relationship of earthworms with crop yields and soil physicochemical properties.

MATERIAL AND METHODS

Description of the experimental site: The current study was done at ICAR-Indian Agricultural Research Institute research farm Block 9B, New Delhi (28° 40' N, 77° 12' E and 228.6 MSL). The experiment was carried out in the long-term no-tillage experimental site (since 2012). The soil type is sandy loam (Typic Haplustept), and the average precipitation of the location is 650 mm per year. Triplicate soil samples were taken from each treatment plot. They were collected before the maize sowing. First, basic soil parameters were analysed using standard (Page 1982). A core sampler was used to collect the soil samples. Then, they were dried, sieved and passed through a 2 mm sieve for soil characterisation (Ghosh et al 1983). The primary soil properties are given in Table 1.

Experimental layout and management practices: The present research includes two years and three cropping seasons: 2018-19 and 2019-20. The experiment has two main plots (MP) and four subplot treatments (SP), each with three replications. Summer mung bean (cv. Pusa Vishal), Kharif maize (cv. PMH 1) and Rabi Wheat (cv. HD 2967) were sown. The main plot treatments are Zero tillage with residue retention (ZTWR) and zero tillage without residue retention (ZTWoR). The subplot treatments are recommended dose of nitrogen (RDN), 33% basal-N followed by Green Seeker N application (33 % N +GS), 50% basal-N followed by Green Seeker N application (50% N+GS), and 70 % basal-N followed by Green Seeker N application (70% N+GS). For crops, the fertiliser rates for Maize-Wheat-Mung bean were 150:60:40, 120:60:40 and 18:46:0, respectively.

Earthworm population sampling: Soil formation is linked with earthworm activity and population (Sandhu et al 2010). Earthworm numbers and activity peaked in the rainy season. Hence samples were taken for three months, where the earthworm activity was high/highest. From each plot, 30×30×30 cm^3 soil blocks were removed using a spade. Then, earthworms were manually sorted from the soil and counted (Martin 1978).

Table 1. Soil physicochemical properties

Parameter	Value	Reference
Soil pH	7.8	Nayak et al 2016
Electrical conductivity	0.42 dS m^{-1}	Nayak et al 2016
Soil organic carbon	4.69 mg kg^{-1}	Walkley and Black 1934
Soil bulk density	1.38	Blake et al 1986
Soil penetration resistance	452.19 kpa	Anderson et al 1980
Soil nitrogen	162.8 kg ha^{-1}	Subbaiah and Asija 1956
Soil phosphorous	15.2 kg ha^{-1}	Olsen et al 1954
Soil potassium	152.2 kg ha^{-1}	Prasad 1998

Statistical analysis: The split-plot design using Microsoft Excel 2016 was used to test the significance (Rangaswamy 2018). Pearson correlation analysis was done using the R programme. The p-values and correlations were obtained using the R-program 1.4.1103 (R Core Team 2013). Finally, principal component analysis and relationship analysis was done using standard procedures (Shankar et al 2019).

RESULTS AND DISCUSSION

Earthworm population: The earthworm population was higher in August, and it gradually decreased in October (Table 2). This is due to rainfall, New Delhi receives good precipitation in August, and starts to decrease in November later winter onsets (Zodinpuii and Lalthanzara 2019). In August, a maximum earthworm population was observed in the ZTWR 50%BN+GS treatment. Overall, the earthworm population was higher in ZTWR plots as the residue was retained in these plots (Dekemati et al 2020). On the contrary, the lowest earthworm population was observed in August under ZTWoR 70% BN+GS treatment. The main plot and subplot treatments are statistically significant in both years. The pooled analysis also reveals a significant difference between the years. The two-way interaction analysis between the main plot (RM) and subplot (PNM) is non-significant; hence there is no interaction between residue management and precision nitrogen management. However, there was a significant difference between a year and residue management and year and precision nitrogen management. The three-way interaction analysis between year × residue management × precision nitrogen management shows non-significant interaction.

Relationship analysis: From the cluster analysis, maize and wheat crop yields are closely related to each other in a single cluster. Soil parameters such as soil bulk density, soil organic carbon and soil penetration resistance are classified into a single cluster. However, the mung bean yields are unclustered. The treatments ZTWoR RDN, WoR 50%BN+GS, and WR 70%BN+GS are classified into a single cluster (Fig. 2). Earthworm, soil bulk density, organic and penetration resistance are closely related to each other, and influenced maize and wheat yields but not mungbean grain yield. Among the treatments, ZTWoR 70%BN+GS are underperformed, and it is reflected in the earthworm population as well.

Correlation analysis: Earthworm population is negatively correlated with soil penetration resistance, soil bulk density and is positively correlated with maize, wheat yield, and mungbean. This has a strong positive correlation with soil organic carbon (soil penetration resistance is soil organic carbon earthworm population, maize yields and wheat yields), mungbean yields and positively correlated with soil bulk density (Fig. 3).

Principal component analysis: All the observed data from the PCA analysis were consolidated into 7 Principal components. Among that, PC 1 and 2 has a variance of 99.04% (Table 3). Hence these two PC were taken for further analysis. Biplot analysis, revealed that the ZTWR 33%BN+GS was considered the best treatment. Mung bean and wheat yields are towards the positive dispersions (Fig. 4). Hence, it is concluded that the earthworm populations positively influence the mung bean and wheat yields with a high confidence level.

Earthworm population was lowest in ZTWoR 70%BN+GS treatment may be due to more nitrogenous fertiliser application in a single dose in the field. However, urea and urea with nitrification inhibitors negatively impact earthworms (Yahyaabadi et al 2018, Zisi et al 2020). Therefore, while applying chemical fertilisers, it is recommended that organic fertilisers be used in conjunction with chemical fertilisers to minimise the detrimental effects on organisms (Dinesh 2017, Dinesh et al 2018). Soil bulk density was positively correlated with soil penetration resistance (Fig. 3). Similar results were reported by Li et al (2020). Mung bean and wheat yields negatively correlate with soil bulk density and penetration resistance and is due to the negative effect of soil bulk density. When soil bulk density is higher, it is difficult for the root to penetrate deeper layers, ultimately affecting crop yields (Diatta et al 2020). Earthworm population is negatively correlated with soil bulk density and soil penetration resistance, but it has a positive relationship with wheat, mung bean yields and soil organic carbon. When the earthworms' population and activity are high, soil compaction, soil bulk density, and soil penetration resistance decrease (Sohrabi et al 2021). Soil organic carbon and crop yields a positive relationship with earthworm populations. Therefore, when

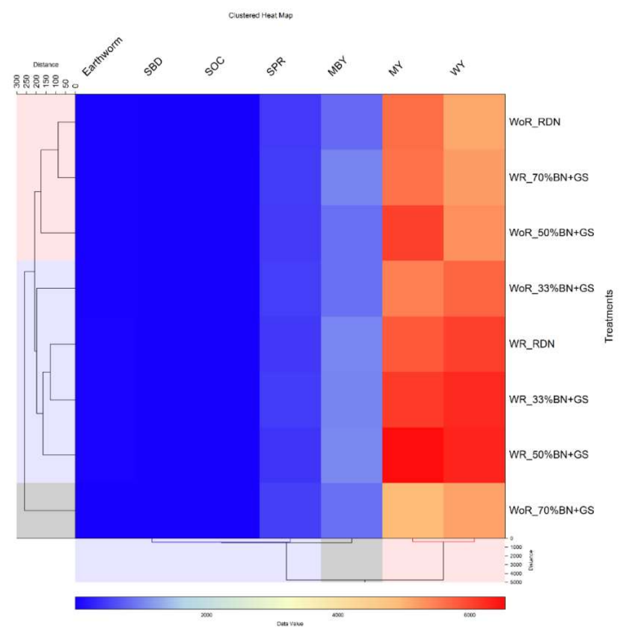

Fig. 1. Relationship between earthworm population with various soil parameters and crop yields

Table 2. Effect of conservation agriculture practices on earthworm population (Nos. ft^{-1})

CRM	PNM	August	September	October	SE	SD
ZTWoR	RDN	5	8	6	0.67	1.17
	33+GS	6	9	5	1.11	1.93
	50+GS	7	14	12	2.16	3.75
	70+GS	3	7	2	1.44	2.50
ZTWR	RDN	16	12	13	1.40	2.42
	33+GS	18	12	14	1.79	3.10
	50+GS	24	17	17	2.51	4.34
	70+GS	14	8	11	1.49	2.59
Treatment		2018-19		2019-20		Pooled
MP: Residue Management (RM)						
CD (p=0.05)		11.03		13.39		1.14
SP: Precision Nutrient management (PNM)						
CD(p=0.05)		1.76		2.06		4.44
Int. RM×PNM		NS		NS		*
Y x RM						1.71
Y×PNM						2.96
Y×RM×PNM						NS

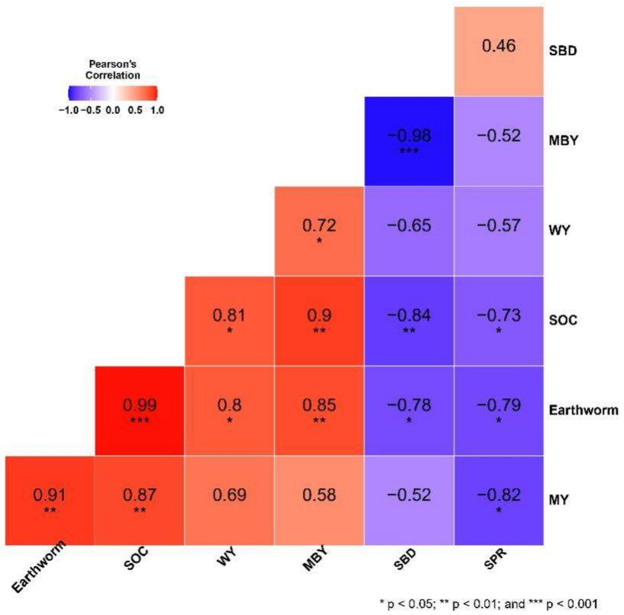

Fig. 2. Relationship between earthworm population and soil physicochemical properties

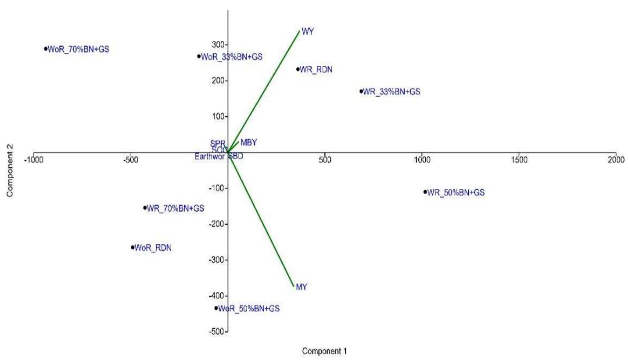

Fig. 3. Effect of conservation agriculture on earthworm population and soil physicochemical properties by Biplot analysis

earthworm populations are high, it will positively influence the crop yields (Johnston et al 2018) and more earthworm activity decomposes the crop residues and converts them into organic carbon-rich humus (Kumar et al 2020, Treder et al 2020). Hence, it can be concluded that SOC improves the earthworm population and eventually increase crop yields.

CONCLUSION

Earthworm populations increased in zero tillage with residue plots than without residues. Earthworm activity peaked in the rainfall season and positively influenced by soil organic carbon. Hence, earthworms improve the maize, wheat and mung bean yields, and are negatively influenced by penetration resistance and bulk density. PCA analysis showed that ZTWR RDN and ZTWR 33%BN+GS were the best treatments for the earthworm. Hence, crop residues retention with appropriate balanced fertiliser is considered as a sustainable practice that improves the soil earthworms and crop yields in the North West Indo Gangetic plains.

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An overview of Automation, Robotics, and Sensor-based Approaches in Weed Detection and Control

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Abstract: Limiting factors of general weed control methods create the situation for the design-development of new approaches based on robotics, automation, and sensor techniques. Several studies have documented the yield loss associated with weed competition and weed discrimination, identification, and control mechanisms. The automatic distinction between crop and weed has importance in weed control applications. Sensor-based approaches, machine vision systems, RTK GPS-based systems, and some other techniques are effective in weed control and help in improving crop yield. Robotic technology could provide a means to reduce the current dependency of agriculture on chemical herbicides by minimizing environmental impacts. The new technologies promise the future improvement of agriculture's few remaining unmechanized and drudgery tasks. So, here we tried to give an overview of these improved technologies in weed control applications.

Keywords: Machine vision, RTK (Real-time-kinematics), GPS (Global positioning system)

Agriculture and weed control or weeding both is old practices. Since the beginning of agriculture, farmers have struggled continuously in their farmland to control weeds. Weed can be considered a pivot issue in farm management practices and is responsible for losses in farm produce of about 45% (Rao et al 2020). Crop yield and quality loss are due to many factors, including crop-weed competition, weed and crop plant density, weed emergence time relative to crop, length of weed existence (roughly one-third of the cycle of a beneficial crop), and weed proximity with the crop plants. Knowledge about biology and the nature of weeds is most important in selecting successful weed control techniques. Weed control tasks with manual, biological, chemical, cultural, mechanical, and thermal methods are more expensive, laborious, tedious, and time-consuming. Manual weeding is the most efficient method of weed control but is a labor and time-consuming process, with more chances of physical injuries associated with this method. (Maurya et al 2020). Mechanical weeding operations are not suitable for all crops and not sufficient for intra-row weeds (Upadhyay et al 2012). Presently weed control methods (in row crops) follow a combination of tillage (mechanical cultivation) with pre-emergence or post-emergence application of chemical herbicides and hand hoeing (Utstumo et al 2018). Consequently, chemical-based weeding can be an effective biological method and economically efficient irrespective of environmental impacts in many circumstances. Increasing regulations on pesticide use on consumer concerns and growing interest in organically produced foods in certain regions limit the chemical-herbicide application's long-term acceptability. Meanwhile, selective post-emergence herbicides are unavailable or ineffective, requiring the hoeing of "in-row" weeds. Thermal weed controlling uses an electric discharge, laser, and flame. Among all rapidly growing weed control techniques, site-specific weed management (SSWM) is taking the top position. It refers to machinery/equipment embedded with technologies that detect weeds growing in a crop and successfully control them without disturbing the beneficial crop. Integrating site-specific weed distribution data, the composition of weed species, size, and impact on crop field is essential to effective site-specific weed management (Chauhan et al 2017). Many advanced research studies are going on weed detection. Some of them are (1). Row guidance system: vision-based automatic row guidance system and RTK-GPS-based row guidance system, (2). Sensor-based and Machine vision recognition of plant species, and (3). GPS mapping systems: automatic RTK-GPS crop seed mapping and automatic GPS and machine vision weed mapping. Among all weed control methods, automatic crop-weed discrimination takes an important role (Abbas et al 2018).

New approaches based on the combination of sensors with different properties and microcontroller processors need to develop that can be used for effective weed control. Since the availability of sensors is not easy for farmers and knowledge of handling them requires some additional effort. Hence, suitable sensor-based systems are not yet widely adopted for practical purposes. The combination of automatic weed detection and mechanical or chemical control is one of the emerging areas in the sustainable crop production field. Therefore, automated or sensor-based weed control is one of the new approaches for non-chemical or low chemical weed control or controlled mechanical weed removal (Chauhan et al 2017).

MATERIAL AND METHODS

Basic weed detection and control system architecture: The selection of weed identification and discrimination method isa high priority for real-time weed detection and control. Sensor-based weed detection and control systems work based on the above strategy. An agricultural robot consists of three key components:

1. a sensing system: measures significant biological and physical properties;
2. a data-processing system: processes the sensor data to know how to manipulate the subsequent system, and
3. a mechanical weeding or chemical spraying unit: actuators manipulated to do the functions accordingly."

Methods like machine vision or image processing, GPS, variable-rate applications, and robotics could provide technological tools to enable robotic weeding.

Working Principles of weed detection and control methods: Andujar et al (2012) designed a weed recognition and control system using the ultrasonic sensor. The system consists of an ultrasonic sensor connected to a power source (12V battery), a data acquisition system (Labjack U12) connected to a laptop through the USB connector, and a robotic operating system (ROS) with a harrowing unit (Fig. 1). Ultrasonic devices works based on the measurement of reflected sound waves. The estimation of the distance is based on the physical principle of time of flight, producing a short burst of sound in a unique direction. After the impact of an object, the wave returns to the receiver.

$$\text{Distance} = \frac{\text{Speed} \times \text{Time}}{2} \quad \text{...... (1)}$$

The device measures the acoustic signal's travel time and transforms it into a voltage signal also possible to convert the output voltage to distance units. The ultrasonic sensor measures the distance between weed mixtures and crop plants. Monitoring weed infestations as a measure of harrowing intensity and these changes were employing previous weed density and tine angle measurements. Weed density classes are defined using fuzzy logic and correlated with ultrasonic measurements. The distance between the sensor and the plants was calculated using Equation (1). A fuzzy set was developed by Rueda-Ayala et al (2015) based on the correlation data of ultrasonic readings (height) with weed densities for laboratory measurements (Table 1).

The intensity classes, controlling the electrical actuator, and harrow tines movement were related to individual ultrasonic measurements. The harrowing intensity uses the angle made by tine with the horizontal and converted to the percentage of maximum angle (90°).Ruiz et al*.* (2014) modeled and built up an intra-row weed detection and control system. The four technologies are driven by the general-purpose autonomous weed control system**:** RTKGPS or machine vision, weed recognition (hyperspectral imaging, machine vision, RTKGPS), precise in-row weeding (micro-spray, cutting, thermal, electrocution), and mapping (GPS & machine vision). RTK-GPS can utilize for auto-guidance in seedbed preparation, with automatic on-the-fly, and geo-positioned mapping during transplanting. This map was used to give input about the location of crop plants to the RTK GPS during the weeding operation. The program was set in the control unit of weeding hoe blades such that except for the crop plant location, it assumes any other plant as a weed. As the intra-row hoes (Fig. 2) pass the plant and reach the exact location, the pneumatic cylinders reposition the hoes to follow the grey dashed lines until they meet in the center of the row. This process was repeated for each plant.

Machine vision-based weed detection and control: The machine vision system is employed to identify the weeds and crops and destroy the weeds by locating them with help of a control unit based on different discrimination factors and the accuracy of some machine vision techniques given in Table2 (Guzman et al 2019). The combined system of automatic weed detection by machine vision and weed control by electrical discharges consists of two machine vision systems and an end effect or for weed identification, location, and control.

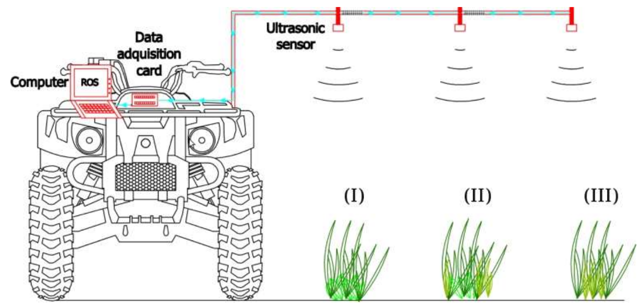

Fig. 1. Ground-based ultrasonic system for weed detection (Andujar et al 2012)

Primary machine vision system: This was designed to detect the individual weeds with the mission to locate weeds on a real-time basis with the forward movement of the robot. The system consists of a color camera connected to a digitizing board inserted into a Pentium-based computer. The vision system needs to process each image captured to the desired pixel quality based on real-time data acquisition and transmission specifications. The developed software was divided into three major tasks: image acquisition, image processing, and transmission of the location of the weeds to the supervisory system and the secondary machine vision system. The information transferred to these systems has to position each weed in the image, the digital signatures of each of the weeds, and a time reference (Blasco et al 2002).

During real-time operation, images were scanned, and each pixel was automatically assigned to a plant or soil class, depending on its RGB (Red, Green & Blue) coordinates. The image is converted from RGB to HSV (Hue-Saturation value) in image pre-processing. The equation below represents the HSV color space and the pixels hue value Ph (i, j), saturation value Ps (i, j), and value Pv (i, j) in the color space and their conversion relationship with the RGB color model (Equation: 2, 3 and 4.)

$$P_h(i,j) = \cos^{-1}\left\{\frac{0.5\left[\left(P_r(i,j)-P_g(i,j)\right)+\left(P_r(i,j)-P_b(i,j)\right)\right]}{\sqrt{\left(\left(P_r(i,j)-P_g(i,j)\right)^2-\left(P_r(i,j)-P_b(i,j)\right)\left(P_g(i,j)-P_b(i,j)\right)\right)}}\right\} \quad \ldots (1)$$

$$P_s(i,j) = \frac{\max\left(P_r(i,j),P_g(i,j),P_b(i,j)\right)-\min\left(P_r(i,j),P_g(i,j),P_b(i,j)\right)}{\max\left(P_r(i,j),P_g(i,j),P_b(i,j)\right)} \quad \ldots (2)$$

$$P_v(i,j) = \frac{\max\left(P_r(i,j),P_g(i,j),P_b(i,j)\right)}{255} \quad \ldots (3)$$

In a second step, the area, the perimeter, and the centroid of each weed are calculated. Objects smaller than the pre-set threshold are considered noise and filtered. Objects larger than another pre-set threshold were considered a crop. The remaining objects were considered weeds (Fig. 3), and t and c ordinates of their centroid were sent to the supervisor and the second vision system. The values of the two above-mentioned thresholds were established during the offline training operation. For each detected weed, a digital signature based on its luminance distribution was also sent to the second vision system.

Secondary vision system (Blasco et al 2002): The objective is to locate the previous weeds, one at a time, provide their actual position, and correct the trajectory of the weeding tool, thus compensating for positioning errors generated by the lack of accuracy of the inertial unit. The module consisted of a monochromatic camera coupled to a specific processing system. The camera is firmly attached to the manipulator and was initially focused on weeds by the primary vision system. At the request of the supervisor, the second vision system grabs an image located on the same weed under its field of view, comparing its signature with that of the primary camera system. Finally transmits the actual coordinates of the weed to the supervisor, and directed the end effector to this position. The end-effector is an electrode that produces electrical discharges of 15 kV and 30mA during 200 ms approximately. The machine was powered by a set of four 24V batteries that provide nearly 40 A. The six degrees of freedom of the robot are implemented by six electrical motors

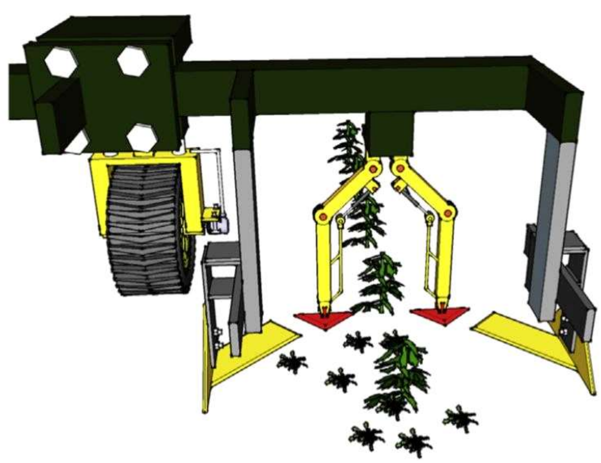

Fig. 2. A miniature co-robotic weeding unit with a pair of intra-row hoes (red triangles) and an odometry sensor (Ruiz et al 2014)

Table 1. Plant height ranges to control harrowing intensity correspond to five discrete classes in Decision Support System (Rueda-Ayala et al 2015)

Class	Min height (cm)	Max height (cm)	Plant density (plants m^{-1})	Harrowing intensity
0	0	10	0-15	None
1	10	15	16-30	Lightest
2	15	20	28-47	Light
3	20	25	45-63	Strong
4	25	77	>60	Strongest

(100W each). At last, the end-effector moves in the trajectory decided by the primary and secondary vision system information (Fig. 4).

RESULTS AND DISCUSSION

The ultrasonic sensor system is used to detect the density of weed infestation in the field. The height data obtained from the ultrasonic sensor was correlated with total biomass and the weed density that were obtained by manual harvesting of weed after the detection process. Weed presence was correctly predicted in more than 92% of the cases. The use of ultrasonic sensor readings proved useful to discriminate grasses (up to 81.1% of success) and broadleaf weeds (up to 98.5% of success). The correlation coefficient was 0.99 for weed height assessed by the ultrasonic sensor and weeding intensity adjusted by the system. Using this method of weed detection Rueda-Ayala et al (2015) developed and system to automatically control the weed. As per the density of weed, the intensity of the tillage was changing. This pre-decides in the system algorithm using fuzzy logic. The harrowing intensity sent by the control unit to the tines to change their angle thus adjusting the harrowing intensity– corresponded well to the change in weed infestation level along the field. The system performed well at high driving speeds needed for harrowing operations (e.g., 12 km/h).

Table 2. Accuracy of different machine vision techniques (Raj and Kavitha 2018)

Method	Accuracy (%)
Spectral reflectance property	85-87
Colour property	50-96
Topology property	83-91
Texture features	30-78
Wavelength transformation	86-94
Pattern matching algorithm	91-97

RTK GPS-based weed detection method was used in association with the intra-row weed control system. The crop (tomato) plants were visually evaluated immediately after the co-robot operation of each row to determine the number of crop plants harmed by the hoes. Field Trials were conducted at 1.2, 1.6, and 2.4 km/h. At the 0.8 and 1.2 km/h travel speeds, no flag contact or damage to the crop plants, respectively, were observed. At the 1.6 km/h speed, flag contact or major damage to the crop plants occurred about 0.5% and 1% of the time, respectively, and increased to 5% in the 4 km/h flag trial and 3% in the 2.4 km/h crop trial. Based on these results, a co-robot travel speed of 1.2 km/h was selected for further study. An 8 h long operational trial was then conducted in the commercial crop field at a travel speed of 1.2 km/h. During this 8 h trial, 0.5% of the crop plants were accidentally killed or received major root damage by the co-robot hoes. The findings showed the feasibility of using RTK-GPS in controlling the path of weeding knives automatically, which is operating between the intra-row region of crop plants in sustainable cropping. This system could save about 57.5%

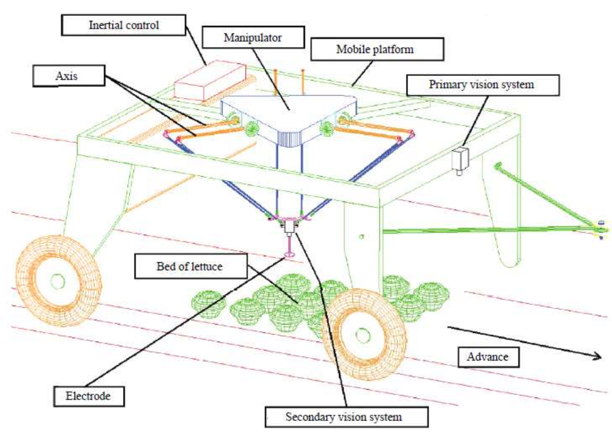

Fig. 4. Electrical control weeding using machine vision system (Blasco et al 2002)

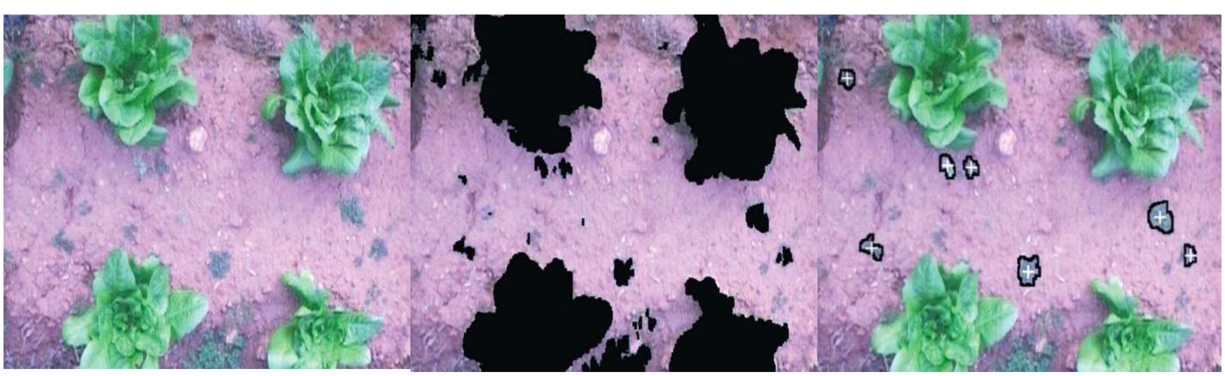

Fig. 3. Weed detection by machine vision system (a) Original field image showing weeds and crop (b) Plant and soil segmentation (c) Detected weeds (Blasco et al 2002)

Table 3. Weed detection and control studies

Researcher name	Source	Research topic	Research approach	Researcher details	Journal name
M. Norremark	Norremark et al (2012)	Evaluation of an autonomous GPS-based system for intra-row weed control by assessing the tilled area	RTK-GPS navigation guided system	Department of Biosystems Engineering, Faculty of Agricultural Sciences, Aarhus University, Denmark e-mail: Michael.Norremark@djf.au.dk	Precision Agriculture: An International Journal on Advances in Precision Agriculture
H.W. Griepentrog	Griepentrog et al (2007)	Autonomous inter-row hoeing using GPS-based side-shift control	RTK-GPS navigation guided system with hoeing attachment	Copenhagen University, Faculty of Life Sciences, Dept. of Agricultural Sciences, Denmark. E-mail: hwg@life.ku.dk	Agricultural Engineering International: the CIGR Journal
D.C. Slaughter	Slaughter et al (2008)	Autonomous robotic weed control systems: A review	---	The University of California, Biological and Agricultural Engineering, Davis, CA 95616, United States	Computers and electronics in agriculture
J. Blasco	Blasco et al (2002)	AE—Automation and emerging technologies: robotic weed control using machine vision	Robotics and Machine-vision systems assisted mechanical weeding	emolto@ivia.es	Biosystems Engineering
R Y VAN DER WEIDE	Van Der Weide et al (2008)	Innovation in mechanical weed control in crop rows	Mechanical weeding: Pneumatic blowing, Torsion weeders, Finger weeders	Applied Plant Research, Wageningen University, and Research Centre, Lelystad, the Netherlands	Weed research
J. Bontsema	Bontsema et al (1998)	Intra-row weed control: a mechatronics approach	Digital signal processor (DSP) with mechanical weed control system	Wageningen, The Netherlands	IFAC Proceedings Volumes
N. D. Tillett	Tillett et al (2008)	Mechanical within-row weed control for transplanted crops using computer vision	Computer-based machine vision guidance along and mechanical weeding attachment	Tillett & Hague Technology Ltd., Greenfield, Bedfordshire, UK	Biosystems Engineering
Zoltan Gabor	Zoltan Gabor (2013)	Mechatronic system for mechanical weed control of the Intra-row Area in Row Crops	Detection system composed of RGB sensor and laser sensor with mechanical weeding tool (hoe, electric driven)	Bavarian State Research Center for Agriculture, Institute for Agricultural Engineering and Animal Husbandry, Germany e-mail: zoltan.gobor@lfl.bayern.de	KI-Künstliche Intelligenz
W. Bond and A. C. Grundy	Bond and Grundy (2001)	Non-chemical weed management in organic farming systems	Selective weed control operations (cultural, mechanical, thermal, and biological methods)	Horticulture Research International, Wellesbourne, Warwick, UK E-mail: andrea.grundy@hri.ac.uk	Weed research
Cointault Frédéric	Frederic et al (2012)	Texture, color, and frequential proxy-detection image processing for crop characterization in a context of precision agriculture	Remote sensing and sensor-based image detection using Proxy-Detection Image Processing	Agro-Sup Dijon, France	Agricultural science
Chung-Liang Chang and Kuan-Ming Lin	Chang and Lin (2018)	Smart agricultural machine with a computer vision-based weeding and variable-rate irrigation scheme	Computer-based machine vision system with multitasking unit	Department of Bio Mechatronics Engineering, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan Email: chungliang@mail.npust.edu.tw	Robotics

Cont...

Table 3. Weed detection and control studies

Researcher name	Source	Research topic	Research approach	Researcher details	Journal name
Bjorn Astrand And Albert-Jan Baerveldt	Astrand and Baerveldt (2002)	An agricultural mobile robot with vision-based perception for mechanical weed control	Machine vision and robotics	Halmstad University, Halmstad, Sweden Bjorn. Astrand@ide.hh.se	Autonomous robots
A. Piron	Piron et al (2011)	Weed detection in 3D images	Machine vision system with video recording	Environmental Science and Technology Department, Gembloux Agricultural University, Gembloux, Belgium e-mail: piron.a@fsagx.ac.be	Precision agriculture
Yun Zhang	Zhang et al (2012)	Robust hyperspectral vision-based classification for multi-season weed mapping	Hyperspectral image-based plant recognition, Machine vision system with a CCD camera and line-imaging spectrograph for close-range weed sensing and mapping.	Department of Biological and Agricultural Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States	ISPRS Journal of Photogrammetry and Remote Sensing
F. Lopez- Granados	Lopez-Granados, F (2011)	Weed detection for site-specific weed management: mapping and real-time approaches	Remote sensing based on multispectral aerial imagery, unmanned aerial vehicles (UAV), and robotic weeding systems	Institute for Sustainable Agriculture/CSIC, P.O. Box 4084, 14080-Córdoba, Spain. E-mail: flgranados@ias.csic.es	Weed research
Gerassimos G. Peteinatos	Peteinatos et al (2014)	Potential use of ground-based sensor technologies for weed detection	Ground-based sensors for weed detection (cameras, distance sensors, spectrometers, fluorometers)	Department of Weed Science, University of Hohenheim, Otto-Sander-Str. 5, 70599 Stuttgart, Germany. E-mail: G.Peteinatos@Uni-Hohenheim.de	Pest management science
Shirzadifar, A. M	Shirzadifar (2013)	Automatic weed detection system and smart herbicide sprayer robot for cornfields	Machine vision algorithm and robotic weeding	Department of Electrical Engineering, Shiraz University, Iran	First RSI/ISM International Conference on Robotics and Mechatronics (ICRoM)
Uri Shapira	Shapira et al (2013)	Field spectroscopy for weed detection in wheat and chickpea fields	Remote sensing-based spectroscopy for weed detection	The Remote Sensing Laboratory, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Beersheba, Israel	International journal of remote sensing
Daniela Stroppiana	Stroppiana et al (2018)	Early-season weed mapping in rice crops using multi-spectral UAV data	UAV imagery for weed mapping with help of a Parrot Sequoia sensor mounted on a quadcopter	Institute for Electromagnetic Sensing of the Environment (IREA), Consiglio Nazionale Delle Ricerche, Milano, Italy	International journal of remote sensing
Christian Frasconi	Frasconi et al (2014)	Design and full realization of physical weed control (PWC) automated machine within the RHEA project	Machine vision-based detection with a micro-sprayer system	Luisa Martelloni, Centro di Ricerche Agro-Ambientali “Enrico Avanzi”, University of Pisa, via vecchia di Marina 6, 56122, San Piero a Grado, Pisa, Italy	In Proc. 2nd Int. Conf. on Robotics and associated High-technologies and Equipment for Agriculture and forestry (RHEA-2014)

of the work required for weeding in intra-row. The model had a determination coefficient (R2) of 0.95 and RMSE (weeds/m2) of 42.3, showing that the method is appropriate for autonomous weed recognition and control.

The comparison of ultrasonic, RTK-GPS, and machine vision-based weed detection and control systems are compared (Table 4). The machine-vision system for recognition of the weed and locating system can be controlled by the electrical discharge method as discussed in the material and methods. The electrical discharges induced

Table 4. Comparison of the technologies discussed

Properties	Ultrasonic sensor-based method	RTK-GPS based method	Machine-vision based method
Sensors used	Ultrasonic sensor	Optical sensor	Camera
Recognition mechanism	Height of plants	Location of plant-based of sowing map	Image processing
Recognition effectiveness	Only predicts density of weed	Only separates crop from others on basis of the map	More effectively recognizes crops and weeds with distinct properties
Accuracy in recognition	Weed presence predicted with 92.8% of success	The correlation coefficient is 0.95	Weed discrimination is 84%
Processing time	195ms	16.7ms	482ms
Weed Control Method	Mechanical online harrow with automatically adjustable tine angle	A pair of intra-row hoes with variable area overage	Electrical discharge type robotic end effector
Advantage	Low-cost system	High accuracy	Medium accuracy but 100 control of recognized weeds
Disadvantage	Low accuracy and no provision for intra-row weed management	High cost of RTK GPS and complex mechanical components	Less recognition percentage and high-power requirement

Table 5. Segmentation process results

Particulars	Classified as soil, %	Classified as plant, %
Real soil pixels	95	5
Real plant pixels	3	97

Table 6. Discrimination capability in lettuce cultures

Particulars	Classified as weeds, %	Classified as lettuces, %
Real weed	84	16
Real lettuces	1	99

Table 7. Average results per image

Process	Time, ms	Time, %
Image acquisition	71	14.7
Segmentation of soil/plant	86	17.9
Filtering	73	15.1
Weed detection	252	52.3
Total	482	100

by the electrode located on the end-effector produced cell plasmolysis in the plants, which could be observed several hours after the treatment. The confirmation of the destruction of the affected tissue was observed after several (3 to 4) days. Different results (Table 7) including soil and plant segmentation process (Table 5), discrimination capabilities between crop and weed (Table 6) of machine vision system are given below.

The machine-vision system can successfully recognize 84% of weeds and 99% of the beneficial crop (lettuce) with an average recognition time of 482ms without causing damage to the beneficial crop. This system can able to eliminate 100% of weeds having less than five leaves or weeds of height less than 20cm (Utstumo et al 2018).

CONCLUSION

Weed detection using the ultrasonic sensor works based on the height and density of foliage coverage. This method is used only for inter-row weed control in terms of the intensity of weed based on the angle of the hoe blade. But the machine vision system could identify the weed and crop between the rows and be used for the intra-row weed control mechanism. While RTK GPS-based weed detection and control system is much more effective and accurate than the above two methods. The problem with using RTK GPS is that one has to use a mapping system during planting operation, and the high cost and the effect of the cloud on GPS accuracy stand as the limiting factor. RTK GPS alone cannot work for weed detection and control as it requires either an optical sensor or a digital camera to get the geo-positioned coordinates of crop plants. The whole study confirms automatic weed detection and control system is a promising technology for sustainable development and crop production. It helps to reduce the chemical applied in the form of herbicides and reduces environmental degradation. These systems demonstrate the promise of robotic weed control technology for reducing the hand labor or pesticide application requirements of existing weed control methods. Additional research is needed to fully optimize the technology for the wide range of conditions found in commercial agriculture worldwide.

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Effect of Sequential Application of Herbicides on Weed Management, Productivity and Nutrient Uptake of Direct Seeded Rice

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Abstract: The investigation was carried out at Bihar Agricultural University, Sabour, Bhagalpur (Bihar) during *kharif*, 2018 to find the impact of chemical weed management on complex weed flora and productivity of direct seeded rice. The dominant weeds in field were *Cyperus rotundus, Echinochloa spp, Eleusine indica* and *Caesulia axillaries*. The lowest weed population, dry weight, highest percent weed control efficiency and productivity of rice were recorded by the hand weeding treatment. Among all the herbicidal treatments, the lowest weeds count, weeds dry weight and highest percent weed control efficiency, high productivity and nutrient uptake of rice were recorded under treatment pyrazosulfuron @ 25 g a.i. ha^{-1} PE fb pyrazosulfuron @ 20 g a.i. ha^{-1} + bispyribac-Na @ 20 g a.i. ha^{-1} at POE which can be recommended to the farmers.

Keywords: Weed flora, Direct seeded rice, Weed population, Weed control efficiency, Nutrient uptake

Rice (*Oryza sativa* L.) is the staple food for more than 60% of the world population, providing energy for about 40% of the world population where every third person on earth consumes rice every day in one form or other (Datta and Khushi 2002). Among several reasons for low rice productivity, the losses due to weeds are one of the most important. Weeds are most severe and widespread biological constrains to crop production in India and weeds alone cause 33% of losses out of total losses due to pests (Verma et al 2015). Direct-seeded rice (DSR) has several advantages over puddled transplanted rice like easier planting, timely sowing, less drudgery, early crop maturity by 7-10 days, less water requirement, better soil physical condition for next crop and low production cost and more profit (Kumar and Ladha, 2011). DSR is severely affected by weed population in *kharif* season which indicates that timely management of weeds is essential for better performance. In the initial stages of the crop, weeds can be effectively controlled by pre-emergence herbicides. However, in later stages of crop, weeds can obstruct the growth and development of crop. Therefore, the present experiment was conducted to find out the effective herbicides or herbicide mixtures for weed control in direct seeded rice.

MATERIAL AND METHODS

A field experiment was conducted at Agriculture research farm, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (longitude 87°2'42" East and latitude 25°15'40″ North at altitude of 46 meters above *mean* sea level in the heart of the vast Indo-Gangetic plains of North India) during *Kharif* season of 2018. The soil of the experimental site was loamy sand in texture having normal soil reaction (pH 7.27) and electrical conductivity (0.27 dSm^{-1}), low in organic carbon (0.46%) & available N (180.61 kg/ha) and medium in available P (22.65 kg/ha) and K (206.88 kg/ha). The experiment comprised of 11 weed management practices (Table 1). The experiment was laid out in randomized block design with three replications. Rice variety *'Sabour Sampannadhan'* (BRR0059) was sown on 16th June 2018 using tractor drawn conventional drill with seed rate of 30 kg /ha in rows spaced at 20 cm. The recommended dose of fertilizers and plant protection measures for insect-pest and disease control were applied. The data on weed density were transformed using square root transformation. Yield and yield attributes were recorded at the time of harvesting.

RESULTS AND DISCUSSION

Weed population: Hand weeding treatments shown significantly lower weeds at all growth stages over rest of the treatments the main reason behind this is timely uprooting of weed species. The significantly higher weed population was obtained in weedy check at 15, 30, 45 and 60 DAS which was significantly superior compared to rest of the treatments at all of the crop growth stages. It was mainly due to uninterrupted growth of all the three types of weeds. This is in conformity with the findings of Chauhan (2013). Among the herbicide applied treatments at 15, 30, 45 and 60 DAS, the minimum weed population of total weeds was recorded. Among the all herbicidal treatments, the minimum weed population was recorded under T_5 (pyrazosulfuron @ 25 g a.i. ha^{-1} PE fb bispyribac-Na @ 20 g a.i. ha^{-1} + pyrazosulfuron @ 20 g a.i. ha^{-1} POE) which was at par with T_4(pyrazosulfuron @ 25 g a.i. ha^{-1} PE fb bispyribac-Na @ 25 g a.i. ha^{-1} POE). This is probably because of capability of the herbicide to impact the cell division, cell growth and competition among the weeds and crop might have resulted in death of some weeds. Herbicidal mixtures decreased the weed density compared to the either solo application of pre or post emergence herbicides. The significant effect of herbicides in combination with hand weeding can be ascribed to the broad spectrum weed control as the herbicides have controlled specific weeds. These results are in conformity with the findings of Tiwari et al (2013).

Weed dry weight: Among the all herbicidal treatments, the minimum weed dry weight (4.91 g m^{-2}) was under T_1 (pyrazosulfuron @ 25 g a.i. ha^{-1} PE) at 15 days after sowing and which was statistically at par with T_2 , T_3 , T_4 , and T_5. The maximum dry weight (7.53 g m^{-2}) of weed was d with T_9 (halosulfuron @ 67.5 g a.i. ha^{-1} + azimsulfuron @ 30 g a.i. ha^{-1} POE). In rest of the growth stages (30, 45 and 60 DAS), the minimum weed dry weight was obtained under T_5 which was statistically at par with T_4 and was significantly superior over rest of the treatments. The maximum dry weight of total was obtained with T_2 (pendimethalin @ 1000 g a.i. ha^{-1} PE). The application of herbicides can be attributed to their efficacy to control wide spectrum of weeds. The integration of herbicides which resulted in broad spectrum weed control over the other treatments was because pre emergence herbicides eliminated the early emerged weeds and post emergence herbicides controlled the later germinated weeds which resulted in lowest weed population as well as weed dry weight. These results are in conformity with the findings of Patel et al. (2018) where application of pyrazosulfuron-ethyl 25 g ha^{-1} fb bispyribac-sodium salt 50 g ha^{-1} at 30 DAS suppressed the weed population and dry weight over control.

Among herbicide applied treatments, the highest WCE was recorded (84.88%) under T_5 at 45 days after sowing whereas minimum WCE (61.94%) was obtained with treatment T_2. The maximum WCE was obtained (87.09%) under T_5 treatment at 60 days after sowing and minimum weed control efficiency (69.00 per cent) was recorded in the treatment T_2. The reducing trend of WCE is because of the decrease of weed dry matter in herbicides treated with advancement of time which is due to decrease in weed dry matter over rest of the weed treatment. The highest WCE with hand weeding was also observed by Singh et al (2014).

Among the different weed treatment, the lowest weed index (1.69%) was with the treatment T_5 followed by T_4

Table 1. Effect of chemical weed management practices on total weed population (no. m^{-2}) at various crop growth stages of direct seeded rice

Treatments	Description	15 DAS	30 DAS	45 DAS	60 DAS
T_1	Pyrazosulfuron @ 25 g a.i. ha^{-1} PE	5.87 (34.03)	7.66 (58.23)	8.48 (71.42)	7.78 (60.05)
T_2	Pendimethalin @ 1000 g a.i. ha^{-1} PE	6.04 (35.93)	7.98 (63.25)	9.02 (80.94)	8.31 (68.59)
T_3	Pyrazosulfuron @ 25 g a.i. ha^{-1} PE fb 2,4-DEE @ 750 g a.i. ha^{-1} POE	6.48 (41.44)	6.98 (48.32)	8.41 (70.30)	7.71 (58.98)
T_4	Pyrazosulfuron @ 25 g a.i. ha^{-1} PE fbbispyribac-Na@ 25 g a.i. ha^{-1} POE	6.34 (39.82)	5.76 (32.70)	6.63 (43.42)	5.42 (28.92)
T_5	Pyrazosulfuron @ 25 g a.i. ha^{-1} PE fbbispyribac-Na@ 20 g a.i. ha^{-1} + pyrazosulfuron @ 20 g a.i. ha^{-1} POE	6.22 (38.26)	5.41 (29.02)	6.20 (38.05)	5.13 (25.90)
T_6	Bispyribac-Na@ 25 g a.i. ha^{-1} POE	8.58 (73.10)	7.28 (52.54)	8.21 (66.95)	7.48 (55.57)
T_7	Bispyribac sodium@ 20 g a.i. ha^{-1}. + pyrazosulfuron @ 20 g a.i. ha^{-1} POE	8.56 (72.64)	6.86 (46.52)	7.64 (57.91)	6.81 (46.0)
T_8	Ethoxsulfuron @ 15 g a.i. ha^{-1} + pyrazosulfuron @ 20 g a.i. ha^{-1} POE	8.87 (78.27)	7.15 (50.64)	7.91 (62.09)	7.06 (49.34)
T_9	Halosulfuron @ 67.5 g a.i. ha^{-1} + azimsulfuron @ 30 g a.i. ha^{-1} POE	8.55 (72.64)	6.42 (40.67)	7.11 (50.22)	6.14 (37.46)
T_{10}	Hand weeding (15,30 and 45 DAS)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)
T_{11}	Weed check	9.61 (91.16)	11.16 (124.07)	12.24 (149.29)	11.57 (133.41)
CD (p=0.05)		(0.39)	(0.43)	(0.51)	(0.54)

Data in parenthesis were transformed to √x+ 0.5 before analysis. The figures in parentheses are the original values

(7.58%) weed management practices. Improved WCE and season long broad-spectrum control of these weed management practices bring about in reduced weed index significantly. These results were supported by Maity and Mukherjee (2008). At the maturity stage, among all herbicides treatments the significantly maximum number of effective tillers (293.7 m^{-2}) were in T_5which was at par with T_4, T_9, T_7 and T_6 and minimum number of effective tillers were recorded in T_2 (236.7 m^{-2}). Irrespective of growth stages, significantly the maximum number of tillers of rice was observed in hand weeding (T_{10}) and minimum number of tillers was observed in weedy check (T_{11}). Highest LAI was observed at 90 DAS in the hand weeding treatment T_{10} (4.84) which were comparable with treatments *viz,* T_4, T_5, T_6, T_7 and T_9 which was due to intercultural operations helping more aeration of the soil. Minimum competition and better WCE which has led to well growth and development finally improved the LAI of the concerned treatments. Lowest LAI with weedy check was reported by Singh et al (2014). Among the herbicide applied treatments, maximum dry matter (1025.7 g m^{-2}) was obtained with T_5 at maturity and minimum was with T_2 (876.3g m^{-2}). It is probably because of application of Pyrazosulfuron as a pre-emergence herbicide and in combination with bispyribac-Na as a post emergence herbicide which resulted in greater reduction of weed population which in turn made the plant-weed competition lowest compared to rest of the treatments. This finding was in conformity with Ramachandran and Balasubramanian (2012).

Among all the herbicide applied treatments, the

Table 2. Effect of chemical weed management practices on total weed dry weight (g/m^2) at various crop growth stages of direct seeded rice

Treatments	15 DAS	30 DAS	45 DAS	60 DAS
T_1	4.91 (23.62)	6.17 (37.56)	6.74 (44.87)	6.90 (47.05)
T_2	5.21 (26.61)	6.54 (42.32)	7.00 (48.57)	7.04 (49.02)
T_3	5.15 (26.13)	5.63 (31.31)	6.17 (40.94)	6.32 (39.50)
T_4	5.08 (25.37)	4.14 (16.70)	4.67 (21.56)	4.82 (24.88)
T_5	5.13 (25.91)	3.82 (14.17)	4.44 (19.29)	4.57 (20.42)
T_6	7.12 (50.18)	5.24 (26.93)	5.78 (32.92)	5.93 (34.70)
T_7	6.81 (45.83)	4.92 (24.04)	5.62 (30.98)	5.86 (33.90)
T_8	7.10 (49.98)	5.53 (30.31)	6.09 (36.65)	6.22 (38.22)
T_9	7.35 (53.59)	4.56 (19.94)	5.28 (27.37)	5.40 (28.72)
T_{10}	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)
T_{11}	8.47 (71.31)	11.23 (104.00)	11.32 (127.62)	12.68 (158.13)
CD (P=0.05)	0.32	0.40	0.24	0.27

See Table 1 for details .Data in parenthesis were transformed to $\sqrt{x+0.5}$ before analysis. The figures in parentheses are the original values

Table 3. Effect of chemical weed management practices on weed index and weed control efficiency of direct seeded rice

Treatments	Weed index	Weed control efficiency (%)			
		15 DAS	30 DAS	45 DAS	60 DAS
T_1	34.48	66.88	63.88	64.83	70.25
T_2	36.39	62.68	59.31	61.94	69.00
T_3	29.13	63.36	69.89	70.55	75.02
T_4	7.58	64.42	83.94	81.54	84.27
T_5	1.69	63.67	86.38	84.88	87.09
T_6	22.75	29.63	74.11	74.20	78.06
T_7	18.06	35.73	76.88	75.65	78.56
T_8	28.42	29.91	70.86	71.28	75.83
T_9	15.06	24.85	80.83	78.55	81.84
T_{10}	0.00	100.00	100.00	100.00	100.00
T_{11}	50.95	0.00	0.00	0.00	0.00

Table 4. Effect of chemical weed management on growth and yield attributes at maturity of direct seeded rice

Treatments	Tillers (m^{-2}) at maturity	Leaf area Index at 90 DAS	Dry matter accumulation ($g\ m^{-2}$) at maturity	Grain yield ($t\ ha^{-1}$)	Straw yield ($t\ ha^{-1}$)
T_1	251.7	4.41	878.7	3.60	4.99
T_2	236.7	4.36	876.3	3.50	5.01
T_3	258.3	4.38	907.7	3.90	5.26
T_4	281.3	4.73	1002.0	5.08	6.35
T_5	293.7	4.75	1025.7	5.41	6.67
T_6	268.7	4.50	990.0	4.25	5.90
T_7	273.0	4.69	992.7	4.51	6.06
T_8	252.0	4.39	928.3	3.94	5.37
T_9	278.7	4.66	998.7	4.67	6.20
T_{10}	307.3	4.84	1058.0	5.50	6.69
T_{11}	194.7	3.82	873.0	2.70	3.97
CD (p=0.05)	33.3	0.32	13.3	0.82	1.31

Table 5. Effect of chemical weed management practices on nutrient uptake by grain and straw in direct seeded rice

Treatments	Nitrogen kg ha^{-1}			Phosphorous kg ha^{-1}			Potassium kg ha^{-1}		
	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
T_1	56.86	19.95	76.82	10.21	9.30	19.51	12.44	70.45	82.89
T_2	55.49	19.96	75.45	9.99	9.41	19.41	12.07	69.49	81.56
T_3	58.57	22.77	81.34	11.02	9.71	20.73	13.34	73.04	86.39
T_4	75.01	23.01	98.02	14.54	11.81	26.36	17.59	88.92	106.98
T_5	78.34	25.33	103.67	15.48	12.45	27.93	18.77	94.26	110.03
T_6	66.64	23.02	89.66	12.23	11.08	23.31	14.69	81.89	96.58
T_7	68.93	22.72	91.65	12.85	11.24	24.08	15.57	84.19	99.76
T_8	55.46	22.56	78.02	11.19	10.04	21.23	13.59	74.35	87.94
T_9	69.57	25.66	95.23	13.27	11.67	24.94	16.06	86.02	102.08
T_{10}	80.09	28.26	108.35	15.57	12.48	28.05	18.96	92.37	111.34
T_{11}	39.74	15.09	54.84	7.66	7.29	14.96	9.32	56.32	65.65
CD (p=0.05)	12.30	4.88	15.15	2.39	2.29	3.54	2.82	17.95	18.57

significantly higher grain yield of (5.41 t ha^{-1}) was produced with T_5which was significant at par T_4 (5.08 t ha^{-1}) and T_9 (4.67 t ha^{-1}). The increased grain yield in these treatments is because of weed density and better WCE along with betterment in yield attributes like number of effective tillers per m^2, panicles length, number of grains per panicle and test grain weight. This corroborates with the findings of Singh et al (2005). The treatment T_5 obtained significantly higher straw yield of (6.69 t ha^{-1}) which was at par with T_4, T_6, T_7, T_8 and T_9. The minimum straw yield was recorded in T11 (3.97 t ha^{-1}) (weedy check). Similar findings were observed by Pratap et al (2017).

Nutrient uptake: Among the different treatments, maximum uptake of 108.35, 28.05 and 111.34 kg ha^{-1} N, P and K respectively was observed with hand weeded plot. The lowest N, P and P uptake was recorded in weedy check plots. The high uptake of nutrients is because of less crop weed competition along with higher nutrient absorption which has led to in higher N, P and K content in grain and straw. Among all herbicidal treatments, the higher value of N (103.67 kg ha^{-1}), P (27.93 kg ha^{-1}) and K (110.03 kg ha^{-1}) was registered in T_5 followed by T_4.

CONCLUSION

Weed management in direct seeded rice is very difficult because of presence of complex weed flora. Usage of herbicidal combinations could be the solution as it controls the all types of weeds effectively and prevents the development of herbicide resistance in weeds. This study was carried out to find the suitable herbicidal combinations in direct seed rice as research on herbicidal combinations in direct seeded rice in India are limited. Based on findings of investigation for one year, it may be concluded that application of pyrazosulfuron @ 25 g a.i. ha^{-1} PE fb bispyribac-Na @ 20 g a.i. ha^{-1} + pyrazosulfuron @ 20 g a.i. ha^{-1} PoE was equally effective to hand weeding thrice in controlling the weeds, improved yield and economically viable option in DSR.

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Changes in Blackgram Seed Quality during Storage as Influenced by Various Packaging Materials and Storage Conditions

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Abstract: A storage experiment was conducted to know the influence of various packaging materials and storage conditions on blackgram seed quality. During the course of the investigation, various packaging materials such as cloth, gunny, high density polythene (HDPE) and vacuum packed bags were used and these bags were kept under ambient and cold storage for 18 months. As the storage period progressed, seed infestation with bruchids (*Callosobruchus* sps.) was observed in cloth, gunny and HDPE bags under ambient condition only after 8 months of storage. At the end of the storage period, there was no such bruchids infestation was seen in a vacuum packed bag of ambient condition and in all the packaging materials of the cold condition. Further, at the end of the storage period, seeds of vacuum packed bag under both ambient and cold conditions only have maintained minimum germination percent as described by the Central Seed Certification Board, Government of India. So, this technology can be effectively used to extend the storage period of blackgram without any storage chemicals i.e., in a chemical-free environment. Hence, along with maintaining seed health for a longer time, a threat to the environment can be completely averted.

Keywords: Ambient and cold conditions, Bruchids infestation, Blackgram, Conventional packaging, Vacuum packaging

The significant agricultural production could be impacted due to the variations in periodicity and intensity of climatic events like floods and droughts, temperature and rainfall patterns (Arun et al 2017). Moreover, a substantial amount of food grains is being damaged after harvest due to a lack of adequate storage and processing facilities. FAO estimated worldwide annual losses of about 10 per cent in stored grains (Parfitt et al 2010). Ensuring global food security requires a range of strategies including the diversification and improvement of staple food crops and the reduction of post-harvest loss. In India, due to insufficient and poor storage facilities, lack of knowledge in post-harvest pulse management and storage, the risk of damage due to post harvest storage (25-30 %) is a major concern in pulses (Anonymous 2017). Controlling these major losses along with other post harvest losses *viz.*, threshing, drying and milling losses can meet the demand for pulses and can reduce the import from various countries. Pulses can remain in edible condition for a long time if, properly stored. However, pulses are more difficult to store compared to cereals (Deshpande and Singh 2001) and suffer much greater damage due to insects and microorganisms resulting in deterioration of quality. Since, pulses have high protein content and are prone to very high insect infestation and thus, undergo heavy losses during storage (Lal and Verma 2007). Among the insects of grain legumes, bruchids (*Callosobruchus* spp.) are very serious pests both in the field and as well as in storage, that can cause considerable economic losses in various pulses such as chickpea, mung, peas, cowpea, lentil and arhar (Srinivasan et al 2008). This insect infestation either originates in the field or in storage and due to their internal feeding behaviour of larvae causes serious loss to the seeds in terms of both quantity and quality (Messina and Jones 2009).

Bruchids are controlled by treating the seeds with carbon disulfide, phosphine or methyl bromide or by dusting with several other insecticides. However, their use by the farmers is criticized worldwide (Williamson et al 2008) as these chemicals are highly toxic and environmentally undesirable and pose a threat to food safety and bio diversity. In addition to chemical control of bruchids, some plant derived extracts *viz.,* plant juice of banana, oil from soybean, maize and neem, hot pepper powder has proven beneficial (Swella and Mushobozy 2007), these plant derived extracts, despite many advantages, have certain limitations such as slow in action, easy degradability (Rozman, 2015), frequent application and importantly affecting non-target organisms to some extent (Trivedi et al 2018). With these limitations of using both synthetic and plant derived chemicals to control bruchids, research on chemical free storage has been conducted with the use of different packaging materials and their storage under different conditions to assess the storability of blackgram seeds.

MATERIAL AND METHODS

A storage experiment was conducted under ambient and cold storage conditions at the University of Agricultural Sciences, Dharwad, Karnataka, India for 18 months (15 November 2019 to 15 May 2021). Average data related to temperature °c and relative humidity (%) that prevailed in the storehouse of ambient condition during the first 15 days of every alternate month were recorded with the help of Anemometer (Table 1), whereas, in the storehouse of cold condition, 5-7°C temperature with 60 ± 2 % relative humidity was maintained throughout the storage period. Freshly harvested healthy seeds of blackgram (DU-1) were collected from farmers' fields and then sun-dried. After sun drying, crops seeds were packed in different packaging *viz.,* cloth bag, gunny bag, HDPE bag and also vacuum packed (wherein the product to be packed is placed in a pouch of suitable material and the air is drawn out from the pack and hermetically sealing it) (Fig. 1). The seeds (3 kgs) were packed in the cloth, gunny and high density polythene (HDPE) bag and replicated 5 times in two storage conditions. However, in the case of vacuum packaging, 1 kg of seeds was packed and 9 bags were packed and replicated 5 times in two storage conditions. Further, stacking of bags was not done, to expose all the bags to prevailing environmental conditions. The characteristics of the polythene bag used for vacuum packaging are presented in Table 2. The machine used for vacuum packaging of different seeds was OLPACK 501/V manufactured by Interprise–Brussels S.A., Bruxtainer Division, Belgium. Seed health parameters such as germination (%), total seedling length (cm), moisture content (%) and **α**amylase activity were recorded. For the germination test, a number of normal seedlings were counted on the final day (on 7th day) (ISTA, 2013) and the average value was expressed in percent. Total seedling length is the summation of shoot length and root length of seedlings on the final day and the average value is expressed in centimeters. Moisture content was measured as per the procedure described by ISTA (2013) and α amylase activity was also measured (Sadasivam and Manickam 1996). Seeds with initial germination (98.00 %), total seedling length (49.8 cm), moisture content (8.10 %) and α amylase activity (54.77 µg maltose released/min/ml) were used for packaging. Again, on the 15th of every alternate month, representative samples were drawn from all treatments and all the above-mentioned seed health parameters were recorded for up to 18 months.

Table 1. Average temperature and relative humidity of storehouse of ambient condition

Months	Temperature (°C)	Relative humidity (%)
January-2020	21.3	68.6
March-2020	24.3	54.6
May-2020	28.5	67.2
July-2020	22.8	86.9
September-2020	22.7	85.4
November -2020	22.1	63.1
January-2021	20.9	74.1
March-2021	24.8	51.7
May-2021	26.6	67.8

Fig. 1. Different packages used for storage of blackgram seeds under ambient and cold conditions

RESULTS AND DISCUSSION

Germination: After 2 months of the storage period, there was no significant difference was observed in germination between the treatments (Table 3). As the storage period progressed, there was an infestation of seeds with bruchids under ambient condition in all packaging's except vacuum packed bags. The infestation of seeds with bruchids under ambient conditions was due to the pervious nature of packaging material that allowed the bruchids to penetrate the packaging and multiply. Similar results of seeds infestation

Table 2. Specifications of the LDPE bag used for vacuum packaging

Characters	Unit	Results	Tested as per (Guidelines of)
Thickness (Microns)	Microns	149.40	IS: 2508
Water vapor transmission rate	g/m^2/24 hrs at 38°C and 90.0 % Relative humidity	0.95	ASTM F 1249
Oxygen transmission rate	cc/(m^2 × day × atm)	0.91	ASTM D 1434-15

with insects in pervious packaging was reported by Patel et al (2018). The infestation of seeds with bruchids resulted in significant decrease in germination due to the internal feeding behaviour of larvae hence, those packaging's were discarded and observations were discontinued in which bruchids infestation were seen. Similar results of a decrease in germination due to infestation of bruchids were reported by Miah et al (2013) and Meenatchi et al (2018).

Before seed infestation with bruchids, the rate of seed deterioration was higher in cloth, gunny and HDPE bags which are kept under ambient storage as compared to cold storage and it might be due to the differential rate of seed respiration as they were kept in different environmental conditions. Similar results of a higher rate of seed deterioration in ambient condition as compared to cold storage condition were reported by Vange et al (2015) and Gupta et al (2017). The rate of seed deterioration was much lower in both ambient and cold conditions in vacuum packed seeds as they were kept in modified atmospheric condition, which enables them to maintain more stable relative humidity inside the package and they were packed in a polythene bag having higher thickness, lower water vapor and oxygen transmission rate. In vacuum packed bag, the seeds were placed under low oxygen concentration and due to the low oxygen concentration in the package, there was very little oxidation of the product and thereby, seeds were less deteriorated as compared to other packaging materials. However, vacuum packed seeds stored in cold condition have performed better as compared to ambient condition and this is because of temperature difference in both conditions as the temperature is also one of the factors which determine the rate of deterioration. Similar results of maintaining the seed viability and vigour for a longer period by vacuum packaging have been reported by Tripathi and Lawande (2014), Khanna et al (2017) and Meena et al (2017). In cold storage condition, as the storage period progressed, there was a decline in viability and vigour of all the seeds in all the pervious packaging due to ageing. As the ageing of seeds is a function of temperature, oxygen and moisture. In cold storage, temperature and moisture are well managed but oxygen has played a major role in seed deterioration. It is well known that, oxygen is an important factor to regulate a series of physiological processes caused by respiration, which was associated with seed deterioration. The results of the investigation are in accordance with the findings of Basavegowda and Arunkumar (2013) and Bhadru et al (2018). Apart from temperature, the main factors of seed deterioration are oxygen and moisture content and maintenance of these constraints will have a greater impact on seed longevity and it can be achieved by vacuum packaging. The beneficial effect of vacuum storage on extending seed longevity has also been reported in sweet corn seeds (Chiu et al 2003). Yeh et al (2005) reported that, the longevity of primed bitter gourd seeds was markedly

Table 3. Influence of packaging and storage conditions on germination (%) at different time intervals of storage in blackgram seeds

Treatments		Storage period (Months)								
		2	4	6	8	10	12	14	16	18
Ambient storage	T_1-Cloth bag	96.8 (79.7)	93.0 (74.7)	90.5 (72.0)	*	*	*	*	*	*
	T_2-Gunny bag	96.5 (79.2)	92.5 (74.1)	90.3 (71.9)	*	*	*	*	*	*
	T_3-HDPE bag	97.0 (80.0)	93.5 (75.2)	90.8 (72.3)	*	*	*	*	*	*
	T_4-Vacuum packed bag	98.0 (81.9)	98.0 (81.9)	97.5 (80.9)	97.5 (80.9)	97.0 (80.0)	96.8 (79.7)	96.5 (79.2)	96.0 (78.5)	96.0 (78.5)
Cold storage	T_5-Cloth bag	97.5 (80.9)	95.5 (77.8)	93.0 (74.7)	90.3 (71.9)	85.8 (67.9)	82.0 (64.9)	78.8 (62.6)	76.0 (60.7)	72.5 (58.4)
	T_6-Gunny bag	97.0 (80.0)	94.8 (76.8)	93.5 (75.2)	90.0 (71.6)	86.3 (68.3)	81.5 (64.5)	78.5 (62.4)	76.3 (60.9)	73.0 (58.7)
	T_7-HDPE bag	96.8 (79.7)	95.3 (77.5)	93.3 (75.0)	89.5 (71.1)	86.0 (68.0)	81.8 (64.7)	79.3 (62.9)	76.5 (61.0)	72.2 (58.2)
	T_8-Vacuum packed bag	98.0 (81.9)	98.0 (81.9)	97.8 (81.5)	97.5 (80.9)	97.3 (80.5)	97.0 (80.0)	96.8 (79.7)	96.5 (79.2)	96.3 (78.9)
	C. D (p=0.01)	1.7	1.0	1.7	1.9	2.2	1.9	1.8	2.0	1.9

Figures in parenthesis are arcsine values
*Observations in cloth, gunny and HDPE bag treatments have been stopped due to bruchids infestation after 8 months of storage period
As per central Seed Certification Board, Department of Agriculture & Co-operation, Ministry of Agriculture, Government of India, the minimum seed germination for black gram is 75 %

decreased after 12 months of storage under ambient oxygen conditions, but seed viability was maintained under partial vacuum storage conditions. Similar results of maintaining seed viability for a longer period by vacuum packaging were reported by Meena et al (2017) and Wang et al (2018). Bruchids last for 29-30 days at 30ºC and 70 per cent relative humidity (Raina 1970) and they have higher fecundity (War et al 2017). Many researchers have investigated the origin of the infestation of bruchids and reported that it either starts in the field or in storage. However, in the present study, it originated in the store because no bruchids were detected after two months of storage; if infested in the field, heavy bruchids were seen due to their short life cycle and high fecundity.

Total seedling length: Deterioration in seed quality is associated with a decrease in seedling length with time. In the present investigation, the seedling length was considerably decreased in cloth, gunny and HDPE bags but not in vacuum packed bags irrespective of storage conditions and seeds (Table 4). Ageing has a damaging effect on enzymes that are necessary for the conversion of food reserve in the embryo to a usable form and ultimately reduced seedling-associated parameters. With the advancement in the storage period, decrease in seedling length was more in cloth, gunny and HDPE bags in cold condition and these treatments were non-significant with each other throughout the storage period. In contrast, a minimal decrease was recorded in vacuum packed bags stored in cold condition as compared to ambient condition and was due to the differential rate of seed deterioration in which seeds are stored. This decreasing trend in seedling length might be attributed to oxidative stress which induced the production of reactive oxygen species that caused lipid peroxidation in terms of malondialdehyde (MDA) as a final product which in turn hinders the cellular metabolism resulting in lower ATP production required for growth and development of the seedling (Blokhina et al 2003). After 18 months of storage, a significant difference was observed

Table 4. Influence of packaging and storage conditions on total seedling length (cm) at different time intervals of storage in blackgram seeds

Treatments		Storage period (Months)								
		2	4	6	8	10	12	14	16	18
Ambient storage	T_1-Cloth bag	49.6	49.0	47.7	*	*	*	*	*	*
	T_2-Gunny bag	49.4	49.2	48.0	*	*	*	*	*	*
	T_3-HDPE bag	49.6	49.1	47.7	*	*	*	*	*	*
	T_4-Vacuum packed bag	49.8	49.8	49.8	49.7	49.5	49.3	49.3	48.9	48.7
Cold storage	T_5-Cloth bag	49.6	49.2	48.9	47.9	47.1	46.7	46.5	45.7	44.2
	T_6-Gunny bag	49.6	49.6	48.9	47.8	47.2	47.2	46.2	46.1	44.7
	T_7-HDPE bag	49.7	49.5	48.9	47.9	47.6	46.8	46.5	45.9	44.6
	T_8-Vacuum packed bag	49.8	49.8	49.8	49.8	49.6	49.4	49.2	49.2	49.0
	C. D (p=0.01)	0.4	0.8	0.5	0.7	0.7	0.5	0.8	0.9	0.7

Table 5. Influence of packaging and storage conditions on moisture content (%) at different time intervals of storage in blackgram seeds

Treatments		Storage period (Months)								
		2	4	6	8	10	12	14	16	18
Ambient storage	T_1-Cloth bag	9.03	8.40	9.13	*	*	*	*	*	*
	T_2-Gunny bag	9.08	8.38	9.00	*	*	*	*	*	*
	T_3-HDPE bag	9.03	8.43	9.05	*	*	*	*	*	*
	T_4-Vacuum packed bag	8.15	8.13	8.18	8.08	8.15	8.20	8.13	8.10	8.13
Cold storage	T_5-Cloth bag	8.73	8.78	8.75	8.73	8.70	8.60	8.68	8.83	8.78
	T_6-Gunny bag	8.70	8.68	8.63	8.68	8.78	8.70	8.70	8.75	8.75
	T_7-HDPE bag	8.60	8.70	8.73	8.80	8.83	8.73	8.65	8.70	8.80
	T_8-Vacuum packed bag	8.13	8.12	8.15	8.13	8.13	8.10	8.10	8.15	8.13
	C. D (p=0.01)	0.17	0.16	0.20	0.17	0.24	0.20	0.24	0.30	0.26

Table 6. Influence of packaging and storage conditions on α amylase activity (µg maltose released/min/ml) at different time intervals of storage in blackgram seeds

Treatments		Storage period (Months)								
		2	4	6	8	10	12	14	16	18
Ambient storage	T_1-Cloth bag	54.34	51.96	50.57	*	*	*	*	*	*
	T_2-Gunny bag	54.30	51.68	50.41	*	*	*	*	*	*
	T_3-HDPE bag	54.16	51.94	51.43	*	*	*	*	*	*
	T_4-Vacuum packed bag	54.77	54.77	54.20	54.47	54.17	54.17	53.96	53.23	53.19
Cold storage	T_5-Cloth bag	54.54	53.30	51.85	50.44	47.92	46.83	43.28	42.91	42.07
	T_6-Gunny bag	54.21	53.17	52.30	50.73	48.47	46.90	44.04	42.80	43.69
	T_7-HDPE bag	54.20	53.31	52.05	50.14	46.89	47.40	44.69	41.70	41.01
	T_8-Vacuum packed bag	54.77	54.77	54.63	54.48	54.24	54.27	53.93	53.64	53.45
	C. D (p=0.01)	0.72	0.90	1.19	1.64	2.04	2.65	3.35	3.81	4.04

between the cloth, gunny, HDPE bags and vacuum packed bags whereas, no significant difference was observed between vacuum packed bags stored in ambient and cold storage conditions. Similar results of higher reduction in seedling length due to ageing were revealed by Meena et al (2017) and Autade and Ghuge (2018).

Moisture content: There was a lot of fluctuation in the seed moisture content (Table 5) stored in cloth, gunny and HDPE bags in ambient condition as compared to cold condition. Seeds are hygroscopic in nature hence, they have absorbed moisture when relative humidity was high and they have lost the moisture when humidity was low under ambient condition (Table 2). Similar results of variation in moisture content under ambient condition in pervious packaging materials were reported by Kumar et al (2017) and Shankar et al (2018). Similarly, when seeds were stored in pervious packaging materials (cloth, gunny and HDPE bags) and kept in cold condition, seeds absorbed the moisture initially but thereafter there was no change in moisture content due to the prevalence of similar storage condition throughout the storage period. Similar results of the initial increase in moisture content when stored in pervious packaging materials and kept in relative humidity same as that of cold storage have been reported by Bakhtavar et al (2019).Hygroscopic nature of seeds holds good only when seeds are packed in pervious packaging materials but not in impervious packaging materials like vacuum packed bags as they have very less water vapour and air transmission rate and higher thickness (Table 2). Due to these special characters of polythene bag used for vacuum packaging, there was no variation in moisture content of the seeds throughout the storage period in cold and ambient conditions. Similar results of no variation in moisture content of seeds when vacuum packed have been reported by Deepa et al(2013) and Meena et al (2017).

α Amylase activity: In seeds, amylase is the major amylolytic enzyme found during germination and is involved in the degradation of endosperm starch. Degradation of starch into soluble sugars is important to support seedling growth and it can be degraded with the help of amylase activity. There was a decrease in α amylase activity as the storage period progressed and this reduction indicates the deterioration of seeds (Table 6). Except at the initial storage period, there was a significant difference in α amylase activity and reduced more in conventional packaging's *viz.,* cloth, gunny and HDPE bags and less in vacuum packed bags under both cold and ambient conditions. After 18 months of storage, a non-significant difference was observed within cloth, gunny and HDPE bags in cold condition and these treatments were significantly differing with vacuum packed bags of both the conditions. No variation in α- amylase activity of vacuum packed seeds is because vacuum treatment enabled seeds to keep low moisture content as oxygen and water were isolated from the storage condition and hence, ageing factor of seeds were controlled in vacuum condition (Wang et al 2018). In general, decreased α amylase activity with an increase in storage period might be due to a reduction in free sugars and amino acids. Further, a direct correlation exists between loss of viability and decline in enzyme activity. Similar results of a decrease in α amylase activity due to ageing have been reported by Bhanuprakash et al (2006). Further, Norastehnia et al (2007) observed the accumulation of aldehyde compounds with the advancement of deterioration especially methyl jasmonate (MeJA) which is a potent inhibitor of alpha-amylase. Higher concentrations of MeJA reduced the concentration of the enzyme protein and consequently inhibition of gibberellin biosynthesis since gibberellin stimulates the alpha-amylase synthesis and its secretion from the embryonic axis into cotyledons.

CONCLUSION

The vacuum packaging technology can be effectively used for the safe storage of blackgram seeds for a longer period without any aid of chemicals.

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Evaluation of *Anabas testudineus* Farming in Natural Ponds and Impact on Subsequent Crop

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***Abstract*:** The climbing perch (*Anabas testudineus*) is a species of amphibious freshwater fish in the family Anabantidae and is widely found across the globe. This fish can survive without water for a short period by resting under the mud during dry seasons. They do aerial respiration using paired suprabranchial labyrinthiform organs. This fish was used as a biocontrol agent against mosquitoes in confined water bodies, including rice fields, as they can feed on insects at the water surface. The performance of monoculture Anabas in natural ponds and impacts on the consecutive carp culture in the same pond are evaluated in the present study. The average production of Anabas recorded was 1.39 Kg per m^2 area of the pond with survival of 57.33%. The significant reduction survival percentage of carps was observed in the second crop. The reduction in carp stock was observed due to the predation by retained Anabas of the previous culture. They resist the toxicity of sea seed cake once hidden in the pond bottom. They might have survived inside the deep layer of clay with the help of respiratory support of the labyrinth and were established later. The study confirms the opportunistic carnivore feeding behavior without other feeds. The study concludes that Anabas is a good candidate fish for places where water availability is an issue. Proper fencing around the ponds needed to check its movement out and through the land. When a different candidate species is planned for the subsequent crop, complete harvest, even from the deeper portions of the pond bottom, must be ensured to prevent surviving Anabas from feeding on the fingerlings of the next crop.

Keywords:

Anabas testudineus (Bloch 1792), belonging to the Perciformes order and Anabantidae family, is widely found across the globe particularly in tropical and subtropical Asian continents (Khatun et al 2019). The peculiar walking/climbing ability makes it a different fish and is commonly called a Climbing perch. This fish is called 'Koi' in Bangladesh and India (Chakraborty et al 2012). It inhabits confined water bodies such as canals, lakes, ponds, swamps, and estuaries (Hossain et al 2015). Anabas possesses the ability to live in low saline water bodies also. The body of Anabas is slender with large scales and spines on the gill cover. This fish has the adaptive ability to survive in adverse climatic conditions such as low dissolved oxygen, high turbid water, extreme pH, high temperature, etc. They can also live without water for a short period by resting under the mud during dry seasons (Tay et 2006, Paliwal et al 2014) and have aerial respiration using paired suprabranchial labyrinthiform organs. It is a carnivorous air-breathing species using a labyrinth organ to take oxygen directly from the atmosphere. Anabas is an omnivore that primarily feeds on fish larvae and shrimps rather than algae (Mustakim et al 2020). This fish was used as a biocontrol agent against mosquitoes in confined water bodies, including rice fields (Bhattacharjee et al 2009) and can feed on insects at the water surface. The seed production of Anabas is standardized, and hatchery-produced seeds are being used for its farming. Anabas breeds at sizes 70-100 mm with the onset of monsoon. Paddy fields and seasonal ponds having low water columns are their breeding grounds. Anabas is farmed commercially in rice fields, ponds, and artificial tanks. Slow-growing local strain and fast-growing Koi strain are the two strains of Anabas popular in India. Local strain reaches only 80-100 g in 8 months, whereas Koi strain reaches 300-400 g in 8 months. Those farmers who take up polyculture are facing issues due to the predation of Anabas on tiny fish larvae and fries. This issue is more predominant in natural ponds than artificial ponds. Complete eradication of Anabas is difficult due to its hibernation ability. Anabas predation is a growing issue for those freshwater fish farmers who are farming in ponds where complete drying is impossible. This study's main objective is to evaluate monoculture Anabas's performance in natural ponds and its impacts on the consecutive Carp culture in the same pond.

MATERIAL AND METHODS

Monoculture of Anabas: Three freshwater ponds, each having dimensions 8m×5m and 0.9m depth, are located at Chenkara, Kothamangalam, Ernakulam district, Kerala, India (10.1170° N, 76.6597° E) for the experiment. The ponds were located at mutual distances of 100-150 m. The ponds were dewatered, excess humus removed, and weed fish eradicated by applying Teaseed cake (Kulakkattolickal 1989) at the rate of 5g per m^2 and pond bottom sun-dried for two days. Subsequently, powdered dolomite was used at 100 g per m^2 as a liming material to correct acidity and enhance phosphorus and carbon contents towards higher phytoplankton production. The ponds were aged for a further three days, and the fresh water was pumped into the pond through a 200-micron mesh screen to prevent the entry of weed fishes. Ponds were fertilized using cow dung groundnut cake and urea at 125, at 7.5 and 1.25 g/m^2 and aged for five days. Koi strain *Anabas testudineus* fingerlings (4.50±0.67 cm & 1.75±1.75 g) 400 numbers sourced from a hatchery were stocked in the ponds.

Formulated floating pellet feed (0.80 mm size) fed three times a day at the rate of 10 percent of body weight and gradually reduced to 2 percent of body weight within six months. The pellet size gradually increased to 1.2 mm, 1.8 mm, and 2.5 mm, respectively, after 1, 2, and 4 months of a stocking to match mouth size. Water quality parameters were recorded regularly. After completion of the seventh month, the harvest was done by entirely dewatering, and fish weight, length, and survival percentage were recorded. One-way Analysis of variance was done using Microsoft excel 2019 toolkit to test the equality of values.

Polyculture of carps: All the ponds were further prepared by following the same standard protocol and refilled using screened water from the same source. Forty numbers of grass carps, Rohu, and Amur carp frys of 3 cm size were stocked in all the ponds. Feeding provided with 0.6mm size formulated feed containing 33 percent protein and 4 percent fat thrice a day for 45 days. Sampling was done at weekly intervals to check survival percentage.

RESULTS AND DISCUSSION

Monoculture of Anabas: The average production of Anabas recorded in the experiment was 1.39 Kg per m^2 area of the pond. This value is on par with the report of Kohinoor et al (2007). The survival percentage was only 57.33, whereas the culture ponds observed no daily mortality. The reduction in survival may be due to the movement of Anabas to other ponds rather than mortality issues. The fishes might have moved using the pelvic fins and spiny opercula, as reported by Liem (1987). The hinged sub-operculars of Anabas allow them for near-upright posture and provide fulcra for vaulting for movement on land. Anabas's eye in a deep bony orbit facilitates rapid water drainage from a corneal surface when the fish leaves the water, aiding vision in the air (Davenport 1990). Sokheng et al (1999) reported that they perform walking movements mainly during low light time due to its negative phototaxis, which may be why such activities are less noticed. The weight gain of 160.56 g and length of 214.4 mm were recorded after seven months of farming (Table 1). Periodical testing was done to monitor changes in water quality and the data is presented in Table 2.

Polyculture of carps: The survival percentage of carps after four weeks was as low as 22 percent (Table 3). Due to the sudden reduction in survival, the existing fish stock was harvested in the fourth week. While catching, Anabas also received a 30 number of considerable size (180 mm).

The reduction in carp stock was observed to be due to the predation by retained Anabas of the previous culture. Even though not stocked any Anabas in the carp culture ponds, Anabas may have been maintained from the earlier stocks by resisting the toxicity of tea seed cake by resting in the pond bottom. They might have survived inside the deep layer of clay with the help of respiratory support of the labyrinth and were established later. Anabas is an omnivore and prefer to feed on carp fingerlings in large quantities due to the

Table 1. Growth *Anabas testudineus* (Mean±SD)

Period (Days)	Length (mm)	Weight (g)
30	45±6.7	1.75±1.75
60	62±11.0	5.88±2.99
90	97.5±9.6	21.30±8.81
120	128.0±14.7	59.80±13.17
150	141.8±11.6	81.12±17.16
180	162.3±22.3	135.12±29.54
210	214.4±46.7	160.56±50.32

Table 2. Water quality parameters in the ponds

Parameters	Range	
	Minimum	Maximum
pH	6.3	8.4
Alkalinity (mg/l)	700	160
Hydroxide (mg/l)	0	0
Total hardness (mg/l)	30	110
Ammonia (mg/l)	0	0.56
Nitrite (mg/l)	BDL*	0.11
Sulfide (mg/l)	BDL	BDL

Table 3. Carp survival during different week

Time	Survival percentage
1st Week	89±3.6
2nd Week	74±1.0
3rd Week	48±5.5
4th Week	22±2.5

abundance of fingerlings in a confined water body. Mustakim et al (2020) reported that Anabas like to eat fish or non-vegetarian rather than vegetarian food, and their intestinal are adjusted for it. Its teeth system is also said to resemble that of predatory fishes. Food and feeding studies confirmed the opportunistic carnivore feeding behavior without other feeds.

CONCLUSION

Anabas is a good candidate fish for places where water availability is an issue. However, using HDPE nets ensure proper fencing to check its movement out of the ponds, even though the land. When a different candidate species is planned for the subsequent crop, complete harvest, even from the deeper portions of the pond bottom, must be ensured to prevent surviving Anabas from feeding on the fingerlings of the next crop.

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Microcosm Study to Assess Allelochemical Potential of *Lantana camara* on *Lactuca sativa*

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Abstract: The allelopathic potential of *Lantana camara* can be deleterious to native diversity and productivity of food crops. The present study aimed to assess the allelopathic potential of *L. camara* on the germination and growth of *Lactuca sativa* through bioassay method. Two pots i.e., control (P_1) and experimental (P_2) were taken for microcosm studies with healthy and *L. camara* invaded soil, respectively. There were differential germination percentage (P_2-80%, P_1-93.33%), germination potential (P_2 0.86, P_1 0.93), germination index (P_2 4.33, P_1 4.66), and germination rate index (P_2 346.66, P_1 435.55). The allelochemicals from *L. camara* adversely influenced the seed germination. However, the growth parameters such as seedling height (32.35cm), root/shoot length (4.75cm/27.6cm), and seedling biomass (1.559g) were higher in *L. sativa*. Thus, *L. camara* exhibited stimulatory as inhibitory effects on *L. sativa*. However, further studies are warranted for explicit elucidation of chemical ecology and allelopathic effects of *L. camara*.

Keywords: Chemical ecology, Plant invasion, Allelopathy, Allelochemicals, *Lantana camara, Lactuca sativa*, Sustainable management

Chemical ecology can manifest remarkable impact on pattern and distribution of biodiversity (Rai and Singh 2020). The distribution and abundance of flora is determined by their allelopathic interactions with invasive alien plants (Enyew and Raja 2014, Vanlalruati and Rai 2022). Allelopathy is influenced by competition for resources and stress from disease, extreme temperatures, and herbicides (Ogunsanya et al 2018). These stresses often increase the production of allelochemicals and accentuate their actions to avoid competition in their environment (Ogunsanya et al 2018, Talhi et al 2020). Due to the production of allelochemicals into the rhizosphere of surrounding crop plants, *Lantana camara* hindered germination, growth, development, and metabolism of food crops (Qasem 2006). *L. camara* interfere with native plants through allelopathy and it is widely distributed in different parts of India (Craig et al 2011, Bhattacharya et al 2020). Further, *L. camara* demonstrates the ability to survive in a wide range of climates and soil types, demonstrating its broad ecological amplitude and strong allelopathy (Day et al 2003; Rai and Singh 2020). The success of *Lantana* in different environmental conditions could be due to its allelopathic effect on other plants which may impair native plants' vitality (Day et al 2003). Maiti et al (2008) reported that the lantana leaf leachates inhibited the seed germination, germination rate, seed viability, and seedling emergence capacity of *Mimosa* spp. Various allelopathic compounds like salicylic acid, gentisic acid, coumarin, ferulic acid, p-hydroxybenzoic acid and 6-methyl coumarin were extracted from *L. camara* (Yi et al 2006). Some other allelochemicals identified in *L. camara* which are cytotoxic in nature (Ma et al 2004) (Table 1). The allelopathic effect of *L. camara* has been reported on many crops such as *Brassica juncea L.*, (Ahmed et al 2007), *Oryza sativa L.* (Hossain and Alam 2010), and *Phaseolus radiatus* (Gantayet et al 2014). In addition, the extract of *L. camara* has also been observed to inhibit the seed germination and seedling growth of many species such as *Salvinia molesta* (Saxena et al 2013), *Brassica campestris*, *Ipomoea aquatica*, *Sorghum bicolor* L. and *Zea mays* (Veraplakorn 2017). Therefore this experiment is carried out in order to find out the effect of the allelochemicals present in *L. camara* on the germination process and seedling growth of the selected food crop (*L. sativa*) using bioassay method.

MATERIAL AND METHODS

The study was conducted in a greenhouse at Mizoram University, Tanhril, Mizoram, India, for 3 months duration (April-June), 2021. Two pots were taken in which soil sample along with leaf litter was taken from the site invaded by *L. camara* . Another soil sample was taken from a healthy forest soil and kept as control. A fast growing crop i.e., *L. sativa* seed was selected and sown in both the pots. *L. sativa* is a fast growing food crop and is commonly grown in the Mizoram, therefore used as bioassay plant. Studies have also indicated that *L. sativa* is responsive to allelochemicals which may clearly visualize the bioassay results (Tadele 2014, Nasrollahi et al 2018). The changes in the morphology of the plant were carefully examined at different time intervals. On the 59th day, when the plants was fully grown, two replicas from each pots were taken out.The plant height (root and shoot length) was measured. The various parameters such as seedling heights (H), root Length and shoot length (RL & SL), biomass (b), germination percentage (GPe), germination potential (GPo). germination index (GI), germination rate index (GRI), vigor index (VI) were estimated (Singh et al 2014).

RESULTS AND DISCUSSION

In comparison to the control pot the germination parameters were higher in the experimental pot (Table 2). This indicates that the

Table 1. Allelochemical compounds present in all parts of *Lantana camara*

Compounds name	Biological activity
β-pinene	Inhibiting the seed germination, growth and antibacterial activity.
Camaric acid	Nematicidal activity
1,8-Cineole	Inhibiting the growth of plant.
Cinnamic acid	Inhibited the activity of plasma H+-ATPase, PPase and inhibit the process of seed germination.
Dipentene	Inhibiting the growth of plant.
Geniposide	Inhibited hepatoxicity and the DNA repair synthesis induced by aflatoxin B1 in rat primary hepatocytes.
Icterogenic acid	Toxic to sheep, cattle, goats.
Lantadene A, B,C D	Death of horses, cattle, sheep, goats and rabbits by failure of liver and other organs.
Lantanilic acid	Nematicidal activity.
Lantanoside	Antimicrobial and Nematicidal activity.
Linaroside	Antibacterial activity
Palmitic acid	Inhibiting the growth of vegetables
ρ-Coumaric acid	suppress root-infecting fungi , root-knot nematode , inhibit the process of seed germination and inhibit the growth of morning glory
Ursolic acid	Inhibitors of human leucocyte elastase,
Verbascoside	Inhibitor of protein kinase and possesses antitumor activity.

Source: Wahab 2004, Gopie-Shkhanna and Kannabiran 2007

seeds were less vital in the experimental soil, ascribed to allelopathic effects on the plants. Gindri et al (2020) also observed that *L. camara* resulted in disruption of cellular membrane and induced the formation of reactive oxygen forms which inhibited the germination of *L. sativa*. Zhang et al (2014) found that aqueous extract of *L. camara* leaves inhibited the seed germination of *L. sativa*. In comparison to the control, the seedling growth of the plants in the experimental pot developed longer roots and shoots which increased their biomass significantly and the experimental pot showed better vegetative growth than the control. This shows that the soil of experimental pot has favorable allelopathic effect on the plant by enhancing the fertility and soil moisture retention property (Sharma and Acharya 2000). This may be due to the fact that allelochemicals are responsible for the suppression of *L. sativa* might be present in deficient amount, which in fact enhance the growth of the bioassay plant. Tadele (2014) also observed that varying concentrations of lantana leaf extract encouraged the growth of food crops (e.g., maize roots and shoots and roots of finger millets), however decreased when present in very high concentrations. Negi and Kandpal (2003) found that lantana mulch or residue improves soil fertility owing to fast decomposition and nutrient release. Various bioassay studies also showed inhibitory effects exerted by aqueous extracts of lantana leaf at variable concentration levels (Bhattacharya et al 2020, Talhi et al 2020). The sustainable management opportunities for lantana may be explored for its sustainable management, especially in biodiversity hotspots (Rai 2012, Rai and Singh 2015, Rai 2015, Rai and Kim 2020, Sakachep and Rai 2021a, b, Vanlalruati and Rai 2021, Rai 2022). In nutshell, plants generally have a stimulatory allelopathic effect on crops at low concentrations of allelochemicals, whereas large or excess amounts can restrict the life cycle i.e., growth and germination of surrounding plants (El-Kenany and El-Darier 2013).

Table 2. Morphological characteristic of *Lactuca sativa* plants grown on *Lantana camara* invaded soil (experimental) and healthy soil (control)

Parameters	Experimental	Control
Number of *L. sativa* seed sown	15	15
Number of seed germinate after 3 days	13	14
Number of seeds germinate after 7 days	12	14
Seedlings heights (H)	32.35 cm	26.4 cm
Root length (RL) (Mean)	4.75 cm	4.8 cm
Shoot length (SL) (Mean)	27.6 cm	21.6 cm
Seedling biomass (BM) (Mean)	1.559 g	0.893 g
Germination Percentage (GPe)	80 %	93.33%
Germination Potential (GPo)	0.86	0.93
Germination Index (GI)	4.33	4.66
Germination Rate Index (GRI)	346.66	435.55
Vigor Index (VI)	6.75	4.16

CONCLUSION

L. camara showed both positive and negative allelopathic effect on *L. sativa.* The Lantana showed negative effect on germination of *L. sativa* by inhibiting the germination process, however, demonstrated positive response on the other growth parameters of the food crop. Thus, low concentrations of allelochemicals in the soil can be beneficial to crop growth, however may be detrimental in higher concentrations. However, further studies are warranted for explicit elucidation of chemical ecology and allelopathic effects of *L. camara*.

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Optimization of Growth Conditions for Enhanced Bacteriocin Production from *Lactobacillus brevis* UN by One Variable at a Time (OVAT) and Response Surface Methodology (RSM)

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Abstract: Lactic acid bacteria have recently caught the attention of scientific researchers. These food associated organisms and transient inhabitant of human gut has potential to curb down the growth of spoilage and disease causing microbes due to its ability to produce bacteriocin, lactic acid, acetic acid, hydrogen peroxide etc. Bacteriocins are biologically derived primary or modified product of bacterial ribosomal synthesis, which have an antibacterial activity. Bacteriocin producing isolates were isolated from traditional and least explored food samples of Himachal Pradesh and North East India by standard microbiological techniques. Isolated strains screened for their bacteriocin production. Growth conditions for bacteriocin production have been optimized by statistical tools viz. One variable at a time method and Response Surface Methodology (RSM). In the present study bacteriocin producing isolate UN was identified and selected for further studies. Various growth conditions viz. growth cycle, effect of incubation temperature, pH, inoculum size were studied to monitor their effect on bacteriocin production. Bacteriocin production was optimized through Classical One Variable at a Time Method (OVAT) and Response Surface Methodology (RSM). Bacteriocin production was maximum at early stationary phase i.e. 34th h, at pH range 4.0-6.5, at temperature 30-35 ^{0}C with an inoculums size 1.0, 1.5, 2.0 OD at 10 %. The production of bacteriocin seems not to be inducible. The incubation conditions and perfect combination of these conditions particularly temperature, pH, inoculum size and time strongly influence the effective yield of active bacteriocin. One Variable at a Time Method and Response Surface Methodology proved to be powerful tools in optimization of bacteriocin production by *Lactobacillus brevis* UN.

Keywords: Antagonism, Bacteriocin, Optimization, Stationary Phase

Diversity of Indian fermented foods is related to incomparable food culture of each community. India, being a huge country has been the home of innumerable religions, human population and diversity in climatic conditions has resulted in a large number of fermented food. Diverse type of ethnic fermented foods and alcoholic beverages are produced either naturally or by adding mixed starter cultures using indigenous and scientific knowledge of food fermentation (Tamang 2021). Food fermentation develops flavour and aroma in food and also detoxify the food and reduce cooking time and fuel requirements. Indigenous food fermentation is one of the oldest process that is dependent upon the microbial activity. Lactic acid bacteria play an important role in the traditional fermentation processes by their functional properties such as bio preservation, bio enrichment of nutritional value, bioavailability of minerals, production of antioxidants, antimicrobial activities and probiotic and postbiotic properties (Gautam and Sharma 2015, Zapasnik et al 2022). The use of lactic acid bacteria and antimicrobial compounds especially bacteriocins is a promising ongoing development in food preservation. Bacteriocins are ribosomal synthesized extracellularly released bioactive peptides or peptide complexes that vary in spectrum of activity, mode of action, molecular weight, genetic organization and considered to be safe bio preservatives since they are assumed to be degraded by proteases in gastrointestinal tract (Gautam and Sharma 2015). Bacteriocin production has been reported to be affected by several factors and fermentation conditions, such as pH, temperature, inoculum size. The optimization of bacteriocin production and enhancement of its activity are economically important to reduce the production cost. Thus, the objective of this paper is to statistically investigate the effect of growth conditions on the maximum bacteriocin production by *L. brevis* UN isolate obtained from Dhulliachar-Traditional Food product of North East India.

MATERIAL AND METHODS

Collection, Isolation and Screening of bacteriocin producing isolates: Diverse traditional least explored food samples of Himachal Pradesh and Sikkim States of India viz., sepu vari, dangal vari, goat meat, chur saag, salori, gundruck, nimboosat, nashasta, chillipickel, dried shrimp, dried fish, rice beer, chaang (wheat fermented), chaang (rice fermented), sauerkraut and dhulliachar were explored for isolation of bacteriocin producing lactic acid bacteria. All samples were collected in clean and sterilized polythene bags or test tubes and stored in refrigerator until further use. In total 16 food samples were taken for isolation of bacteriocin producing lactic acid bacteria. The isolation was carried out by serial dilution method on de Man Ragosa sharpe (MRS) agar (Hi Media Laboratory Pvt. Ltd. Mumbai, India) under anaerobic conditions. The bacterial colonies obtained on MRS agar were further streaked and the pure lines were obtained. Pure cultures were further preserved on stabs and 40 % glycerol in deep freezer (-20^{0}C). Screening of isolates were performed on the basis of morphological, physiological, biochemical and antagonistic pattern. In total, 53 bacteria were isolated from diverse food sources and were differentiated and observed on the basis of morphological, cultural and biochemical characteristics.

All bacterial isolates which showed catalase test -ve were tested against indicators by Bit/Disc method (Gautam and Sharma 2015) and well diffusion method (Gautam and Sharma 2015). Serious food borne pathogens/food spoilage bacteria i.e. *Listeria monocytogenes* MTCC 839 and *Leuconostoc mesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Lactobacillus plantarum* CRI, *Bacillus cereus* CRI, *Clostridium perfringens* MTCC 1739*, Pectobacterium caratovorum* MTCC 1428, *Escherichia coli* IGMC and *Staphylococcus aureus* IGMC*, Aeromonas hydrophila* IGMC were used in screening of bacterial isolates for bacteriocin production. The test strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh, Central research Institute, Kasualli and Indira Gandhi Medical College (IGMC), Shimla. All these test strains were revived twice for 24 h at 37^{0}C before performing the experiments, as all these indicators were preserved in 40 % glycerol at -20°C. On the basis of bit disk method and well diffusion method isolate UN isolated from dhulliachar was finally selected for further study. On the basis of 16S rRNA gene technique UN identified as *Lactobacillus brevis.* The sequences so obtained were submitted in National Centre for Biotechnology Information (NCBI) to get an accession number. *L. brevis* UN registered under the accession no JX046150.

Estimation of activity units per ml (Arbitrary units - AU/ml) of culture supernatant of *L.brevis* UN: The activity units of culture supernatant of *L. brevis* UN was calculated by serial two fold dilution method. AU/ml was calculated as reciprocal of the highest dilution of bacteriocin containing sample that gave a minimal visible inhibition zone (Noorozi et al 2019)

Optimization of process parameters for bacteriocin production by using classical one variable at a time method (OVAT): Various growth conditions viz. incubation temperature, pH, inoculum size, incubation time were studied to monitor their effect on bacteriocin production.

Bacteriocin production during growth cycle of L. brevis UN: MRS broth (pH 6.5$\pm$ 2) was seeded with active bacterial isolate *L. brevis* UN @10 % (1.0 OD). Bacterial isolate was incubated in orbital shaker at 35$\pm$2°C with a shaking speed of 120 rpm for 60 h on rotatory shaker. OD of isolate was noted down periodically after every 2 h at 540 nm. To detect bacteriocin production, the culture of *L. brevis* UN was centrifuged after every 2 h at 18,000 rpm at 4°C for 20 min. The cell free supernatant was neutralized to pH 7.0 (with sterilized 1N NaOH), catalase was added @ 10 mg in 100 ml and well diffusion method was repeated with these crude preparations of isolate against test indicators. The plates were kept for incubation at 35$\pm$2°C for 24 h and results were observed as clear halos of inhibition formed around the wells against three selected standard indicators i.e. *L. monocytogenes, S. aureus* and *C. perfringens.*

Effect of temperature: The optimum temperature for bacteriocin production for *L. brevis* UN was determined by inoculating 24 h old culture of each bacterial strain separately in Erlenmeyer flasks followed by their incubation at different temperature i.e. 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C, 45^{0}C, 50 ^{0}C. The inhibitory activity of *L. brevis* UN determined after 34th h of growth by well diffusion assay.

Effect of pH: The 24 h old culture of *L. brevis* UN was inoculated separately on MRS medium of varying pH in the range of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and effect of different pH on inhibitory activity of *L. brevis* UN was studied after 34h of growth against three standard indicators viz. *L. monocytogenes, S. aureus* and *C. perfringens* by well diffusion method.

Effect of inoculum size: Different inoculum size viz. 0.5, 1.0, 1.5 and 2.0 (O.D.) with 10 % of *L. brevis* UN was added in flask containing MRS broth having best studied pH. The growth of bacterial isolate was achieved by incubating them at best optimized temperature of previous experiments. The antagonistic pattern of *L. brevis* UN was determined after 34 h of growth by well diffusion assay against three reference targets.

Optimization of bacteriocin production by using Response Surface Methodology (RSM) Central Composite design: RSM was carried out using central composite design, optimize for further process to identify the interactions between the significant factors obtained from OVAT. The 4 variables chosen in this experiment were temperature, pH, inoculum size and incubation time with 5 coded levels (−α,-1, 0, +1, + α) were used for their combined influence on bacteriocin production. Central composite design constituted of 30 experimental trials which were carried out with 16 factorial points, 8 axial points with α = 2 and 6 replication of central points.

In developing regression equation, the test factors were coded according to the Eq. (1)

$$X_i = (X_i - X_0)/\delta X_i$$

Where x_i is the dimensionless coded value of the i^{th} independent variable; X_i the natural value of the i^{th} independent variable; X_0 the natural value of the i^{th} independent variable at the centre point and δX_i is the difference in effect. The response data obtained from the design were fitted with a second order polynomial. The general polynomial equation is as follows in Eq. (2)

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+ \beta_{11}x_{12}+_{\beta22}x_2^2+\beta33x_3^2 +\beta44x_4^2+\beta12x_1x_2+_{\beta13}x_1x_3+_{\beta14}x_1x_4+_{\beta23}x_2x_3+_{\beta24}x_2x_4+\beta_{34}x_3x_4$$

Where, Y is the predicted response i.e. bacteriocin produced (AU/ml), β_0 is the model constant, β_1, β_2, β_3, β_4 the linear coefficients, β_{11}, β_{22}, β_{33}, β_{44} the squared coefficients and β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} the interaction co-efficient.

Data analysis: Design expert 8.0.7.1 (Stat-Ease, Inc., Minneapolis, USA) was used for the regression analysis of the experimental data obtained. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the significance of the model, an optimum value of parameters was assessed by the determination coefficient, correlation coefficient and statistical testing of the model was made by Fisher's test Myers et al (2016).

RESULTS AND DISCUSSION

Initial screening of 53 isolates was done on the basis of catalase test. Out of 53 isolates 41 isolates were short listed depending upon being catalase negative in nature (tentatively identified as *Lactobacilli*) and 12 isolates were discarded as these were catalase positive (tentatively identified as *Bacilli*). The above selected 41 catalase negative isolates were further screened for antagonistic activity by bit/disk method against test pathogens. The isolates which showed maximum percent inhibition against indicators were subjected to final screening by well diffusion method. Out of 41 isolates 8 isolates were selected for well diffusion assay as these suppressed maximum number of test pathogens. In well diffusion assay, UN exhibited highest percent inhibition i.e. 60% against highest number of test pathogens, thus finally screened for further characterization and preservation related experiments. The 16S rRNA sequences of the isolate have been submitted to GenBank databases. *L. brevis* UN is registered under the accession number JX046150.

Optimization of process parameters for bacteriocin production by using classical one variable at a time method (OVAT): Bacteriocin production is influenced by growth factors. Effect of incubation time, temperature, pH, inoculum size influence the yield of active bacteriocin significantly. Optimum conditions for bacteriocin production have been set by statistical tools i.e. one variable at a time approach and response surface model. Bacteriocin production is growth associated.

Production of bacteriocin during growth cycle: The growth curve of the isolates followed a sigmoid curve pattern based on measuring bacterial turbidity level OD_{540} nm (Fig. 1, 2). The bacterial cultures were incubated at 37^oC in MRS broth (6.5 pH) for different interval of time (24 to 60 h). Optical density and inhibition zones of bacterial culture were measured after 2h of interval at 540 nm. *L. brevis* UN was quite effective against all the three tested indicators viz. *L. monocytogenes*, *S. aureus* and *C. perfringens* within 32nd to 62nd h of production time in the presence of organic acids but after ruling out the acidity of the supernatant it was observed

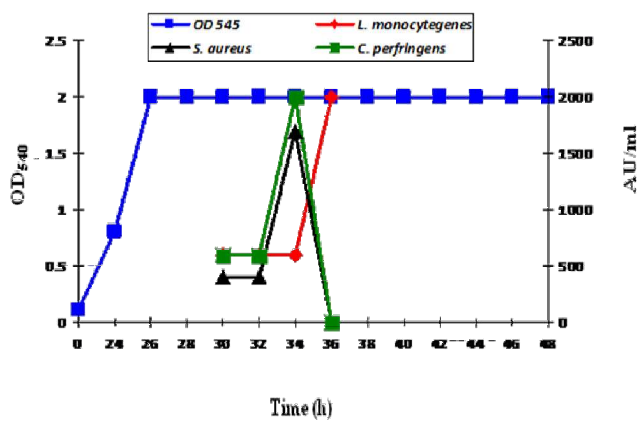


Fig. 1. Growth curve of *L. brevis* UN showing production pattern of bacteriocin

Fig. 2. Effect of different inoculum size on bacteriocin production by *L. brevis* UN

that *L. brevis* was effective against test indicators at 34th h of production time. Maximum bacteriocin production by isolate *L. brevis* UN was measured at 34th h having activity units of 6×10^2 AU/ml against *L. monocytogenes*, 1.7×10^3 against *S. aureus* and 2×10^3 AU/ml against *C. perfringens*. Bacteriocin is produced at a particular time of growth phase so it is necessary to find out that period of growth cycle where bacteriocin is produced. Maximum bacteriocin production by isolate *L. brevis* UN was measured at 34th h. After wards the decrease in bacteriocin production at the end of stationary phase may be due to protein aggregation, proteolytic degradation by specific or nonspecific enzymes and re-adsorption of bacteriocin to the producer cell surface at low pH (Gupta et al 2010).

Effect of temperature: *L. brevis* UN showed best antagonism against all tested indicators at 35°C (Table 1). At this temperature, *L. brevis* UN inhibited the activity of *L. monocytogenes*, *S. aureus* and *C. perfringens* by forming zones of 3mm, 10mm and 10mm with bacteriocin activity 6×10^2, 2×10^3 and 2×10^3 AU/ml. Bacteriocin of *L. brevis* UN failed to inhibit *L. monocytogenes* and *C. perfringens* at growth temperatures 25°C, 45°C and 50°C. In contrast to these test indicators *S. aureus* was inhibited by the same bacteriocin produced at 25°C, 45°C and 50°C. Temperature is a major factor that influences the antagonistic activity of bacterial strain. Growth at optimal temperatures usually results in optimal bacteriocin production but temperature stress and growth at suboptimal temperature may result in decrease in its yield.

Effect of pH: The maximum antagonism and bacteriocin activity by *L. brevis* UN was observed at pH 4.0 and 4.5 (Table 2). The inhibitory zone size and bacteriocin activity (AU/ml) at pH 4.0 and 4.5 against *L. monocytogenes*, *S. aureus* and *C. perfringens* were 3.0 mm (6×10^2 AU/ml), 10mm (2×10^3 AU/ml) and 10 mm (2×10^3 AU/ml), respectively. No inhibition/activity was observed below pH 4.0 and above 6.5 against *L. monocytogenes* and above 7.5 for *S. aureus*. The zone size of 10mm each with 2×10^3 AU/ml were observed against *S. aureus* and *C. perfringenes* while zone of inhibition of 8mm and bacteriocin activity 16×10^2 at pH 4.0 and 4.5

Table 1. Effect of temperature on bacteriocin production by *L. brevis* UN through classical one variable at a time method

Temperature (°C)	*L. monocytogenes*		*S. aureus*		*C. perfringens*	
	Zone size* (mm)	AU/ml#	Zone size* (mm)	AU/ml #	Zone size * (mm)	AU/ml#
25	-	-	10	2×10^3	-	-
30	1	2×10^2	10	2×10^3	-	-
35	3	6×10^2	10	2×10^3	10	2×10^3
40	1	2×10^2	10	2×10^3	5	1×10^3
45	-	-	10	2×10^2	-	-
50	-	-	7	14×10^2	-	-

*Bacteriocin production in terms of inhibitory zone size
#AU/ml: Arbitrary unit (reciprocal of highest dilution forming detectable zone of inhibition) expressed in the form of AU

Table 2. Effect of pH on bacteriocin production by *L. brevis* UN through classical one variable at a time method

pH	*L. monocytogenes*		*S. aureus*		*C. perfringens*	
	Zone size* (mm)	AU/ml#	Zone size* (mm)	AU/ml #	Zone size * (mm)	AU/ml#
3.0	-	-	1	2×10^2	-	-
3.5	-	-	7	14×10^2	-	-
4.0	3.0	6×10^2	10	2×10^3	10	2×10^3
4.5	3.0	6×10^2	10	2×10^3	10	2×10^3
5.0	3.0	6×10^2	8	16×10^2	7.0	14×10^2
5.5	3.0	6×10^2	8	16×10^2	7.0	14×10^2
6.0	3.0	6×10^2	5	1×10^3	5.0	1×10^3
6.5	3.0	6×10^2	5	1×10^3	3.0	6×10^2
7.0	-	-	5	1×10^2	3.0	6×10^2
7.5	-	-	5	1×10^2	-	-
8.0	-	-	-	-	-	-

* Same as in Table 1
# Same as in Table 1

AU/ml was observed against *S. aureus* and 14x10^2 AU/ml against *C. perfringens* at pH 5.5. Every microorganism has a minimal, maximal and optimal pH for growth and metabolism. Microbial cells are significantly affected by the pH of their immediate environment because they apparently have no mechanism for adjusting their internal pH (Bhattacharya and Das (2010). Thus, studying the effect of pH on growth of isolate is an important criterion of the study. In the present investigation it is observed that maximum bacteriocin production by lactic acid bacteria took place at acidic pH. It is a well-known fact that lactic acid bacteria optimally grow under slightly acidic conditions, when pH is between 4.0 to 6.4. Genes and proteins involved in pH homeostasis and cell protection or repair play a role in acid adaptation mechanism of lactic acid bacteria (Yang et al. 2018). The three main systems involved in pH homeostasis in lactic acid bacteria i.e., the ADI system i.e. urease and arginine deiminase pathways, the H - ATPase proton pump, and the GAD i.e. the lysine, arginine, and glutamate decarboxylases (GAD). In addition to all these above mentioned mechanisms LABs are also capable of inducing an acid tolerant response to mild-acid treatment. The systems induced by this response include pH homeostatic, protection, and repair mechanisms (Cotter 2003).

Effect of inoculum size: Highest bacteriocin production by *L. brevis* UN was with an inoculum of 1.5 OD (Fig. 3). Bacteriocin of *L. brevis* UN with inoculum size of 1.5 OD inhibited *L. monocytogenes*, *S. aureus* and *C. perfringens* by forming zones of 5mm (1×10^3), 10 mm (2×10^3) and 10 (2×10^3) respectively. Though, bacteriocin of *L. brevis* UN was produced with an inoculum size of 2.0 OD inhibited all the three test indicators but potency of inhibition was less as it form zones of 3mm (6×10^2), 8mm (16×10^2) and 7.5 (15×10^2) against *L. monocytogenes*, *S. aureus* and *C. perfringens*. *L. brevis* UN did not inhibit *L. monocytogenes* and *C. perfringens* when seeded with inoculum of OD 0.5 and 1.0. Whereas *L. brevis* UN produced bacteriocin with each tested inoculum i.e. OD 0.5, 1.0, 1.5, 2.0 that was found active against *S. aureus* and formed zones of 3 , 7.5, 10 and 8 mm with bacteriocin activity of 6×10^2, 15×10^2, 2×10^3 and 16×10^2 AU/ml, respectively. Increase or decrease in bacteriocin production by changing the inoculum size may be explained on the basis of ratio of number of nutrient molecules to the number of microbial cells. At lower concentrations all the nutrients seemed to be occupied by the available microbial cells and some of the nutrients remained without access to the cells by increase in the inoculum size, thus the biosynthesis of bacteriocin is often an inducible trait that depends on the cell density of the cell culture Usmiati and Marwati (2009). Therefore, in the present study the improved

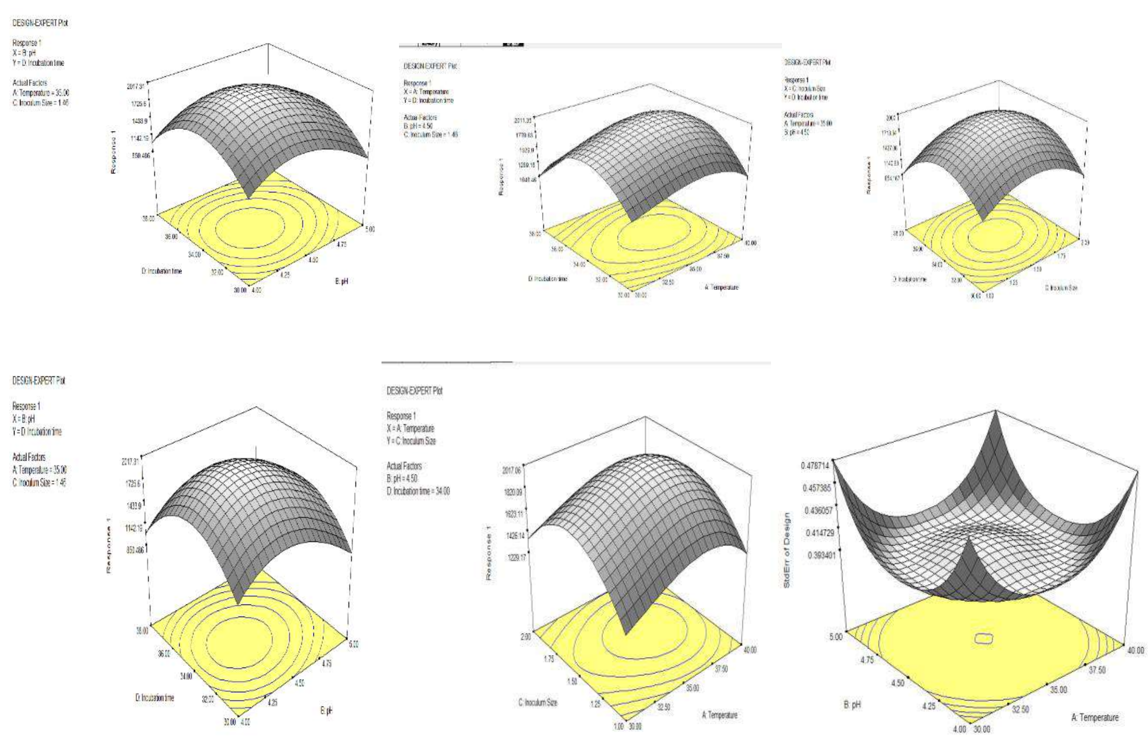


Fig. 3. Response surface curves for bacteriocin activity as influenced by the level of pH, incubation temperature, inoculum level and incubation time

bacteriocin production was achieved by optimizing the inoculum size of both the isolates.

Optimization for bacteriocin production by using Response Surface Methodology: The variables temperature, pH, inoculum size and incubation time were added for the optimization by RSM. The results of 30 run from CCD experiments for studying the effects of 4 independent variables on bacteriocin production are represented in Table 3. The maximum experimental value for bacteriocin production was 2000 AU/mL based on RSM. The regression analysis data were fitted to a quadratic model and the second order regression equation obtained was full actual model on bacteriocin production is shown in Eq. (3)

$$Y = + 2000 + 141.67^{*}A - 208.33^{*}B + 41.67^{*}C - 58.33 -^{*}D - 257.29^{*}A^{2} - 432.29^{*}B^{2} - 432.29C^{2} - 582.29^{*}D^{2} - 43.75^{*}A^{*}B - 31.25^{*}A^{*}C + 43.75^{*}A^{*}D - 56.25^{*}B^{*}C + 18.75^{*}B^{*}D + 56.25^{*}C^{*}D$$

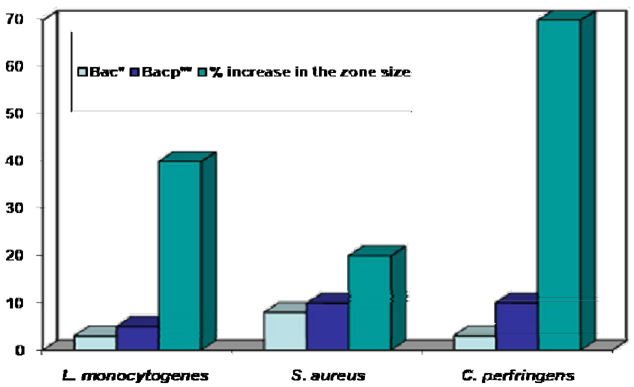
Where Y is bacteriocin activity AU/ml. A is temperature, B is pH, C is inoculum size and D incubation time. The statistical significance of equation 3 was checked by F-test implies model is highly significant. There is only a 4.20% chance that a "Model F-Value" this large could occur due to noise. The goodness of the model can be made by the determination coefficient (R^2) and the correlation coefficient (R). Values of "Prob > F" less than 0.0500 indicate model

Table 3. Bacteriocin activity of the culture *L. brevis* UN cultivated with different levels of pH, temperature, inoculum size and incubation time

Temperature	pH	Inoculum size	Incubation time	Bacteriocin activity
33	4.30	1.70	32	600
37	4.70	1.30	32	600
35	4.50	1.50	34	2000
31	4.50	1.50	34	800
35	4.50	1.90	34	600
35	4.50	1.50	34	2000
35	4.50	1.50	34	2000
35	4.50	1.10	34	600
33	4.30	1.30	32	600
37	4.30	1.70	32	600
33	4.30	1.30	36	400
35	4.50	1.50	38	0
35	4.50	1.50	34	2000
37	4.70	1.70	36	600
37	4.30	1.70	36	400
33	4.30	1.70	36	400
35	4.10	1.50	34	400
33	4.70	1.30	32	800
37	4.30	1.30	36	1000
39	4.50	1.50	34	0
35	4.50	1.50	34	2000
37	4.30	1.30	32	600
33	4.70	1.30	36	600
35	4.50	1.50	30	0
35	4.90	1.50	34	2000
37	4.70	1.50	36	1000
33	4.70	1.30	36	600
37	4.70	1.70	32	800
33	4.70	1.70	32	400
35	4.50	1.50	34	2000

Table 4. R -Squared, Adj R-Squared, Pred R-Squared and Adeq Precision value of the model

Std. Dev	466.88	R-Squared	0.9201
Mean	636.67	Adj R-Squared	0.6689
C.V. (%)	73.33	Pred R-Squared	-10.5099
Press	2.197E+008	Adeq Precision	5.698



Bac* = Initial production, Bacp** = Production after optimization

Fig. 4. Per cent increase in bacteriocin production of *L. brevis* UN after optimization incubation conditions through classic one variable at a time method

terms are significant. In this case A^2, B^2, C^2, D^2 are significant model terms. A negative "Pred R-Squared" implies that the overall mean is a better predictor of response than the current model."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 5.698 indicates an adequate signal. This model can be used to navigate the design space (Table 4). Response surface representation provides a method to show relation between the response and experimental levels of each varabile. This technique has been widely adopted for optimizing the process of bacteriocins of *Pediococcus pentosaceous* Sanna (Upendra et al 2021). The increase in size of zones of inhibition after optimization is directly associated with the increase in bacteriocin production.

Per cent increase in bacteriocin production after optimization: The zone of inhibition of bacteriocin of *L. brevis* UN against test indicators increased after optimizing various parameters. The inhibitory zone size of bacteriocin of *L. brevis* UN increased 40% against *L. monocytogenes*, 20% against *S. aureus* and 70% against *C. perfringens* (Fig. 4).

CONCLUSIONS

The increase in bacteriocin production after optimization revealed that the conditions of incubation particularly incubation time, temperature, pH, inoculum size influence the yield of active bacteriocin significantly. Bacteriocin production was maximum at early stationary phase i.e. 34th h, at pH range 4.0-6.5, at temperature 30-35 °C with an inoculums size 1.0, 1.5, 2.0 OD at 10 %. It is necessary to optimize various process parameters of bacteriocin producing isolates to achieve maximum bacteriocin production. Response surface methodology (RSM) results indicated all the four factors studied have significant effect on bacteriocin production and proved to be a powerful tool in optimizing the culture conditions.

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Cryotherapy: An-innovative Tool for Eradication of Pathogens in Horticultural Crops

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Abstract: Cryotherapy has a wide range of applications in agriculture, horticulture, biotechnology and breeding programs. Cryopreservation can be used as an alternative preservation method for various crops. Ultralow temperatures can be employed not only for germplasm storage but also for the eradication of plant infections. It may potentially have applications in the protection of endangered plant species germplasm. A wide variety of horticultural crops, including fruits, vegetables, and ornamental plants, have cryopreservation techniques that can be utilized as such or adapted for cryotherapy, which intensifies the eradication of pathogens in infected plant tissues. Several pathogens in some of the horticultural crops such as banana, *Citrus spp.,* grapevine*, Prunus spp.,* raspberry, potato and sweet potato have been eradicated using cryotherapy. Disease-free stock plants are necessary for propagation and healthy materials are required for the exchange of germplasm across nations or regions via quarantine programs. In addition, plant gene banks seek to keep germ-plasm collections free of pathogens. To tackle this challenge cryotherapy can act as an innovative method for the elimination of plant pathogens. This review analysed several methods and procedures for cryopreservation of plant germplasm and how it is helpful in eradicating pathogens.

Keywords: Pathogen, Cryotherapy, Germplasm, Horticultural crops, Viruses

Cryopreservation is accomplished by immersing plant materials at extremely cold temperatures, typically liquid nitrogen at -196 °C or liquid nitrogen vapour at -165ºC to -190ºC (Zhao et al 2019) utilized to eradicate viruses, phytoplasmas, and bacteria from plant meristems. Cryopreservation has various advantages for storing live plants for long periods of time, including minimal storage space and maintenance requirements, as well as maximum stability of phenotypic and genotypic traits of saved germplasm. Cell dehydration is regarded as an important step before immersing tissues in liquid nitrogen. Cryopreservation has been used effectively on a wide variety of plant species, including herbaceous plants from both tropical and temperate regions, as well as woody species. Viruses are commonly recognized to spread unevenly throughout plants, and meristems may be virus-free or have a low viral concentration. Cryopreservation of shoot tips involves the use of liquid nitrogen, which causes the cells in the lower area of the apical dome to be destroyed. Only a few of the cells in the top layers of the dome are able to survive. Therefore, there is a possibility that viruses can be eradicated from cryopreserved shoot tips (Wang et al 2009).

Cryotherapy of shoot tips using cryopreservation: Cryotherapy of shoot tips is a potential strategy for eradicating plant pathogens that may be easily applied to a wide range of species and cultivars using existing cryopreservation protocols (Fig. 1). It can be performed in a tissue culture laboratory with minimal equipment, exhibits promising results in the creation of pathogen-free regenerants, and reduces the danger of genetic alterations during therapy compared to traditional approaches. More than 10,000 accessions of in vitro propagated crop plants are currently safely cryopreserved for the long term, with more than 80 percent of these belonging to five major crops (potato, cassava, bananas, mulberry, and garlic). Cryotherapy is a novel biotechnology method that is based on cryopreservation (Wang et al 2014, Wang et al 2009). According to Wang et al (2008) cryopreservation of shoot tips is a technique in which shoot tips are induced to ultra-low temperature, stored and regenerated for multiplication. Shoot tips subjected to cryopreservation or cryotherapy are typically the size consisting of apical or lateral shoot meristem (1–1.5 mm in size) with three to four leaf primordia (Benson, 2007). Meristem cells are subjected to highly packing to leave without intercellular cavities, are small and isodiametric in structure and consist of a large nucleocytoplasmic volume ratio. Sizes of cells and vacuoles increase and the nucleocytoplasmic ratio decreases with increasing distance from the apical dome (Wang et al 2008).

Need for plant pathogen eradication and preservation:

Since the dawn of time, pathogens such as viruses, viroids, and phytoplasmas have posed a threat to horticultural growth (Zhao et al 2019). All crops must have access to pathogen-free planting materials in order to produce excellent yields and quality. There are many economically important crops that are usually propagated through the vegetative method so it makes susceptible to pathogen attack (Skoric 2017). Many economically important horticultural crops, such as citrus, pome and stone fruit trees, berry crops, and many ornamental plants have been attacked by different pathogens namely viruses, mycoplasma, and rickettsia diseases of fruit trees. According to Hogenhout et al (2008), few infections attack only tissues that enable their independent mobility and are irregularly distributed. Infected plants domes or the youngest part of the meristematic tissue, are usually either pathogen-free or have a very low concentration of viruses and phytoplasmas.

Cryopreservation of plant germplasm: There are many ways to preserve plant germplasm including seeds, tubers, roots, bulbs, corms, rhizomes, buds and cuttings. Since all biochemical and most physiological processes are halted at -196°C, cryopreservation or freeze-preservation, which entails storing the germplasm at ultra-low temperatures, is the approach of choice for long-term conservation. Recently, several plant species have been tested for this technology with a specific protocol like banana, citrus spp Grapevine, raspberry, sweet potato, etc.

Cryopreservation methods: The existing cryogenic strategies rely on air-drying, freeze dehydration, osmotic dehydration, the addition of penetrating cryoprotective substances and adaptive metabolism (hardening) or combinations of these processes.

Air drying: This is the most frequent approach for reducing cell water content in hydrated tissues, and it is sometimes referred to as the desiccation technique. This technique was mostly used on pollen, seeds, and embryogenic axes. The second common method of dehydration is air-drying, in which water is eliminated by airflow. In any case, dehydration is necessary for efficient cryopreservation to prevent intracellular freezing and permanent cell damage caused by ice crystal formation (Kaviani et al 2011).

Classical freezing protocol and preculture: Slow freezing and one-step freezing are the two most prevalent methods of cryopreservation (Kalaiselvi et al 2017). Samples must be prepared with cryoprotectants such as DMSO, sucrose, etc. before freezing slowly (also known as the two-step freezing procedure). They are then instantly submerged in liquid nitrogen and chilled to 40°C at the rate of 0.03–0.5°C/min. One-step freezing, on the other hand, does not necessitate any additional equipment. Cryogenic techniques like vitrification, encapsulation and cryoplates have recently been created despite the fact that older methods have been employed successfully on a variety of plant materials.

Vitrification: This approach is one of the most commonly used for plant cryopreservation since it is relatively simple to implement, does not require specialized equipment, and typically yields a high percentage of recovery. The formation of an amorphous glassy structure from intracellular solutes is the cornerstone of vitrification (Kaviani et al 2011). The dried material is then immersed in liquid nitrogen and rapidly frozen. PVS1, PVS2, and PVS3 are the three most commonly reported glycerol-based vitrification solutions. Two basic vitrification-based techniques, encapsulation vitrification and droplet vitrification have been developed

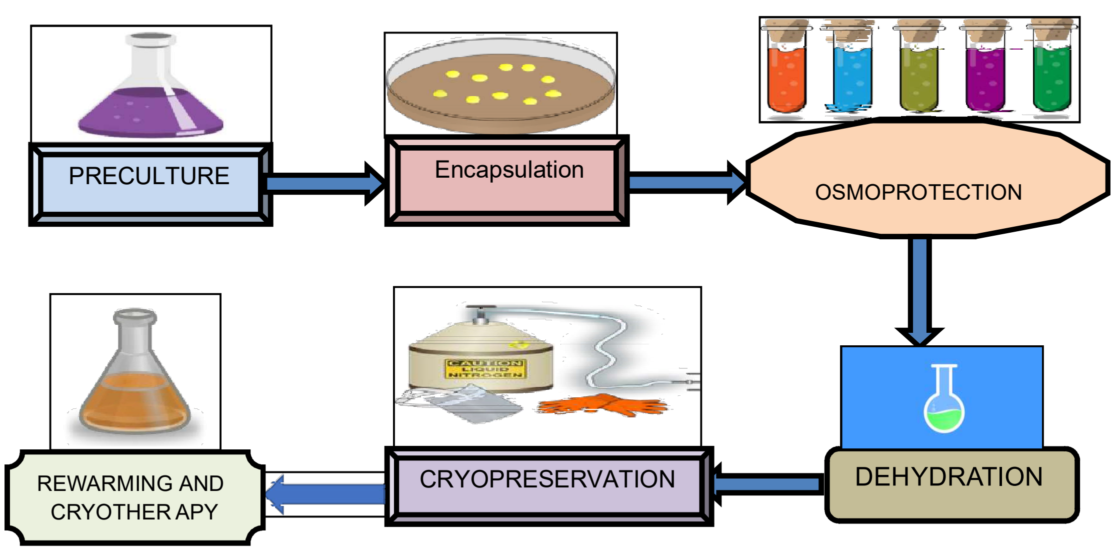

Fig. 1. Cryopreservation protocols for the cryotherapy of shoot tips

through the modification and optimization of vitrification procedures.

Cryo-Plate methods: Cryoplates are utilized in the most advanced cryogenic methods. On the basis of the dehydration operation, two basic cryoplate approaches may be identified: the V and D cryoplate methods (Matsumoto 2017). Explants are dehydrated using the V technique on cryoplates utilizing PVS2 vitrification (Yamamoto et al 2011), whereas explants are dehydrated using air using the D technique on cryoplates (Ninno et al 2013). Shot tips are put into tiny wells of an aluminum cryoplate loaded with alginate beads and dehydrated using either a PVS2 solution or air flow in a laminar flow cabinet, depending on the technique. The cryo plate is then submerged in liquid nitrogen instantly. Cryoplate methods are mostly user-friendly since manipulating samples on aluminum plates is straightforward (Yamamoto et al 2011, Ninno et al 2013). Several plant species, including the strawberry (Yamamoto et al 2012) and date palm (Salma et al 2014), have been successfully cryopreserved utilizing cryoplate techniques.

Dehydration: The first stage in cryopreservation is dehydrating tissues to remove water that can freeze. The water content of less than 0.25 g H_2O g/dm (dm; dry mass) is typically referred to as "unfreezable" water, and plant cells holding 0.25-0.4 g H_2O g/dm (dry mass) typically survive exposure to liquid nitrogen (Volk et al 2006). Proper dehydration can be achieved osmotically by administering extremely concentrated solutions; in this scenario, the concentration gradient between the solution and intracellular fluid provides the driving force for dehydration. The second common method of dehydration is air-drying, in which water is extracted by airflow. In any case, dehydration is necessary for efficient cryopreservation to prevent intracellular freezing and permanent cell damage caused by ice crystal formation (Kaviani et al 2011).

Pathogen eradication from economically important horticultural crops: There has been a lot of research done on cryotherapy employing shoot tips for pathogen eradication (Table 1). All *Prunus* spp. are susceptible to Sharka pox virus (Damsteegt et al 2007). The success rate was higher in cryo treated shoot tips, with 96 percent of the regenerated plants virus-free, compared to only 12 percent in meristem culture (Wang et al 2008). They also created a new technique for virus elimination in which RBDV-infected shoot tips (1 mm) were first treated to thermotherapy under *in-vitro* conditions, followed by cryotherapy. After 28 days of heat treatment, the survival (36%) and regeneration (30%) of cryo treated shoot tips were obtained using this method. Wang et al (2008) discovered that thermotherapy followed by cryotherapy resulted in virus-free plantlets in 33-35 percent of cases. According to studies, Ribavirin is the most important antiviral agent for controlling potato plant viruses when used alone or in combination with other treatments such as meristem culture, cryotherapy, and electrotherapy (Kushnarenko et al 2017). According to Waswa et al (2017), thermotherapy is one of the efficient strategies for eradicating the potato virus, which relies on the kind of virus, potato cultivar, and the length of heat treatment. Chemotherapy is ineffective when administered at low doses (Yang et al 2013), but when used at high doses, it generally inhibits potato plant development. As a result, by combining the two therapies, the regeneration capacity of plantlets can be improved.

Effects of combined treatments: Thermal, cold and chemotherapeutic treatments have been utilized in the past to remove infections from various plant species, and cryotherapy has also been used to enhance pathogen eradication (Jeon et al 2016, Barba et al 2017, Kushnarenko et al 2017, Zhao et al 2018). Combining thermotherapy and cryotherapy improves pathogen eradication because the pathogen-free regions are larger and fewer shoot tip cells

Table 1. Cryotherapy techniques' effectiveness in eliminating viruses from horticultural plant species

Plant species	Pathogen	Pathogen-free (%)		References
		Cryopreserved shoot tips	Shoot tip culture	
Malus x domestica	Apple stem grooving virus (ASGV)	30-100[a]	22	Zhao et al (2018)
Malus x domestica	Apple stem pitting virus (ASPV)	100	0	Bettoni et al (2018)
Vitis vinifera	Grapevine fanleaf virus (GFLV)	78	82	Marković et al (2015)
Prunus armeniaca	Plum pox virus (PPV)	100	-	Sekar et al (2015)
Solanum tuberosum	Potato virus M (PVM)	100[b]	-	Kushnarenko et al (2017)
Solanum tuberosum	Potato virus S (PVM)	100[b]	-	Kushnarenko et al (2017)
Allium sativum	Garlic common latent virus (GCLV)	80	13	Vieira et al (2015)
Allium sativum	Leek yellow strip virus (LYSV)	100[b]	35	Vieira et al (2015)

[a]Thermo+cryo; [b]Chemo+cryo

survive the cryotreatments (Wang 2008, Zhao et al 2018). Thermotherapy has some additional advantages as it reduces virus tire and also enhances virus-induced gene silencing (Liu et al 2016, Zhao et al 2018). As discussed earlier the combination of cryotherapy and thermotherapy resulted in 33 to 36% of the recovered plants being free of RBDV (Raspberry bushy dwarf virus (Wang et al 2008). Around 30 to 100% ASGV-free plants for four apple cultivars and rootstocks were obtained in a combination of thermotherapy and cryotherapy but this was not possible when shoot tips were treated only with cryotherapy treatment (Zhao et al 2018). Cold therapies have advantages when combined with thermotherapy because they limit viroid replication and reduce viroid mobility in the hosts (Barba et al 2017; Jeon et al 2016, Zhang et al 2016). Jeon et al (2016) discovered that cold therapy followed by cryotherapy improves CSVd (Chrysanthemum stunt viroid) removal from in vitro shoot tips. This combination (cold and heat treatment) resulted in 20% CSVd-free plants. However, only 13% of plants were viroid-free after being treated with cryotherapy alone (Zhao et al 2019). Aside from thermotherapy and cold therapy, chemotherapy has the potential to be useful since it suppresses viroid RNA production, reduces viroid titer, and expands viroid-free zones in the shoot tips (Barba et al 2017). 'Asana' and 'Nikitka' potato cultivars were free of PVM in 20% and 57 % of cases, respectively, however both cultivars were not free of PVS. It was estimated that 100 percent of the plants that had been regenerated were free of PVM and PVS after 135 days of in vitro ribavirin treatment (Kushnarenko et al 2017).

Advantages and limitations of cryotherapy: Using cryotherapy to remove diseases from numerous plant species can improve or perhaps replace some of the more traditional approaches. Studies have shown that cryotherapy methods are simple to implement and do not require specific tools or equipment beyond those found in a plant tissue culture facility. Furthermore, it makes it easier to process vast quantities of samples (Bhojwani and Dantu, 2013). It produces pathogen-free plants with a high frequency, minimizing the challenges associated with the excision of extremely small meristems. The vegetative and root growth of virus-free plants was substantially greater than that of virus-infected plants (Li et al 2018a).

CONCLUSION

Cryotherapy is a viable, well-organized approach for the successful removal of plant diseases in a wide range of horticulture species. Cryogenic techniques limit the amount of viable contaminated tissue in several fruits, vegetables, and ornamental plants, cryopreserved shoot tips may be regarded as safer for exporting germplasm between regions and countries. For instance, post-thaw regeneration of major agricultural species remains quite low. In this study, we demonstrate that much of the work in the field of plant cryobiotechnology has already been completed. However, significant obstacles continue to impede the expansion of cryopreservation's use. Variations in morphological and yield variance in micro propagated bananas have been found under *in vitro* conditions. As a result, the phytosanitary status of regenerated plants must still be determined. Plants regenerated after cryotherapy should be tested for true-to-type since tissue culture can cause genetic instability in plants. More plant species and cultivars will necessitate the optimization and modification of existing techniques in the foreseeable future.

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Estimating Volume and Mass of Tomato Fruits by Image Processing Technique

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Abstract: A technique based on image processing was developed to determine volume and mass of tomato fruits. The images of different grades of tomato were attained using a digital camera. A MATLAB based algorithm was developed to enumerate and process these digital images. The geometric characteristics such as axial dimensions, mass, volume, density, sphericity, aspect ratio and ellipsoid ratio were recorded. The size of tomato varied from 30.00-77.62 mm. The bulk density was maximum (0.26 g/cc) for tomato of size 57-66 mm. The aspect ratio was higher than unity for tomatoes of all grades indicating the variation in length with respect to width. The differences between two methods were normally distributed and predictable to lie between M - 1.96SD and M + 1.96SD, known as 95% limits of agreement. The paired samples t-test results showed that parameters observed with image processing method was not significantly different from the measured using vernier caliper. A linear relationship between mass of tomato and the projected area, volume and axial dimensions was also developed for calculation of mass using image processing. This information can be used to design and develop sizing systems.

Keywords: Tomato, Image processing, Volume, Mass, Grading

The knowledge of engineering properties of horticultural products is very important in developing new consumer products, evaluating and retaining the quality of products and designing of machines, processes and controls. The physical properties of food materials (raw, unprocessed and processed) include particle size and shape, density, surface area and porosity. Among all the important quality parameters, fruit size and shape are one which is mostly preferred by consumers i.e. consumer prefers fruits of uniform size and equal weight. So, the determination of fruit size and shape is very important in meeting the standards of quality parameters which results in monitoring the increasing market growth. The determination of physical properties such as size, unit mass, volume, sphericity and density using advanced technologies were reported by many researchers. In recent researches, it has been estimated that there is a huge loss of crop before consumption because of poor handling, storage, transportation and marketing practices. One of the major reasons for this loss is the time-consuming manual grading. Grading of the agricultural produce is considered very important as it improves handling, packaging, transportation and other post-harvest operations. The process of grading of fruit starts with the sorting of fruits by size, shape and colour by humans assessed by sight and feel. The increasing cost of labour and also demand for consistency in quality of products lead to mechanization of the sorting process (Tabatabaeefar and Rajabipour 2005, Lorestani and Tabatabaeefar 2006, Shahi-Gharahlaretal 2009, Adebowale et al 2011, Seyedabadiet al 2011, Ercisli et al 2012, Shahbazi and Rahmati 2013).

The application of image processing-based methods in agricultural activities has been developed for years (Lak 2011). Nowadays, commercial fruits such as strawberries, orange, peaches, tomato and apples have been graded by the use of image processing and machine-vision technology (Spreer 2009). The main components of machine-vision technology consist of a color CCD camera with an image capturing device and a lighting source to evaluate fruit based on different engineering properties such as size, shape, color and defection. Due to the availability of infrastructures, the automated sorting/ grading in various food industries had suffered substantial growth in the developed and developing countries. The application of computer in field of agriculture and food industries has resulted in better sorting, grading of fresh crops, recognition of defects such as cracks, dark spots and bruises on fresh fruits and seeds. So, automation is one of the most important aspects of agricultural mechanization. It allows improvement in efficiency, increase in capacity and protection to human workers from tedious and hazardous activities. The digital image processing has become very important and more probable to many areas including agricultural industry (Yimyam et al 2005, Chalidabhongse 2006, Bulanon 2012). The use of machine vision knowledge for quality examination, classification, sorting, and grading agricultural products is becoming more fascinating (Teoh 2010). This technology has been extensively used in the agricultural and food industry for evaluation and inspection as they offer suitable, quick and economic assessment. The automated examination of produce using this technology not only results in labor savings, but also advances inspection objectivity (Valente 2009, Spreer 2009). The development in hardware and software for digital image processing and their use in analysis of food crops have inspired several studies for the development of the system which can assess the quality of different foods. So as the manual and mechanical system which is currently being used for sorting/grading is labour intensive and also the new technology of image processing has not been fully explored in India for up gradation of the sorting/grading equipment, there is a need to develop such system which can meet the demand of the products in market without compromising the quality parameters. The present study has been planned to develop a system based on image processing technology to perform functions of sorting/grading based on volume and mass with greater precision at a faster rate without any drudgery.

MATERIAL AND METHODS

The tomato of variety *Punjab Chhuhara* was considered for the present study. A total of 25 fruits each of different grades of tomato were selected at random from the storage piles. The mass of each fruit was measured by an electronic weighing scale with an accuracy of ±0.1g. The axial dimensions (length, width and thickness) of tomato were measured using a digital vernier caliper with an accuracy of 0.01mm. The other parameters like shape, density, sphericity and true volume were determined using standard methods (Mohsenin 1986). Based on the shapes of tomato i.e. considering them to be ellipsoid, volume of fruits was calculated using three axial dimensions of fruits and geometric mean diameter. The following equations were used in calculations.

$$\text{Volume of ellipsoid} = \frac{4\pi}{3} \times L \times W \times T \ (\text{mm}^3)$$

Where L, W T are length, width, thickness in mm

$$\text{Change in volume}(\%) = \frac{\text{True volume - Calculated volume}}{\text{True volume}} \times 100$$

The same parameters were determined using image processing technique also. The setup of image processing consisted of a digital camera (AF-S DX NIKKOR 18-55mm f/3.5-5.6G VR II) connected to computer having image processing software. The camera was mounted on the image acquisition box for keeping the standardized distance. The height of optical lens of camera was tested for three different heights based on the size of tomato of different grades and height with the best results was selected. For tomato, the different heights of optical lenses tested were 145mm, 160mm and 175mm. Among them, height of 160mm was selected for capturing images of tomato. Images were captured in natural illumination. The camera was mounted on the tripod stand for keeping the distance of the fruit from the camera uniform. The analysis of the images of tomato for different grades was done using Image processing software (MATLAB R2013a, version 8.1). The flowchart showing image analysis algorithm used for processing of different images of tomato is given in Figure 1. The fruit images were calibrated for axial dimensions and other geometric properties by using images of a square sheet of black colour of size 5cm x 5cm under similar conditions. The data related to geometrical features attained by image processing of tomato was compared with the measured physical parameters and a correlation was studied between measured parameter (size, volume etc.) and those analyzed from image. The analysis was based on plots between different physical parameters like axial dimensions and volume determined by both the methods. The mass of tomato was also predicted on the basis of volume.

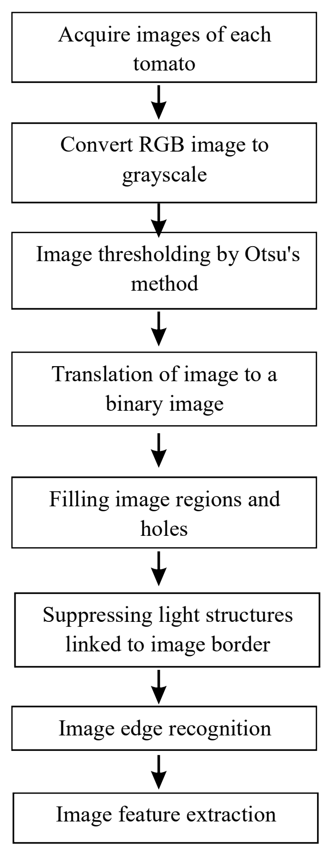

Fig. 1. Flow diagram for image processing and analysis

Statistical analysis: The statistical analysis was performed using Descriptive Statistics at 95% confidence level for mean in Microsoft Excel 2007. The paired t-test and the Bland-Altman plots (the mean difference confidence interval approach) were used to compare the engineering characteristics determined by image processing technique and the standard method. The paired t-test was used to test the significance of difference between the two measurements. A correlation was developed between parameters measured by both the methods. The Bland-Altman approach was used to plot the agreement between the values measured by both the methods.

RESULTS AND DISCUSSION

Tomato fruits of variety *Punjab Chhuhara* were separated into six grades viz. Grade A (30-34 mm), Grade B (35-39 mm), Grade C (40-46 mm), Grade D (47-56 mm), Grade E (57-66 mm) and Grade F (67-81 mm). A total of 150 fruits were evaluated for each parameter. The length of the tomato fruit was considered as the major criteria for grading categories. The length of tomato fruits varied(mm) between 30.00-33.99, 35.04-39.23, 40.19-46.00 48.17-55.90, 57.34-65.85 and 67.11-79.48 for grades A, B, C, D, E and F respectively. The true volume of different grades of tomato was determined using water displacement method (Table 1). The true volume of smallest grade of tomato was 4666. mm^3 whereas it was 38760 mm^3 for largest grade.

Linear relationship was observed between calculated volume and true volume for ellipsoid shape of tomatoes (Fig 2). In addition to this, the value of R^2 for all the samples of tomatoes of respective grades was greater than 0.9. This was in agreement with by Kumar et al (2013) for selected dry beans. Therefore, both the methods of volume measurement i.e. either by using equivalent diameter or by the three different axial dimensions can be used successfully. The percent change in volume was 89.74, 5.41, - 30.61, - 135.43, - 57.89 and - 68.1 for respective grades. The negative values indicated that calculated volume was higher as compared to that of true volume.

Engineering parameters of tomato by image processing: The physical properties of tomato fruits were also extracted from images with the help of MATLAB software. The parameters like major axis diameter, minor axis diameter, equivalent diameter were extracted from the images. The fruit images were calibrated by taking images of a black colour square sheet of size 5cm x 5cms from the standard height i.e. 160 mm selected for tomato mm.

For tomato, 1mm = 17 pixels

The volume calculated from the images of tomato (longitudinal and lateral view) of different grades also showed correlation with the measured true volume (Fig. 3). The value of R^2 for respective grades was observed to be 0.814, 0.915,

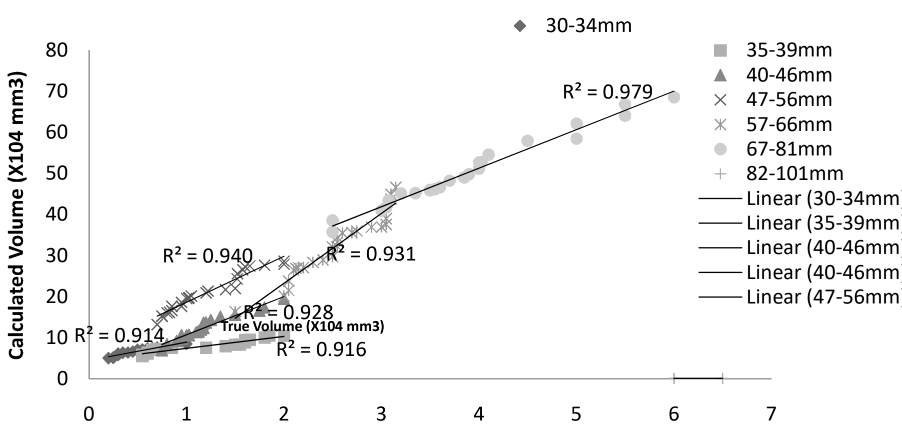

Fig. 2. Relationship between true volume and calculated volume for ellipsoid shape of tomato

Table 1. Volume of different tomato

Characteristics	Grade A (30-34 mm)	Grade B (35-39 mm)	Grade C (40-46 mm)	Grade D (47-56 mm)	Grade E (57-66 mm)	Grade F (67-81 mm)
Volume of ellipsoid tomato (mm^3)						
Mean	65010.15	78455.96	123993.80	210424.60	324000.10	500562.50
Standard deviation	12174.01	16944.40	30896.90	46718.50	83630.18	88059.16
True volume (mm^3)						
Mean	4666.67	11777.78	11779.00	12207.00	25170.00	38760.00
Standard deviation	2135.30	5341.41	3030.17	4042.89	4324.54	9283.02

0.910, 0.971, 0.893 and 0.963 for longitudinal view and 0.907, 0.920, 0.897, 0.969, 0.961 and 0.932 for lateral view. The relationship between the volumes of overall tomato measured with both the methods was also developed and is shown in Figure 4. The value of R^2 was 0.923.

Bland-Altman plot of mean values and differences of volumes for tomato obtained by image processing method and water displacement method were also plotted to check the agreement between both the methods (Fig. 5). The volumes determined by both the methods for tomato were normally distributed and was M = 14438.01 mm^3 (95% confidence interval). The 95% limits of agreement for comparison of volumes for tomato measured with both the methods were e -4848.25 mm^3 and 33724.27 mm^3 (Fig. 5).

The same pattern was observed by Khojastehnazhand et al (2009) for oranges, Rashidi et al (2007) for kiwifruit volume and Soltani et al (2010) for banana volumes. The paired samples t-test results showed that volume obtained with image processing method was not significantly different from the parameters measured with water displacement method for tomato (Table 2). Hence the images of tomato captured with digital camera can be efficiently used for the study of geometrical properties.

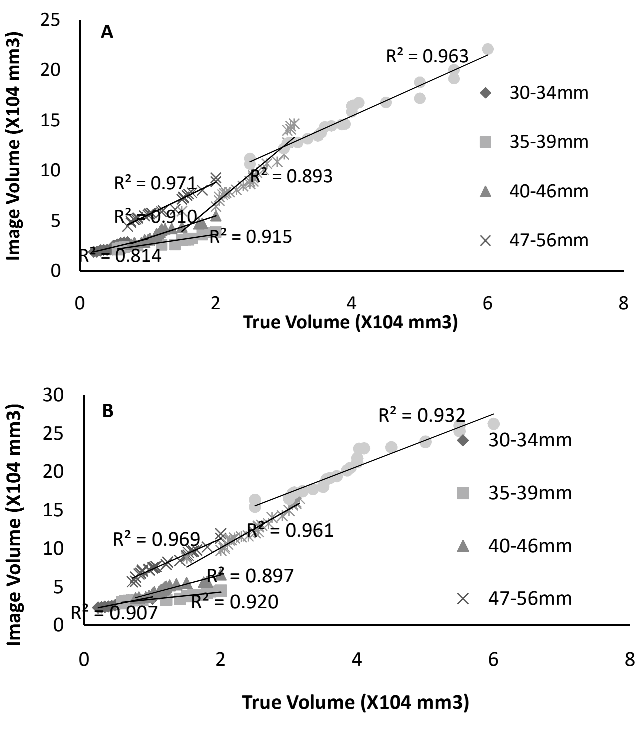

Fig. 3. Relationship between true volume and volume worked from images of different grades of tomato (a) Longitudinal view (b) Lateral view

Prediction of mass based on volume: The connection between mass of the fruits and the volume estimated by both water displacement and image processing method was also studied (Fig. 6). The R^2 for water displacement method was 0.971 whereas was 0.984 for volume determined by image processing method. The volume and mass are highly

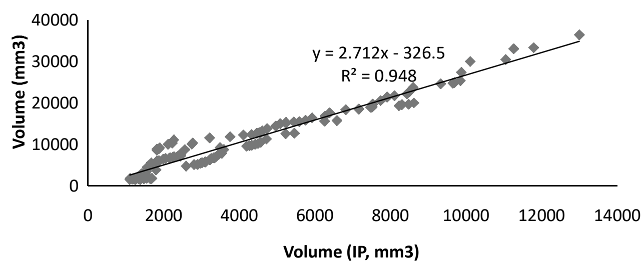

Fig. 4. Relationship between the volume of tomato measured with water displacement method and calculated from image

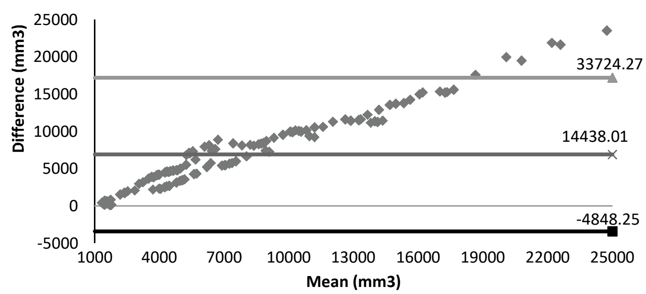

Fig. 5. Bland-Altman plot for the assessment of volume of tomato computed with image processing (IP) method and measured by water displacement method

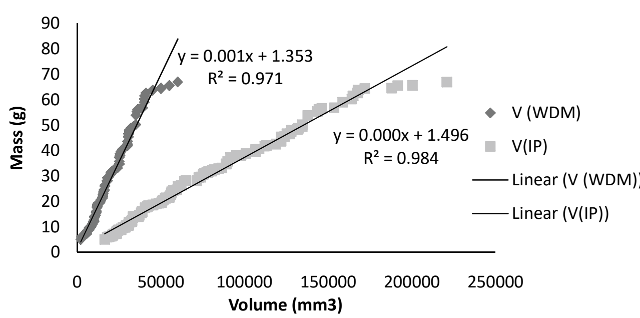

Fig. 6. Relationship between mass and volume of tomato

Table 2. Paired sample t-test analyses on a comparison of volume measurement methods for tomato

Parameter	Size	Average difference (mm^3)	Standard deviation of difference (mm^3)	P value	95% confidence intervals for the difference in means (mm^3)
Tomato	150	6879.79	5249.52	0.723	6031.98, 7727.61

correlated. The same result was obtained by Omid et al (2010) while estimating mass and volume of citrus fruits using image processing. The data was concluded to be best fit for mass determination based on volume of fruit using image processing technique.

CONCLUSIONS

The two methods were presented for estimating the volume of tomatoes. Both the methods were relatively general and may be applied for volume calculation of other ellipsoidal agricultural crops such as peaches, eggs, onion, lemons and kiwifruit etc. The volume calculated from the images of different grades of tomato showed good correlation with the volume measured by water displacement method. Also, the results for various tomato fruits showed that the volume and mass are highly correlated. Mass can be predicted easily using image processing data. The paired samples t-test results showed that parameters determined by image processing method was not significantly (P>0.05) different from the those measured using vernier caliper. This information can be used to design and develop sizing systems.

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Response of Potato (*Solanum tuberosum* L.) Varieties to Different Levels of Nitrogen under Agroclimatic Conditions of Meghalaya

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***Abstract*:** Nitrogen (N) is a very dynamic yield-limiting plant nutrient and excessive N fertilization beyond crops demand has led to soil acidification and major environmental issues. Nitrogen use efficiency of potato cultivars is vary widely and Nitrogen use efficiency was found to be very low for some cultivars. We investigated the response of potato varieties (Kufri Girdhari and Kufri Himalini) to different levels of nitrogen (0, 70, 140,1 75 kg N ha^{-1}) under Meghalaya conditions. The results of the two-year experiment indicated that total tuber yield was significantly influenced by both potato variety and N fertilizer treatment. Compared to Kufri Girdhari, Kufri Himalini recorded 26 percent higher tuber yield than its counterparts. Regardless of variety, the application of 175 kg N ha^{-1} with RDP and RDK resulted in the highest total potato tuber yield (21.84 t ha^{-1}). The agronomic nitrogen use efficiency decreased linearly as N doses increased. Kufri Himalini was more responsive to nitrogen fertilizer than Kufri Girdhari as it recorded higher Nitrogen use efficiency and Partial factor productivity in all nitrogen levels. Being more responsive to fertilizer nitrogen, Kufri Himalini is cultivated with 175 kg N $ha^{-1}$to produce the highest net return.

***Keywords*:** Potato, Varieties, Nitrogen fertilizer, Tuber yield, NUE, BCR

Adequate nutrient management with a successful agronomic strategy is essential throughout the growth period of potatoes. Due to shallow roots and high nutrient demand, potatoes require more fertilizer than other crops (Munoz et al 2005). According to Tehran et al (2008), the fertilizer use efficiency for the potato to NPK fertilizers is 40-50, 10-15 and 50-60%, respectively. Among the major nutrients, Nitrogen (N) is a critical nutrient in potato production. Nitrogen is the main component for synthesizing chlorophyll, nucleic acids, amino acids, coenzymes, proteins *etc*., (Ahmed et al 2015). The potato plant is sensitive to deficiencies and excess nitrogenous fertilizer levels, affecting the tuber's growth, quality and quantity. Excessive nitrogen application in the early stages prolongs the vegetative phase and interferes with tuberization. Excess nitrogen application in mid-season reduces tuber bulking in favor of vegetative growth (Waddell et al 1999). Low nitrogen rates reduce canopy growth, tuber yields, quality, increased disease and insect susceptibility and premature senescence due to early nitrogen translocation from leaves to tubers. The outcome of low N fertilizer use efficiency and excessive application of N fertilizer causes nitrate leaching and groundwater N contamination (Plosek et al 2017). Furthermore, potato yield response to nitrogen (N) fertilizer varies with cultivar and soil type. ICAR-Central Potato Research Institute, Shimla (India) has developed high-yielding potato varieties for the North-eastern hilly region, which vary in their response to nitrogen (Dubey et al 2012). Among the varieties, Kufri Himalini and Kufri Girdhari are the high-yielding, late blight-resistant potato varieties suitable for cultivation in the Indian hills, including Meghalaya. Therefore, it is necessary to determine the optimal nitrogen fertilizer application rates for maximizing potato yields while minimizing nitrogen pollution. The present study was undertaken to determine the nitrogen requirement of the promising potato varieties and to work out N use efficiency (NUE) in the north-eastern hilly region of India.

MATERIAL AND METHODS

The study was conducted at ICAR Central Potato Research Institute Regional Station, Shillong during the summer season of 2019-2021. Shillong is located in Meghalaya at 25.54 °N Latitude and 91.85 °E Longitude with an altitude of 1738 m above mean sea level. The soil of the experimental field was sandy loam with an acidic pH (4.6), 319 kg available N ha^{-1}, 26 kg available P_2O_5 ha^{-1} and 266 kg available K_2O ha^{-1}. The experiment was laid out in Factorial Randomized Block Design with two potato varieties, Kufri Girdhari and Kufri Himalini and four levels ofN (0, 70, 140 and 175 kgN ha^{-1}) were replicated three times. The basal application of 120 kg P_2O_5 and 60 kg K_2O per hectare and the half rate of the N fertilizers per treatment. Forty-five days after planting, the remaining dose of nitrogen was applied as per the treatments. Urea (46% N), Single superphosphate (16% P) and Muriate of potash (60% K) were used as fertilizer sources for NPK. The well-sprouted, medium-sized (30-45 g) tubers were planted on ridges 60cm apart with a spacing of 20cm. Potato varieties were planted in the last week of February. All recommended packages of practices were followed to raise a healthy crop.During the growth period, germination percentage (40 days after planting), plant height (cm), number of leaves and shoots (60 days after planting) were recorded. Six plants from each plot were tagged for growth observation. To estimate tuber yield, all the plants in the net plot area were harvested manually. Shade-dried tubers were weighted and graded as very small (< 25 g), small (25-50 g) medium (50-75 g) and large (>75 g). The tuber yield of each plot was calculated and converted into tonnes per hectare. Nitrogen use efficiency (NUE) and Partial factor productivity(PFP)were calculated as below:

i. NUE= (Yf-Yc)/Na - expressed in kg yield per kg N applied
ii. PFP = Yf/Na - expressed in kg yield per kg of N applied.

Where Yf, Yc and Na are yield in the N-fertilized plot, yield in the control plot and quantity of fertilizer N applied (kg/ha), respectively (Dua et al 2007).

RESULTS AND DISCUSSION

Growth parameters: The final plant stand (40 DAP) was more than 90 % in all the treatments, indicating that potato varieties and nitrogen (N) levels did not affect plant emergence (Table 1). Similar results were reported by Kumar et al (2008) and Das *et al.* (2015). Varieties, nitrogen (N) levels, and their interactions had a significant effect on plant height. Variety Kufri Himalini was taller (46.57 cm) than Kufri Girdhari (43.31cm), indicating their inherent varietal difference. An increasing N rate resulted in increasing trends in plant height. The application of 175 kg N ha^{-1} recorded the highest plant height (49.53 cm) and shortest plant height (39.07cm) was recorded at 0 kgN ha^{-1}. Regarding variety by N rate interactions, KufriHimalini recorded the highest plant height (52.46 cm) with 175 kg N ha^{-1} followed by the same variety with 140 kg N ha^{-1} with a mean plant height of 49.97 cm. The lowest plant height (37.21 cm) was recorded with Kufri Girdhari grown in a control plot. A proportional increment in potato plant height in response to the increasing nitrogen applications has also been reported by Biruk (2015). Different N levels significantly increased the number of shoots and leaves per plant compared to control. The highest number of leaves per plant (40.11) and shoots per plant (3.38) were with 175 kg N ha^{-1} although it was at par with the application of 140 kg N ha^{-1}. The varieties and interaction effects were insignificant, indicating that both the varieties responded similarly to increased doses of N. Results of the present experiment agree with the finding of Udom et al (2012) and observed significant effect of increased nitrogen doses on stem number of potato. Increased nitrogen levels had a significant impact on plant height, number of shoots and leaves, which could be due to the role of nitrogen in cell division, cell enlargement and protein synthesis, all of which promote vegetative growth.

Grade-wise tuber yield of potato as influenced by the cultivars and levels of N: Kufri Himalini recorded significantly higher non-marketable tuber yield (3.51 t ha^{-1}) and marketable tuber yield (15.44 t ha^{-1}) (Table 2). Therefore Kufri Himalini would be more acceptable to farmers in terms of marketable tuber yield, as it produced a 26.18% higher total tuber yield (18.94 t ha^{-1}) than Kufri Girdhari (15.01 t ha^{-1}). The differential response of cultivars reflects the genetic variation between the cultivars as the environmental conditions during the growing period was similar. Tehran (2006) and Jatav et al (2013) reported that potato varieties differ widely in their nitrogen requirements. Nitrogen is a critical macronutrient for potato yield and yield components. The application of nitrogen had a significant effect grade-wise as well as on potato yield. Among the nitrogen levels, the application of 175 kg N ha^{-1} recorded the highest yield of non-marketable tuber (4.30 t ha^{-1}) and marketable tuber (17.54 t ha^{-1}). In comparison, the highest non-marketable yield of tuber (2.43 t ha^{-1}) was with 0 kg N ha^{-1}, which has at par effect

Table 1. Effect of varieties and nitrogen application rates on potato growth parameters

N levels (kg/ha)	Germination (%)			Plant height (cm)			No. of leaves/ plant			No. of shoots/plant		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
0	91.94	91.39	91.67	40.92	37.21	39.07	29.66	30.38	30.02	2.77	2.81	2.79
70	90.56	90.28	90.42	42.92	43.80	43.36	35.29	32.19	33.74	2.92	2.54	2.73
140	91.11	90.83	90.97	49.97	45.63	47.80	36.84	38.75	37.80	3.05	3.04	3.05
175	90.28	90.56	90.42	52.46	46.59	49.53	38.31	41.92	40.11	3.19	3.56	3.38
Mean	90.97	90.77		46.57	43.31		35.02	35.81		2.98	2.99	
Factors	V	N	V X N	V	N	V X N	V	N	V X N	V	N	V XN
CD (0.05)	NS	NS	NS	0.86	1.22	1.73	NS	3.62	NS	NS	0.39	NS

V_1 : Kufri Himalini, V_2: Kufri Girdhari with 70 kg N ha^{-1}. Increased nitrogen application increased the number of stolons, number of tuber (Zabihi et al 2010), tuberization and duration of tuber bulking (Kotsyuk 1995), which would have led to large-sized tubers (Zebarth and Rosen 2007) with a higher yield. Maitiet al (2004) and Marguerite et al (2006) reported that tuber yield per unit area increased with increasing nitrogen fertilizer to an adequate level.In this study, potato yield increased with increasing N levels. This result was similar to other findings (Dubey et al 2012, Jatavet al 2013, Das et al 2015, Banerjee et al 2015). Among all treatments, N fertilization produced the highest tuber yield than *non*-fertilized control plots. The highest tuber yield (21.84 tons ha^{-1}) was recorded at 175 kg N ha^{-1} followed by 140 kg N ha^{-1} with a mean tuber yield of 19.90 tons ha^{-1}. The lowest tuber yield (10.29 tons ha^{-1}) was in control. The relationship between N rates and tuber yield fit a convex quadratic function (Fig. 1), while mean tuber yield and N rates increased linearly. Variety Kufri Himalini had a significantly higher yield than Kufri Girdhari at all N levels. This suggests that the Kufri Himalini variety has better environmental adaptability than Kufri Girdhari. The interactions between the varieties and the N rates resulted in significant differences in the total potato tuber yield. Among nitrogen levels, Kufri Himalini with 175 kg N ha^{-1} had the highest total tuber yield (24.59 t ha^{-1}) followed by the same variety (22.14 t ha^{-1}) with the application of 140 kg N ha^{-1} and Kufri Girdhari with 175kg N ha^{-1} (19.08 t ha^{-1}).Several reports confirm this variation in total tuber yield of varieties under different N levels (Dubey et al 2012, Jatav et al 2017, Das et al 2015 Banerjee et al 2015, Jatav et al 2018).

Nitrogen uptake efficiencies of potato: Optimal N rate and timely N application are effective tools for increasing Potato Nitrogen use efficiency (NUE). NUE decreased linearly with increasing N level and maximum (79.77 kg tubers yield /kg N) at 70 kg N ha^{-1} and minimum (66.02 kg tuber/kg N) at the highest N dose of 175 N ha^{-1} (Fig. 2). The most efficient treatments received less N, indicating that NUE was inversely proportional to N amount. Potato grown in a nitrogen-limited environment extracted more nitrogen from the soil, confirming the findings of Cabello et al (2009), Trehan, 2009 and Banerjee et al (2014). NUE was higher in 'Kufri Himalini' potato at all N levels compared to Kufri Girdhari, indicating Kufri Himalini was the most efficient in NUE. Numerous studies have revealed that NUE varies by variety (Kumar et al 2008, Trehan 2009, Jatav et al 2013, Das et al 2015) and maturity class (Errebhi et al 1998, Zebarth et al 2004). Banerjee et al (2015) observed that Kufri Himalini had a higher nitrogen use efficiency under West Bengal

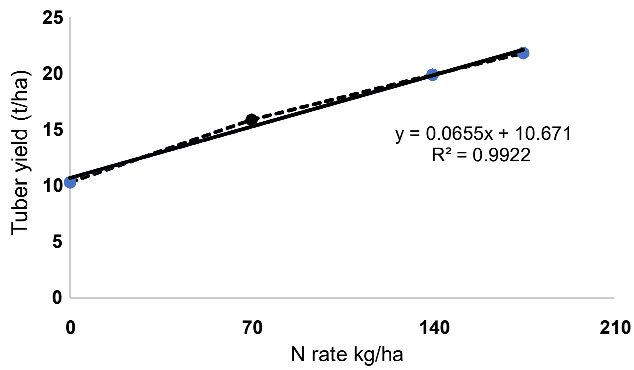

Fig. 1. Relationship between N rates and total tuber yield

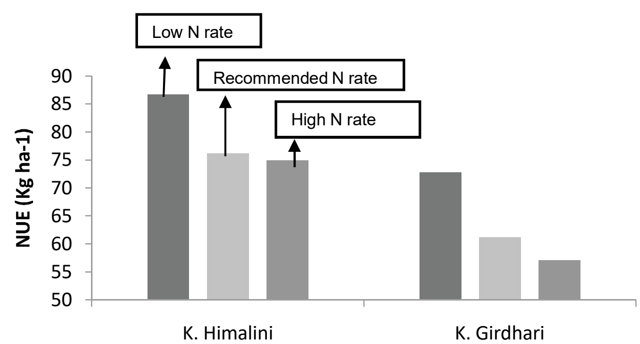

Fig. 2. Nitrogen use efficiency (NUE) under three N levels with two potato varieties

Table 2. Effect of varieties and different nitrogen application rates grade-wise and total tuber yield of potato

N levels (kg/ha)	Non-marketable tuber yield (t/ha)			Marketable tuber yield (t/ha)			Total tuber yield (t/ha)		
	V_1^*	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
0	2.82	2.03	2.43	8.66	7.06	7.86	11.48	9.09	10.29
70	3.12	2.62	2.87	14.43	11.57	13.00	17.54	14.19	15.87
140	3.52	3.41	3.47	18.63	14.25	16.44	22.14	17.66	19.90
175	4.56	4.03	4.30	20.02	15.05	17.54	24.59	19.08	21.84
Mean	3.51	3.02		15.44	11.98		18.94	15.01	
	V	N	V X N	V	N	V X N	V	N	V X N
CD (p=0.05)	0.40	0.57	NS	1.64	2.31	NS	0.69	0.97	1.37

*V_1: Kufri Himalini, V_2: Kufri Girdhari

Table 4. Economics, net return and B:C ratio of potato crops due to different N levels

N levels (kg/ha)	K. Himalini				K. Girdhari			
	Cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	B:C ratio	Cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	B:C ratio
0	179250	229600	50350	1.28	179250	181800	2550	1.01
70	184570	350900	166330	1.90	184570	283800	99230	1.54
140	189855	442800	252945	2.33	189855	353200	163345	1.86
175	192550	491800	299250	2.55	192550	381600	189050	1.98

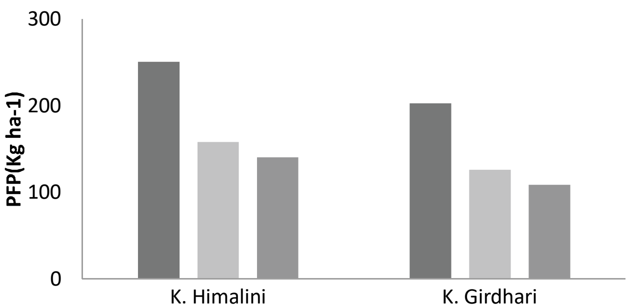

Fig. 3. PFP of N under three N levels with two potato varieties

conditions. Partial factor productivity of N in potato (PFP_N) decreased as the applied N level increased; the rate of decrease varied between varieties (Fig.3). The mean PFP_N was 226.67 kg tuber/kg N when used at 70 kg Nha^{-1}, which declined to 124.28 kg tubers/kg N when applied at 175 kg N/ha. This pattern reflects the law of diminishing returns as N increases and the response to N decreases. A similar trend was observed during the different decades (Dua et al 2007). PFP_N of varieties was compared, Kufri Himalini had the highest PFP_N (250.62) at 70 kg N/ha while Kufri Girdhari had the lowest (202.72).

Economics of potato cultivation: The cost of cultivation, net returns per hectare and B:C ratio increased with increasing nitrogen dose (Table 4). Cultivation without nitrogen had the lowest cost cultivation. K. Himalini and K. Girdhari at a higher N dose (175 kg N ha^{-1}) had the highest gross and net returns due to increased marketable and total tuber yield. Higher tuber yields increased gross return, net return, and cost-benefit ratio.K. Himalini had the best cost-benefit ratio (2.55 and 2.33)with applications of 175 kg and 140 kg of N followed by K. Girdhari with an application of 175 kg of N (1.98).

CONCLUSIONS

In terms of highest marketable and total tuber yield, NUE and net returns, 'Kufri Himalini' is a more profitable variety for cultivation under Meghalaya conditions. Kufri Himalini is more responsive to nitrogen fertilizer and can be grown with 175 kg N ha^{-1} to maximize net return.

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Microbial Biofertilization to Improve Yield and Quality of Strawberry

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Abstract: Microorganisms inhabiting the soil and rhizosphere take part in biogeochemical cycles and enhance the availability of macronutrients leading to improved soil productivity and enhanced food production. This study was conducted to examine the effect of inoculation of plant growth-promoting Rhizobacteria (PGPR) on phenological data, yield per plant and fruit quality characteristics of strawberry (*Fragaria x ananassa* Duch) cv. 'Chandler' during 2016 and 2017. Root inoculations with rhizobacteria significantly increased yield per plant (1.59–25.03%) and average fruit weight (1.00-12.86%) compared to control, whereas the bacterial inoculations did not affect dry weight of plant, fruit size and moisture content in strawberry cv. 'Chandler'. The bacterial inoculations also increased soluble solid content (SSC),ascorbic acid and anthocyanin content in strawberry. The ascorbic acidand anthocyanin contents of fruits ranged from 38.70 ml /100ml (control) to 41.41 ml/ 100ml (CPS67), 39.90 ml/ 100ml (control) to 42.96 ml/ 100ml (CPS67), while TSS varied between 6.26% (control) and 7.45% (HMM57). Thus, inoculation ofrhizobacterial strains JMM15, HMM57, HMM92 and CPS67 have the potential to increase the yield and growth of strawberries and could provide a promising sustainable strategy to improve strawberry growth in low fertility soils.

Keywords: Strawberry, PGPR, Root inoculation, Growth, Yield

Strawberries (*Fragaria* × *ananassa* Duch.) are unique in their highly desirable taste and flavor. Most of its cultivars are octaploid with 2n= 56. Its fruits are rich in bioactive phytochemicals especially phenolic compounds with high antioxidant capacity. It also contains vitamins and minerals making the daily diet beneficial to human health. In India, the cultivated area under strawberry is mainly located in Maharashtra, Himachal Pradesh, Uttar Pradesh, Haryana and the Nilgiri hills is nearly 1,000 ha with a production of 5,000 tonne, and in Haryana, is around 150 ha with a production of 2,010 tonne (Rao and Saxena 2017). The modern strawberry cultivation that warrants high yield and quality requires extensive use of chemical fertilizers, which not only disrupt the balance of nature but also reduce economic efficiency. The continuous use of chemical fertilizers not only affects soil health and environment adversely but also reduces the productivity of crops. This situation emphasized the need for the development of alternate production systems, which are environment friendly and is more judicious to manage the soil health. More recently, the use of plant growth promoting rhizobacteria (PGPR) has been exploited in fruit crops (Sindhu et al 2010) and proved a major tool in fruit production. Plant growth promoting rhizobacteria may improve plant growth and yield by means of producing plant growth regulators (auxin, gibberellins, cytokinins), solubilizing/mineralizing the organic phosphate or other nutrients, fixing atmospheric nitrogen and facilitating the uptake of nutrients (Sharma et al 2018, Sindhu and Sharma 2019). Various research workers found that plant growth promoting rhizobacteria could stimulate plant growth and increase yield of apple, sweet cherry, citrus, raspberry, high bush blueberry, mulberry and apricot. Inoculation of PGPR species could increase the growth attributes like leaf area, dry weight of both the root and aerial parts of the inoculated plants, resulting in better development. The application of PGPR strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) through roots enhanced the quality of strawberry fruits by increasing the total soluble solids, total sugars and reducing sugars significantly, and decreasing the titratable acidity (Pirlak and Kose 2009). PGPRs like *Bacillus*, *Pseudomonas*, *Azotobacter* and *Azospirillum* promote the synthesis of growth promoting substances like auxins, gibberellins, cytokinins and antibiotic metabolites, which in turn improve resistance against biotic and abiotic stresses (Tomic et al 2015). The plant rhizosphere bacteria of genera *Pseudomonas* and *Bacillus* have been recognized as early root colonizers, which enhance plant growth by increasing seed emergence, plant weight and crop yield. The environmental benefits of this innovative approach reduces the use of agro-chemicals and fitting with sustainable management practices. Recent progress in understanding the biological interactions that occur in the rhizosphere and the need for inoculants formulation and delivery may facilitate its commercial development (Mohanram and Kumar 2019). The objective of this study was to investigate the growth-promoting effects of PGPR strains inoculation under greenhouse conditions in relation to phenological data, total yield and fruit quality characteristics of strawberry.

MATERIAL AND METHODS

Study site and planting material: The study was conducted on 'Chandler' strawberry plants during the consecutive seasons (2016-2017 and 2017-2018) in greenhouse at CCS Haryana Agriculture University, Hisar (29º10' N latitude and 75º 46' E longitudes). The experiment was replicated twice in completely randomized design. The treatments contained four fertilizer types, *Pseudomonas* strains namely, JMM15, HMM57, HMM92 and CPS67 (containing 10^8 bacterial cell/ml), whereas untreated soil served as control. Total 60 plants with four replications were inoculated with plant growth promoting rhizobacteria. The planting was done in the last week of October during the both years. Single uniform runner was planted in each pot after treating with carbendazim (0.1%). Each pot was filled with 4 kg of soil. Basal dose of fertilizers was added to soil in each pot along according to per plant requirement as per basal dose. The seventy five per cent fertilizers *viz.,* urea, liquid NPK (19:19:19), sulphate of potash and murate of potash were given at weekly interval as per the recommendation.

Treatments: Four PGPR strains HMM57, HMM92, JMM15 and CPS67were obtained from Dept. of Microbiology. These bacteria were reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens for different cereal and oil seed crops (Table 1). Bacterial cultures were maintained on Luria Bertani (LB) medium slopes (Sambrook et al 1989).

Estimation of IAA, ALA, salt tolerance and phosphate solubilization: Rhizobacterial isolates were tested for their ability to produce indole acetic acid using Salkwoski's Reagent (Gordon and Weber 1951). Indole acetic acid (100 µg/ml) was used as standard and results were expressed as µg IAA produced per ml of culture supernatant. Uninoculated LB broth served as control. For α-aminolevulinic acid isolates were tested using the method as described by Mauzerall and Granick (1955). The concentration of ALA in the culture supernatant of different rhizobacterial isolates was determined by using standard curve. Screening of rhizobacterial isolates for growth at different salt concentrations: selected rhizobacterial isolates were checked for their ability to grow at different concentrations of NaCl upto 8% (w/v) on LB medium containing 20 Mm HEPES (N-2- hydroxyethane- sulphonic acid) buffer (Marsudi et al 1999). Medium plates were spotted with 20 µl growth suspension of different bacterial isolates. The plates were incubated for 3-4 days at 28±2°C in a BOD incubator. The susceptibility or tolerance to NaCl was recorded by observing the growth as a positive or negative result and colony size was measured. Phosphorus solubilization by rhizobacterial isolates was studied on Pikavskaya's medium plates by spot test method (Sharma et al 2011). Phosphorus solubilization by different rhizobacterial isolates were scored based on their ability of solubilization zone formation.

Growth, yield and fruit quality traits: Growth parameters such as height of plant was measured individually with a measuring scale, Plant spread was calculated by measuring the canopy of plant in East-West and North-South direction with the scale and the average of both was expressed as plant spread, Number of leaves per plant was counted from the time of transfer to the end of growing season (November-March) at fortnightly interval and the average number of leaves per plant, crown diameter of plant was measured with the help 'Inox' vernier scale (±0.05 mm accuracy), Fresh weight of plant recorded at after harvesting of fruits and plants taken for fresh weight were dried at room temperature and there after dried in oven at 45°C for five to six days until the reduction in weight became constant. Yield parameter fresh weight of ten fruits in each replication were randomly selected to determine average fruit weight and the data were expressed in g per fruit. Fruit dimensions (mm) (length and breadth) were also determined in the samples. Quality traits, viz., TSS, acidity, ascorbic acid, anthocyanin content and moisture content of fruits were measured at commercial maturity stages. Total soluble solids (%) was determined using hand refractometer having a range of 0 - 32 (ERMA) by putting a drop of juice and taking the readings. The titratable acidity (%) and ascorbic acid (ml/ 100ml) was determined as per the method suggested by AOAC (1990) and anthocyanin content (ml/ 100 ml) was determined using pH differential method. The moisture content (%) of fruits was taken for fresh weight. Then fruits were dried in oven at 55°C up to 15 days until the reduction in weight became constant.

Statistical analysis: The data was subjected to analysis using OP STAT statistical computer package (OP Sheoran 2004, CCS HAU Hisar).

RESULTS AND DISCUSSION

Phosphate-solubilizing capacities, salt tolerance and production of IAA and ALA by PGPR strains: All the rhizobacterial strains showed IAA and ALA production (Table 1). Significant phosphate solubilization was observed in JMM15 and HMM92 strains. All the rhizobacterial strains showed salt tolerance upto 8% NaCl concentration.

Effect of PGPR inoculation on the yield, growth and fruit quality: Root inoculation of strawberry plants with different rhizobacterial strains showed significant effect on the growth parameters, *i.e.,* plant height, number of leaves, plant spread, fresh weight and dry weight of plant, except dry weight of plants (Table 2). All the growth parameters increased significantly with the application of *Pseudomonas* strain CPS67 in association with 75% RDF. Wani et al (2007) also reported that *Pseudomonas* spp. secrete organic acids and enzymes tha tact as mineralization of immobile form of phosphates and also produces amino acids, vitamins and growth-promoting substances, resulting in promotion of plant growth. The quality of fruits in terms of total soluble solids, ascorbic acid and anthocyanin content increased significantly with the application of different *Pseudomonas* strains in strawberry plants.

The ascorbic acid content and anthocyanin content (Table 3) was recorded in fruits of strawberry plants inoculated with *Pseudomonas* strain CPS67 with lowest value for acidity in fruits, whereas maximum TSS was recorded in strain HMM57. Anuradha et al (2020) recorded maximum TSS and ascorbic acid in strawberry fruits harvested from the plants inoculated with biofertilizers. Biofertilizers increased the accumulation of carbohydrates, which converted into disaccharides leading to higher sweetness. Moreover, root inoculation of strawberry plants with *Pseudomonas* strain CPS67 had significantly positive effect on the yield parameters, *i.e.,* number of fruits per plant, fresh weight of fruit and yield of fruit, except size of fruits (length and breadth). The maximum number of fruits per plant, fresh weight of fruits and yield of fruits per plant was obtained in plants inoculated with *Pseudomonas* strain CPS67 (Table 4). The number of fruits per plant was the highest in CPS67 treatment (20.75) and followed by HMM57 (20) and control (18.75). The highest fruit weight was in treatments having inoculation with *Pseudomonas* strain CPS67 (10.44 g) followed by HMM57,JMM15, HMM92 and control (Table 4). In brief, the bacterial inoculation increased the fruit weight by 1.00-12.86% compared to control. The bacterial treatments increased yield per plant by 1.59-25.03% compared to control. All the bacterial strains revealed higher yield values than the control that varied from 176 to 216 g in bacterial treatments.

The bacterial strain CPS67 was most effective treatments for growth, yield and quality among all treatments. Statistical differences were not recorded for dry weight of plant, acidity, moisture content and fruit size between any tested bacterial strain and control. The present study showed that root inoculation with CPS67 and HMM57 promoted plant growth and yield of Chandler strawberry cultivar, but growth responses were strain-specific. The positive effects of PGPR application on growth, yield and quality of apricot, raspberry, tomatoes,, sugar beet, apple, sweet cherry and barley were explained by promoting abilities of these bacteria for auxin and cytokinin production, N_2-fixation, phosphate solubilization and antimicrobial substance production

Table 1. Beneficial characteristics of PGPR isolates

Bacterial cultures	Relevant characterstics				Reference for source
	ALA (µg/ml)	IAA (µg/ml)	***P	Salt tolerance 8%	
JMM 15	17.45	12.24	+++	+++	Phour, 2016
HMM 57	11.74	4.62	-	++	Phour, 2016
HMM 92	17.53	0.99	+++	+++	Phour, 2016
CPS 67	7.5	50.25	+	++	Phour, 2012

Table 2. Effect of plant growth promoting rhizobacteria on growth traits of strawberry plants

Treatments	Plant height (cm)	Plant spread (cm)	Number of leaves per plant	Crown diameter (mm)	Fresh weight of plant (g)	Dry weight of plant (g)
JMM 15	11.35	20.58	9.94	11.12	40.13	10.58
HMM 57	11.54	21.27	10.27	11.30	40.96	11.07
HMM 92	11.28	20.46	9.69	11.03	39.83	10.50
CPS 67	12.24	22.73	11.18	12.19	42.54	11.72
Control (75%)	11.20	20.22	9.49	10.86	39.44	10.39
100 %	13.82	23.18	11.85	12.83	45.00	12.73
CD (p=0.05)	0.21	0.46	0.23	0.16	0.63	NS

NS= non significant

Table 3. Effect of plant growth promoting rhizobacteria on quality parameters of strawberry fruits

Treatments	TSS (%)	Acidity (%)	Ascorbic acid (ml/ 100ml)	Anthocyanin content (ml /100ml)	Moisture content (%)
JMM 15	6.77	0.84	40.18	41.01	94.23
HMM 57	7.45	0.81	40.57	41.50	93.95
HMM 92	6.44	0.83	39.49	40.48	94.28
CPS 67	7.34	0.75	41.41	42.96	93.21
Control (75%)	6.26	0.86	38.70	39.90	94.43
100 %	7.57	0.72	45.03	46.75	92.58
CD (p=0.05)	0.08	NS	0.64	0.79	NS

NS= non significant

Table 4. Effect of plant growth promoting rhizobacteria on fruit size and yield of strawberry plants

Treatments	Fresh weight of fruit (g)	Length of fruit (mm)	Breadth of fruit (mm)	Number of fruits	Yield per plant (g)
JMM 15	9.46	38.31	27.34	19.25	182.11
HMM 57	9.71	38.60	27.61	20.00	196.10
HMM 92	9.34	38.18	27.24	18.88	176.20
CPS 67	10.44	39.88	28.54	20.75	216.84
Control (75%)	9.25	37.91	27.06	18.75	173.43
100 %	10.90	40.74	29.17	22.87	243.05
CD (p=0.05)	0.18	NS	NS	0.31	3.21

NS= non significant

(Cakmakci et al 2001, Esitken et al 2006, Orhan et al 2006, Pirlaket al 2007, Aslantus et al 2007, Karlidag et al 2007). The yield and growth enhancement effects of bacteria used in this study on strawberry could be explained by similar reasons.

Rhizobacterial isolates used in this study were analyzed for growth promoting hormones IAA, ALA and solubilize bound phosphorus (Phour and Sindhu 2019).The hormones of auxin group have positive effect on fruit setting in different fruit species. In our study, higher yield obtained by bacterial inoculation of strawberry plants may therefore be explained by IAA producing capacity of strain CPS67. The presence of high number of bacteria in the rhizosphere is also important since they may mineralize bound form of nutrients to plant utilizable form.

CONCLUSION

The beneficial activities of PGPR inoculants in the rhizosphere of inoculated plants may provide promising solutions for sustainable and environment-friendly agriculture. The different rhizobacterial strains in association with 75% RDF were found to have great potential to increase the production of strawberry. From the present study' the recommendation can be made that application of chemical fertilizers may be reduced by inoculation of biofertilizers strains which not only improve the yield but also provide to obtain health and environment-safe products. Further studies are required under field conditions for application of these PGPR as biofertilizers and for sustainable agriculture.

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Micro-Climatic Variations in Naturally Ventilated Polyhouse Under Cucumber Cultivation

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Abstract: The experiment was carried out to investigate the seasonal variations of climatic parameters inside a naturally ventilated poly house during various stages of cucumber crop growth. Micro-climatic parameters such as ambient temperature (T), relative humidity (RH), light intensity (LI), and carbon dioxide (CO_2) were analyzed on daily basis in the polyhouse's vertical, spatial, and temporal scales. These parameters fluctuated at various stages of crop development. The weekly average maximum temperature of 38.73 °C was recorded in the polyhouse during the afternoon. The weekly average relative humidity (84.40%) was higher in the morning and lower in the afternoon (44.96 %). The light intensity inside the polyhouse was higher in the afternoon (43 klux) and lower in the morning and evening. The variations in CO_2 levels inside the polyhouse at different locations were observed to be very small. Micro-climate changes varied spatially and temporally within the polyhouse during crop growth. For every one-meter increase in height, the vertical gradient of temperature increased by 0.1°C and RH decreased by about 1%.

Keywords: Micro-climate, Cucumber, Naturally ventilated, Temporal & Spatial

The impact of biotic stresses and climatic variations on the crop in the open field is a challenge to overcome. As a result, adopting protected cultivation practices such as polyhouses, shade nets, tunnels, etc. could be an alternative crop cultivation option (Pattnaik and Mohanty 2021). Polyhouse cultivation is the best method for dealing with all biotic and abiotic stresses on plants. In natural ventilation, pressure is created mainly due to temperature and wind differences between the outside and inside of a polyhouse. Natural ventilation occurs as a result of the pressure difference. It also regulates temperature and humidity within the polyhouse and allows for adequate air exchange. Photosynthesis-induced growth requires a number of climatic parameters, including temperature, relative humidity, carbon dioxide (CO_2), and light intensity, all of which influence plant growth at every stage. Specific climatic changes can alter yield, productivity, plant characteristics (quantitative and qualitative), and disease development (Egel and Saha 2015), which is due to the non-uniformity of microclimate inside the polyhouse (Qian et al 2015). A distributed monitoring system will be useful for monitoring and modifying the microclimate within the polyhouse. Knowledge of the effects of climatic factors such as light, temperature, soil, and water on plant growth provides an insight into how to increase crop yield. By monitoring these variables, one can learn more about how each element affects crop growth and how to maximize agricultural productivity.

The polyhouse remains heterogeneous with irregular temperature, relative humidity, CO_2 concentration, and irradiation distributions that require management. A single measurement cannot represent the entire polyhouse or provide complete information on the distribution of temperature and relative humidity (Korner et al 2007). Spatial and temporal variations of climatic parameters within a polyhouse lead to the careful consideration of sensor placement, data processing, and maintenance. The planning of an automated or internet-connected irrigation system will be possible with the knowledge of microclimatic fluctuations within the polyhouse. This allows the identification of ideal sensor locations as well as the calculation of the number of sensors required for accurate system operation and maintenance. The primary goal of this research is to detect and analyse spatial and temporal variations in climatic parameters inside a naturally ventilated polyhouse used for cucumber cultivation in a subtropical climate, as well as to investigate the possibility of detecting difficult situations for the plants and developing environmental control approaches for better micro-climate management.

MATERIAL AND METHODS

Study area: The current study was carried out in a naturally ventilated ridge-vent polyhouse at the ICAR-Central Institute of Agricultural Engineering in Bhopal (23.31° N, 77.40° E). Bhopal's climate is classified as sub-humid. Winter temperatures range from 1°C to a maximum of 25°C, while summer temperatures range from 22°C to 48°C. The average annual rainfall is 1160 mm. The soil in Bhopal is categorized as vertisols.

Description of polyhouse: In cucumber cultivation, a 375 m^2 naturally ventilated polyhouse with dimensions of 25m (L) × 15m (W)× 6m (H) was used to monitor microclimate parameters. The polyhouse cladding material is a 200μ thick translucent polyfilm with an 80 percent diffusivity. Rollable curtains on all four sides of the polyhouse were used to control micro temperature. Insect-proof nylon nets with a mesh size of 40 were used as side curtains to allow for natural airflow while protecting the plants from insect/pest entry. Hundred percent evapotranspiration (ET) based irrigation was given to the crop by using a low-pressure inline drip irrigation system with a discharge rate of 2 lph was used. 24-day-old hybrid cucumber (KCUH-10) seedlings were transplanted in a randomized block design.

Methodology: Temperature and relative humidity were measured using a hygrometer (Make: Testo, Model:623). The instrument had relative humidity measuring range of 0 - 100 %. The CO_2 was measured using a portable indoor air monitor (Make: Rotronic, Model: CP11) with a range of 0-5000 ppm. With a 3 % accuracy, a light meter (Make: Ideal, Model: 61-686) was used to measure light intensity up to 200,000 lux. The variation of micro-climatic parameters such as temperature, relative humidity, light intensity, and carbon dioxide was observed for a month before crop sowing and three months during crop cultivation. These parameters were recorded daily at 8:00 a.m., 1:00 p.m., and 4:30 p.m. (IST) and at nine different locations within the polyhouse at three different heights, namely ground level (GL), one meter, and two meters above the GL. Figure 1 depicts the layout of these locations and heights. For the meteorological weeks of July

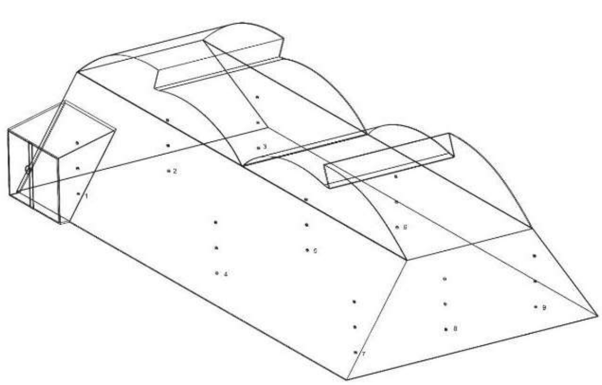

Fig. 1. Layout of the polyhouse under experiment

to September, the average weekly temperature, relative humidity, carbon dioxide, and light intensity were assessed inside a naturally ventilated polyhouse at various heights (ground level, 1m, 2m above ground) and times of day (8 am, 1pm, 4:30 pm). The weekly average weather parameters inside and outside the polyhouse were investigated for various weeks during plant growth. At monthly intervals, the spatial and temporal variability of temperature and relative humidity was examined for nine locations. The following metrics were used to investigate these variations.

Maximum average difference (MAD): The maximum average difference of monthly average values was calculated by using

$$MAD = (V_m)_{max} - (V_m)_{min}$$

Mean relative deviation (MRD): The mean relative deviation (MRD) of the monthly average values is calculated as follows.

$$MRD = \frac{\sum_{i=1}^{N} |V_i - V_m|}{N.V_m}$$

Where, N – Number of measurements of a particular variable, V_i – The i^{th} measurement, V_m – Average value of all N measurements.

The first two metrics depict the average magnitude of measurement variability, whereas MRD is a uniformity metric, with lower values indicating greater uniformity.

RESULTS AND DISCUSSION

Ambient temperature: The weekly average temperature in the polyhouse during the crop period was be highest (38.73 °C) in the afternoon of the 39^{th} week, followed by the afternoon of the 29^{th} week (36.90 °C), and lowest (28.15 °C) in the 30^{th} meteorological week. Higher temperatures were observed in the afternoon, which might be due to short-wave radiation trapping in the polyhouse under partially closed conditions. A temperature increase of 0.1 °C was observed with every 1 m increase in height at different locations of the polyhouse, which is similar to the findings of earlier researchers (Soni et al 2005, Suay et al 2008, Singh et al 2017). The temperature was increased along the length and width of a polyhouse. A similar pattern was observed for temperature variations when no crop was present inside the polyhouse (Fig. 2)

Relative humidity: The polyhouse had the lowest relative humidity in the afternoon (1 pm) during the entire crop growing period (Fig. 2). In polyhouse, relative humidity was highest (84.4%) in the morning of the 37^{th} week, followed 34^{th} week, and lowest (65.7%) in the 29^{th} week. The afternoon relative humidity reached maximum (64.7 %) in the 37^{th} week

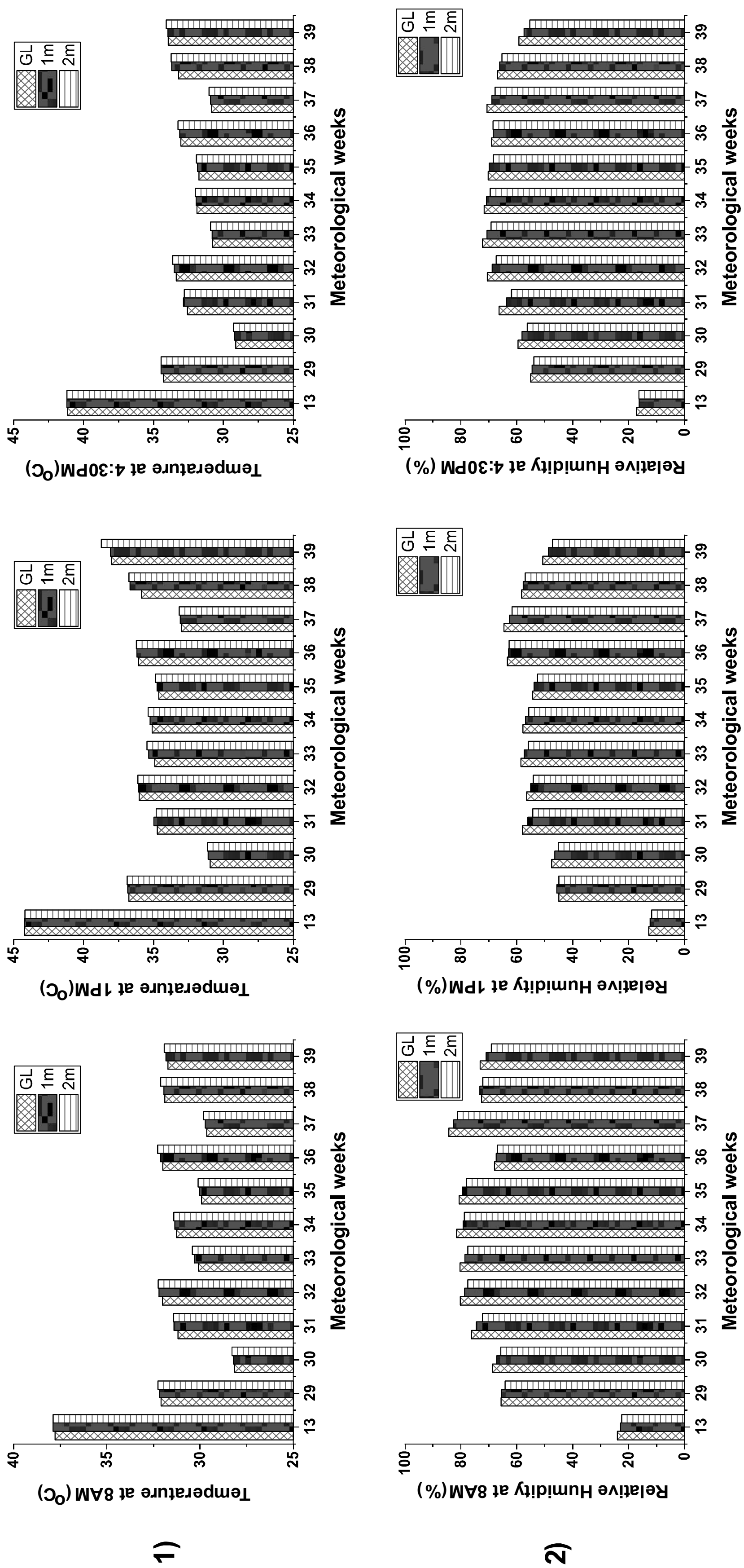

Fig. 2. Weekly average 1) temperature 2) relative humidity 3) light intensity 4) carbon dioxide at 8:00 AM b) 1:00 PM, c) 4:30 PM at different heights

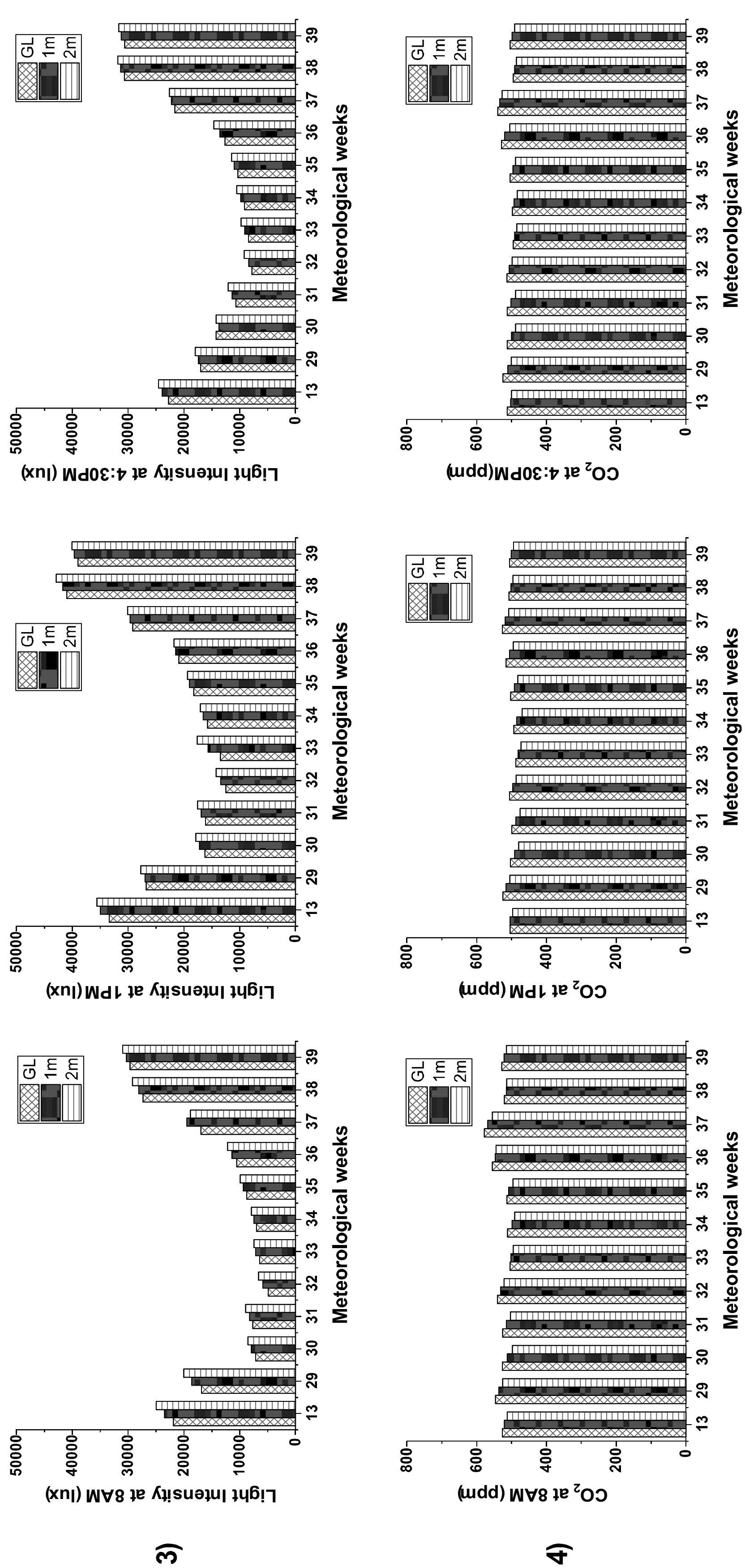

Fig. 2. Weekly average 1) temperature 2) relative humidity 3) light intensity 4) carbon dioxide at 8:00 AM b) 1:00 PM, c) 4:30 PM at different heights

and its minimum (45 %) in the 29th week. The findings were in accordance with Zorzeto et al. (2014). The relative humidity drops by 1% for every 1m increase in height which may be due to the increased temperature. The current findings revealed that RH ranged between 45 and 84.4 % during the crop period in which the plant growth was found to be optimum which was also similar with Deogirikar et al (2005).

Light intensity: During the 38th meteorological week, the weekly mean light intensity in the polyhouse peaked at around 43 klux in the afternoon, then fell to 40 klux in the 39th meteorological week. Morning light intensity reached a peak of 31 klux in the 39th week and dropped to 5 klux in the 32nd week. The 38th week had the highest evening light intensity (32 klux) and the 32nd week had the lowest (8 klux) (Fig. 2). Umesha et al (2011) observed similar results where light intensity was low in the morning and evening hours. The intensity of the light increased with height i.e for every 1 m increase in height light intensity increased by about 1000 lux. From morning to evening, the light intensity was increased along the length of the polyhouse i.e. from east to west, as well as across the width of the polyhouse i.e. from north to south. Crop growth and yield were found to be improved as a result of the light intensity fluctuations.

Carbon dioxide: The 37th week had the highest morning weekly average CO_2 (570 ppm), followed by the 36th week (554 ppm). During the afternoon, CO_2 levels were found to be lower (470 ppm) in the 34th week because of the higher temperatures, low relative humidity, and adequate light which might have boosted photosynthesis causing more CO_2 uptake. Because plants release CO_2 during the night as a result of respiration, the CO_2 levels in the polyhouse were highest in the morning. The lack of solar radiation and the use of UV stabilized sheets reduce the amount of CO_2 escaping from the polyhouse throughout the night and less photosynthesis in the early morning. This effect lasted only an hour after sunrise because the polyhouse was naturally ventilated. CO_2 levels decreased by 10 ppm for every 1 m of height when the crop was grown in the polyhouse (Fig. 2). Jerszurki et al (2021)observed similar trend of CO_2 reduction from the bottom to the plant canopy's top. The results showed that CO_2 levels varied between 470 and 570 ppm, which is the optimal range for crop growth (Xu et al. 2015) and that no CO_2 supplementation was required. The four stages of plant development followed a similar pattern.

Spatial variability of temperature and relative humidity: The largest temperature heterogeneity occurred during the afternoon in September (maximum difference of 2.4°C with a 0.72 standard deviation of average temperatures between the nine locations and an MRD of 0.01), followed by the morning in July (maximum difference of 2.16°C with a 0.72 standard deviation of average temperatures between the nine locations and an MRD of 0.01) (Table 1). The temperature variations in the polyhouse could be caused by the opening of the curtains or the orientation of the polyhouse, or they could be caused by unexpected changes in the weather during the experimental period. Other researchers (Jerszurki et al 2021) found similar or even higher air temperature differences, indicating that the polyhouse's natural ventilation system worked well. The corresponding values were considerably lower in the mornings of September and the mornings, afternoons, and evenings of the remaining months (a maximum average temperature difference of approximately 1.5°C); thus, variability during these periods was much smaller. During July and August, the greatest spatial variation in relative humidity (Table 2) occurred (maximum differences of 9.99 % and 8.99 %, and MRD around 0.04). The findings are similar to those of Tzounis et al. (2015) and Zorzeto et al. (2014). The smallest differences occurred in the afternoons during March (maximum difference of 1.94 %and MRD around 0.04).This may be due to the absence of a crop within the polyhouse. During this month, relative humidity variation appeared to be mainly distributed along the polyhouse longer (north-south) axis. In the afternoons and evenings, relative humidity measurements were fairly consistent (in March, July, August, and September). Because there was no airflow inside the polyhouse at night, there were significant differences in the morning compared to the afternoon and night. Temperature and relative humidity distributions inside the polyhouse are influenced by the intensity of light in relation to the height above the ground surface, i.e. when the light intensity is high, the highest humidity and temperature gradients occur. Changes in microclimate have a positive impact on cucumber growth and yield characteristics in polyhouses.

Comparison of weather parameters of inside and outside of polyhouse: During the crop period, the weekly average ambient temperature at 1 p.m. ranged from 31.06 °C to 38.27 °C inside the polyhouse and from 31.53 °C to 39.40 °C in open fields (Fig. 3). The findings are similar to those of Thipe et al (2018). At 1 p.m., the average weekly relative humidity inside the polyhouse ranged from 45.2 % to 63 %, while outside in the open field, it ranged from 50.6 to 62.6 % (Fig. 3). Under both climatic conditions, relative humidity displayed a more or less inverse relationship with temperature. In contrast to the temperature pattern, relative humidity in the polyhouse followed the inverse pattern as that of ambient temperature, i.e., it was lower in the polyhouse than in the open field at the beginning and higher or nearly similar in the later stages. The weekly average relative humidity difference within and outside the protected structure

Table 1. Average temperature and standard deviation of each location in the morning, afternoon and evening for the different months

Location of reading	March			July			August			September		
	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening
1	36.78±1.36	44.01±3.74	40.7±3.20	28.98±2.42	33.23±3.78	31.09±3.65	30.52±1.32	34.43±0.93	31.94±1.20	30.21±1.98	35.10±2.76	31.96±1.97
2	37.47±1.39	44.41±2.97	40.80±2.75	29.43±2.88	33.65±3.86	31.57±3.75	31.05±1.44	35.00±1.07	32.43±1.24	30.73±2.03	35.60±2.87	32.32±1.99
3	37.81±1.27	44.82±2.73	41.00±2.12	30.06±3.36	33.96±3.99	31.91±3.93	31.30±1.46	35.38±1.00	32.59±1.15	31.10±2.08	35.89±2.88	32.69±1.93
4	38.49±1.39	44.61±2.08	40.58±1.65	30.20±3.66	34.11±3.95	31.96±3.97	31.00±1.47	35.04±1.15	32.48±1.28	30.98±2.03	34.10±7.77	31.50±4.89
5	38.48±1.59	44.09±2.17	40.57±1.76	30.65±3.86	34.40±4.04	32.32±4.04	31.20±1.44	35.18±0.92	32.78±1.19	31.41±1.92	35.79±2.93	32.80±1.91
6	38.51±1.24	44.30±1.91	40.73±2.06	30.86±3.82	34.56±4.08	32.28±3.78	31.31±1.35	35.37±0.88	32.88±1.15	31.63±2.01	36.07±2.93	33.05±2.00
7	37.80±1.15	43.71±1.57	40.50±1.99	30.75±3.99	34.55±4.13	32.08±3.91	31.17±1.49	35.45±1.11	32.67±1.16	31.60±2.10	36.01± 3.0	33.09±1.97
8	37.57±1.20	43.70±1.44	40.51±1.71	31.06±4.26	34.40±3.95	32.36±4.17	31.47±1.49	35.40±1.17	32.83±1.15	31.94±2.22	36.26±3.08	33.35±2.12
9	37.77±1.28	44.31±1.51	40.56±v2.05	31.15±4..36	34.65±3.99	32.53±4.39	31.54±1.50	35.70±0.96	33.13±1.20	32.17±2.32	36.53±3.10	33.56±2.26
MD	1.74	1.12	0.50	2.16	1.41	1.45	1.01	1.28	1.19	1.96	2.42	2.06
ASD	0.57	0.38	0.16	0.75	0.48	0.45	0.30	0.37	0.34	0.62	0.72	0.67
AMRD	0.01	0.01	0.003	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02

Table 2. Average relative humidity and standard deviation of each location in the morning, afternoon and evening for the different months

Location of reading	March			July			August			September		
	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening
1	27.20±3.55	13.25±1.31	16.75±3.38	73.06±7.13	49.77±6.59	60.25±6.23	81.37±3.67	61.62±2.15	72.59±6.28	77.55±9.03	61.85±9.40	68.32±8.73
2	24.53±3.83	12.58±1.37	16.88±2.24	70.42±6.84	48.79±6.67	58.08±7.50	79.90 ±3.32	59.78±2.99	70.95±6.22	75.79±8.22	60.29±9.68	67.30±8.63
3	24.43±3.52	12.12±0.70	15.69±1.40	68.32±7.30	46.81±6.55	57.14±6.95	78.63±±4.22	57.90±1.95	69.33±5.82	74.68±9.63	59.30±9.55	66.48±8.61
4	21.60±4.05	11.31±1.08	15.48±2.06	64.83±9.20	45.09±5.70	55.75±7.74	75.66±6..04	56.34±1.92	68.20±5.83	73.40±8.44	58.70±9.16	65.63±8.38
5	21.35±4.19	11.97±1.47	16.49±3.40	63.65±9.40	45.12±6.61	54.85±7.74	75.62± 4.62	57.17±2.08	66.783±6.53	72.86±8.52	57.38±9.13	64.88±8.40
6	22.38±4.45	11.90±1.12	17.46±4.16	64.13±8.80	43.94±7.10	54.44±7.54	75.66±2.27	54.40±2.49	65.84±6.90	73.50±8.53	56.97±9.09	63.91±8.19
7	22.25±4.14	12.63±1.56	16.79±1.66	63.07±8.84	43.54±6.26	55.38±7.87	76.12±4.14	52.63±3.30	66.42±7.07	71.79±9.92	56.24±9.73	64.19±9.02
8	22.59±4.79	12.78±1.49	17.66±3.78	64.07±10.4	44.99±6.30	55.23±8.96	76.72±4.89	53.06±3.85	65.97±6.69	71.00±9.70	56.74±10.32	63.80±9.51
9	22.46±4.19	12.64±0.99	16.50±2.64	63.32±9.99	44.19±4.86	53.96±7.82	76.45±5.62	53.21±	65.05±6.82	71.20±9.28	56.16±10.91	63.63±9.60
MD	5.86	1.94	2.18	9.99	6.23	6.29	5.75	8.99	7.54	6.55	5.69	4.69
ASD	1.86	0.58	0.72	3.62	2.19	2.20	2.11	3.18	2.57	2.18	1.99	1.69
AMRD	0.06	0.04	0.03	0.04	0.03	0.02	0.02	0.04	0.03	0.02	0.03	0.03

Note: Location is indicated by *x-y* coordinates: *x*& *y* indicates the polyhouse length and width showed in the Fig. 1 for both Table 1 & 2

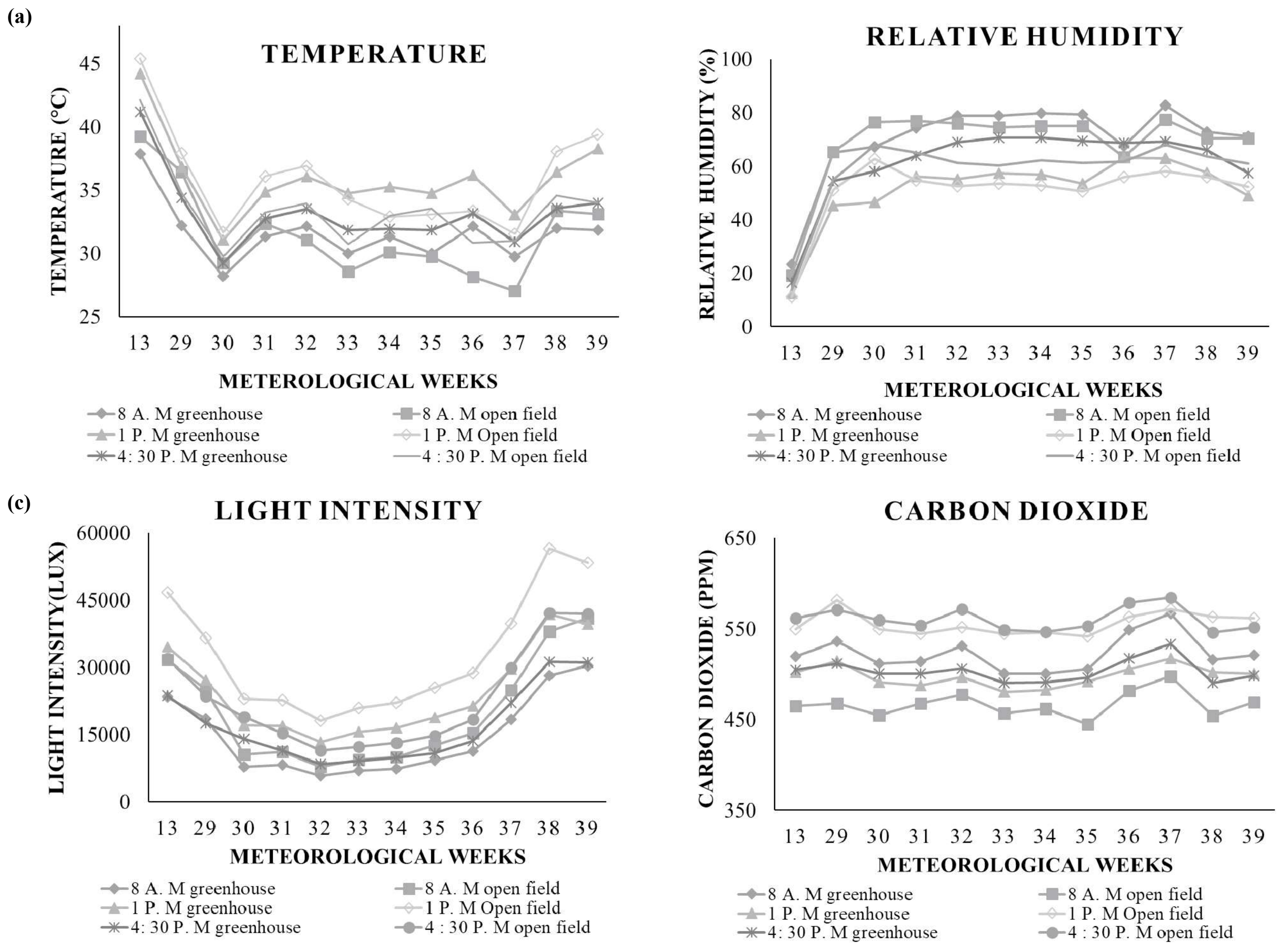

Fig. 3. Weekly average a) temperature b) relative humidity c) light intensity d) carbon dioxide in polyhouse and open field

ranged from 1.5 % to 5%. Omid and Shafaei. (2005), Ahmed et al. (2011) and Kumari et al.(2015) showed similar variations .

Inside the polyhouse, the weekly average light intensity at 1 p.m. ranged from 13 klux to 42 klux, while outside in the open field, it ranged from 18 klux to 56 klux (Fig. 3) .Plants within the polyhouse received 35% less light intensity than those outside, resulting in lower evapotranspiration. Because of the type of cover sheet used, the receiving amount of light intensity within the polyhouse was reduced. Inside the polyhouse, weekly average carbon dioxide levels were 20% lower at 1 p.m. than outside (Fig. 3). CO_2 levels within the polyhouse ranged from 480 to 518 ppm, whereas in the open field, CO_2 levels ranged from 582 to 542 ppm. The CO_2 concentration results are similar to Boulard et al (2017).

Vegetative and yield parameters of the crop: The average crop production was 57.81 t/ha under evapotranspiration (ET) based irrigation. At the final stage of the crop average height of the plant was 2.1m with 65 leaves with a 3cm girth. The average weight of the fruit was 279.3 g with average length and width of the fruit were 21.2 and 6.53cm, respectively. The fruits TSS was 5 brix. The yield and vegetative parameters were found to be satisfactory under these micro-climatic conditions (Rahil and Quanadillo 2015).

CONCLUSIONS

The goal of the experiment was to look at the seasonal variations of micro-climatic parameters in a naturally ventilated polyhouse during the cucumber crop's growth phases. Daily micro-climatic parameter changes, such as temperature, relative humidity, light intensity, and carbon dioxide, were measured. Temperature raised by 0.1 °C, RH reduced by 1%, LI increased by around 1000 lux, and CO_2 levels decreased by 10 ppm for every 1 m increase in height. Under plant growth, changes in temperature and relative humidity varied spatially and temporally along and across the polyhouse which will influence the irrigation scheduling. The average monthly temperature and relative humidity changes were 2.4°C and 9.9%, respectively, with the highest variability in September and July. A similar trend of spatial variability and vertical gradient was seen when no crop was grown inside the polyhouse. The current study revealed that the variations of micro-climatic parameters are negligible in the vertical direction, whereas horizontal variations are noticeable. Finding these variances will aid in finding the best sensor installation location for precise irrigation scheduling. Choosing the right place for sensor installation will allow for accurate real-time interpretation of operational system characteristics. Plants can grow properly in the polyhouse without being stressed during their development because of the ideal environment. Based on the current data, it can be stated that in a naturally ventilated polyhouse in a subtropical climate, putting a sensor for precise irrigation should be done in the centre of the polyhouse. All of these findings can be used to create more precise and efficient irrigation and environmental control systems. This results in highly uniform plant development circumstances, thus enhancing the quantity and quality of produce.

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AUTHORS' CONTRIBUTIONS

Lakshmi Poojitha Challa, Ph. D student, conceptualized, carried out the experimental work, and prepared the manuscript, data curation, and written-original draft preparation. C.D Singh: Chairperson, Advisory committee, conceptualization, methodology, reviewing, and editing. K.V Ramana Rao, extended field facilities, reviewed and edited the manuscript.

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Influence of Insecticides on Food Consumption and Utilization Behaviour of Different *Leucinodes orbonalis* Populations from Punjab

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Abstract: *Leucinodes orbonalis* Guenee is a monophagous and serious pest causing high damage in the brinjal crop. The effect of the different insecticides viz. emamectin benzoate 5 SG, chlorantraniliprole 18.5 SC, deltamethrin 2.8 EC, spinosad 45 SC, fenvalerate 20 EC, cypermethrin 25 EC on food consumption and utilization in different *L. orbonalis* larval populations of Punjab were observed in a laboratory. Emamectin benzoate showed the most potent effect on the feeding of *L. orbonalis* larvae and lowest relative consumption rate (RCR), relative growth rate (RGR), the efficiency of conversion of ingested food to body substance (ECI) and efficiency of conversion of digested food to body substance (ECD) with highest approximate digestibility (AD). The nutritional indices value except AD was higher for *L. orbonalis* larvae fed on untreated food followed by deltamethrin, fenvalerate, chlorantraniliprole, cypermethrin, spinosad and emamectin benzoate treated food. The RCR, RGR, ECI, ECD were maximum for larval populations of the Amritsar region followed by Kapurthala, Malerkotla, Ludhiana and susceptible populations. The approximate digestibility (AD) was higher for larvae of the susceptible population followed by Ludhiana, Malerkotla, Kapurthala and Amritsar populations of *L. orbonalis.* The findings of present study would be helpful in designing management strategies for *L. orbonalis*.

Keywords: Insecticides, *L. orbonalis*, Nutritional indices, Punjab

The brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is the key pest widely distributed throughout the world (CABI 2018, Islam et al 2019). It has high reproductive potential, rapid turnover of generations and cause significant damage during both rainy and summer seasons (Jhala et al 2007, Kaur et al 2014, Singla 2014). Brinjal shoot and fruit borer could cause losses as high as 70 to 92 per cent and even 100 per cent if no control measures are applied (Chakraborti and Sarkar 2011). The synthetic conventional insecticides such as organophosphates, synthetic pyrethroids and carbamates are used generally for the management of this insect pest, but their sole dependency and excessive use has resulted in insecticide residue problems resistance development in *L. orbonalis* (Srinivasan 2008, Kaur et al 2014, Latif et al 2010, Saraswathi et al 2020). The understanding about the nutritional indices of insects such as the rate of ingestion, digestion assimilation and conversion of food would be useful in studying the impact of insecticides on the physiology and behavior of an insect pest (Rashwan 2013). The amount quality and rate and of food consumed by insect's larval stage have a high influence on various aspects of insects such as growth, development, survival and fecundity in adults impacting the overall physiology of insects (Rashwan 2013). The present investigation was designed to study food consumption and utilization in *L. orbonalis* populations from different regions of Punjab concerning various insecticides.

MATERIAL AND METHODS

Culture of *Leucinodes orbonalis*: The infested shoots and fruits of brinjal were collected from vegetable fields of Amritsar, Kapurthala, Malerkotla and Ludhiana regions. The larvae of *L. orbonalis* were extracted from brinjal fruits and reared in the insect physiology laboratory. Larvae of *L. orbonalis* procured from brinjal fruits were transferred to the glass jar (10 ×15 cm) containing fresh pieces of brinjal fruit and placed in an environmental chamber (MAC) at 27° C and 70 per cent relative humidity. The food was changed daily in the morning hours to prevent fungal contamination till reached the fifth instar larvae. The pupae were then shifted to another glass jar, with a moist sponge at the base and the jar was covered with muslin cloth for pupation. On the same day the emerging adults were transferred into new jars for mating and oviposition on the same day. A cotton swab dipped in 10 per cent honey solution was provided food to the adults. The leaf containing eggs was removed daily and replaced with a new leaf to facilitate further oviposition. The population of *L. orbonalis* was collected from untreated infested fruits of the brinjal sown at Entomological Research Farm of Punjab Agricultural University, Ludhiana and reared for twenty generations to develop the susceptible population.

Test insecticides: The commercial preparations of emamectin benzoate 5 SG, chlorantraniliprole 18.5 SC, deltamethrin 2.8 EC, spinosad 45 SC, fenvalerate 20 EC, cypermethrin 25 EC used in toxicity bioassay.

Food consumption and utilization behaviour of *L. orbonalis:* Fresh fruits of brinjal were cut into uniform pieces and treated with LC_{50} concentrations (Table 1) of test insecticides (Chandi and Kaur 2021). The single-piece was placed in an individual plastic cup. Single larva of uniform weight from different populations (Amritsar, Malerkotla, Kapurthala and Ludhiana) was placed in each plastic cup. Ten cups of each treatment replicated thrice were maintained. The initial weight of the larvae was taken before releasing them in cups. After four days of feeding, the weight of these larvae was again recorded to see the larval weight gain. Simultaneously, the weight of food (brinjal fruit) was also recorded for estimation of loss in weight due to evaporation. Based upon these, the corrected weight of the leaf eaten was worked out. Excretal pellets were collected after four days and the weight of the same was recorded. The experiment was replicated thrice. Various consumption and utilization indices were determined (Waldbauer 1968)

i. Relative consumption rate (RCR) = F/TA

Where, 'F' is fresh weight (mg) of food eaten

'T' is duration of feeding period in days

'A' is geometric mean fresh larval weight (mg) during feeding period

ii. Approximate digestibility (AD) = $\frac{F-E}{F} \times 100$

Where, 'F' is fresh weight (mg) of food eaten

'E' is weight (mg) of faeces

iii. Relative growth rate (RGR) = $\frac{G}{TA}$

Where, 'G' is fresh weight (mg) gain of larva

'T' is duration of feeding period in days

'A' is the geometric mean fresh larval weight (mg) during the feeding period

iv. Efficiency of conversion of ingested food to body substance (ECI) = $\frac{G}{F} \times 100$

Where, 'G' is fresh weight (mg) gain of larvae

'F' is fresh weight (mg) of food eaten

v. Efficiency of conversion of digested food to body substance (ECD) = $\frac{G}{F - E} \times 100$

Where, 'G' is fresh weight (mg) gain of larva

'F' is fresh weight (mg) of food eaten

'E' is weight (mg) of faece

Statistical analysis: One-way analysis of variance was done using SPSS software (standard version 16.0). A “P” value of 0.05 was selected as a criterion for statistically significant differences.

RESULTS AND DISCUSSION

The relative consumption rate (RCR), was maximum for Amritsar larval populations of *L. orbonalis* ranging from 1.847 to 9.800 followed by Kapurthala, Malerkotla, Ludhiana and susceptible populations (Table 2). The RGR also showed the same trend (Table 3). Amritsar populations showed maximum ECI ranging from 5.588 to 12.755 followed by Kapurthala, Malerkotla, Ludhiana and susceptible (1.578 to 6.774) populations (Table 4). ECD was maximum for larvae of Amritsar population ranging from 5.692 to 13.888 followed by Kapurthala, Malerkotla, Ludhiana and susceptible populations (1.600 to 7.118) (Table 5). AD was higher for larvae of the susceptible population (95.161 to 98.684) followed by Ludhiana, Malerkotla, Kapurthala and Amritsar populations (Table 6).

RCR was maximum for larvae of *L. orbonalis* fed on untreated food (4.558 to 9.800) followed by deltamethrin-treate, fenvalerate-treated, chlorantraniliprole–treated, cypermethrin-treated, spinosad –treated, and emamectin benzoate-treated food (Table 2). RGR was maximum for larvae of *L. orbonalis* fed on untreated food (0.308 to 1.250) followed by deltamethrin-, fenvalerate, chlorantraniliprole,

Table 1. LC_{50} concentrations of different test insecticides with respect to different regions of Punjab

Insecticides	LC_{50} (%)				
	Amritsar	Kapurthala	Malerkotla	Ludhiana	Susceptible
Emamectin benzoate	0.000046	0.000035	0.000027	0.000023	0.000015
Spinosad	0.000083	0.000058	0.000034	0.000024	0.000016
Cypermethrin	0.0094	0.0049	0.0028	0.0011	0.0009
Chlorantraniliprole	0.0134	0.0117	0.0088	0.0069	0.0046
Fenvalerate	0.0219	0.0176	0.0122	0.0086	0.0065
Deltamethrin	0.0332	0.0275	0.0267	0.0241	0.0198

cypermethrin, spinosad and emamectin benzoate-treated food (Table 3). ECI was maximum for larvae of *L. orbonalis* fed on untreated food (6.774 to 12.755) followed by deltamethrin, fenvalerate, chlorantraniliprole, cypermethrin, spinosad , and emamectin benzoate-treated food (Table 4). ECD was maximum for larvae of *L. orbonalis* fed on untreated food (7.118 to 13.888) followed by deltamethrin, fenvalerate, chlorantraniliprole, cypermethrin, spinosad and emamectin benzoate treated food (Table 5), while AD was recorded more for larvae fed on emamectin benzoate-treated food (98.684 to 98.176), followed by those fed on spinosad, cypermethrin, chlorantraniliprole, fenvalerate, deltamethrin and untreated food (Table 6). The trend was similar for all populations.

The larvae fed on food treated with insecticides showed a decrease in nutrition indices and utilization of food as compared to the untreated one. Among all insecticides, emamectin benzoate was most effective insecticide which hindered the feeding in a significant manner in all populations of *L. orbonalis* collected from different regions of Punjab. The results of the present study are in line with observations of other research workers where emamectin-benzoate, chlorantraniliprole and indoxacarb significantly decreased the nutrition indices and utilization of food in larvae of the cotton leafworm, *S. littoralis* (El-Naggar 2013, El- Sheikh 2015, El-Dewy 2017). Similarly, Xu et al (2016) also reported a decrease in RCR, RGR, ECD and ECI indices but an increase in AD when 4th instar larvae of *Agrotis ipsilon* were fed on an insecticide-treated artificial diet and concluded that cyantraniliprole had antifeedant activity and it prevented insects from feeding which could lead to nutrient insufficiency for normal growth and proper metabolic functioning of insects (Hanning et al 2009, Xu et al 2016). The persistence of food in the digestive tract and slow passage of food through it could have resulted in higher approximate digestibility in *L. orbonalis* in the present study (Nathan 2006, Xu et al 2016). The decreased rates of excretion in treated *L. orbonalis* populations could also have resulted in higher AD. Decrease in consumption index, relative growth rate and efficiency of conversions of either ingested or digested food to body tissue of *S. littoralis* larvae when fed on abamectin and sumialfa was also reported by El-Malla and Radwan (2008). Similarly, Nathan (2006) and Nathan et al (2005) observed significant reduction in RCI, RGR and ECI of *Cnaphalocrocis medinalis*

Table 2. Effect of insecticides on the relative consumption rate (RCR) of different populations of *L. orbonalis*

Treatment (LC_{50})	Relative Consumption Rate (RCR)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Emamectin benzoate	1.250	1.341	1.511	1.666	1.847
Spinosad	1.571	1.687	1.890	2.302	3.571
Cypermethrin	1.918	2.697	2.894	3.187	3.783
Chlorantraniliprole	2.916	3.257	3.357	3.684	4.285
Fenvalerate	3.257	3.642	4.296	4.687	5.312
Deltamethrin	4.032	4.500	4.750	5.000	7.083
Untreated	4.558	5.156	6.250	7.666	9.800

Populations F (4,30)= 1.6144 (*NS) , Insecticides F(6,28)= 13.7692 (*NS)
**NS indicate values are non-significant as P>0.05

Table 3. Effect of insecticides on the relative growth rate (RGR) of different populations of *L.orbonalis*

Treatment (LC_{50})	Relative growth rate (RGR)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Emamectin benzoate	0.0197	0.042	0.063	0.072	0.103
Spinosad	0.0428	0.075	0.097	0.138	0.241
Cypermethrin	0.0930	0.138	0.177	0.206	0.256
Chlorantraniliprole	0.166	0.189	0.216	0.243	0.307
Fenvalerate	0.189	0.221	0.281	0.3125	0.390
Deltamethrin	0.2415	0.291	0.316	0.342	0.570
Untreated	0.308	0.390	0.482	0.716	1.250

Populations F (4,30)= 1.9301 (*NS), Insecticides F(6,28)= 6.9302 (*S)
*S indicate values are significant as P<0.05
*NS indicate values are non significant as P>0.05

fourth instar larvae upon treatment with *Melia azedarach* and biopesticides (Xu et al 2016). The reduced RGR in the present study might be due to the result of irreparable damage to the cellular surface of the midgut lumen and it is also indicated the treated food was unsuitable for the insect (Jansen and Groot 2004, Schoonhoven et al 2005, Xu et al 2016). Spinosad was also an effective antifeedant after emamectin benzoate in this study. Cypermethrin, chlorantraniliprole and fenvalerate moderately affected nutritional and food utilization indices in all *L. orbonalis* populations, while deltamethrin was least effective. The sublethal concentrations of insecticides give the insight to check the effectiveness and potential of insecticides in integrated pest management programmes by providing valuable data regarding the physiology and behaviour of insect-pest (El-Dewy 2017). Among different populations of *L. orbonalis* susceptible population showed the least RCR, RGR, ECI and ECD values but higher AD, while a trend was

Table 4. Effect of insecticides on the efficiency of conversion of ingested food to body substance (ECI) of different populations of *L. orbonalis*

Treatment (LC_{50})	Efficiency of conversion of ingested food to body substance (ECI)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Emamectin benzoate	1.578	3.181	4.230	4.333	5.588
Spinosad	2.727	4.444	5.161	6.000	6.750
Cypermethrin	4.848	5.121	6.136	6.470	6.785
Chlorantraniliprole	5.714	5.813	6.446	6.607	7.166
Fenvalerate	5.813	6.078	6.545	6.666	7.352
Deltamethrin	6.000	6.481	6.666	6.857	8.058
Untreated	6.774	7.575	7.714	9.347	12.755

Populations F (4,30)= 3.0904 (*S); Insecticides F (6,28)= 6.9828 (*S); *S indicate values are significant as $P<0.05$

Table 5. Effect of insecticides on the efficiency of conversion of digested food into the growth (ECD) of different populations of *L. orbonalis*

Treatment (LC_{50})	Efficiency of conversion of digested food into growth (ECD)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Emamectin benzoate	1.600	3.233	4.303	4.411	5.692
Spinosad	2.770	4.518	5.252	6.109	6.882
Cypermethrin	4.984	5.303	6.428	6.832	7.169
Chlorantraniliprole	5.899	6.067	6.808	6.981	7.610
Fenvalerate	6.097	6.378	6.923	7.104	7.836
Deltamethrin	6.302	6.835	7.063	7.361	8.670
Untreated	7.118	8.064	8.346	10.141	13.888

Populations F (4,30)= 2.9567 (*S) , Insecticides F (6,28)= 7.5161 (*S)
*S indicate values are significant as $P<0.05$

Table 6. Effect of insecticides on approximate digestibility (AD) of different populations of *L. orbonalis*

Treatment (LC_{50})	Efficiency of conversion of digested food into growth (ECD)				
	Susceptible	Ludhiana	Malerkotla	Kapurthala	Amritsar
Emamectin benzoate	98.684	98.409	98.307	98.233	98.176
Spinosad	98.454	98.370	98.258	98.200	98.075
Cypermethrin	97.272	96.585	95.454	94.705	94.642
Chlorantraniliprole	96.857	95.813	94.680	94.642	94.166
Fenvalerate	95.348	95.294	94.545	93.833	93.823
Deltamethrin	95.200	94.814	94.385	93.142	92.941
Untreated	95.161	93.939	92.428	92.173	91.836

Populations F (4, 30)= 3.0356 (*S) , Insecticides F(6,28)= 8.9845 (*S)
*S indicate values are significant as $P<0.05$

vice versa for the Amritsar population. The difference could be due to heterogeneity in the insecticidal exposure history of *L. orbonalis* in a different region of Punjab, which could be a result of variations in insecticide spray patterns (Chandi and Chandi 2019). The rate of insecticide application for insect-pest control in any region along with other physical and biological factors plays a vital role in the development of pest resistance (Helps and Van den Bosch 2017, Chandi et al 2019, Chandi and Kaur 2019, Chandi and Kaur 2021). Chandi (2015) reported no significant difference in RCR, AD, RGR and ECI values for the larvae of a susceptible and resistant population of *Plutella xylostella* respectively but ECD for susceptible was significantly more as compared to resistant *P. xylostella* larvae.

CONCLUSION

The insecticides exerted the sublethal influences on the *L. orbonalis* in the form of the change in feeding behavior. The current study could be implied to screen the insecticides for their inclusion in various Integrated Pest Programmes of *L. orbonalis*.

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Elimination of Organophosphorus Pesticides from Wastewater on Fiber Cotton Waste: Kinetic Models Approach

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***Abstract*:** The objective of this work is therefore, to study the removal of three organophosphate pesticides (diazinon, fenthion and malathion), by their adsorption on waste cotton. The waste fiber cotton was characterized using FTIR, XRD, BET and SEM techniques. The effects of pH, contact time, adsorbent dose, initial concentration, temperature and ionic strength were evaluated and the removal efficiency of each parameter was established. The adsorption isotherms on waste cotton fibers, for concentrations of malathion and fenthion chosen for the initial experimental data, were interpreted by the Redlich-Peterson and Langmuir models. The Redlich-Peterson and Langmuir models for malathion retention for grafted cellulose. The results of the kinetic study for all pesticides showed that retention was extremely rapid. In fact, the linear regressions showed that the pseudo second order model monitors both the kinetics for diazinon and the pseudo first order model monitors fenthion, thus, malathion. This was clearly confirmed by the corresponding correlation factor values for each model.

Keywords: Solid waste, Cotton, Adsorption, Wastewater, Textile, Kinetics equilibrium, Pesticides, Organophosphorus

The reduction of pollution sources is, a major obligation for the environmental protection, this to encourage industrials for improving the development of new processes of anti-pollution and new control methods (Dubey et al 2015, Po-Hsing et al 2021). The use of water for human consumption, irrigation or discarded in the wild become major concern for all national and international organizations. The discharge of industrial wastewater containing toxic residues increasingly complex for the natural environment and threat the ecosystem and biotopes (Mhara et al 2021, Jill et al 2019, Theano et al 2021) and especially the human being. to preserve environment for future generations we must comply with international standards and regulations. Among these harmful chemicals, pesticides are used for control of weed. insects and diseases which are responsible for losses (Joop et al 2018, Rajveer et al 2019, Imran et al 2019, Hassaan et al 2020, Feng-Jiao et al 2020). Organophosphorus pesticides have replaced organochlorinated compounds this is due to their long-term stability (Bumbăcilă et al 2020). The removal of organophosphorus compounds at low concentration levels from water always constitute a technological and environmental problem, Because these pesticides contain different ratios of carbon , nitrogen, sulfur, and oxygen atoms in their structure and they are connected to a central phosphorus atom. Among the methods tested are oxide surface catalyzed hydrolysis (Sinar et al 2020). Many techniques have been developed for pollutant removal, such as electrochemistry (Sughosh et al 2018), biodegradation (Purbasha et al 2020) membrane techniques (Saravanann et al 2021), photocatalysis (Thien et al 2020), oxidation (Harald et al 2019) and adsorption (Cosgroveet al 2019, Costantino et al 2020, Iman et al 2020). Among these removal methods, the adsorption has been recently considered a highly attractive technique for the removal of organophosphorus pesticides, as it is easy, low-cost and ecologically friendly. In a standardized atmosphere has high capillarity, cellulose in various physical forms can absorb up to 10% moisture, has a great absorbency. The cellulose has a high affinity for water (hydrophilic) and is the most abundant organic substance in nature (Lei et al 2019). The homopolysaccharide composed of β-D-glucopyranose units linked together by a glycosidic bond β- (1- 4) (substitution of a hydroxyl group (OH) monovalent radical) of the hemiacetal of a sugar with a hydroxyl group of an alcohol of another sugar (Algarra et al 2019, Ibrahim et al 2019). Pesticides are highly toxic, hazardous to the environment and human health when they are transport-ted by sewage (Shalaby et al 2019, Sabran et al 2021) and currently, their separation is the subject of very serious concern. In this study, on absorption and retention of organic pollutants, mainly organophosphorus pesticides malathion (Ma), diazinon (Di) and fenthion (Fe), which are used now, because they are less harmful environment, the influence of physicochemical parameters on the adsorption capacity of pesticides .this according to the spatial chemical structure and physicochemical characteristics (weight-mass-dimensions-volume etc.) of our biomaterial. In addition, tested the material under real conditions after physico-chemical characterization of wastewater (STEP Reghaia-Algeria).

MATERIAL AND METHODS

Adsorbents: Malathion [Ma] [O,O-dimethyl-S-(1,2-dicarbethoxy) ethyl phosphorodithioate] is a widely used non-systemic insecticide and acaricide that exhibits low mammalian toxicity is an pesticide with a molecular formula of $C_{10}H_{19}O_6PS_2$, and is a derivative of phosphor-di-tuning acid (Ravindran 2019), 31(Rani and al 2017) (Fig. 1 and Table 1).

Diazinon [Di] [O, O-diethyl O-(2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate] is a moderately persistent organophosphorus pesticide largely used in agriculture (Eunseon and al 2016a). Fenthion [Fe] O, O-dimethyl O-[3-methyl-4-(methyl-thio) - phenyl] phosphorothioate is one of OP insecticides. It is also moderately toxic to mammals (Wismer 2018) (Eunseon 2016b)

Adsorbent: The choice of cotton is also motivated as raw cotton contains about 87% cellulose, but after treatment it rises to 98-99%, and it has a very high quality hydropyle and material available and inexpensive (Pelegrini and al 2019, Ortega. and al 2019). Pesticides have a linear structure and planar synthesized with sulfonic groups to increase their solubility in water, they are particularly valued for dyeing cellulosic fibers (Fig. 2), this behavior allows the dye to bind on cellulose chains in cotton fiber, often by intermolecular bonding (including hydrogen). The specific area of cotton is 10-3 $km^2.kg^{-1}$- 13, 4.10-3 km^2. Kg^{-1} (Sudong and al 2019, Mäkelä. and al 2019)

For our study, used waste fiber cotton (WFC) from the textile factory DBK (Algeria). They are recovered at the beginning of the process of manufacture l (raw cotton) and after cutting the fabric, in the raw state, among the waste recovered at the unit DBK (carding waste, waste willow) as they contain less impurities, about 10 percent.

Cotton surface structure: The surface of the structure of cotton (Fig. 3), seen under an electronic microscope Dotopon Electronic, 5 MP USB 8), shows that the material has a certain porosity of different sizes, especially mesopores and micropores distributed unequally And which denotes its capacity to adsorb medium and large diameter molecules, which facilitates the flattened physical adsorption and

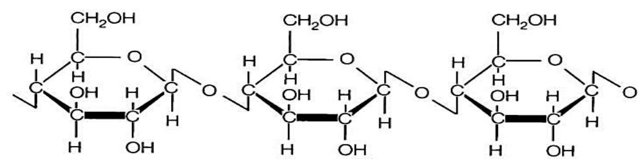

Fig. 2. Structural formula of cellulose

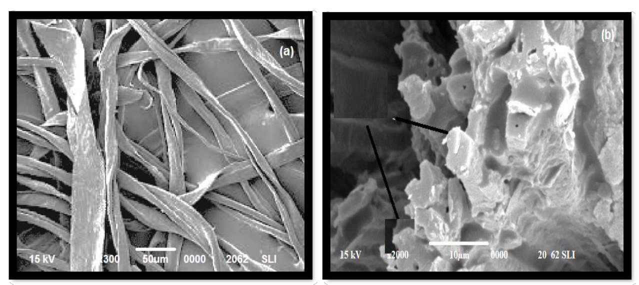

Fig. 3. Micrographics of fibers waste cellulose

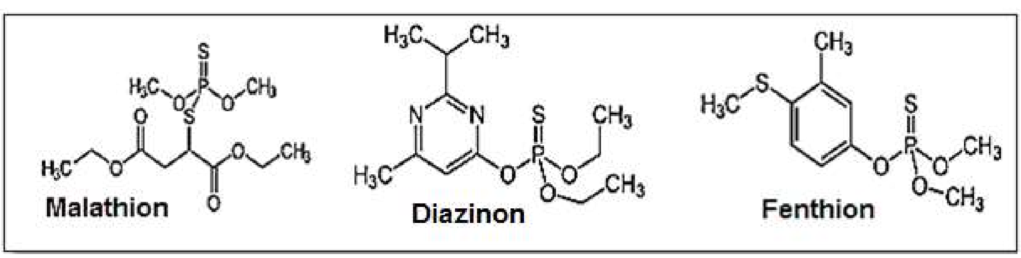

Fig. 1. Chemical formula of studied pesticides

Table 1. Characteristics of the three pesticides

Pesticides	Principal characteristics of pesticides				Topological polar
	Formule	Masse molaire	Masse volumique	Apparence	
Malathion	$C_{10}H_{18}O_6PS_2$	330.338	1.2	Liquid limpid –yellow to brown	128
Diazinon	$C_{12}H_{21}N_2O_3S_2$	304.346	1.1	Liquid colorless -oily	85.6
Fenthion	$C_{10}H_{15}O_3PS_2$	278.328	1.25	Liquidcolorless pur	85.1

exhibits a great increase in the area available for adsorption. This is probably due to the increase of porosity and the number of active sites leading to a slight swelling, allowing optimum permeability. This approach is confirmed by Lei Yang and Ferrero (Ferreira 2020, Jakob et al 2021, Mikko et al 2021a), without any real increase in the volume polymer used in the case where the adsorption is used in fixed bed.

Waste water characteristics: The raw wastewater was processed directly by adsorption with cotton waste. The TOC and COD and the Hydrocarbons largely decreased, without, reduction of the dissolved salts, a negligible decrease of the turbidity, probably due to the presence of very fine solids particles or oil drops (Table 2). The removal of pesticides in raw water and noted their concentrations before and after treatment (Table 3).

Determination of Zeta potential: To estimate the consequences of the modification of the interfacial properties of cellulose (surface tension) caused by the grafting agent "acrylic acid", Measurements of the zeta potential were carried out using Zetameter "Malvern Instruments", which measures the speed movement of a particle in a liquid within an electric field.

Determination of specific surface area: It was measured with a micromeritics ASAP 2420. Approximately 100 mg of WFC was kept at 105°C for minimum of 12 hours. The nitrogen adsorption method was conducted using a Perkin Elmer Sorptometer Model 21 2 C. Nitrogen adsorption and desorption were conducted at -196°C. The analysis of the specific surface area was calculated with the Brunauer, Emmett, Teller (BET) method (Mikko et al 2021b)

Table 2. Characteristics of wastewater (STEP of Reghaia-Algeria)

Parameters	Units	Values before treatment	Values after treatment
Temperature	°C	23.56	23.55
pH		6.55	6.99
COD	Mg/L	33500	201.23
TOC	Mg/L	1288	71.45
Hydrocarbons	Mg/L	54.56	28.12
Dissolve salts	Mg/L	897.23	895.12
Turbidity	NTU	721	87.56
Conductivity	u.s/cm	189.65	172.25

Effect of physicochemical parameters on the three pesticides: All the chemicals used for analysis were have an analytical grade with absolute purity (99.9%). To study the retention of pesticides by adsorption on FPC and GC, the experimental parameters studied are:

- Influence of physicochemical parameters (contact time, pH, the initial concentration, ionic strength of the retention of the three pesticides by varying one parameter at a time).
- Determination of the nature of the adsorption isotherm.
- Determination of the adsorption kinetics.
- The kinetics of adsorption was carried out by repeating the equilibrium study described previously, differing time of contact. The knowledge of equilibrium time was necessary for establishment of kinetic for adsorption models.

RESULTS AND DISCUSSION

Zeta potential: Zeta potential of pure cellulose is -24.34 mV. In addition, the effective charge of the cellulose fibers has gone from (−24.34 mV originally) to (+28.53 mV).

Specific surface area: The specific surface area measurements give a surface area varying from 25 to 28 g/m²

Characterization of WFC with Fourier Transformed Infrared Ray (FTIR): The results were confirmed by spectrophotometry infrared (Perkin Elmer 225) ,with the pelletizing technique in "KBr". Characteristic bands of the studied monomers for our samples confirmed the measurements (Fig. 1) confirmed these results. The absorption "peaks" (or bands), present in the transmission diagram, for our WCF is characterized by the common molecular bonding types and functional groups shown below. The WCF has cyclic bonds (weak acid) and unsaturated groups (double bonds) which are very suitable for the attachment of foreign elements to the structure.

The peaks are listed and detailed below.

3330- OH characteristic of the hydroxyl (OH) groups of

Table 3. Pesticides concentration before and after treatment on fiber waste cellulose with and without extraction and without settle able solids

Pesticides (mg/l)	Units	Before treatment	PA after treatment on cotton fiber waste				
			Percent	With settleable solids and extraction	Percent	Without settleable solids and extraction	Percent
Di	mg/l	75.821	68,45	20..801	91,52	2,256	97,02
Fe	mg/l	45.80	62,98	20.74	80,87	8,564	96,52
Ma	mg/l	41.256	39,81	14.5	52,15	0,076	93,95

cellulose, lignin (Deng 2022)

2897-CH_2- , =CH_2,methyl stretching vibration of C-H in cellulose and hemicellulose(Elisa 2020)

1621-The presence of water in the fibers at the band (Juntao and al 2018)

1428 -CH_2 symmetric bending of the cellulose

1364 bending vibrations of the C-H and C-O groups, respectively, of the aromatic rings in polysaccharides cellulose (Ornaghi 2020)

1315 -Hydrophobic alkyl radical

1033 -Primary alcohols the (CO) and (OH) stretching vibrations

895 -The presence of β-glycosidic linkages between monosaccharides (Balaji et al 2017)

X-ray diffraction analysis of WFC: X-ray diffractometer (D500 Siemens, Germany)was used in this study, operated at 40 kV and 40 mA at a scanning rate of 0.2°/min at ambient temperature (20°C). Figure 5 shows that for XRD plots, the maximum intensity peak is obtained at 22.7 of the 2h, which relates to the cellulose structure (Lizandro et al 2017, Orrabalisa et al 2019). The peaks at 14-16 and 34.4 are characteristics of cellulose (Kargarzadeh et al 2017,Tanpichai et al 2019),which corresponds to the (110), (0 0 2) and (0 0 4) diffraction patterns, these are characteristic of peaks of the Iβ form of cellulose 4 (Jankowska et al 2019, French et al 2020).

Effect of Physicochemical Parameters on the Three Pesticides

Effect of contact time on adsorption: The results presented in Figure 6, show that the retention of the three pesticides occurs in two distinct steps, the first step is slow, second is faster. The adsorption of Diazinon is fast, higher and reaches, Qe ranges, respectively, of 40 and 95 mg/g of retention, then it becomes stable when the equilibrium state is reached in 190 mn, For Fenthion , the retention is practically linear with a very low gradient and the rate of retention is really very low and does not exceed 45 mg/g after 200 mn.The retention rate for malathion is extremely low (10 mg/g), then a desorption process is observed. The spatial chemical structure and dimensions of the pesticides are probably responsible for this behaviour

Effect of initial concentration on adsorption: The initial concentration of pollutant has an important influence on the removal capacity of the solid matrix (De Gisi et al 2016, Aliyeh et al 2018). Forstudy, used concentrations of 5, 10, 20, 30, 40, 50, 60 and 100 mg/l.Ma,Fe and Diretention follows a same pattern, showing three steps adsorption, the first is very fast there , almost instantaneous (from 0 to 1 mg/g), the second step is parabolic and the third shows a saturation ,there is no substantial difference and seems to stabilize for malathion and fenthion (4.5 and 14 mg/g) and decreases appreciably for diazinon (Fig. 7). This can be attributed to the number of active sites of the adsorbent, which is constant (saturated pores) in increase in the number of molecules of polluting pesticides , except for malathion whose chemical structure presents very weak chemical bonds. This is due to molecules and the mode of attachment of the molecules which is "plane" by vertical by bonds (-O-, OH, ester,) at first

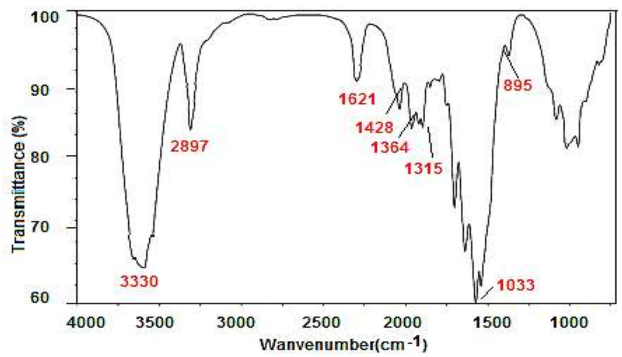

Fig. 4. Fourier Transformed Infrared Ray (FTIR) of fiber waste cotton

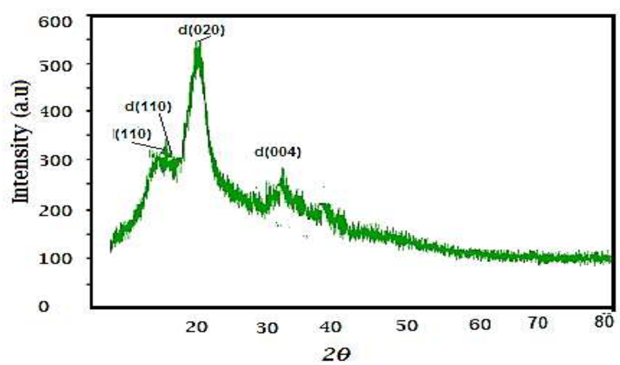

Fig. 5. XRD diffraction patterns of waste fiber cotton

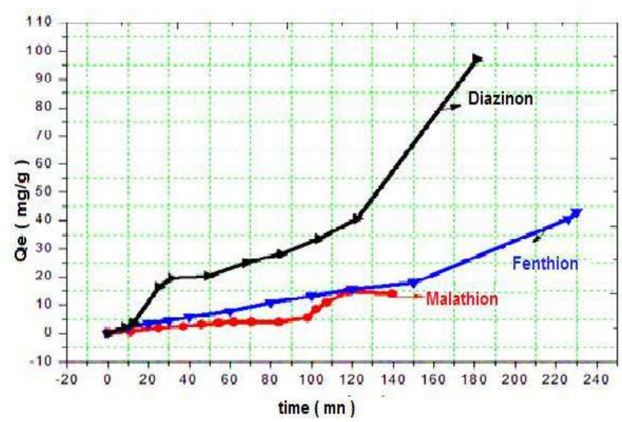

Fig. 6. Effect of contact time on the retention of Ma; Di, FeT ° = 20 ± 2 ° C, V agitation = 350 ; rpm, V = 25 ml, Time = 60 min, pH = 5.33

and in a second phase binds by double bonds and releases carboxylic ions; C-O, in the structure of the carboxylate and OH.(Brian et al 2017, Jiusheng et al 2017).

Influence of pH on the adsorption: The pH has a major influence on the removal process of pesticides from aqueous solutions, as it directly influences both the charge of the ionic species of the adsorbents and the nature of the surface (Fig. 8). The Figure 5, shows that in general the adsorption of pesticides is optimum at pH5 and diazinon is most influenced by pH, compared to the other two pesticides. The equilibrium removal capacity of diazinon increases with pH from 2 to 4.5, peaking at pH 4 and decreasing linearly to pH of 8. This is favoured by the presence of amide groups and protonation of the hydroxyl group on the adsorbent and the protonation of nitrogen atoms on the diazinon pyrimidine groups (Malakootian etal2021). In two other pesticides the influence of pH is very slight, the adsorption is between 2, 4 and 4.5 mg/g for fenthion and for malathion was 1.5 and 3.5 mg/g, showing a very low rate of elimination regardless of pH.It seems that the affinity of the studied organophosphorus pesticides towards the examined FWC decreases in the order diazinon- fenthion-malathion, this is due to their different physico-chemical characteristics (amide groups, surface-area) and this behaviour is influenced by the interference of chemical bonds in the active sites of the adsorber pore .

Influence of ionic strength on the adsorption : The efficiency of pesticide retention under the effect of ionic strength was studied as a function of the dose of sodium chloride salt (NaCl: 10, 30, 60, 120, and 180mg/l) added to solutions of three different ionic strengths (1, 2 and 3 Mole/l).The increase in ionic strength led to a decrease in the number of adsorbed pesticide molecules resulting from the competition between these pesticides and salt ions(Yong-Gu et al 2021, Magdalena et al 2021). The reduction in uptake is probably due to the activity of Na+ ions, which inhibit the approach of Ma, Fe, and Di, to the active sites and the affinity of the WFC adsorbent for Na+ ,as the electrical charge of sodium favours its fixation in microspores (Fig. 9).

Effect of temperature on adsorption : The adsorption of the three pesticides as a function of temperature (Figure 10)shows, that when varying the temperature in the interval 30-36°C at a concentration of 10 mg/L, for each pesticide, the adsorption initially increases, then decreases with increasing

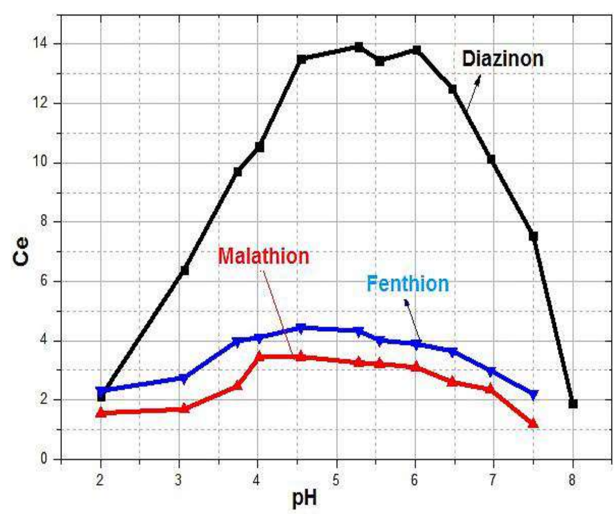

Fig. 8. Effect of the pH of the retention of pesticides (Ma, Di, and Fe). T ° = 20 ± 2 ° C, V agitation = 350 ; rpm, V = 25 ml, Time = 60 min, the retention of Copper ions: T ° = 20 ± 2 ° C, V agitation = 350

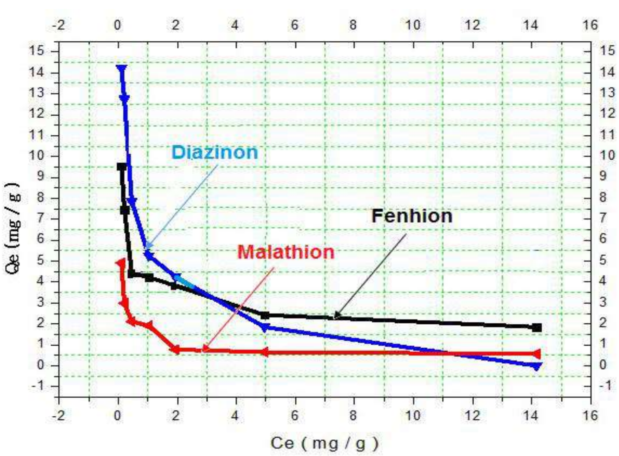

Fig. 7. The effect of concentation on the retention of (Ma, Di, Fe). T ° = 20 ± 2 ° C, V agitation = 350 ; rpm, V = 25 ml, Time = 60 min, pH = 5.33 the retention of Copper ions: T ° = 20 ± 2 ° C, V agitation = 350

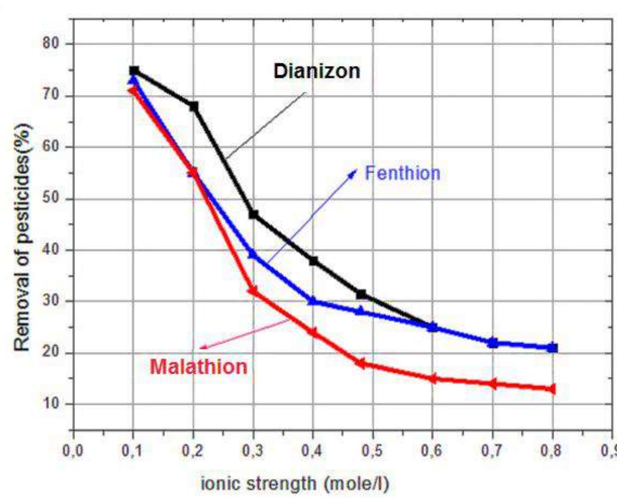

Fig. 9. Effect of pesticides rétention (Ma, Di, Fe). T ° = 20 ± 2 ° C, V agitation = 350 rpm, V = 25 ml, Time = 60 min, pH = 5.33

temperature, which suggests that their adsorption is an exothermic process. The followed by fention are the most adsorbed as temperature increases (35°C) and this may be due to their Topological Polar Surface Area Å² (85.1 , 85.6 and 128) (Table 1).

Validation of kinetic models: Kinetic data obtained for the adsorption process were analyzed based on the three most used models. In order to identify the retention mechanism for initial concentrations (C0 = 5 to 100 mg / L). The equilibrium concentrations of pesticides solutions were measured spectrophotometrically. The amount of pesticide adsorbed per unit mass of carbon-cloth, « qe », was calculated by Eq. (1),

$$q_e = V.\frac{C_0 - C_e}{m} \quad eq1$$

Where «V» is the volume of pesticide solution in l, «C_0» and Ce» are the initial and equilibrium concentrations, respectively, of the pesticide solutions in mg l^{-1} and m is the mass of the carbon-cloth in g. Eq. (1) gives «qe» in mg pesticide adsorbed per g carbon-cloth.

In order to make a detailed analysis for the adsorption data of the present work, some probable kinetic models were applied. These models include intraparticle diffusion, which can be formulated as.

$$q_t = k_i - t_2^1 \quad eq2$$

Pseudo-first order, which can be formulated as

$$\ln c - \ln c_0 = k_1 t \quad eq3$$

and pseudo-second order which can be formulated as

$$\left(1/C\right) - 1/C_0 = k2.t \quad eq4$$

Where qt is the amount of adsorbate adsorbed at any time, «C_0» is the initial concentration of adsorbate, «C» is the concentration of adsorbate at any time, « it» is time and «ki, k_1 and k_2»are rate constants for diffusion, pseudo-first order and pseudo-second order models, respectively. «q_t» is obtained from «c_0» -and «c» values by the following equation:

$$qt = \frac{(C_0 - C).V}{m} \quad eq5$$

The kinetic data obtained for the adsorption process was analyzed for the six models in Table 4

- **First-order pseudo model:** The validity of the Lagergren equation is first tested by plotting ln (qe -qt) against t. The results obtained follow perfectly the linear variation given by the equation for pseudo first order kinetics, and the k1 constant for cellulose
- **Pseudo second-order kinetics model:** Based on the characteristic equation of pseudo second order kinetics and its linear form, the K2 value can be plotted by qt /t as a function of t.
- **Intra-particle diffusion model:** The use of the intra-particle diffusion model, plotting qt as a function of t0.5, indicates the application of the intra-particle diffusion model to the experimental data, controlling the adsorption kinetics. .

The D–R isotherm model was developed to account for the effect of the porous structure of the adsorbents (Qili et al 2018) and was based on the adsorption potential theory and assumed that the adsorption process was related to micropore volume filling as opposed to layer-by layer adsorption on pore walls (Dan et al 2022). The D–R isotherm model was superior to the Langmuir isotherm since it did not consider a homogeneous surface or constant adsorption potential (Kaur et al 2015). All isotherm curves pass through the one point (Cs, q_{max}) and the maximum adsorption capacity q_{max} was obtained when the equilibrium concentration is equal to the solubility. Freundlich isotherm is applicable to adsorption processes that occur on heterogonous surfaces which defines the surface heterogeneity and the exponential distribution of active sites and their energies (Ayawei et al 2015a, 2015b). The Freundlich and Langmuir adsorption isotherm models are used for acceptable error ranges. The shape of isotherms in this study, indicates, that fenthion and malathion are adsorbed as a monolayer, as the pesticides contaminants.

Adsorption isotherms of the three pesticides (Ma-Di-Fe): There was strong affinity of adsorbent for adsorbate, which is often observed when adsorbing in nanometer pore; type I observed for microporous adsorbents such as our WFC for which the porosity consists mainly of pore smaller than 2-5 nanometers, followed by a very significant reduction in the diffusion rate of adsorbate molecules towards the solid surface (Inglezakis et al 2020,Saraswati et al 2020). This is due to the mode of attachment of pesticide molecules which appears to be segmental at the first level (hydrogen bonding) and planar at the end of the process (Kärger et al 2016, Sang and al 2021).

Diazinon isotherms adsorption: The adsorption isotherms of diazinon on WFC (Table 4, Fig. 11) indicates that the

Table 4. Parameters of kinetic models studied fordiazinon

Kinetic models	Constants	Values
Kinetics of the first order	K1 (min^{-1})	1.,022
	Qe (mg/g)	6,8829
	R²	0,9724
Kinetics of the 2nd order	K2 (min^{-1})	0,01749
	Qe (mg/g)	8,2027
	R²	0,9531
Diffusion intra particle	kint (mg/g.$min^{0.5}$)	0,062
	R²	0.784

cellulose support has a very high affinity for Da and capacity of which increases with the initial concentration where two distinct zones are noted: for the first, the adsorption is very quickly, Qe increase from 0 to (4.5 and 5 mg/l) when Ce reached 50 mg/l ,This is explained by the formation of a monolayer segmentaire fixation (vertical) and the second zone the retention is too low(saturation) the adsorption is plane this is due to physical property (surface area of Diazinon, the isotherms obtained are similar to the L type according to Gill classification of isotherms.

Fenthion isotherms adsorption: The adsorption isotherm of fenthion on the cellulose (Table 5, Fig. 11) shows good affinity for. However, the curve seems at first sight to be linear or parabolic and the adsorption capacity increases with the initial concentration from 0 to 100 mg/g, until a level is reached corresponding to a saturation of the adsorption sites, which corresponds to a mono-layer adsorption resulting from the fixation mode of the fenthion molecules on the solid matrix. The isotherms obtained are similar to the L type, in accordance with the Gills classification of isotherms.

Malathion adsorption isotherms: The adsorption isotherm of malathion had a good affinity on the cellulose substrate, where can see a linear curve shaped by two distinct zones: the first, adsorption capacity increased very quickly, with the initial concentration and the second, linear, again, but with a very low gradient and the saturation plateau is reached very rapidly corresponding to saturation of the adsorption sites (Table 6 , Fig. 11). The adsorption operates in a double monolayer, the first one seems to show a plane adsorption in the macro-pores and the second stage is segmental (hydrogen bond and those due to the breaks of the double bonds). The isotherms obtained are similar to the C type

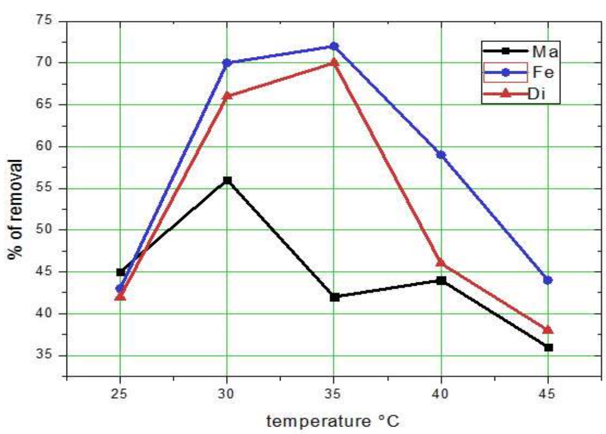

Fig. 10. Effect of temperature of retention of pesticides (Ma, Di, and Fe). C, V agitation = 350 rpm, V = 25 ml, Time = 60 min, the retention of Copper ions: T ° = 20 ± 2 ° C, V agitation = 350

Table 5. Parameters of kinetic models studied for fenthion

Kinetic models	Constants	Value
Kinetics of the first order	K1 (min^{-1})	1.,022
	Qe (mg/g)	6,8829
	R^2	0,9724
Kinetics of the 2nd order	K2 (min^{-1})	0,01749
	Qe (mg/g)	8,2027
	R^2	0,9531
Intra particle	kint (mg/g.$min^{0.5}$)	0,062
	R^2	0.784

Table 6. Parameters of kinetic models studied for malathion

Kinetic models	Constants	Values
Kinetics of the first order	K1 (min^{-1})	0..0153
	Qe (mg/g)	06.43904
	R^2	0.77774
Kinetics of the 2nd order	K2 (min^{-1})	0.00606
	Qe (mg/g)	5.11644
	R^2	0.3362
Diffusion intra particles	kint mg/g.$min^{0.5}$)	1.3911
	R^2	0.9421

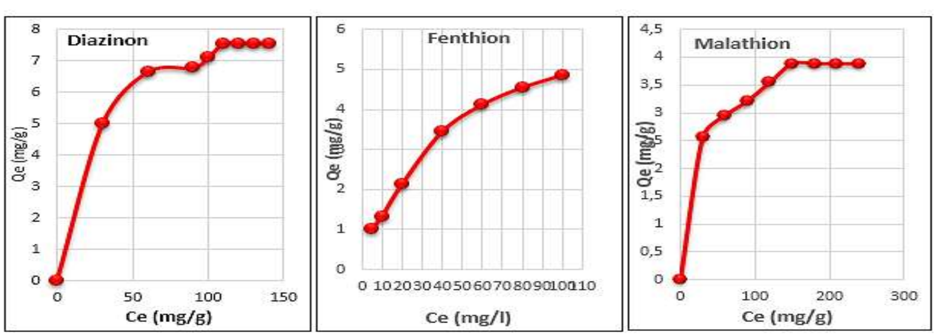

Fig. 11. Isotherm of adsorption ofdiazinon fenthion and malathion. T°= 20±2°C, V agitation= 350 tr/min, V= 25 ml, Time =60 min, pH =5.33

according to the classification of Gills classification of isotherms.

Kinetic studies adsorption: To identify the mechanism retention of pesticides on waste fiber cotton, by varying the initial concentration "C_0"=5 to "C_f" =100 mg/l), six theoretical models were tested (Freundlich, Langmuir, Temkin, Elovich ,Dubinin-Radushkevich (D-R) and Redlich-Peterson. For dianizon follows from the theory of Langmuir and Redlich-Peterson that in formation of more than a monolayer adsorption on the surface of the solid matrix. May be possible and sites are heterogeneous with differing mounting energy (Table 7). The shape of the isotherms indicates, that fenthion and, malathion are adsorbed as a monolayer, as the pesticides contaminants. In recent years, the analysis of linear regression, has been one of the most frequently used methods, to identify, the most, adequate adsorption models, as it can quantify the adsorbate distribution, estimate the adsorption process and test the theoretical assumptions of the adsorption isotherm model The Langmuir equation can be expressed in the form of a linear equation (Sang et al 2017).

$$\frac{C_e}{q_e} = \frac{1}{qmK \backslash E} + \frac{C_e}{q_m} \quad eq6$$

Where Ce is concentration of adsorbate at equilibrium (mg. g^{-1}).

K_L is Langmuir constant related to adsorption capacity (mg. g^{-1}), which can be correlated with the variation of the suitable area and porosity of the adsorbent which implies that large surface area and pore volume will result in higher adsorption capacity. The essential characteristics of the Langmuir isotherm is expressed by a dimension less constant called the separation factor (Somaia et al 2020).

$$R_L = \frac{1}{1 + KL + CO} \quad eq7$$

Where C_0 is initial concentration of adsorbate (mg g^{-1}). R_L values indicate the adsorption to be unfavorable when R_L>1, linear when R_L= 1, favourable when 0<R_L<1, and irreversible when R_L = 0.

Freundlich isotherm is applicable to adsorption processes that occur on heterogonous surfaces. This isotherm gives an expression, which defines the surface heterogeneity and the exponential distribution of active sites and their energies.

$$log_{qe} = log_{CF} + 1/n\ logC_e \quad eq8$$

The linear form of the Freundlich isotherm is as follows: where KF (L/mg) is adsorption capacity and 1/n is adsorption intensity; it also indicates the relative distribution of the energy and the heterogeneity of the adsorbate sites.

Dubinin-Radushkevich isotherm model is an empirical adsorption model that is generally applied to express adsorption mechanism with Gaussian energy distribution onto heterogeneous surfaces. It is usually applied to differentiate between physical and chemical adsorption, Dubinin-Radushkevich isotherm (Agustín et al 2021).

$$lnq_e = lnq_m + \beta E_2 \quad eq\ 9$$

$$e = RT \ln\left(1 + \frac{1}{C_E}\right) \quad eq10$$

$$E = \frac{1}{\sqrt{2B}} \quad eq11$$

Table 7. Correlation coefficients of isotherms adsorption of the three pesticides

Type d'isotherme	Constantes	Diazinon	Fenthion	Malathion
Langmuir	q_m (mg/g)	19,8019	11,0619	2,003205
	K_L (L/mg)	0,246101	0,53937	0,920014
	R²	0,997	0.8117	0.948
Freundlich	K_f (mg^{1-n}. l^n. g^{-1})	4,81193	3,735568	0,84806
	$1/n_f$	0,4521	0,7165	0,3071
	R²	0,897	0.966	0,917
Dubinin-Radushkevich	q_{mDR} (mg/g)	11,01105	1,5666	1,30343
	β	0,59696	6,4215	1,8623
	R²	0,7245	0,751	0,8024
Redlich-Peterson	n	1.999	2.525	1. 692
	ln (K_L^{-n}/q_m)	-2,86749	9,859.10^{-02}	-0,5347
	R²	0,895	0,7473	0,9956
	β	1.605	1,232	1,142
Temkin	B_T (mg/g)	2,6043	3,2423	2,9043
	K_T (L/mg)	0,7894	0,3524	0,3764

Where ϵ is Polanyi potential, β is Dubinin-Radushkevich constant R, is gas constant (8.31 $Jmol^{-1} k^{-1}$), is absolute temperature T, and E is mean adsorption energy.

The Redlich-Peterson isotherm is a mix of the Langmuir and Freundlich isotherms. The numerator is from the Langmuir isotherm and has the benefit of approaching the Henry region at infinite dilution (Mohammad et al 2020). This model is defined by the following expression:

$$q_e = \frac{AC_e}{1 + BC_e^B} \quad eq12$$

Where A is Redlich-Peterson isotherm constant (Lg−1), *B* is constant (Lmg^{-1}), β is exponent that lies between 0 and 1, is equilibrium liquid-phase concentration of the adsorbent (mgl^{-1}), and qe is equilibrium adsorbate loading on the adsorbent (mg g^{-1}).

The linear form of the Redlich-Peterson isotherm is expressed as follows (Abdoulaye Demba and al 2019).

$$\ln \frac{C_e}{q_e} = \beta \ln C_e - \ln A \quad eq13$$

A plot of Ce/qe versus Ce enables the determination of Redlich-Peterson constants, whereβ is slope and A is intercept.

The Temkin isotherm is valid only for an intermediate range of ion concentrations. The linear form of Temkin isotherm model is given by the following:

$$q_e = \frac{Rt}{b} \ln K_T + \frac{Rt}{b} \ln C_e \quad eq14$$

Where b is Temkin constant which is related to the heat of sorption (J). The linear forms of the Elovich model are expressed as mole $^{-1}$ and K_T is Temkin isotherm constant.

Elovich isotherm. The equation that defines this model is based on a kinetic principle, which assumes that adsorption sites increase exponentially with adsorption and this implies a multilayer adsorption. The equation was first developed to describe the kinetics of chemisorption of gas onto solids.

$$\frac{qe}{qm} = Ke.Ce.e\frac{qe}{qe} \quad eq15$$

However, the linear form is expressed as follows

$$\ln \frac{qe}{Ce} = \ln Ke.qm - \frac{qe}{qn} \quad eq16$$

Elovich maximum adsorption capacity and Elovich constant can be calculated from the slope and intercept of the plot of lg (qe/Ce) versus Ce.

The adsorption isotherms of diazinon were studied, and the experimental data follow the Langmuir isotherm. Adsorption of diazinon could be well described by pseudo-first and second order kinetic models, the (R^2 = 0.997) of the Langmuir isotherm is higher than the Freundlich and D-R isotherms respectively (R^2 = 0,897 and 0,895). The removal of diazinon by the cotton waste showed the favourable and spontaneous nature of the adsorption process. This adsorbent can be considered, therefore, as a potentially efficacious adsorbent for the elimination of diazinon from aqueous solutions. For fenthion, The Langmuir constants are in relation with the adsorption capacity and the affinity of the binding areas. The correlation coefficient value (R^2 = 0.966) shows that the Freundlich isotherm fits the experimental data better than the Langmuir R^2 = 0.8117 and Temkin models. The maximal adsorption capacity of fenthion in all conditions), and is occurred on KT. The factor 1/n accounts for the non-linearity of the adsorption isotherm. When the Freundlich coefficient 'n' is around one, the adsorption would be linearly proportional to the equilibrium solution concentration and this would be used as the 'kf' distribution coefficient. The values of the distribution coefficient were not as high as those of Freundlich constant and was observed that the values of 'n' varied from 1.10 to 2.06 for cotton waste. For malathion, the linear regression approaches to determining the isotherm data appear to lead to acceptable fits to the analytical data with good coefficient of regression values (R^2). The linearized Redlich-Peterson and Langmuir, isotherms should also provide a better fit to the experimental data than the linearized Freundlich and Tempkin isotherms. Additionally, the value of the Redlich-Peterson exponent being higher than 1 (β > 1) at 25 ◦C suggests that no strain was fixed on the Redlich-Peterson exponent (Table 9) .The kinetic analysis using varying concentrations indicated that the pseudo-second order model.

CONCLUSION

Material's (cotton fibre waste) primary physiochemical characterizations were used to evaluate its absorbent properties. The infrared fourier transform spectroscopy validates the cellulose-specific bands. Under a scanning electron microscope, an extremely porous morphology of the fibre was observed, which assisted in the aqueous solution's flow. This promotes the growth of more amorphous, less structured areas for higher absorption. The retention of metallic ions on cellulose occurred relatively quickly; equilibrium was reached for copper after 60 minutes and for cadmium and chromium after 120 to 150 minutes for each (unbleached and grafted).The range between pH 4.5. and 6.5 of the organic matter removal rate is a function of pH. The effect of initial concentration, as well as the solid/liquid ratio for pesticides, was also examined, and diazinon was still the most affected. The binding mechanism with a high probability of adsorption is due to the formation of a water bridge by H-bonding between the surface of the biomaterial and the charged electronic side of the examined pesticide molecules, containing O and P. The adsorption isotherms of cotton fiber waste, for the selected malathion and fenthion concentrations, were interpreted by Redlich-Peterson and Langmuir models. Redlich-Peterson and Langmuir models are chosen for retention of malathion on grafted cellulose kinetics for all pesticides showed retention to be extremely rapid. The values of the linear regression factors for each showed that the pseudo second order model monitors both diazinon kinetics and the pseudo first order model monitors fenthion, and malathion. This was clearly confirmed by the corresponding correlation model.

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Resistance to Diamide Insecticides in Stem Borers of Rice in Bangladesh

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***Abstract*:** The study was conducted from September 2019 to December 2020 to know the efficacy of the diamide products, namely Virtako 40 WG (Chlorantraniliprole + Thiamethoxam) and Coragen 18.5 SC (Chlorantraniliprole) against rice yellow stem borer (*Scirpophaga incertulas*) and dark headed stem borer (*Chilo suppressalis*). Larvae of the stem borers were collected from four locations of Bangladesh, and then the diamide products with the concentrations of 0.1, 0.5, 2, 10, 50 and 200 ppm were applied along with untreated control to know the mortality of the larvae at 96 hours after treatments. Diamide products showed differing mortality effects on the larvae based on different concentrations. After the application of Coragen 18.5 SC, the highest rate of mortality of larvae of *S. incertulas* and *C. suppressalis* at the four locations were 55.0 to 68.8% and 51.4 to 59.5%, respectively with the concentration of 200 ppm. In Virtako 40 WG, also the concentration of 200 ppm showed the highest mortality of the larvae of the four locations ranging from 60.6& to 71.3% in *S. incertulas* and 59.8 to 70.9% in *C. suppressalis*. The LC_{95} value of the Coragen 18.5 SC ranged from 337.2 to 494.6 ppm for *S. incertulas* and 435.2 to 557.0 ppm for *C. suppressalis.* LC_{95} value of Virtako 40 WG ranged from 312.1 to 488.1 ppm for *S. incertulas* and 405.6 to 514.0 ppm for *C. suppressalis*.

***Keywords*:** *Chilo suppressalis*, Coragen 18.5 SC, Mortality, *Scirpophaga incertulas,* Virtako 40 WG

Rice is the staple food for more than three billion people in developing countries of Asia (Deepa 2020). It is the highest consumed cereal crop occupying more than 80% of the acreages in Bangladesh (Ullah and Tuhin 2018). The annual production of rice in Bangladesh is 35.3 million tons covering an area of 11.8 million hectares of land (BBS 2020). The geographical, climatic and edaphic conditions of Bangladesh are favorable for year-round rice production. However, in Bangladesh, the average yield of rice is about 3.2 t ha^{-1} (BBS 2020) which is very low compared to other rice growing countries of the World (Akando et al 2020). The infestation of the insect pests, stem borers is the major constraint for rice production, which cause up to 20% yield loss (Rahman et al 2004). Management of stem borers in rice field of Bangladesh is solely relied on chemical insecticides, and the diamide products have been using for a decade. The diamide insecticides exhibited selective activity against Lepidopteran insects and showed significant reduction of the abundance and infestation of stem borers in rice field. Now-a-days, repeated use of diamide products with recommended concentrations did not show satisfactory result against yellow stem borer and dark headed stem borers. Farmers of Bangladesh are applying higher doses of diamide products in the rice field, thus creating disruptions of the rice ecosystem. Understanding the susceptibility of insect populations to insecticides plays a key role in insecticide resistance management and for developing new strategies for pest control. Considering this, the study was done to know the resistance of diamide products, Virtako 40 WG (Chlorantraniliprole and Thiamethoxam) and Coragen 18.5% SC (Chlorantraniliprole) in yellow stem borer *Scirpophaga incertulas* and dark headed stem borer *Chilo suppressalis* of rice through the assessment of the mortality rate of the larvae of the pests.

MATERIAL AND METHODS

The study was conducted during September 2019 to December 2020 in the Laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Egg masses of yellow stem borer *Scirpophaga incertulas* and dark headed stem borer *Chilo suppressalis* were collected from four different locations of Bangladesh (Table 1).

Cut-stem bioassays against stem borers with different treatments: Eggs and larvae of the stem borers were reared in the laboratory at 25 ± 2°C in dark at 70% relative humidity providing fresh stems of rice plants. The rice stems were collected from insecticide treatment free rice field of BSMRAU at the tillering stage and cut into 5-6 cm long pieces. Then the cut pieces were treated with the diamide products Virtako 40 WG (chlorantraniliprole + thiamethoxam) and Coragen 18.5 SC (chlorantraniliprole) separately at 0.1, 0.5, 2, 10, 50 and 200 ppm concentrations. Ten fifth-instar larvae of *S. incertulas* and five of *C. suppressalis* were taken for each replication of the treatments. The larvae with five pieces of treated rice stems were placed in glass jars, and the opening of the jars was closed using cloth. The experiment was conducted following completely randomized design. Experiments of both of the diamide products were constituted with seven treatments (six doses and control) and three replications. Control treatment did not receive any diamide products.

Observation and calculation of the mortality of larvae: Mortality data of the larvae of the stem borers were recorded at 96 hours after treatment (HAT). Number of dead larvae inside of each glass jar was counted and calculated into percentage. The mortality data at different doses of insecticides were corrected using control mortality according to Schneider-Orelli (1947).

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality (\%)} - \text{Control mortality (\%)}}{\text{100- Control mortality (\%)}} \times 100$$

Statistical analysis: The toxic effects of the insecticides on the larvae were analyzed following Probit statistic through the dose-mortality response at 95% fiducial confidence intervals, and the LC_{50} and LC_{95} values with their fiducial limits were estimated. The analyses were performed using IBM SPSS 20.0.

Table 1. Geographic locations of the regions of Bangladesh from where the egg masses of yellow stem borer and dark headed stem borer were collected

Name of the location	Latitude	Longitude
Gazipur	24.0	90.42
Tangail	24.25	89.91
Kishoregonj	24.43	90.78
Bogura	24.84	89.37

RESULTS AND DISCUSSION

The corrected mortality rates of the larvae of *S. incertulas* of the four locations at different treatment concentrations of the diamide products are presented in Table 2. The mortality rates of the larvae of different locations among the treatment concentrations of Coragen 18.5 SC and Virtako 40 WG showed differences. The mortality of the larvae of Gazipur, Tangail, Kishoregonj and Bogura among the treatments of Coragen 18.5 SC ranged from 10.6 to 68.8, 10.6 to 61.9, 17.8 to 60.0 and 11.3 to 55.0%, respectively. The mortality of larvae with of Virtako 40 WG ranged from 10.0 to 71.3, 12.1 to 69.4, 10.0 to 65.0 and 10.6 to 60.6%, respectively at Gazipur, Tangail, Kishoregonj and Bogura, respectively. Mortality rate of *S. incertulas* larvae of the four locations was the highest at 200 ppm concentration of both diamide products (Table 2). The toxicity of the Coragen 18.5% SC on the larvae of *S. incertulas* at Tangail, Gazipur, Kishoregonj and Bogura showed the LC_{50} and LC_{95} values ranging from 125.2 to 227.1 and 337.2 to 494.6 ppm, respectively (Table 3). Due to the application of Virtako 40 WG LC_{50} and LC_{95} against the larvae of *S. incertulas* at these four locations ranged from 115.7 to 213.4 and 312.1 to 488.1 ppm, respectively (Table 3). Due to different concentrations of Coragen 18.5 SC and Virtako 40 WG the mortality of the larvae of *C. suppressalis* of different locations varied (Table 4). The mortality rates of the larvae of Gazipur, Tangail, Kishoregonj and Bogura among the doses of Coragen 18.5 SC ranged from 16.1 to 59.5, 10.8 to 51.4, 10.3 to 52.4 and 6.3 to 57.5%, respectively. In contrast, the mortality rates of the larvae of Gazipur, Tangail, Kishoregonj and Bogura among the treatments of Virtako 40 WG ranged from 20.6 to 70.9, 11.1 to 66.7, 13.6 to 65.0, 12.3 to 59.8%, respectively. Mortality rate of the larvae of all the four locations was the highest at 200 ppm concentration irrespective of the diamide products.

The toxic effects of the Coragen 18.5 on the larvae of *C. suppressalis* at Tangail, Gazipur, Kishoregonj and Bogura showed the LC_{50} and LC_{95} values varying from 159.6 to 256.6 and 435.2 to 557.0 ppm, respectively. In case of the toxic

Table 2. Effects of different concentrations of Coragen 18.5 SC and Virtako 40 WG on the corrected mortality rate (%) of *Scirpopgaga incertulas* larvae collected from different locations of Bangladesh

Treatment concentration (ppm)	Coragen 18.5 SC				Virtako 40 WG			
	Gazipur	Tangail	Kishoregonj	Bogura	Gazipur	Tangail	Kishoregonj	Bogura
200	68.8	61.9	60.0	55.0	71.3	69.4	65.0	60.6
50	45.0	40.6	39.4	38.1	53.1	40.0	40.6	46.9
10	30.6	26.9	33.1	32.5	35.0	30.0	31.3	32.5
2	25.0	18.8	21.9	23.8	26.3	21.3	25.6	23.1
0.5	20.0	13.1	16.3	18.1	19.3	15.6	20.0	18.9
0.1	10.6	10.6	17.8	11.3	10.0	12.1	10.0	10.6

Table 3. Toxic effects of different concentrations of Coragen 18.5 SC and Virtako 40 WG on the larvae of *Scirpopgaga incertulas* collected from different locations of Bangladesh

Location	Insecticide	LC_{50} (Lower limit-Upper limit)	LC_{95} (Lower limit-Upper limit)	Slope±SE	χ^2 (df)
Gazipur	Coragen 18.5 SC	125.2 (117.8-353.3)	337.2 (258.5-429.0)	-0.4±0.2	49.3 (38)
	Virtako 40 WG	115.7 (103.9-323.9)	319.0 (270.0-430.1)	-0.4±0.2	47.8 (38)
Tangail	Coragen 18.5 SC	151.1 (115.3-290.5)	385.0 (267.1-543.3)	-1.2±0.2	56.2 (38)
	Virtako 40 WG	131.9 (117.4-219.9)	380.6 (261.9-563.9)	-0.3±0.2	53.3 (38)
Kishoregonj	Coragen 18.5 SC	138.3 (117.6-328.3)	386.6 (262.3-569.8)	-0.3±0.2	52.7 (38)
	Virtako 40 WG	122.5 (109.5-227.8)	312.1 (275.3-584.7)	-0.1±0.1	56.5 (38)
Bogura	Coragen 18.5 SC	227.1 (114.9-362.6)	494.6 (366.0-707.1)	-0.2±0.2	54.0 (38)
	Virtako 40 WG	213.4 (189.2-333.2)	488.1 (365.7-649.8)	-0.3±0.2	49.3 (38)

Concentrations are expressed as ppm. Figures in parentheses indicate the range

Table 4. Effects of different concentrations of Coragen 18.5 SC and Virtako 40 WG on percent mortality rate of *Chilo suppressalis* larvae collected from different locations of Bangladesh

Treatment concentration (ppm)	Coragen 18.5 SC				Virtako 40 WG			
	Gazipur	Tangail	Kishoregonj	Bogura	Gazipur	Tangail	Kishoregonj	Bogura
200	59.5	51.4	52.4	57.5	70.9	66.7	65.0	59.8
50	45.8	32.4	39.2	42.5	52.5	50.0	45.9	43.1
10	32.5	24.2	26.4	31.3	41.9	38.8	32.5	29.7
2	26.9	21.6	23.3	27.5	31.1	25.0	24.4	21.7
0.5	21.4	13.1	15.6	13.8	26.1	19.4	16.1	18.9
0.1	16.1	10.8	10.3	6.3	20.6	11.1	13.6	12.3

Table 5. Toxic effects of different concentrations of diamide products on the larvae of *Chilo suppressalis* collected from different locations of Bangladesh

Location	Insecticide	LC_{50} (Lower limit-Upper limit)	LC_{95} (Lower limit-Upper limit)	Slope±SE	χ^2 (df)
Gazipur	Coragen 18.5 SC	159.6 (142.5-362.0)	435.2 (289.5-564.3)	1.3±0.2	7.7 (22)
	Virtako 40 WG	149.7 (121.1-275.3)	405.6 (207.6-501.1)	-1.3±0.2	8.7(22)
Tangail	Coragen 18.5 SC	171.9 (103.3-405.4)	455.6 (281.4-565.5)	-1.2±0.2	10.8 (22)
	Virtako 40 WG	154.7 (106.1-369.4)	430.9 (203.8-591.9)	-1.2±0.2	6.2 (22)
Kishoregonj	Coragen 18.5 SC	244.1 (136.91-357.2)	545.0 (384.4-664.2)	-1.1±0.2	8.9 (22)
	Virtako 40 WG	211.5 (143.2-366.0)	508.9 (398.5-685.4)	-1.2±0.2	7.1 (22)
Bogura	Coragen 18.5 SC	256.7 (144.9-387.8)	557.0 (312.9-703.9)	-0.9±0.2	19.1 (22)
	Virtako 40 WG	247.7 (140.3-361.0)	514.0 (390.0-669.1)	-1.2±0.2	7.6 (22)

Concentrations are expressed as ppm. Figures in parentheses indicate the range effects of Virtako 40 WG on the larvae of *C. suppressalis* collected from those four locations, the LC_{50} and LC_{95} values ranged from 149.7 to 247.7 and 405.6 to 514.0 ppm, respectively (Table 5).

The findings of the present study showed that the recommended dose (200 ppm) of the diamide products, Coragen 18.5 SC and Virtako 40 WG revealed variations in the mortality of the larvae collected from different geographic locations and due to the use of insecticides in different patterns. The highest mortality rates of the larvae of *S. incertulus* at the recommended dose (200 ppm) of Coragen 18.5 SC and Virtako 40 WG were 68.8% and 71.3%, respectively. The highest mortality rates of the larvae of *C. suppressalis* at the recommended dose (200 ppm) of Coragen 18.5 SC and Virtako 40 WG were 59.5 and 70.9%, respectively. The results of the recommended dose of the diamide products indicated that the efficacy of the insecticides against the stem borers was poor. Richardson et al (2020) reported high resistance to diamide products in Lepidopteran insects. Population of *C. suppressalis* collected from different geographic locations of China depicted low level of resistance to Chlorantraniliprole (Gao et al 2013). A six years study by Ahmad and Gull (2017) showed that the Chlorantraniliprole revealed low level of resistance in the larvae of *Spodoptera litura.* Nozad-Bonab et al (2021) showed that the LC_{50} value of Chlorantraniliprole was 0.02 (0.002-0.03) and LC_{90} was 0.28 (0.17–0.61) on lepidopteran larvae. But our study showed higher levels of LC_{50} and LC_{90} values which indicated that the recommended dose of the diamide products did not remain sufficiently toxic to the larvae of the stem borers.

CONCLUSION

The bioassay data of rice stem borers, collected from different locations of Bangladesh, revealed low levels of mortality with the recommended dose (200 ppm) of the tested diamide products. According to the current findings, Coragen 18.5 SC at the recommended dose revealed 55.0% to 68.8% mortality of *S. incertulas,* and 51.4 to 59.9% of *C. suppressalis*. The Virtako 40 WG at the recommended dose exerted 60.6% to 71.3% mortality of *S. incertulas* and 59.8 to 70.9% of *C. suppressalis* larvae.

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Species Composition and Conservation Risk Factors for Bumblebees (*Bombus* spp.) Across the Chitwan Annapurna Landscape, Nepal

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***Abstract*:** The study was conducted in Chitwan Annapurna Landscape(CHAL) to explore the species composition of bumblebee (*Bombu*s spp.) and their conservation risk factors along elevation gradients. The field surveys were conducted in a range of different habitats along an altitudinal gradient (500 to 3500 m asl) in the Kaligandaki, Marsyangdi, and Budhigandaki river basins of study area. A total of 656 *Bombus* specimens were identified comprising 16 different species with eight new records (*Bombus grahami, B. pressus, B. branickii, B. cornutus, B. novus, B. turneri, B. lepidus, and B. asiaticus*) from this region. The highest relative abundance was of *B. haemorrhoidalis* followed by *B. festivus*. The major survival risks factors for bumblebee were habitat loss, ecosystem alternation by invasive plants, pesticide application and nesting sites destruction by many human activities. The severity of conservation risk factors varies along elevation gradient that determine on species filtering of bumblebee along the CHAL.

***Keywords*:** Bumblebee, Altitudinal gradient, Species distribution, Conservation implication

Bumblebees (Hymenoptera: Apidae) are an important group of pollinators in the alpine and subalpine regions of the world (Bingham and Ranker 2000, Yu et al 2012, Streinzer et al 2019). Although, the understanding of their dispersal limitation along altitudinal gradients is vastly lacking from many parts of the world. In mountainous regions, it is difficult to predict how bumblebees interact with local and landscape feature (Fourcade et al 2019). It is known that the diversity and distribution of bumblebee is strongly influenced by elevation gradients and the geographical location of the mountain, as well as the specific ecological adaptations of each species and their thermal tolerance (Burkle and Alarcón 2011). Beside this is also influenced by the human disturbance and other limiting ecological factors such as habitat alternation. The Himalayan range is a hotspot of bumblebee (Williams et al 2009, Bhusal 2020). However, taxonomic richness, vulnerability to climate change and other anthropogenic pressures remain poorly known (Williams 2009, Williams et al 2010, Saini et al 2015, Streinzer et al 2019), particularly, in heterogeneous landscapes. The declines of bumblebees were some have also shown the influence of landscape composition on bumblebee populations (Vray et al 2019). Similarly, the conservation risks of bumblebees are thought to be driven by a range of interacting human-induced threats, including habitat loss (and the associated loss of food and nesting resources), pesticide use, the introduction of new pathogens and non-native species, and the increasing threat posed by the climate change (Biesmeijer et al 2006, Goulson et al 2015, Potts et al 2016). Though the threats facing bumblebees and the impacts of their decline have been relatively well characterized in some parts of the world, they remain largely understudied in many parts of the world where the threats are often rapidly increasing and the impacts of insect pollinators decline are expected to be more severe (Timberlake and Morgan 2018). Within the Himalayan region, bumblebees have been intensively studied along altitudinal gradients, particularly in the West Himalaya (Saini et al 2015), but such studies are poorly documented in the Central and Himalaya (Williams et al 2010, Saini et al 2011). In the recent days, this region is facing the increasing threat of climate change and growing human pressure that directly and indirectly affects the biodiversity of this region, particularly, the high altitude ecosystem of this region are now threatened by intensive grazing, expansion of agricultural land and other rapid land use change (Telwala et al 2013, Sharma 2016). Furthermore, this region has high conservation risks of bumblebees that to be driven by a range of other human-induced threats, including loss of food and nesting resources, increasing pesticide use, the introduction of new pathogens and non-native species, and climate change (William et al 2010, Streinzer et al 2019, Bhusal 2020). However, the conservation issues of Bumblebee have been poorly reported from central Himalaya Nepal (Bhusal 2020). The present study was conducted in the Chitwan Annapurna Landscape (CHAL) in the central Nepal where altitudinal and climatic gradients are apparent, giving rise to a range of distinct ecological zones, each with their own unique assemblage of flowering plant species.

MATERIAL AND METHODS

Study area: This study was carried out along an altitudinal gradient (from 500 to 3500 m asl.) in three river valleys of the Chitwan Annapurna Landscape (CHAL): Kaligandaki (Mustang site 28◦87' 65.15" N - 83◦79' 47 65" E), Marshyandi (Manang site, 28◦57' 52. 62" N - 84◦18' 66. 28" E), and Budhigandaki (Gorkha site, 28◦18' 36.44" N - 84◦85'15.79" E) (Fig. 1). The CHAL region contains ranges from a subtropical monsoon climate with very high rainfall in the south (below 1000 m) to a temperate climate in the mid-hills (1000-4000 m) and an alpine/arctic climate with very low rainfall above 4000 m (Chhetri et al 2017). The area hosts diverse habitat types, including agriculture, forested, grassland, and human settlements. The study area is rich in biodiversity and includes the Annapurna conservation area which is an important transit route for migratory birds, as well as

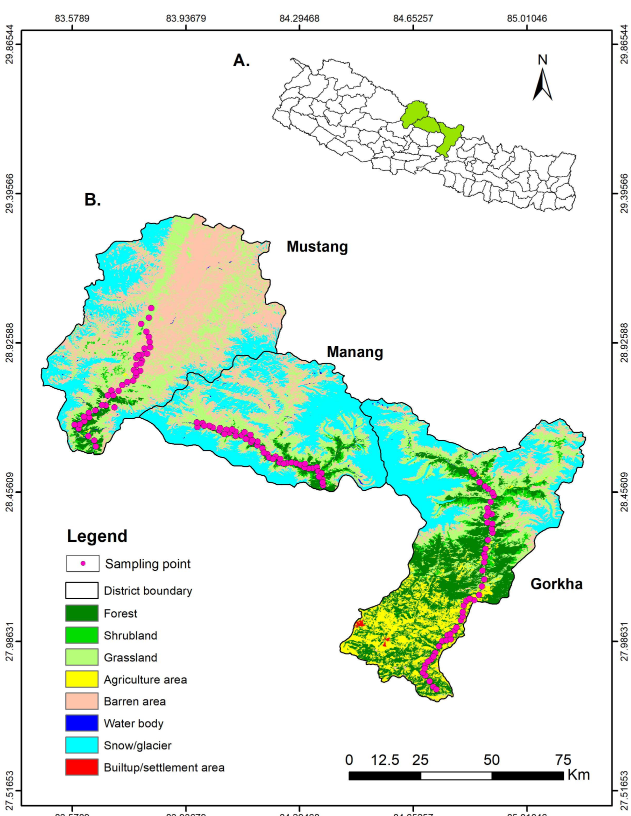


Fig. 1. Study area showing sampling points for bumblebee with in Kaligandaki, Marsyangdi and Budhigandaki River basins

supporting populations of various endangered species including the snow leopard, red panda, and the Himalayan black bear (Adhikari et al 2019, Chetri et al 2019). The landscape has a rich cultural heritage, with over four million people who have a high dependency on forest resources and ecosystem services for their livelihoods and well-being.

Bumblebee surveying and identification: Field surveys were conducted throughout the entire flowering season between April and November 2019 and followed three accessible walking routes (transects) along the river valleys of the Kaligandaki, Marshyandi, and Budhigandaki Rivers (Fig. 1). Opportunistic surveys were conducted along the three transects from 500 to 3500 m (Goulson et al 2005). Whenever a bumblebee was detected at a particular point along the route, we stopped and observed this point for up to one hour, or until the observer was satisfied that all possible species on the site were completely collected at a point for thirty minutes. Those individuals only collected which area foraged only in the floral parts. The survey was carried out between 9 am and 6 pm when rain was absent and wind speeds were low. *Bombus* species were captured using an entomological net and immediately killed using ethyl acetate. During the survey, habitat characteristics, host plant species, species frequency, and altitude and GPS location were recorded. Specimens were stored in airtight containers with a few layers of tissue and the addition of a few drops of ethyl alcohol to prevent the growth of mold during transport. Specimens were subsequently dry-mounted using standard insect pins and deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, and Kathmandu. The collected specimens were observed under stereoscopic microscope and identified using published identification keys for adjacent regions, e.g., Kashmir (Williams 1991), Nepal (2009), Sichuan (Williams et al 2009), North China (An et al 2014) and India (Saini et al 2015).

Conservation risk assessment for the bumblebee: Two approaches were applied to identify risk factors for the conservation of bumblebee in this area: (i) performed direct observation of habitat characteristics and noted presence and absence of particular threats in the local sampling sites across the altitudinal gradients and ii) conducted a household survey in the sampling sites which assessed local residents' experience-based perception of changes in local habitat characteristics, trends and patterns, perceived risk to pollinators, and attitudes towards pollinators. The data based semi structured questions about the history, severity and impact of these threats was collected. The survey was conducted on a total of 540 people, with 180 people from each site equally spread across the altitudinal range of our study area. The survey included respondents above 25 years of age and prioritized local farmers, teachers, social workers, shepherds and community forest user groups.

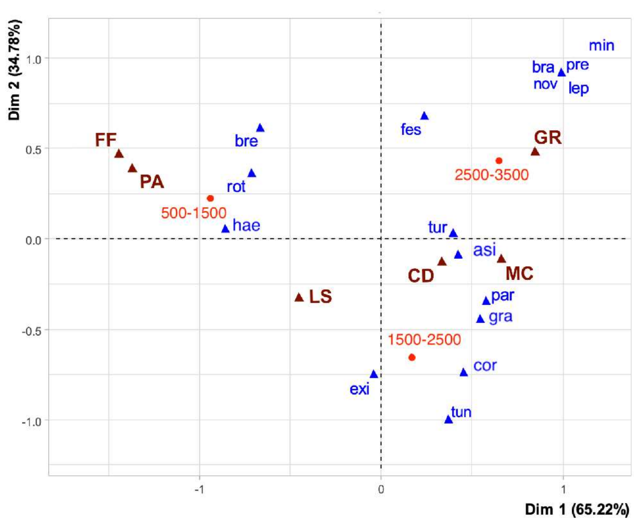
Data analysis: The relative composition of 16 Bombus species was determined. The non-parametric data from the habitat assessment were quantified using the cross tabulation (two-way contingency) to determine the frequency distribution of each threat within the three elevation gradients. The relative number of risk factors was produced and the thus produced data was used to performed for correspondence analysis (CA) among *Bombus* species, severity of threat, and three elevation gradients. All data were analyzed using R program (R core Team 2022).

RESULTS AND DISCUSSION

Species composition in study area: A total of 656 *Bombus* specimens were collected comprising 16 different species. In this study, eight species (*Bombus grahami, B. pressus, B. branickii, B. cornutus, B. novus, B. turneri, B. lepidus, and B. asiaticus*) were new records for the CHAL. In study area, the highest mean abundance (Levene's test: n = 136, df = 15, F = 3.40, P = 0.001) was observed *in B. Pressus*, *B. cormutus* and followed by *B. lepidus* and *B. branickii* (Table 1). Similarly, a lower mean abundance was observed in *B. tunicatus, B. eximius* and followed by the lowest mean abundance of *B. haemorrhoidalis.* Some species such *as B. festivus*, *B. grahami*, *B. asiaticus* and *B. lepidus* had a higher frequency variation within the study area (Fig. 2). The correspondence analysis (CA)was performed to determine the pattern of species, altitudinal gradients and associated risk factors. The species were well ordinated along the altitudinal gradients with specific associated risk factors present thereby. The highest species richness was in high altitude (16 species) followed by in mid altitude region (10 species), whereas, only 7 species recorded from the lower altitudinal gradients. Five species including *B. branickii*, *B. lepidus*, *B. miniatus*, *B. novus* and *B. pressus* were limited to the high elevation of the study area. Similarly, four other species (*B. cornutus*, *B. ghrami*, *B. parthenius* and *B. tunicatus*) were limited to mid and high elevations, with *B. tunicatus* most dominant at the mid elevation of the study area. Seven species *B. asiaticus*, *B. eximius*, *B. festivus, B. haemorrhoidalis, B. rotundiceps*, *B. turneri* were observed from all elevational gradients. The major survival risks for bumblebee were habitat loss by emerging invasive plants (IV), increasing trends of pesticide application (PA), colony destruction by local people (CD), increasing monocultures (MC), habitat loss landslides (LS), forest fire (FF), and intensive grazing (GR). However, the relative severity of each risk factors differed across the elevation gradient. The intensity (Friedman chi-square -X^2 = 33.807, P = 0.053) of these threats at each elevation was determined. The forest fire, pesticide application and invasive species (in particular *Ageratina adenophora* and *Parthenium hysterophorus*) were the most substantial threats at lower elevations (500- 1500 m) level of this landscape. Similarly, colony destruction by

Table 1. Relative abundance (%) of *Bombus* species and their mean elevation in sampling area and individual collection

Sites	WSA (%)	Mean elevation (m. above sea level) recorded
Bombus asiaticus	4.35 (29)	2556
B. branickii	0.72 (5)	2660
B. breviceps	5.07 (34)	2399
B. cornutus	2.17 (15)	2700
B. eximius	18.84 (124)	2257
B. festivus	19.57 (129)	2443
B. grahami	3.62 (24)	2452
B. haemorrhoidalis	20.29 (134)	2189
B. lepidus	3.62 (24)	2673
B. miniatus	0.72 (5)	2592
B. novus	0.72 (5)	2536
B. parthenius	2.17 (15)	2353
B. pressus	0.72 (5)	3000
B. rotundiceps	5.8 (39)	2121
B. tunicatus	5.07 (34)	2309
B. turneri	5.07 (34)	2353



Elevation level (m asl): (500-1500, 1500-2500, 2500-3500), The Risk factors are codded as: PA- pesticide application, IV- invasive plants, PA-pesticide application, CD- colony and nesting site destruction, MC- monocultures, LS-landslides, FF- forest fire, GR- intensive grazing

Fig. 2. Corresponding analysis (CA) for *Bombus* species abundance, specific elevation range and associated risk factors

local shepherds, emerging monocultures tendencies and pesticides application in commercial vegetables farms were the major risk factor at the mid elevation (1500-2500 m). Beside this, wider range of landslides occurrence was also recorded at the mid elevation region whilst at high elevations (2500- 3500 m) over-grazing and intense habitat loss by some human activities during herbal collection are notable risk factors for the important host plant for bumblebee. These factors impacting on species composition of bumblebee (Hoiss et al 2012, Sydenham et al 2015, Miller-Struttmann and Galen 2014). Furthermore, this might be linked with the critical thermal limits (Martinet et al 2015, Oyen et al 2016) of bumblebees which determine their altitudinal distribution (Dudley et al 2017). In this study, some of the species such as *B. asiaticus, B. eximius, B. festivus, B. haemorrhoidalis, B. rotundiceps, B. turneri* exhibited a particularly wider range i.e. from lower to higher altitudinal range in this study. Furthermore, this altitudinal variation in the distribution of *Bombus* species might be link with the critical thermal limits of these species driven by environmental temperatures (Oyen et al 2016), and habitat selection process along altitudinal gradients (Carvel 2002, Saini et al 2012, Diaz-Forero 2013, Goulson et al 2015). Similarly, the climate and land-cover change in this landscape probably alter the bumblebee species richness and community composition in CHAL (Fourcade et al 2019). The most abundant three species, *B. rotundiceps, B. haemorrhoidalis, B.eximius,* were observed at relatively low mean elevations as also reported by Williams et al (2009) and Streinzer et al (2019) in other parts of Himalaya. In case of *Bombus breviceps,* its mean altitudinal distribution in eastern sites was found to be at lower elevations, these species were recorded up to a similar altitudinal level in the western Himalaya (Saini et al 2015). Some of the species were confined to a relatively narrow altitudinal zone, or from specific sites, demonstrating their high specificity in this study area. For example, *B. asiaticus, B. branickii, B. cornutus, B. pressus, B. novus.* these species may be particularly adapted to unique microhabitats and vegetation types as they are restricted to a very limited altitudinal range in our study sites. In summary, altitude appears to act as an important environmental filter for the community assembly of bumblebees in area. In addition, vegetation dynamics, micro climatic variation, topographic factors and anthropogenic disturbance are also likely to be influencing bumblebee communities in this landscape.

Some of the species such as *B. breviceps, B. rotundiceps,* and *B. haemorrhoidalis,* appear mostly with lower altitudes having threats like FF, PA. At mid-altitudes, species *B. asiaticus, B. cornutus, B. parthenius, B. grahmi, B. tunicatus* are most prevalent, corresponding with CD and MC. At high altitudes, species *B, festivus, B. branicki, B. novus, B. pressus, B. Lepidus* and *B. miniatus* are most common, and the threats most likely to be encountered was GR. Many authors suggested, the habitat destruction by infrastructure development and the resulting changes in landscape configuration and permeability is likely to reduce the availability of food resources, hibernation and nesting sites for bumblebees (Kells and Goulson 2003, Otterstatter and Thomson 2008, Osborne et al 2008, Wermuth and Dupont 2010). At mid-altitudes, the colony destruction evidence, and extensive monocultures, were the important threats to bumblebee observed in recent years from CHAL region (Bhusal 2020). Periodic forest fires are a natural phenomenon in this region their increasing frequency and intensity as a result of human activities is a cause for concern, given their potential to destroy bumblebee nests and foraging habitat particularly at lower elevations. In the mid altitude region, shepherds frequently destroy bumblebee colonies using fire, to obtain protein sources from their larvae. Ultimately, this will have a negative impact on the habitats and food resources for bumblebees.

An additional threat identified from the lower to mid elevation gradients of our study area is the spread of rapidly-growing invasive plants species, such as *Ageratina adenophora and Parthenium hysterophorus*. In the last 10 years, these species have proliferated across the CHAL, particularly at lower elevations (below 1500 m), with negative effects on the local biodiversity (Sheathe et al 2019, Maharjan et al 2019) including native flora including for host plants of bumblebees. Some of the study from other parts of the world have listed invasive plants as an important potential driver of bee declines bee communities (Fiedler et al 2012, Morales et al 2013); however, the exact nature of their impacts upon bee populations and the mechanisms by which this occurs, remains unclear. Further studies are required to clarify the extent of the threat posed by invasive plants in this region and identify the likely effects on bumblebees. At high altitudes, species *B. festivus, B. branicki, B. novus, B. pressus, B. lepidus,* and *B. miniatus* are most common even in the high livestock grazing sites. The intense livestock grazing in the mid to high altitude regions of the study area were found to be degrading the important grassland ecosystem, likely reducing the availability of floral resources for bumblebees). Indeed, a recent study in the in the Western Himalaya showed that livestock grazing patterns can shift the abundance and community composition of grassland flora, with likely knock-on effects for insect pollinators (Hatfield and LeBuhn 2007, Hatfield 2007). The collection of the medicinal herb such as caterpillar fungus (*Ophiocordyceps sinensis*) is also being a high risk factor for the natural habitat of bumblebee bee of that region. While collecting this *O. sinensis,* thousands of people are deployed in the flowering seasons and search this herb digging on ground that may destroy suitable and specific foraging flowering plant for bumblebee and other bee communities leading to crisis on food plants.

CONCLUSION

It attributes the microclimatic and food resources change along the elevation gradient that majorly limit the shaping of distribution and diversity of Bombus species in study area. The severity and type of risk factors for the survival of bumblebees in this region vary along altitudinal level that affecting in species composition and abundance. The further identification species specific risk factors along elevation level and suitable mitigation approaches for the sustainable conservation of bumblebee and pollinator communities from CHAL is recommended.

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Groundwater Quality Assessment for Sri Sathyasai Puttaparthy Region of Andhra Pradesh using Geographical Information System

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Abstract: The study area falls under scarce rainfall zone of Andhra Pradesh, groundwater is the major source of irrigation, two hundred and thirty one (231) groundwater samples were collected and were analyzed for water reaction, conductivity, ionic composition. The suitability was correlated based on the EC, sodium adsorption ratio (SAR), residual sodium carbonate (RSC), permeability index (PI), Kelly's ratio (KR), soluble sodium percentage, irrigation water quality index (IWQI) . Inverse distance weightage interpolation method used in geographical information system to develop spatial variability maps of pH, EC, SAR, RSC and groundwater irrigation quality. The analysis of groundwater showed the pH of groundwater falls in the range of 6.8-8.4, EC 0.1-4.2 dSm^{-1}, RSC -19.4 to 8.0, SAR 0.45-8.59, KR 0.20-3.69, PI 20.32-105.63 and SSP 12.6-71.78. The irrigation water quality index (IWQI) falls in the range 14.9-192.34. As per the AICRP criteria 64.50% reported good, 20.78% marginally saline, 0.43% saline, 6.93% marginally alkaline, 7.36% alkali in quality for irrigation.

Keywords: Groundwater quality, Spatial variability, SAR, RSC, IWQI, GIS

Irrigation water quality has major role in agriculture production and soil sustainability**.** Sri Sathyasai Puttaparthy region comes under scarce rainfall zone of Andhra Pradesh and predominantly dominated by shallow red soils using ground water as major source for irrigation. In arid and semiarid areas marginal and poor quality waters constitute a greater part of phreatic groundwater resources (Gupta et al 2019). The soil health is majorly affected by the use of poor quality groundwater over a period of time. The evapo-transpiration exceeds the rainfall and basin level natural drainage remains either absent or insufficient, it is necessary to assess groundwater quality of arid and semi-arid region for irrigation. The groundwater quality for irrigation also depends upon the mineral constituents present in the water and is essential to maintain higher productivity. It is a prerequisite to assess the groundwater for sustainable agricultural development and crop production (Vinothkanna et al 2020). It also helps in planning and implementation of groundwater management strategies for better crop production. Therefore, a database on groundwater quality and spatial variability maps of groundwater quality of Sri Sathyasai Puttaparthy region can help the farmers and policy makers for implementation and adoption of efficient crop production practices for profitable returns. Keeping this in view a study was conducted to assess the groundwater quality for irrigation in Sri Sathyasai Puttaparthy region of Andhra Pradesh.

MATERIAL AND METHODS

Study area: The Sri Sathyasai Puttaparthy region lies in between 13.68' and 14.65' of Northern latitudes and 76.88' and 78.47' Eastern longitudes occupies southern part of Andhra Pradesh (Fig. 1). The annual rainfall of the district is 535 mm through South-West and North-East monsoons. The maximum temperature varied 35^oC to 46^oC during summer and the minimum temperature of 23^oC to 25^oC during winter.

Analysis of groundwater: Two hundred and thirty one (231) ground water samples were collected from different sources like bore wells and open wells with GPS coordinates (Fig. 2). Sampling was carried out using preconditioned clean high density polythene bottles. The pumps were run for 5-6 minutes prior to collection of water samples, immediately after collection of water samples toluene was added to avoid microbiological deterioration. Standard procedures were followed to analyze the quality of water. pH in water samples was determined by potentiometrically using pH meter (Jackson 1973). Electrical conductivity was determined by using Conductivity Bridge (Willard et al 1974). Chlorides (Mohr's method), carbonates and bicarbonates (double indicator method) and calcium and magnesium (versenate method) were determined by adopting the procedures given by Richards (1954) . Similarly the sulphates by turbidimetric method, sodium and potassium in ground water samples were determined by using flame photometer (Richards 1954). Sodium Adsorption Ratio (SAR), RSC were calculated by using the formulas given by Richards (1954) such as SAR = Na/ $(Ca^{2+}+Mg^{2+})/2)^{0.5}$ and RSC = $(CO_3^{2-}$ + H $CO_3^-)$ - $(Ca^{2+}$ $+Mg^{2+})$. The Na^+, Ca^{2+} and $Mg2^+$ are in m e L^{-1}. RSC, CO_3^{2-}, H CO_3^-, Ca^{2+} and Mg^{2+} are in meq L^{-1}. The RSC, SAR, KR, SSP, PI was computed for assessing irrigation water quality index (IWQI).

Soluble sodium (%): Sodium concentration in groundwater is a very important parameter in determining the irrigation quality. The formula used for calculating the sodium percentage was

$$Na\% = (Na^+ + K^+)/(Ca^{+2} + Mg^{+2} + K^+ + Na^+) \times 100$$

Where all ionic concentrations are in meq/L.

Kelley's ratio: Kelley's ratio was used to classify the irrigation water quality (Kelley 1963), which is the level of Na^+ measured against calcium and magnesium. The formula for calculating the Kelley's is as follows

$$KR = \frac{Na^+}{(Ca^{+2} + Mg^{+2})}$$

Where the concentration of ions are in mg/L

Permeability index: Long-term use of irrigation contains Na^+, Ca^{+2}, Mg^{+2} and HCO_3^- ions greatly influence the soil permeability. Doneen (1964) expressed the degree of soil permeability in terms of permeability index (PI).

$$PI = \frac{(Na^+ + \sqrt{HCO_3^-})}{(Ca^{+2} + Mg^{+2} + Na^+)} \times 100$$

Where all ionic concentrations are in meq/L.

Statistical analysis and mapping: Range, mean, standard deviation and standard error, correlation coefficient of water properties were computed. Spatial distribution of groundwater quality was depicted in figures using inverse distance weightage interpolation method of Q-GIS 3.16.10.

RESULTS AND DISCUSSION

Water reaction (pH): The pH of ground water varied from 6.8 to 8.4 (Table 1) with a mean of 7.37, variation in pH of groundwater in the study area are very small, the low pH may be due to presence of forest areas in certain pockets. Higher pH of ground water may be due to dominance of Na^+, Ca^{+2}, Mg^{+2} and CO_3^- and HCO_3^- ions (Bhat et al 2018). The spatial variability in pH of groundwater is depicted in Figure 3**.** Gupta et al (2019) reported that pH of groundwater should be 6.5-8.4 for better performance of crops, all the samples of ground water of the study area is within this limit can be used for sustainable crop production.

Electrical Conductivity (EC): The conductivity values of groundwater samples ranged from 0.1 to 4.2 dS m^{-1} with a mean of 1.51 dS m^{-1} (Table 1). Electrical conductivity is customarily used for indicating the total concentration of the

Table 1. Descriptive statistics of quality parameters in groundwater

Parameter	Range	Mean	Standard deviation
pH	6.8-8.4	7.37	0.26
EC (dSm^{-1})	0.1-4.2	1.51	0.69
CO_3^{2-} (me L^{-1})	0.0-1.0	0.19	0.26
HCO_3^- (me L^{-1})	1.6-13.8	7.07	1.97
Cl^- (me L^{-1})	0.8-21.6	5.00	4.20
SO_4^{2-} (me L^{-1})	0.27-10.55	2.39	1.86
Ca^{2+} (me L^{-1})	0.8-18.4	4.32	2.39
Mg^{2+} (me L^{-1})	0.8-12.0	3.72	2.21
Na^+ (me L^{-1})	0.7-17.2	5.08	2.80
K^+ (me L^{-1})	0.001-3.02	0.12	0.26
RSC (me L^{-1})	-19.4-8.0	-0.78	4.24
SAR	0.45-8.59	2.61	1.36
KR	0.20-3.69	1.00	0.65
PI	20.32-105.63	60.98	14.77
SSP	12.6-71.78	38.84	12.58
IWQI	14.9-192.34	102.67	31.22

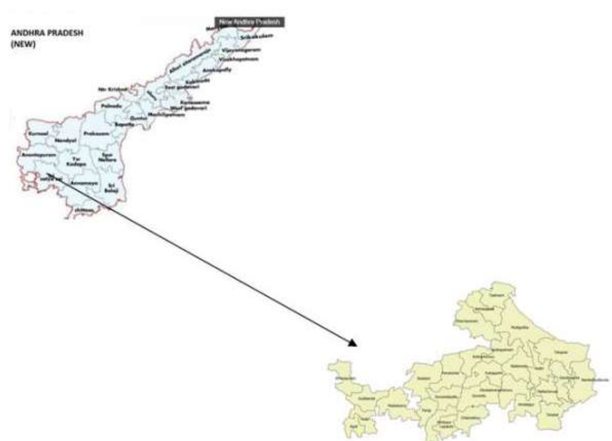

Fig. 1. Location map of Puttaparthy

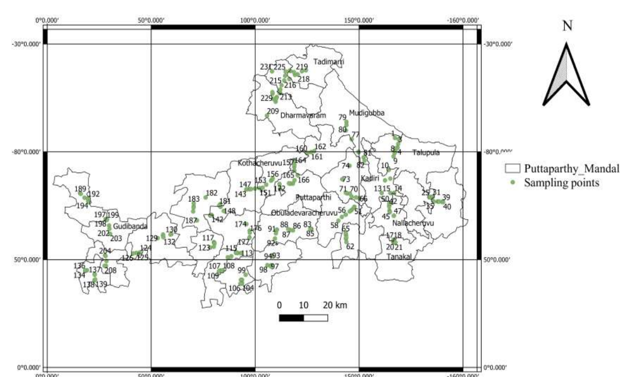

Fig. 2. Ground sampling points

ionized constituents of natural water. The spatial variability of EC in groundwater is depicted in Figure 4. The EC of groundwater indicated that 80.52 % samples are good, 19.05% samples are marginally saline and only 0.43% samples are highly saline (Table 2). The variation in EC may be due to variation in hydro-geological conditions and the anthropogenic activities in the region. Kumar and Balamurugan (2019) reported similar results with Omalur taluk of Tamilnadu.

Sodium adsorption ratio (SAR): The SAR of groundwater in the study area is safe for irrigation (Fig. 5). Presence of calcium and magnesium minimizes the sodicity danger of groundwater for irrigation (Naidu et al 2020).

Residual sodium carbonate (RSC): The residual sodium carbonate (RSC) of groundwater varied from -19.4-8.0 meq L^{-1} with a mean of -0.78 meq L^{-1}. The spatial distribution of residual sodium carbonate was depicted in. The RSC was higher in groundwater of few places due to its high Na^+ and HCO_3^- ion activity than calcium and magnesium ions (Fig. 6). Based on RSC (Table 3) 83.12% samples have no sodium hazard, 8.66% samples are with moderate hazard, 8.23% samples with severe hazard. Majority of the samples are with low sodium hazard indicated that dissolved calcium and magnesium contents are higher than carbonate and bicarbonate contents in groundwater (Kumar and Kumar 2021).

Table 2. Ground water quality based on electrical conductivity (dSm^{-1})

EC (dSm^{-1})	No. of samples	Per cent of samples
0-2	186	80.52
2-4	44	19.05
4-5	1	0.43

Table 3. Classification of ground water based on RSC (mel^{-1})

Residual Sodium Carbonate (mel^{-1})		No. of samples	Per cent of samples
Class	Value		
None	<2.5	192	83.12
Slight to moderate	2.5-4.0	20	8.66
Severe	>4.0	19	8.23

AICRP classification of groundwater for irrigation: The groundwater was classified into 6 classes for irrigation purpose (Minhas and Gupta 1992). The 64.50% samples were of good quality, 20.78% were of marginally saline, 0.43% of saline, 6.93% of marginally alkali and 7.36% of alkali (Table 4, Fig. 7). Similar results were also reported by Subbaiah et al (2020) with Chittoor district of Andhra Pradesh

Kelley's ratio (KR) for irrigation: Kelley's ratio for all the groundwater samples is calculated. Kelley's ratio value (Table 5) less than one is suitable for irrigation (63.20%) and more than one is unsuitable (36.80% samples). Though sodium is dominant ion in groundwater, presence dissolved calcium and magnesium minimized its effect on irrigation water quality in more number of samples

Permeability Index (PI): Based on permeability index irrigation water classified into Suitable (17.32%), marginally suitable (82.25%) and un suitable (0.43%) for irrigation (Table 6). Long-term use of irrigation water contains high salts may affect the permeability index of soils

Soluble sodium per cent (SSP): Soluble sodium per cent values (Wilcox 1955) classification of groundwater resulted 4.33% excellent, 53.68% good, 34.63% permissible, 7.36% samples doubtful. Overall majority of the samples are with less sodium hazard

Irrigation water quality index (IWQI): Irrigation water quality index resulted 2.16% samples are excellent, 50.22% samples are good, and 47.62 percent samples poor in quality for irrigation

Correlation coefficients: The groundwater quality mainly depends upon its ionic composition, presence of one ion may have correlation with other and together influence the quality of groundwater. Correlation coefficients of different ion are given in Table 9. The EC of groundwater has significant

Table 4. Classification of ground water and their management (Minhas and Gupta 1992)

Rating	EC (dSm^{-1})	SAR	RSC (me L^{-1})	Number of samples	Per cent Samples
A.Good	<2	<10	<2.5	149	64.50
B. Saline					
Marginally saline	2-4	<10	<2.5	48	20.78
Saline	>4	<10	<2.5	1	0.43
High SAR Saline	>4	>10	<2.5	0.0	0.0
C. Alkali Water					
Marginally alkaline	<4	<10	2.5-4.0	16	6.93
Alkali	<4	<10	>4.0	17	7.36
Highly alkaline	Variable	>10	>4.0	0.0	0.0

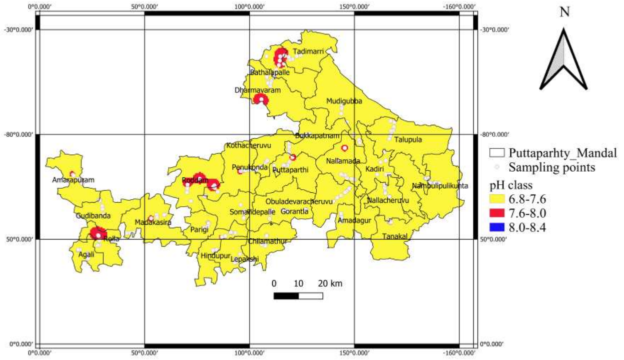

Fig. 3. Spatial distribution of pH in groundwater

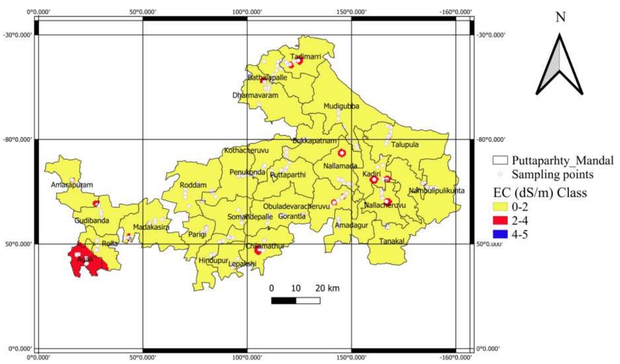

Fig. 4. Spatial variability in EC (dS/m) of groundwater

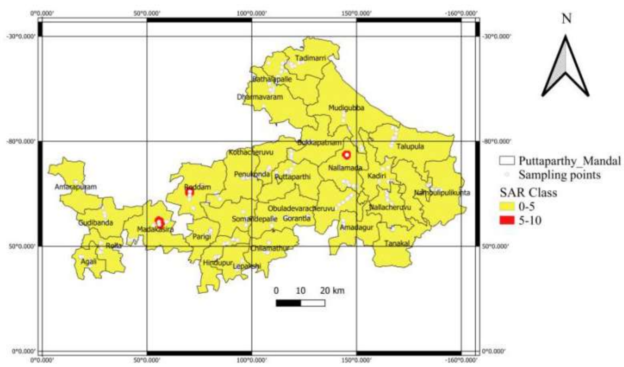

Fig. 5. Spatial variability in SAR (dS/m) of groundwater

Table 5. Classification of groundwater for irrigation based on Kelly's ratio (Kelly 1963)

Kelly's ratio	Suitability	Sample	
		Numbers	Per cent
<1.0	Good	146	63.20
>1.0	Not good	85	36.80

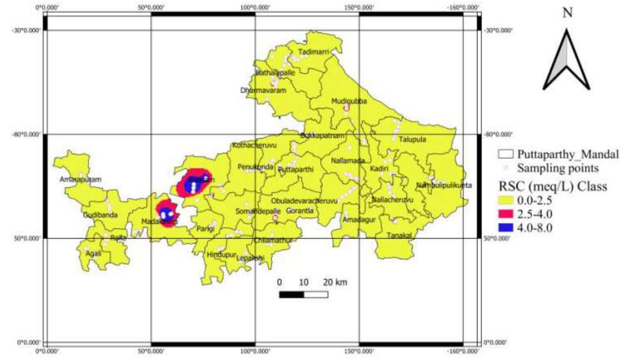

Fig. 6. Spatial variability in RSC (dS/m) of groundwater

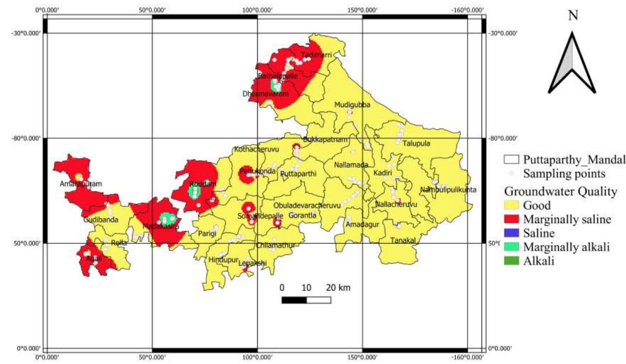

Fig. 7. Spatial variability in groundwater quality

Table 6. Classification of groundwater based on permeability index (PI) for irrigation (Doneen 1964)

Classification of PI	Permeability	Suitability	Sample	
			Numbers	Per cent
I	>75	Suitable	40	17.32
II	25-75	Marginal	190	82.25
III	<25	Unsuitable	1	0.43

Table 7. Grouping of groundwater based on % Na values (Wilcox 1955)

% Na (After Wilcox 1955)	Classification	Total no. of samples	Percentage
<20	Excellent	10	4.33
20-40	Good	124	53.68
40-60	Permissible	80	34.63
60-80	Doubtful	17	7.36
>80	Unsuitable	0.0	0.0

Table 8. Grouping of groundwater based on IWQI for irrigation

Water value range	Water quality	No. of samples	Per cent samples	Sustainable state
<50	Excellent	5	2.16	Sustainable
51-100	Good	116	50.22	Sustainable
101-200	Poor	110	47.62	Slightly unsustainable
201-300	Very poor	0.0	0.0	Unsustainable
>301	Very bad	0.0	0.0	Highly unsustainable

Table 9. Correlation matrix among the chemical constituents of the groundwater

	pH	EC	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-2}	HCO_3^{-}	Cl^{-}	SO_4^{-2}	RSC	SAR	SSP	PI	KR	IWQI
pH	1															
EC	-0.219	1.000														
Ca^{+2}	-0.455	0.717**	1.000													
Mg^{+2}	-0.153	0.810**	0.621	1.000												
Na^{+}	-0.050	0.799**	0.277	0.494	1.000											
K^{+}	-0.089	0.387	0.139	0.208	0.345	1.000										
CO_3^{-2}	0.280	0.172	-0.094	0.128	0.312	0.021	1.000									
HCO_3^{-}	-0.189	0.470	0.107	0.265	0.671**	0.314	0.149	1.000								
Cl^{-}	-0.175	0.952**	0.712**	0.824**	0.705**	0.342	0.173	0.288	1.000							
SO_4^{-2}	-0.260	0.602**	0.567	0.619**	0.454	0.151	0.030	0.186	0.537	1.000						
RSC	0.267	-0.597	-0.845**	-0.741**	-0.081	-0.039	0.119	0.277	-0.687**	-0.554	1.000					
SAR	0.126	0.461	-0.110	0.101	0.871**	0.278	0.362	0.678**	0.349	0.160	0.348	1.000				
SSP	0.233	0.145	-0.391	-0.203	0.637**	0.225	0.331	0.552	0.054	-0.100	0.605**	0.910**	1.000			
PI	0.310	-0.361	-0.699**	-0.597	0.173	-0.035	0.197	0.306	-0.415	-0.431	0.861**	0.588	0.834**	1.000		
KR	0.286	0.118	-0.391	-0.178	0.599	0.162	0.351	0.544	0.022	-0.095	0.589	0.907**	0.945**	0.803**	1.000	
IWQI	0.288	-0.171	-0.616**	-0.464	0.378	0.084	0.266	0.446	-0.252	-0.314	0.815**	0.755**	0.940**	0.969**	0.902**	1

Note: SAR= Sodium Adsoprtion Ratio; KR = Kelly's Ratio; CR= Corrosivity Ratio; PI= Permeability Index
IWQI= irrigation water quality index; ** Significant at *r*>0.6

Table 10. Quality of irrigation water in various mandals of Puttaparthy region

Name of the Mandal	pH		EC (dSm^{-1})		RSC (me L^{-1})		SAR		Groundwater type
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Amarapuram	7.52	7.2-8.1	1.83	1.1-2.8	-0.95	-4.6 to 4.4	2.85	1.96-3.94	Na^{+}-HCO_3^{-}
Parigi	7.37	7.1-7.5	1.3	0.6-2.2	2.2	0.4-6.4	3.12	1.08-4.67	Na^{+}-HCO_3^{-}
Roddam	7.58	7.1-8	1.24	0.7-2.1	3.62	-2.6 to 8.0	3.88	2.08-6.32	Na^{+}-HCO_3
Lepakshi	7.43	7.1-7.8	1.71	0.8-3	-0.2	-4.4 to 4.4	2.92	1.5-4.15	Na^{+}-HCO_3^{-}
Penukonda	7.54	7.3-7.9	1.8	1.1-2.6	0.875	-5.4 to 8.0	3.52	1.1-6.0	Na^{+}-HCO_3^{-}
Gornatla	7.26	6.9-7.6	1.09	0.5-1.7	0.96	-2.4 to 4.4	2.26	0.8-4.09	Na^{+}-Ca^{+2}-HCO_3^{-}
Bukkapatnam	7.26	7.1-7.5	1.01	0.6-1.5	1.42	-0.4 to 3.0	1.82	1.03-2.59	Na^{+}-HCO_3^{-}
Kadiri	7.18	7-7.3	1.66	0.9-2.8	-2.56	-19.4 to 1.8	2.75	1.0-6.5	Ca^{+2}-Na^{+}-HCO_3^{-}
Nallacheruvu	7.22	6.9-7.6	1.8	0.7-4.2	-1.44	-17.0 to 4.2	2.88	1.59-4.0	Na^{+}-HCO_3^{-}
Dharmavaram	7.4	7.1-8.1	1.92	1.1-3.6	-0.11	-14.2 to 5.6	3.96	1.47-5.89	Na^{+}-HCO_3^{-}
Mudigubba	7.24	7-7.6	1.15	0.8-1.7	0.73	-2.2 to 4.2	2.52	0.85-4.17	Na^{+}-HCO_3^{-}
Talapula	7.19	6.9-7.6	1.44	0.5-2.8	-2.8	-12.6 to 1.8	1.98	0.75-3.27	Ca^{+2}- Na^{+}-HCO_3^{-}
Gandlapenta	7.29	7.1-7.6	1.61	0.9-3.1	-1.75	-7.8 to 1.0	2.38	1.0-4.93	Na^{+}-Ca^{+2}-HCO_3^{-}
Tanakal	7.31	6.9-7.5	1.57	0.9-3.1	-3.04	-10.2 to 0.8	1.95	1.30-3.10	Ca^{+2}-HCO_3^{-}
O.D.Cheruvu	7.28	7.2-7.5	1.93	0.9-3.3	-4.0	-9.2 to 0.6	2.93	1.20-5.04	Na^{+}-HCO_3^{-}
Amadgur	7.27	6.8-7.7	1.17	0.7-1.6	-1.02	-5.4 to1.2	2.16	1.08-3.04	Ca^{+2}-HCO_3^{-}
Hindupur	7.4	7.1-7.9	1.23	0.4-2	0.34	-3.6to4.4	2.26	1.34-3.32	Na^{+}-HCO_3^{-}
Chilamthur	7.12	6.9-7.3	1.88	1.0-3.4	-5	16to-0.2	1.88	1.20-2.72	Ca^{+2}-HCO_3^{-}
Madakasira	7.4	7.0-7.7	1.86	1.5-2.5	1.0	-7 to 6.8	4.23	1.9-6.3	Na^{+}-HCO_3^{-}
N.P. Kunta	7.3	7.1-7.7	1.12	0.6-1.5	-0.8	-5.6 to 3.4	1.89	1.11-4.22	Ca^{+2}-HCO_3^{-}
Gudibanda	7.34	7.0-7.5	1.64	0.5-2.7	-2.68	-8.2 to 0.8	1.96	1.1-2.34	Ca^{+2}-HCO_3^{-}
Rolla	7.6	7.0-8.4	1.35	1.0-1.8	0.46	-2.4 to 3.0	2.72	1.7-4.0	Na^{+}-HCO_3^{-}
Agali	7.34	7.0-7.5	2.45	1.3-3.4	-5.7	-14 to 7.8	3.09	1.97-6.03	Na^{+}-HCO_3^{-}
Bathalapalli	7.6	7.1-8.0	1.32	0.6-1.9	-2.37	-6.2 to 3.0	1.85	0.45-4.53	Na^{+}-Ca^{+2}-HCO_3^{-}
Tadimarri	7.53	7.2-7.7	1.66	0.8-3.3	-1.05	-3.2 to 2.2	2.5	0.8-5.36	Na^{+}-Ca^{+2}-HCO_3^{-}
Kothacheruvu	7.43	6.9-7.8	1.22	0.7-2.1	0.08	-6 to 3.0	2.08	1.05-3.73	Na^{+}-HCO_3^{-}
Puttaparthy	7.5	6.9-8.1	1.48	0.7-2.7	-2.42	-9.2 to 1.0	1.70	0.87-2.64	Ca^{+2}-HCO_3^{-}
Nallamada	7.36	7.1-7.8	1.34	0.7-2.7	-0.42	-4.4 to 1.8	2.53	1.04-8.59	Ca^{+2}-HCO_3^{-}
Somandepalli	7.4	7.2-7.5	1.46	0.8-3.1	0.53	-7.4 to 3.2	2.50	1.41-3.99	Na^{+}-HCO_3^{-}

positive correlation with Na^+ , Ca^{+2} , Mg^{+2} , Cl^- and SO_4^{-2} Ca^{+2} positively correlated with Cl and negatively correlated with RSC , PI and IWQI . Mg^{+2} is positively correlated with Cl^- and SO_4^{-2} and negatively correlated with RSC indicates presence calcium and magnesium minimized the sodium and bicarbonate hazard of groundwater (Pal et al 2018). Na^+ positively correlated with HCO_3^- Cl^- , SAR , SSP. HCO_3^- is positively correlated with SAR (0.678**) indicates sodium hazard of groundwater in few places of the study area, IWQI is positively correlated with RSC , SAR , and KR . KR is positively correlated with SAR, SSP and PI where *r* value is approaches to unity (r>0.6). The presence of minerals which contain sodium, calcium, magnesium, bicarbonates, chlorides and sulphates in the study area may be the reason for their higher amounts in groundwater.

Mandal wise groundwater quality: The mandal wise groundwater quality is given in Table 10, indicated that the presence of high RSC resulted dominance of Na^+ - HCO_3^- type of groundwater in parts of the mandals *viz.,* Amarapuram, Parigi, Roddam, Lepakshi, Penukonda, Gorantla, Nallacheruvu, Dharmavaram, Mudigubba, O.D. cheruvu, Hindupur, Madakasira, Rolla, Agali, Kothacheruvu, Somandepalli. This might due to dominance of sodium and bicarbonate containing minerals might have impact on groundwater quality. Dominance of Na^+-Ca^{+2}-HCO_3^- ions in groundwater was observed in mandals (with RSC <4) *viz.,* Bukkapatnam, Gandlapenta, Bathalapalli, Tadimarri. Ca^{+2}-Na^+-HCO_3^- ions were observed in Kadiri, Talapula. Ca^{+2}-HCO_3^- type groundwater observed in Tanakal, Amadgur, Chilamathur, N.P. Kunta, Gudibanda, Puttaparthy, Nallamada. The variation in groundwater type may be due to variation hydrogeological conditions of the study area (Shalini and Bhardwaj 2017).

CONCLUSION

The ground water quality in Srisathyasai Puttaparthy region varied from place to place. The dominance of major ion was in the order of $Na^+ > Ca^{+2} > Mg^{+2} > K^+$ for cations and $HCO_3^- > Cl^- > SO_4^{-2} > CO_3^-$ for anions, which indicated the quality of groundwater used for irrigation is Na^+-HCO_3^- type, presence of calcium and magnesium in sufficient quantities minimized the sodium hazard of groundwater in majority of the samples, very few samples which have excess sodium and bicarbonate ions resulted high sodicity due to residual sodium carbonate. More than 50 per cent of groundwater quality of the region is good for irrigation can be used for sustainable crop production without affecting soil health, poor quality water is around 40 percent, use of this water limits the crop production. However, adoption of proper management practices are needed in case of poor quality ground water. The spatial maps of different parameters, prepared using GIS could be valuable for policy makers for initiating groundwater quality monitoring of the area as well as for suggesting management plans. Assessment and mapping of quality of irrigated groundwater may help the farmers in selection of suitable crops and other agronomic management practices for getting profitable yields without affecting the soil health.

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Assessing the Impact of Super Cyclone Amphan on Indian Sundarban Biosphere Reserve

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Abstract: Evaluation of a natural disaster's impact is essential for formulating preparedness, mitigation, and response plans. This study evaluates the impact of super cyclone Amphan, which hit the Indian Sundarban Biosphere Reserve on May 20, 2020. Pre- and post-cyclone land use/land cover (LULC) maps were created using LANDSAT 8 OLI, while flood inundation was mapped using Google Earth Engine (GEE) cloud platform and Sentinel-1 A C band Synthetic Aperture Radar (SAR). The study revealed that tropical cyclone Amphan had a significant impact on dense/mangrove vegetation (5%) and agricultural (2%), as well as an increase in flooded areas (40%). The proximity of 3% (290.62 km^2) of the area near the path of the cyclone was impacted by flooding, particularly in low elevation areas (5m). The investigation observed that cyclone Amphan caused extensive damage to open vegetation, agricultural lands, and urban areas. These findings provide a quantifiable estimate of cyclone Amphan's damage and will help decision-makers improve disaster preparedness. Native-based intervention initiatives, including natural ecosystem-based disaster risk reduction strategies, can be implemented to promote and conserve mangroves, rehabilitate human populations from highly vulnerable areas to safer areas, and ensure their livelihood security in cyclone-prone regions.

Keywords: Amphan Cyclone, Mangrove forests, LULC, Sundarban Biosphere reserve, Sentinel-1

Over the years, the regular occurrence of cyclones in the Bay of Bengal (BoB) has caused severe damage to eastern India, especially the states of Odisha and West Bengal, had faced the wrath of the various cyclones incurring heavy financial and environmental losses (Kumar et al 2020). The Super Cyclonic Storm (SuCS), Amphan, hit as a Very Severe Cyclonic Storm (VSCS) at Sundarbans with 155-165 kmph (maximum 224 kmph) wind speed on 20^{th} May after landfall (IMD, July 13, 2020). Amphan damaged ~1200 sq km of reserve forest, including ~28% of mangrove forest, and affected the floral diversity through increased soil salinity (Singh 2020). The forest ecosystem's functionality, diversity, and composition get altered due to natural disturbances, including cyclones (Xi 2015). Tropical cyclones continually affect the structure of tropical forests (Ibanez et al 2019). Many studies performed globally on the impact of natural disasters on forest ecosystems suggest that natural disaster events have an intense effect on forests leading to altered forest composition, fire hazards, and decreased carbon sink (Lin et al 2017). Loss of vegetation is one of the major contributors to climate change, resulting in high temperature and precipitation variability, and rising sea levels that accelerate runoff and sedimentation flow (Al-Nasrawi et al 2018). Global warming has severely impacted the ecosystem through the frequent occurrence of natural disasters leading to enormous loss of life and property (Einsty 2017). The oceans absorb over 90% of the heat trapped by anthropogenic greenhouse gas and abruptly increase ocean surface temperatures (Guldberg et al 2018).

Previous research reported the effective use of remote sensing technology in understanding the critical role of cyclone monitoring, and effective planning in response, mitigation, and damage assessment. The concurrent availability of satellite images through open archives significantly increased the efficiency of analyzing and determining forest habitat (Zanndouche et al 2022) land cover variation due to any hazardous event (Jamali and Kalkhajeh 2020). Lal et al (2020) analyzed flood inundation in the lower Indo-Gangetic-Brahmaputra plains (IGBP) using C-band Sentinel-1A SAR using Google Earth Engine. Pheri et al (2020) used object-based image analysis (OBIA) to understand the effect of cyclone Idai in Mozambique, employing a threshold method for flood inundation.

The existing literature suggests that the studies on the impact of cyclone-induced floods concerning elevation range are limited and need further understanding. Another major issue that researchers face is the processing of remote sensing data sets. Google Earth Engine (GEE) provides a reliable and efficient platform for accurate and fast processing of remote sensing data (Mutanga and Kumar

2019, Jamali et al 2022). We intend to fill this gap and better understand the cyclonic impact in the present investigation through the present study. The present study analyzed the impact of super cyclone Amphan on the Indian Sundarban Biosphere Reserve using geospatial tools, including the Google earth engine platform. The Sundarban Biosphere Reserve is a World Heritage site because of its ecological significance (UNESCO). The region is prone to cyclones and has a long history of cyclones causing destruction and loss of life and property. The present study was carried out with the objectives to (i) study the changes in different LULC due to the Amphan, (ii) map cyclone-induced flood inundation and its impact, and (iii) investigate the relationship of elevation with flood-affected areas.

MATERIAL AND METHODS

Study area: Sundarban is the world's largest contiguous mangrove forest and delta, originating from the Ganges, Brahmaputra, and Meghna rivers in the Bay of Bengal (Ghosh et al 2015). The present study was conducted in the Indian Sundarban Biosphere Reserve (SBR), encompassing six blocks of North 24 Parganas and thirteen blocks of South 24 Parganas district of West Bengal. The word 'Sundarban' is made up of two Bengali words, one is 'Sundar' which means beautiful, and the other is 'Ban,' which means forest. The study area lies between 88° 01' E to 89° 5' E and 21° 40' N to 22° 35' N, covering a geographical area of approximately 9630 km^2 (Fig. 1). Of these, ~4264 km^2 falls under wetlands and dense mangrove forests, while the remaining ~5366 km^2 area is under inhabited lands. The term 'Sundarban' originates from one of the significant mangrove tree species, 'Sundari' (*Heritiera fomes*) (Sahana et al 2015) and has a prominent distribution of primary mangrove species, including *Avicennia marina*, *Avicennia officinalis, Avicennia alba, Ceriopsdecandra, Excoecariaagallocha, Xylocarpus mekongensis, Nypafruitcans, Aegicerascorniculatum, Sonneratiacaseolaris, Rhizophora apiculata, Sonneratia apetala, Xylocarpus granatum, Bruguieragymnorrhizaetc.*(http://naturewildlife.org).

Data collection: The land use/ land cover of the SBR was prepared using the LANDSAT 8 OLI images for the pre-Amphan period (acquired on 6th April (Path 138, Row 44,45) and 13th April 2020 (Path 139, Row 45) and post-Amphan period (acquired on 27th July and 15th October 2020) processed in Google Earth Engine (GEE) cloud platform. Using two months post-Amphan data enabled to address the possible overestimation of vegetation loss due to mud or floodwater. While C band Sentinel-1A Synthetic Aperture Radar (SAR) ground range detected (GRD) Interferometric Wide (IW) swath mode datasets were used to map flood inundation. The Sentinel-1A VH (Vertical transmit and Horizontal receive) and VV (Vertical transmit and Vertical receive) polarization were also acquired and processed in GEE throughout the pre-cyclone (28^{th} April and 4^{th} May 2020) and post-cyclone (28^{th} May 2020) periods. Shuttle Radar Topography Mission (SRTM) digital elevation model (DEM) data was also downloaded from the USGS earth explorer site to prepare the relief map of the study area. Secondary data collection, including the administrative boundary maps of the selected districts of West Bengal, was acquired from the census commissioner of India website (https://censusindia.gov.in/). We used 225 random points generated from the pre-cyclone LULC map for validation using Google earth pro software. For the post-cyclone LULC map and flood map, we collected 50 and 18 ground observation points.

Data processing: GEE was used to reduce the error of topographical distortion, atmospheric, geometric corrections, and cloud masking for LANDSAT 8 OLI data. The simple composite cloud score algorithm to overcome the cloudy condition in the post-Amphan dataset was applied. The classified map for the pre and post-Amphan period were generated using a supervised classification approach. Data processing for the Sentinel-1A dataset was done using GEE instead of SNAP software as it does not require high configuration devices and satellite data download like SNAP software. For preprocessing, the Sentinel-1A data employed Apply orbit file, thermal and border noise removal, terrain correction, and conversion backscatter coefficient. The binary flood extent raster layer at 1.25 threshold to assess the cyclone-induced flood map was created. The threshold method (binarization) was applied to verify the flooded and

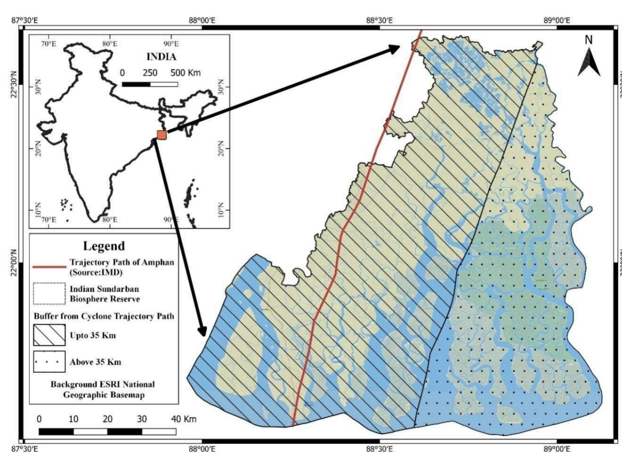

Fig. 1. Location map of Indian Sundarban Biosphere Reserve with the trajectory path and proximity of Tropical Cyclone Amphan up to 35 km and above 35 km zone (May 2020) overlaid on ESRI national geographic base map

non-flooded areas and validate the flood map using 18 ground truth points' data, where 14 points fell under the flood area, and the rest of the points fell in the non-flood class (Fig. 2).

Land Use/ Land Cover mapping: The LULC maps of the pre and post-Amphan scenarios were prepared using Random Forest (RF), a supervised classification approach. Six major LULC classes, *i.e.,* agricultural land, built-up land, water bodies, waterlogged, open vegetation, and dense vegetation/Mangrove, were generated for the pre and post-Amphan periods. The LULC map was validated for accuracy and measurement of map errors using randomly chosen 225 reference points from Google earth pro software for the pre-Amphan period. For the post-Amphan scenario, we collected 50 random points through a field survey. The overall accuracy of classified maps was 89 and 80%, while the Kappa coefficient value was 0.87 and 0.75, respectively.

RESULTS AND DISCUSSION

The pre-Amphan LULC mapping showed that the large geographical area of SBR was occupied by the water bodies (~31% of the geographical area related to the Bay of Bengal), followed by dense vegetation/mangroves (~23%). There were significant changes in the LULC pattern of SBR during the post-Amphan scenario. The notable change was observed as a considerable increase in the waterlogged area (~40%; from 342.24 km^2 to 482.36 km^2), open vegetation (~3%), and water bodies (~0.5%), in contrast to a marked decrease in dense vegetation/ mangrove (~5%) and agriculture land (~2%) during post-Amphan compared to pre-Amphan (Fig. 3- 4). Sentinel-1A C band SAR-based flood inundation in SBR was overlaid on the LULC map of pre-Amphan periods to deduce the impact of cyclone-induced flood inundation on land use/ land cover in SBR (Fig. 6). The comparison of the bimodal histograms generated for the VH polarization images for pre and post-cyclone (Fig. 5). The intensity (dB) of the VH polarization is high in the post-cyclone image. The study showed that 240.91km^2 (2.50% of total area) was affected due to the cyclone-induced flood, primarily in the northern parts of SBR after landfall. The study revealed severe impacts of tropical cyclone Amphan on agricultural lands (182.14 km^2; 75.61%), open vegetation (36.30 km^2; 15.07%), and built-up land (22.47 km^2; 9.32%). The impact of flood inundation was analyzed over the relief zones of the SBR, which is located up to 46 m relief zone in the Indian subcontinent. The elevation-wise analysis of the flooded area demonstrated that the zones having elevation <33 m were affected by the flood, which constitutes about ~3% of the total geographical area. The majority of the flood (~99%) occurred under a <9 m elevation zone due to the low-lying landscape of the SBR (Fig. 7).

The current study found significant changes in vegetation and waterlogged areas in Indian Sundarban reserve forests. A significant proportion of mangrove forests (5.71 %) have been severely damaged, resulting in the fragmentation of the vegetative cover. The loss in mangrove vegetation supports

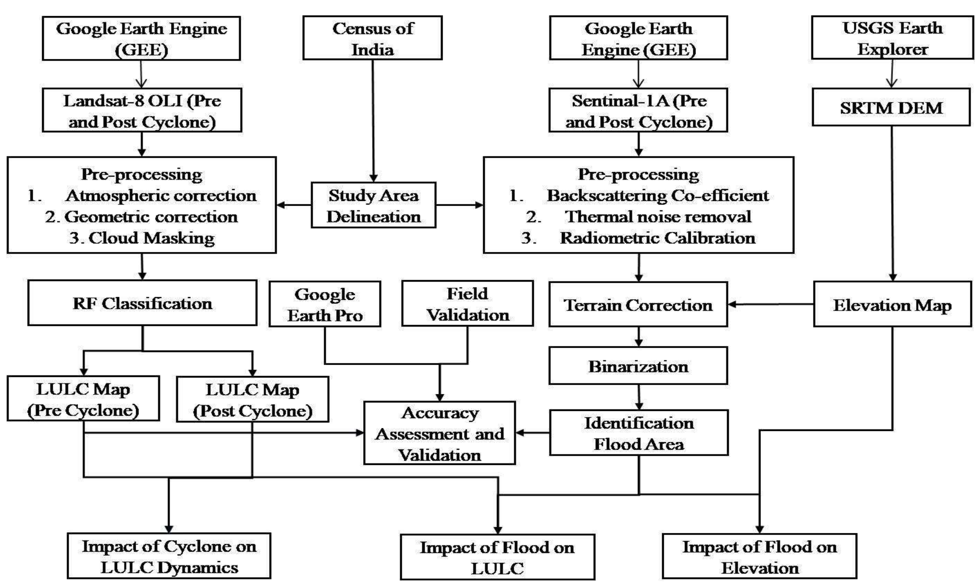

Fig. 2. Flow chart of the methodology depicting the procedure of impact assessment of cyclone Amphan on LULC and assessing the impact of flood over different land cover and elevation range

the conclusion that 13.4% of mangroves in the Indian Sundarban region were damaged by Cyclone Amphan (Acharyya et al 2021). The damage to the dense mangrove patches is of great concern as mangroves act as a barrier to cyclones (Sakib et al 2015) and preserve significant biodiversity. It significantly reduces the cyclonic wind speeds and breaks the storm waves due to the compact structure of

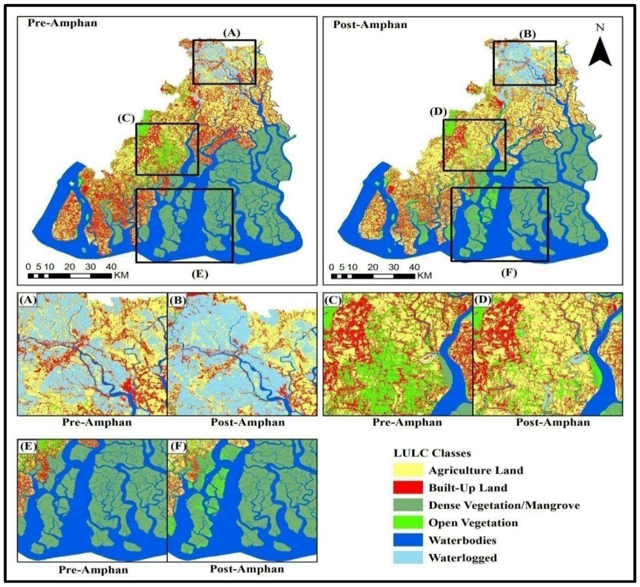

Fig. 3. Map showing the comparison between pre and post-Amphan LULC map of Sundarban Biosphere Reserve and revealed (A & B) expansion of the waterlogged area in the northern part of the study area including Minakhan, Sandeshkhali, Nyazat, and Jibontola, (C & D) damaged open vegetation area at Kultuli and Tulshihata, (E & F) lose of the dense mangrove forest in the island of Ajmalmari, Bulchery and Holiday

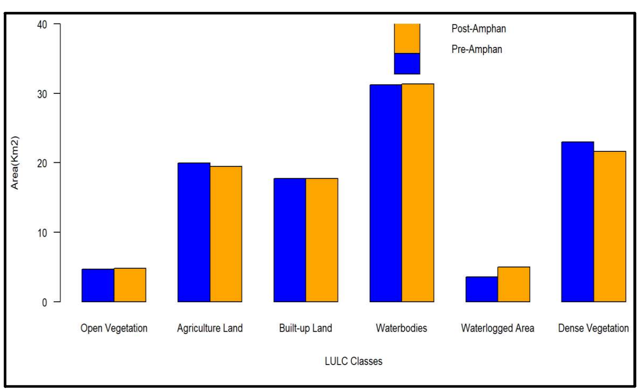

Fig. 4. Graph shows the area of different LULC classes for pre-Amphan and post-Amphan period. The graph clearly indicate the damage to open vegetation, Agricultural landscape, dense vegetation, and increase in water bodies and water-logged area due to the cyclone over the Indian Sundarban Biosphere Reserve

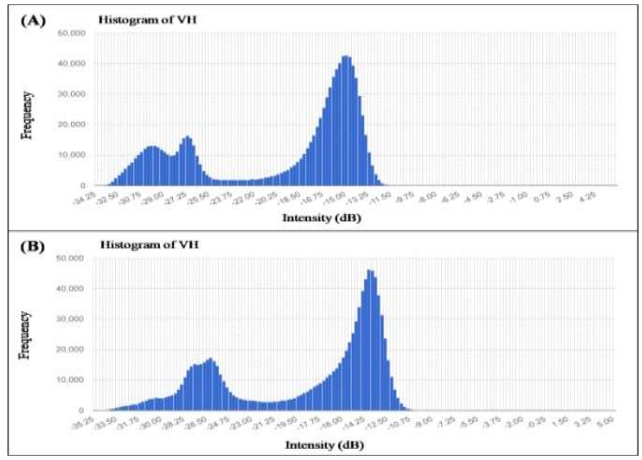

Fig. 5. Biomodal histogram of VH polarization images of (A) pre- Amphan cyclone and (B) post-Amphan cyclone, depicting the distribution of the intensity over SBR generated using the Google Earth Engine

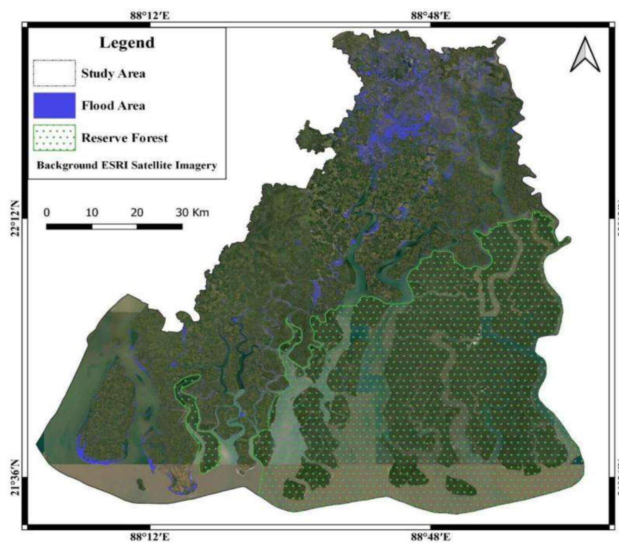

Fig. 6. Water inundation map of Indian Sundarban Biosphere Reserve during the super cyclone Amphan depicting the northern and southern part of the projected area flooded due to the cyclone

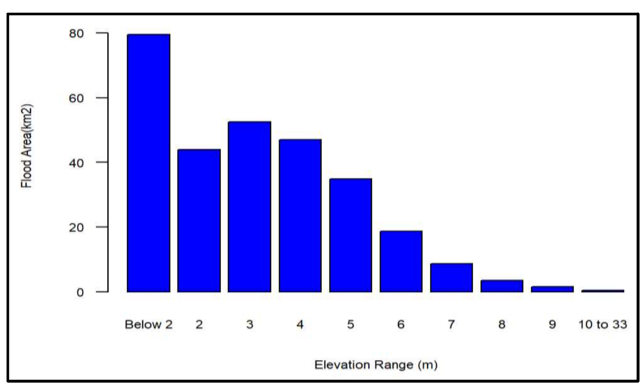

Fig. 7. Graph showing the distribution of flood areas to different elevation ranges. The graph shows the low elevation areas <5m are highly impacted by the flood

the trees (Kumar et al 2021b). Several numerical and physical model-based studies found that mangroves reduce the intensity of cyclones and tsunamis (Darryl and William 2015). Numerous important mangrove species thrive in this region, providing shelter and favourable niches for diverse ocean faunal communities. The extinction of these essential species can have devastating effects on the region's ecological equilibrium and marine biodiversity. The severity of the destruction caused by Cyclone Amphan was greatest within 35 km of the cyclone's trajectory and gradually diminished gradually away from the path. Tropical storm Amphan wreaked havoc on the dense mangrove forest and agricultural crops in the vicinity with varying degrees of severity, as observed during post-Amphan field surveys and validation. The study's outcome revealed expansion in the saturated areas due to damage to farmland and vegetation. In addition, the open vegetation class, including roadside and agricultural bund vegetation, was badly devastated (Fig. 8).

The cyclone Amphan-induced flood caused substantial damage to vast portions of existing agricultural lands (~76 % of agricultural land), constituting ~1.89 % of the Sundarban reserve forests' entire geographical area. The sudden increase in rainfall can be one of the causes of flash floods (Jha et al 2021,Bharath and Venkatesh 2022). In conformity with prior studies, the majority of agricultural and fallow land was inundated in the coastal districts as a result of the Amphan (Behra et al 2021, Kumar et al 2021a) and crops were damaged (~66 %) in Odisha, West Bengal, and the western coast of Bangladesh (Ahammed and Pandey 2021). The damage to vegetation poses severe consequences on the ecosystem and ecosystem services and affects socio-economic composition (Zommers et al 2016). The study demonstrated a comparatively very small built-up area, 0.23% of the total geographical area got impacted by the flood. Few previous studies also reported the flood's comparatively limited influence on urban areas (Dadhich et al 2019; Lal et al 2020). The change in the green urban environment in built-up areas has resulted in a substantial increase in maximum floodwater discharge (Parsasyrat and Jamali 2015). The SAR data perfectly describes all weather conditions and flood mapping (Hassan et al 2020). VH polarisation shows considerable spatial linear association in pre and post-cyclone image histograms, but not VV (Zhang et al 2018). The results indicated that flood severity was greater in low lying areas with an elevation of 5 meters or less (Phiri et al 2020). Consequently, these are the regions where the majority of the population resides, and agriculture is one of the primary means of subsistence for the inhabitants. The flood led to an increase in waterlogged areas, resulting in a diminished agricultural and plant landscape. Due to the flood, particularly in low-elevation areas, flood-induced salinity affected agricultural production (Shrivastava and Kumar 2015) and impacted drinking water quality (Nahian et al 2018). Thus, the promotion of salt-tolerant agricultural crops, particularly the indigenous rice varieties, could be a viable option for enhancing the region's population' food security (Shultanaet al 2020).

In SBR, the rising sea level and the degradation of the ecosystem's terrestrial component are of more concern. Increasing anthropogenic intrusions are also a significant concern, as they impose pressure on the natural resources and threaten the region's ecological equilibrium (Mondal et al 2021). Thus, a well-planned strategy focused on educating the populace, enhancing their skills, and relocating the populace

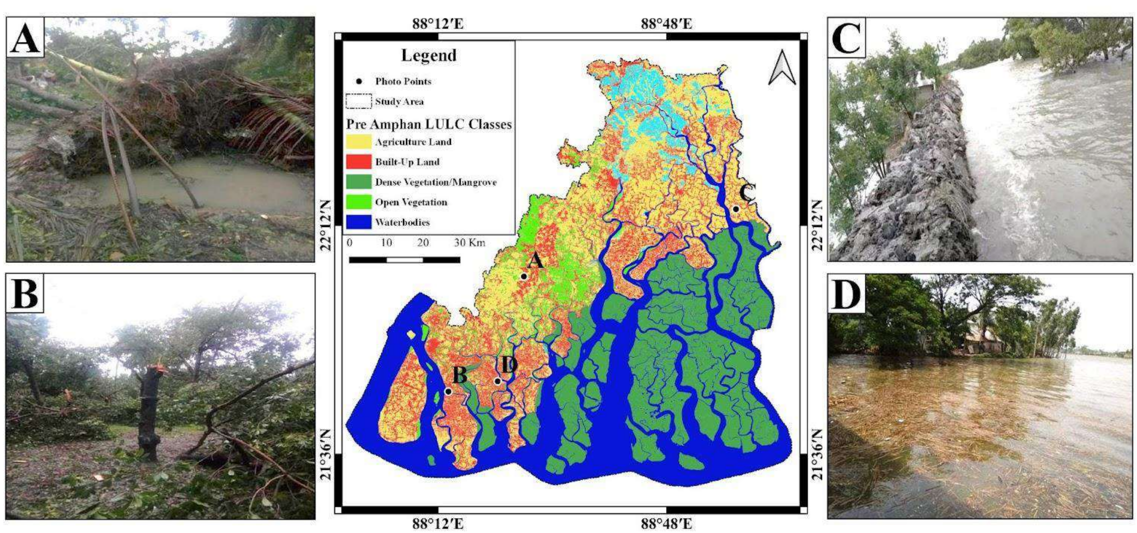

Fig. 8. Images showing damage caused due to the super cyclone Amphan in different parts of Indian Sundarban biosphere reserve at (A) Khari, (B) Namkhana, (C) Jogesh Ganj, and (D) PatharPratima

from extremely vulnerable areas to safer areas with greater employment or work opportunities will be the preferable option. Consequently, a well-planned approach centered on educating the people, enhancing their skills, and relocating the population from extremely vulnerable areas to safer areas with greater employment or work opportunities will be the preferable option, particularly for youth (Danda 2020). The approach will enable more terrestrial space for mangroves to flourish, resulting in an improved ecosystem and protection from natural disasters. A study of the effects of Cyclone Fani on the coastal region of Odisha revealed that non-native species suffered the most and is less resilient to natural disasters than native species (Nandi et al 2020). To encourage the expansion of mangrove forests in the region's natural range, the state's nodal agency must adopt native-based conservation and promotion measures immediately. In total, the findings of the study indicate wide-scale damage to mangroves and agricultural landscapes of the Indian Sundarban Biosphere Reserve due to the cyclone Amphan. This study highlighted the consequences of the cyclone Amphan-induced flood on various classes based on the elevation range and the sabotage of the mangroves. The outcome of the study strengthens our understanding of the impact of cyclones on diverse landscapes and elevation ranges. One major outcome of the study is the elevation-wise understanding of flood impact. It indicates that the lowland areas especially <5m, are at a higher risk of damage due to cyclonic events. These findings are vital for developing strategies to combat such occurrences and urge for greater focus on vulnerable elevation ranges. A key strength of the present study was the comprehensiveness of the adopted methodology to estimate the damage caused by the cyclone and the cyclone-induced flood. The implications and applications of this study pertain to the elevation-based detection of locations susceptible to severe damage from comparable catastrophes. This allows policymakers to prioritise future settlement and support of native flora programmes. Another implication of the study is the replication of the adopted methodology in other areas for similar evaluations. Cloud-free data availability was one of the difficulties. However, the basic composite cloud score technique based on the GEE addresses the post-cyclone cloud analysis. The availability of cloud-free data was one of the issues. This contributes to the scientific merit of the current study and strengthens the usage of the cloud-based GEE platform for simple, accurate, and rapid processing. The study enables for the use of GEE-based analysis to various investigations.

CONCLUSIONS

The present study set out to assess the impact of tropical cyclone Amphan on the Indian Sundarban Biosphere Reserve encompassing the UNESCO world heritage mangrove sites. The study showed wide-scale damage due to the cyclone and notable changes in the LULC of Sundarban Biosphere Reserve. The outcome showed that the severity of damage was excessive within the proximity (~35km) of the trajectory of Amphan. The study exhibits that the low land areas, especially <5m relief are at a greater damage risk to similar events. The flood-induced salinity caused wide-scale damage to agricultural crops. These findings have significant implications in assessing the extent of damage and initiatives to prioritize areas for conservation interventions and combat future threats due to similar cyclonic events. It is recommend native species-based conservation and promotion approach to strengthen and extend the natural distribution of mangroves in the region. Rehabilitation of the population from high-risk sites to safer sites, as well as people skill development programmes for better employment opportunities, should be encouraged in order to ensure socioeconomic security.

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Vulnerability of Water Resources to Climate Change in Indian Himalayan Region

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***Abstract*:** Climate change has become a proven threat to humanity. The unprecedented increases in the climate-related phenomenon and its impacts on nature, people, and animals are glaring examples of it. Water resources and the agriculture sector are going to be hit hard. The Indian Himalayan Region (IHR) is the most vulnerable region to climate change due to its peculiar geographical location, threatening food and nutritional security of millions of people whose livelihood options are primarily agriculture-based. This paper reviews the current status of water resources in IHR, the indicators of climate change in the region, and the implications of climate change on water resources. There are several issues that challenge the acquisition of data and sharing information concerning climate change, water resources, and agriculture in IHR. Various researchable, developmental, and policy issues have been raised to address the challenges associated with the vulnerability of water resources to climate change.

Keywords: Climate change, Indian Himalayan Region, Water resource, Vulnerability

The unprecedented increases in climate-related phenomenon such as warming of atmosphere and oceans, intense and frequent heat waves, declining snow in the Arctic region, reduced or lost permafrost, rising sea levels, increased glacial lake outburst floods (GLOF), torrential rainfall events, hurricanes, typhoons, storms, cloud bursts and flash floods, alarming forest fires, severe droughts, new insect pests and diseases, etc. are the strong evidence of climate change (Tiwari et al 2015, Dimri et al 2021). In addition, the impacts of climate change on nature, people, animals and marine life have become increasingly visible. Climate change brings different interactions with the water stored in the mountain cryosphere that even bring shifting of dominant communities in agro-ecosystems. This sometimes leads to threats and new opportunities in some areas, but the overall impacts in the long term are negative. The Intergovernmental Panel on Climate Change (IPCC) also discussed the long-term impacts of climate change that have also been given in the report on mountain ecosystem on Hindu Kush Himalayan Monitoring and Assessment Programme (HIMAP) (IPCC 2014, Cramer et al 2014, HIMAP 2019). Water insecurity will increase, leading to increased water conflicts among states and nations. It threatens economic growth and stability worldwide (Boretti and Rosa 2019). Recognizing the seriousness of climate change impacts, several countries worldwide have joined under Sustainable Development Goals (SDG) (Target 13.3) to establish climate forecasting systems, improve coverage of meteorological warnings, disaster prevention, and reduction systems, and strengthen climate resilience. It has also been recognized that coupling local and national mechanisms with appropriate communication and coordination systems, vitalizing local institutions, and appropriate policy instruments are necessary to achieve the desired goal of disaster risk reduction. The Indian Himalayan Region (IHR) is highly vulnerable to climatic disturbances due to its typical geographical location, lack of adequate infrastructure and poverty (Upgupta et al 2015).

Agriculture is the mainstay of people in this region. The risks of food and nutritional security and farming-based livelihood options for millions of people in the region have, thus, multiplied. According to a climate assessment report released by the International Centre for Integrated Mountain Development (ICIMOD), the IHR is warming much faster than the global average rate, resulting in significant changes in agro-ecology of the region (Anonymous 2020). The most glaring example is the disappearance of apple cultivation in the Kullu valley of Himachal Pradesh, where the rapid temperature rise has resulted in the inadequate chilling requirement for apple crop (Sahu et al 2020). Apple cultivation has shifted from lower altitudes (Kullu valley) to higher altitudes (Lahaul & Spiti districts) in Himachal Pradesh. NITI (National Institution for Transforming India) Aayog (Indian government's think tank) in 2018 expressed serious concern about social and environmental damages of economic activities in the IHR and future demands on the IHR rivers. A need has been felt to assess future water availability to the dependent highly densely populated areas downstream. Such assessments in the Himalayan range are a real challenge. Understanding of hydrology in IHR, with all threats and potentials, is needed for policy planning and initiating action to sustain agro-ecology and livelihood security in the region. This paper reviews the extent and pace of climate change and its possible threats to hydrology, water resources, agriculture, and livelihood options of the local people in IHR. Necessary steps for mitigating threats of climate change on water resources have also been discussed. Although due care has been taken to compile data only from IHR, few data from Hindu Kush Himalayas (HKH) have also been referred to understand the impact trends. The climate change studies conducted in IHR are few and sporadic.

The Himalayas: Himalayas are the highest and the youngest mountain chain on the planet Earth which is still evolving and is known as the 'water tower of Asia'. The Himalayas, formed about 50 million years ago (Kious and Tilling1996), are the world's third-largest storehouse of ice and snow. They account for 70 percent of the world's non-polar glaciers (Nandy et al 2006). Himalaya is the source of the 10 largest rivers in Asia, viz. Ganges, Brahmaputra, Indus, Irrawady, Mekong, Amu Darya, Salween, Tarim, Yangtze, and Yellow Rivers. These rivers cover an area of more than 4,192,000 km^2 (Fig. 1) (Wester et al 2019). They serve as a source of fresh water for drinking, irrigation, and power for over 1.3 billion people across eight South Asian territories of Afghanistan, Pakistan, China, India, Nepal, Bhutan, Bangladesh, and Myanmar, which is nearly 23.7 percent of the world's population (Hirji et al 2017).

The Indian Himalayan region: The Indian Himalayan Region (IHR) refers to the Indian part of the Himalaya. It stretches about 2,400 km across the northern border of India, between the eastern border of Pakistan in the west and the frontiers of Myanmar in the east. The geo-dynamically complex young mountains of the IHR occupy a special place in the world's mountain ecosystems (Singh 2006). Geographically, the IHR between Indus and Brahmaputra river systems covers about 5,33,604 km^2 area (16.2% of the total geographical area of the country) and is inhabited by about 40 million people (about 3.5% of the total population of the country) in a little over the 73,000 villages and nearly 500 towns (Singh 2013). The IHR covers partially or fully twelve states in the country viz., Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya and hill regions of two states i.e., Assam (Karbi Anglong and Dima Hasao) and West Bengal (Darjeeling and Kalimpong) (Table 1). As per a recent FAO report (IMI-FAO Report 2019), nearly 70 and 30 percent population in IHR is rural and urban, respectively. Around half of the water needs of people in IHR are met through three major rivers, viz., Indus, Ganga and Brahmaputra, fed by snow-melt and glacier-melt waters. The Planning Commission of India (1989) has divided IHR into five agro-climatic zones (Table 2).

The hydrology in IHR: The hydrology in IHR is complex and

Table 1. The geographical distribution of states in IHR (adapted from: Wastelands Atlas of India 2000 & FSI 2000)

States/Regions	Geographical area (km^2)	Percent (%) of area under		
		Agricultural land	Wastelands	Forest lands
Jammu and Kashmir	222,236[a]	4.7	64.6	9.6
Himachal Pradesh	55,673	14.5	56.9	25.8
Uttarakhand	55,483	12.5	30.1	44.8
Sikkim	7076	16.1	50.3	45.0
West Bengal hills	3149	43.5	2.2	69.7
Meghalaya	22,429	48.2	44.2	69.5
Assam hills	15,322	10.5	56.6	79.8
Tripura	10,486	29.6	12.2	67.4
Mizoram	21,081	21.2	19.3	83.0
Manipur	22,327	7.3	58.0	75.8
Nagaland	16,579	38.4	50.7	80.5
Arunachal Pradesh	83,743	3.5	21.9	81.3
India	3,287,263	55.8	20.2	20.6

Source: Kumar et al (2021)

highly variable, dominated by precipitation (rains and snow), glaciers, rivers, and springs. The significance and contribution of each of these components vary with the location in the region.

Precipitation: Two weather systems operate in the Himalayan region:

i. Indian Summer Monsoon (ISM) system (June-September) (Fig. 2) (Hodges 2006), and
ii. The winter westerly disturbances (WD) (December to March).

Both the systems show a significant impact on water resources in the region. A downward gradient exists in rainfall from the east to the west and in snowfall from west to east in IHR. A wide variation exists in the amount of rainfall received. Mawsynram of Khasi Hills in Meghalaya (NE India) is the wettest place in India and the world, with the highest annual rainfall recorded as 11,872 mm. It is closely followed by Cherrapunji, also on the slopes of Khasi Hills, with a record annual rainfall of 11,619 mm. Only 75 to 150 mm of rainfall is received at places such as Skardu, Gilgit, and Leh in the Kashmir portion of the Indus valley in the North of the Great Himalaya. The average annual rainfall in IHR varies between 1,530 mm in the western Himalaya and 3,050 mm in the eastern Himalaya. The sustainability of glaciers and the replenishment of water resources in IHR largely depend on the snowfall input by WD. Important glaciers in IHR are enlisted in Table 3. The cryosphere -snow, ice, and permafrost is an important part of the water supply in the extended Hindu Kush Himalayan region including IHR. The changes in the cryosphere affect the timing and magnitude of stream-flows across the region, with proportionally more significant impacts upstream. Cryospheric change is expected to have modest impacts on total annual stream-flows in large river systems but will strongly affect the timing and seasonal distribution of runoff, which is relevant for both ecology and economy of the region.

Rivers: In the Himalayan region, the river basins are characterized into three types viz., (1) rainfed basins, which exclusively depends upon the rainfall (2) snowfed basins, where the runoff is generated through both rainfall–runoff as well as snowmelt and the (3) glacierfed basins, runoff is primarily generated from the melting of snow and glaciers. The rivers flow in the Himalayan region is highly sensitive to climatic conditions, precipitation and evapotranspiration. Climate change effect the hydrological regime of the river basin. Three major rivers, viz., the Indus, Ganga and the Brahmaputra, originate in the IHR. They cover a basin area of around 321,289 km^2 (34%), 861,452 km^2 (82%) and 194,413

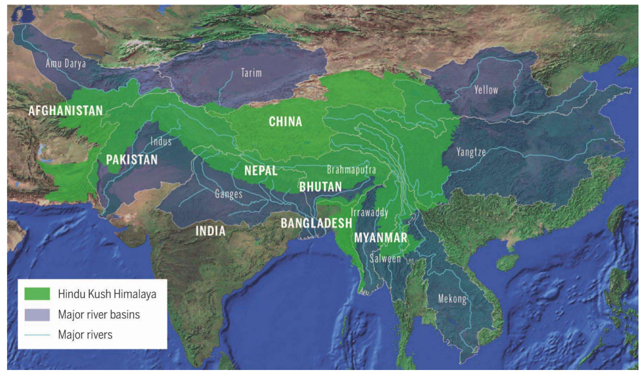

Fig. 1. Major Himalayan rivers (Wester et al 2019)

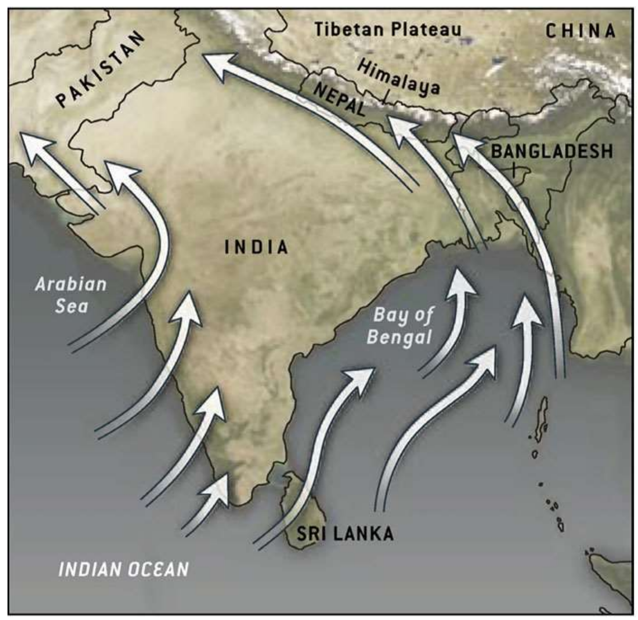

Fig. 2. Indian summer monsoon system in Himalaya (Hodges 2006). Monsoon flow from the Arabian Sea carries moisture to Indian subcontinent indicated by white arrows

Table 2. Agro-climatic zones of IHR

Agroclimatic zone	Climate	Rainfall (mm)	State/regions
Zone I	High altitude temperate (humid to cold arid)	<1200	Jammu and Kashmir
Zone I	Hill temperate (per humid to subhumid)	1200-1800	Himachal Pradesh and Uttrakhand
Zone II	Per humid to humid	1800-2200	Nagaland, Mizoram, Manipur and Tripura
Zone II	Per humid to humid	2200-2800	Sub-Himalayan West Bengal, Sikkim, Assam and Meghalaya
Zone II	Per humid to humid	>2800	Arunachal Pradesh

Source: Agro-climatic Regional Planning, Planning Commission (1989) km^2 (34%), respectively (Qazi et al 2020). The glacial and snow melt waters feed these rivers. Around 50% of country's total utilizable surface water resources are contributed by these three rivers (Srivastava and Misra 2012). The climate models project reductions in annual snowfall by 30–50 percent in the Indus Basin, 50–60 percent in the Ganges Basin, and 50–70 percent in the Brahmaputra Basin by 2071–2100 (Viste and Sorteberg 2015). It has been associated with the upward shift of mean elevation where rain changes into the snow (the rain-snow line).

Water springs: Seasonal and perennial water springs, are the primary water source for rural households in the IHR. Human settlements in the inaccessible regions in IHR are found nearby the spring sources. In the non-snow fed and rain-shadow watersheds, springs are the only potable water source. Nearly 80-90 percent of the population in Meghalaya, Sikkim, and Uttarakhand depends on springs for freshwater (Tambe et al 2009). Around 64 percent of irrigated areas in India are fed by springs (Rana and Gupta 2009). Further, springs also play a crucial role in generating streamflow for non-glaciated catchments and in maintaining winter and dry-season flows across numerous IHR basins. The base flow of many rivers in the region is contributed by mountain springs. A few studies conducted in small and scattered areas in IHR suggest a strong relationship among precipitation, recharge and spring discharge (Negi and Joshi 2004, Vashisht and Sharma 2007, Tambe et al 2012, Tarafdar 2013, Sharma et al 2016). However, the results are variable across the region. While spring-discharge variation appeared to be consistent with rainfall in Sikkim in the east (Tambe et al 2012) and Uttarakhand in the central-western Himalaya, it showed an inverse pattern with monthly rainfall in the western Himalayan springs of Kashmir (Negi et al 2012). The groundwater aquifers also play an important role in charging the water springs (Mahamuni and Kulkarni 2012). A case study in the western Himalaya shows that spring discharge during the rainy season is very high for *Karst* springs and much lower for alluvium (fluvio-lacustrine) and *Karewa* (glacio-fluvio-lacustrine) springs (Jeelani 2008). Several other factors, such as human activities, changes in land use, soil erosion, and other causal local factors also significantly impact spring water flows (Valdiya and Bartarya 1989, Panwar 2020).

Table 3. Important glaciers in IHR

Glaciers	Location
Siachin	Indus basin, Karakoram
Rulung	Indus basin, Trans-Himalaya
Neh-Nar	Sind basin, Great Himalayan range
Thanak-Lungpa	Suru basin, Zansakar range
Braham Sar	Pir Panjal range
Harmukh	Sind basin, North Kashmir range
Gara	Tirung Khad basin
Gor Garang	Baspa basin
Bara Shigri	Chenab basin, Great Himalayan range
Shaune Garang	Baspa basin
Gangotri	Alaknanda basin, Kumaon Himalaya
Pindari	Alaknanda basin, Kumaon Himalaya
Chorabari	Alaknanda basin
Dunagiri	Alaknanda basin
Shangme-Khangpu	Sikkim Himalaya
Zemu	Sikkim Himalaya

Source: ENVIS Monograph 3

Climate change indicators in IHR

Warming of air and earth surface: The Himalayas are continuously warming faster than the nearby Indian landmass (Sabin et al 2020). The western and eastern Himalayan river basins in IHR are showing progressive warming, with rise in minimum temperatures slightly higher than the maximum temperatures (Rajbhandari et al 2015). The IHR has warmed at a rate of about 0.1-0.7°C per decade depending on elevation during the last four decades (Singh et al 2018). The warming rate has been slower (<0.2°C per decade) at low-elevation sites (<500 m) than at high elevations (>2000 m) (Liu et al 2009, Ren et al 2017). According to the Ministry of Earth Sciences (MoES), even with the Indian government's commitment to mitigate greenhouse gases by 2030, the Indian Himalaya could warm by 2.6–4.6°C by 2100. By the end of the 21st century, the average temperature over India is projected to rise by approximately 4.4°C compared with the 1976–2005 average. The temperature rise accelerates the melting of glaciers, in addition to the increased frequency of heat waves and more severe droughts, severe cyclonic storms, and floods in Himalayan river basins.

Changing precipitation patterns: Although visible changes in the amount and pattern of precipitation have been observed during past few years, the precipitation data are too scarce to conclude any particular precipitation trends in IHR. Studies conducted by Snow and Avalanche Study Establishment (SASE) indicated a decrease in the snowfall with a concurrent increase in the rainfall, but the total precipitation showed a decreasing trend in NW Himalaya during 2001-2015. Bhutiyani et al (2010) observed a statistically significant downward trend in monsoon rains in the western IHR from 1866 to 2006. A similar downward trend in winter precipitation in Jammu and Kashmir and Uttarakhand of Himalayan region during 1901–2003 (Guhathakurta and Rajeevan 2008). The monsoon rains are delayed and their typical characteristic feature of 7-10 days of continuous downpour has almost disappeared. Similarly, the winter rains have also become unpredictable and declined in quantity. Negi et al (2012) reported extreme drought events and shifts in the rainfall regime in Kullu valley, resulting in failure of crop germination and fruit set.

Melting of glaciers: Receding glaciers is one of the most reliable indicators of climate change. During the Pleistocene era (2 million years ago) glaciers occupied about 30 percent of the total area of the Earth, which presently has reduced to around 10 percent due to glacial melt (Bahadur 1998). Thinning and squeezing glaciers at increased rates since the mid-19th century has been observed globally and the Himalayas are no exception. Due to climate change, Himalayan mountain glaciers have been experiencing a significant decline over the past several decades, although detailed information is available only for a few glaciers (Rai and Gurung 2005). Kulkarni and Karyakarte (2014) have reported large-scale melting of snow and retreat of glaciers in IHR during the past five decades. Kulkarni and Pratibha (2018), based on remote sensing data and *in situ* observations from 83 glaciers, reported a loss in glacier extent by 12.6 ± 7.5 percent for the past 40 years. Mass balance studies of Singh et al (2018) in IHR (Gor Garang and Shaune Garang of Baspa Basin and Chhota Shigri glacier of Chandra Basin) have revealed continuous negative mass balance (with few exceptions for a year or two) with an increasing trend in recent decades. This has coordination with a continuous increasing pattern of temperature and decreasing pattern of precipitation.

The 'First-ever Assessment of Climate Change' report of ICIMOD, Kathmandu, Nepal indicated an increase in the number of glaciers in the Himalayan area during the last five decades due to the fragmentation of larger glaciers into smaller ones. Studies indicate that the melting of small glaciers is faster than the large glaciers. The Eastern Himalayan glaciers are shrinking faster than glaciers in the central or western Himalaya (ICIMOD 2011). The average glacial retreat has been between 0.1 and 1.0 percent per year. Studies on selected glaciers in IHR indicate that glaciers have been retreating discontinuously since post-glacial time. The 25 km long Gangotri glacier in 1930s, one of the major and important Glaciers in the Himalaya, has shrunk to about 20 km during the last six decades (Hasnain 1999). The average recession rate of this glacier between 1985 and 2001 has been around 23 m/y (Hasnain 2002), which increased to around 30 m/y during the recent decade (Rai and Gurung 2005). The Siachen & Pindari Glaciers retreated at the rates of 31.5 m/y and 23.5 m/y, respectively (Vohra 1981). Dobhal et al (1999) observed shifting of snout of Dokriani Bamak Glacier in the Garhwal Himalaya at a rate of 17.2 m/y during 1962-1997. Geological Survey of India studied the Gara, Gor Garang, Shaune Garang, Nagpo Tokpo Glaciers of Satluj River Basin and observed an average retreat of 4.2-6.8 m/y (Vohra 1981). The Bara Shigri, Chhota Shigri, Miyar, Hamtah, Nagpo Tokpo, Triloknath and Sonapani Glaciers in Chenab River Basin retreated at the rate of 6.8-29.8 m/y. A massive glacial retreat rate of 178 m/y was observed in Parbati Glacier in Kullu District from 1962 to 2000 (Kulkarni et al 2004). The Indian Space Research Organization (ISRO) recently monitored the glacial advance and retreat of 2,018 glaciers in IHR, using satellite data from 2000-2001 and 2010-2011. This study further concluded that 12 percent of glaciers retreated, 1 percent glaciers advanced and 87 percent glaciers remained unchanged. Glaciers in Sikkim are melting faster than in other parts of Himalayan region. The melting of snow and glaciers feeds river and spring flows, but if not compensated by matching/increased snowfall may lead to loss of water reserves in the Himalayas. Maximum runoff from snowmelt occurs of June and July. The increased glacial melt is also projected to increase flood frequencies in Indus, Ganges, and Brahmaputra rivers in the 21st century, risking the livelihood security of 220 million people (Hirabayashi et al 2013, Wijngaard et al 2017, Wester et al 2019).

Formation of glacial lakes and rising GLOF threats: The glacial melt waters may accumulate in natural depressions in hills giving rise to glacial lakes. The fast melting and retreating (at rates between a few meters to hundreds of meters per year) of Himalayan glaciers have been resulting in an increase in the number and size of glacial lakes and a concomitant increased threat of glacial lake outburst floods (GLOFs) (Chalise et al 2006, Bajracharya et al 2007). Some glacial lakes are expanding at a dangerous rate (Bajracharya et al 2008). Studies indicate that glacial lakes were almost non-existent in Nepal prior to the late 1950s, when they started forming and rapidly increasing in number and size (Chalise et al 2006). The investigation carried out by ICIMOD between 1999 and 2005 documented 8790 glacial lakes covering a total of 801.83 km^2 in the HKH, out of which 203 lakes were potentially dangerous for the GLOF in future (Ives et al 2010, Bolch et al 2019).

Abandoned moraines form the majority of the glacial lakes in Himalaya. (A moraine is generally soil and rock material left behind by a moving glacier). The moraine-dammed lakes are highly unstable and susceptible to outburst, triggered by ice or debris falls, strong earthquake tremors, internal piping or overtopping waves that exceed the shear resistance of the dam (Richardson and Reynolds 2000, Westoby et al 2014). As a result, they breach suddenly and release huge volumes of sediment-laden floods at rates

>100 km downstream within minutes to hours (Richardson and Reynolds 2000, Bajracharya et al 2007). The GLOFs are independent of hydrometeorological floods and are a potential threat downstream to lives (humans and livestock) and property (cultivated lands, forests, buildings, bridges, dams, hydro-power projects, roads, etc.). Studies have shown that the occurrence of GLOFs in the Eastern Himalayas is about 3 times higher than in any other Himalayan region (Veh et al 2020).

Changes in river flows: The river flows are linked with snow and glacier melt and rainfall events in the mountain regions. The river basins in the eastern and central IHR (i.e. Ganges and Brahmaputra Rivers) are primarily fed by the summer monsoon rains (ISM), while snow and glacial-melt during winter-time western disturbances (WD) feed river basins originating in the western Himalayan region (i.e. Indus River). Thus, rainfall contributes more to the flows of the Ganga and Brahmaputra, while snow and glacier melt contribute more to Indus flow (Alford and Armstrong 2010, Immerzeel et al 2010, Mukhopadhyay and Dutta 2010). The contribution of snow and glacial melt in Ganga, Brahmaputra, and Indus river flows was observed to be around 9, 19, and 60 percent, respectively. Therefore, a consistent increase in streamflow at large scales has been projected for the upstream reaches of the Indus, Ganges, and Brahmaputra rivers until at least 2050; in the Indus river due to increased glacial melt, and in Ganges and Brahmaputra rivers due to increased precipitation (Immerzeel et al 2013, Lutz et al 2014).

Studies also indicate that the average annual downstream flows in Ganga and Brahmaputra Rivers are unlikely to show change for quite some time, and it is unlikely that these rivers shall become seasonal rivers (Miller et al 2012). In case of the Indus River, however, increased glacier melt will provide short-term increases in river flow, but in the long run, the river flow is likely to decrease due to a decline in the potential melt-water stores (Miller et al 2012).

Receding mountain springs: There is increasing evidence of springs drying, reducing discharge and deteriorating water quality in many parts of the Himalayan region. Springs in IHR are reported to be drying up, although the status of most springs in the region is still unknown due to paucity of data. According to Rana and Gupta (2009) around 50 percent of perennial springs in the IHR have dried up or become seasonal. Around 30 percent of springs in one of the mid-hills districts in Nepal have been reported to be completely dried up in the last decade due to a combination of biophysical, technical, and socio-economic factors (Sharma et al 2016). Spring discharges have also significantly declined (Sharda 2005). Valdiya and Bartarya (1989) reported around 25 percent spring flow decrease by late 1980s in Gaula river basin; over 35 percent decrease during 2000s in Sikkim was reported by Tambe et al (2012). Several factors have been responsible for the drying up of springs or decrease in spring flows including, climate change, deforestation, grazing, exploitative land use, depletion of shallow water table because of reduced infiltration due to crust formation and high-intensity rainstorms, etc (Vashisht and Bam 2013, Scott et al2019). Spring flows are also impacted by rapid socio-economic growth, demographic changes, and infrastructural developments, such as dams and road construction (Mahamuni and Kulkarni 2012, Vashisht and Bam 2013).

Deposition of black carbon on glaciers: Black carbon (BC) has been deposited on snow and glaciers in the Himalayan region due to atmospheric pollution by CO (particulate pollution) and CO_2 (biomass pollution). The forest fires have also been responsible for it. The first historical record of BC deposition of about 80 ng m^{-3} was found in Mt. Qomolangma (Everest) in the high Himalaya from 1951 to 2001 (Ramanathan and Carmichael 2008). The increasing levels of BC in South Asia caused BC deposition in Tibetan Plateau. Kuniyal (2010) observed BC concentration as high as 15,657 ng m^{-3} hourly at Mohal-Kullu (H.P.) in the northwestern IHR. Deposition of BC cover on glaciers increases the rate of snow and glacial melting by decreasing albedo and increasing absorption of solar radiation (Ming et al 2008). According to Ramanathan and Carmichael (2008), solar heating from BC at high elevations in the Himalayan region may be as important as carbon dioxide in melting snow packs and glaciers.

Implications of climate change on water resources: The implications of climate change on the availability of water resources-spatial distribution, temporal dynamics and water security, in general, are extremely significant. Important ones are briefly described below:

Shrinkage of water resources: The changes in precipitation coupled with the continuous melting of glaciers is expected to reduce the storage of snow and ice in the region with the future implication of shortage of fresh water availability in the snow-glacier fed river systems. The recent review of the past literature reveals a huge cumulative loss of glacial ice equivalent to the 443±136 Gt out of the total 3600–4400 Gt stored glacial water in the IHR (Kulkarni and Buch 1991, Kulkarni and Karyakarte 2014). Local communities start to relocate as the water resources decline in many villages of HKH (Kulkarni et al 2021). This has threatened regional water security and livelihood of the local inhabitants.

Change in river flows: A consistent increase in stream flow is expected at large scales in all three major rivers viz. Indus, Ganges, and Brahmaputra in IHR, until at least 2050.

Increased glacier melt will provide short-term increases in the contribution to discharge but will likely lead to decreased water supply in the future, impacting the water availability downstream (https://www.ipcc.ch/srocc/about/faq/faq-chapter-2/).

Drying of mountain springs: Drying of springs or progressive decline in spring flows is a matter of grave concern to hill people who mainly depend on spring waters for their household needs and irrigation. The drying of mountain springs can also lead to an environmental crisis in the loss of indigenous flora, fauna and human migration.

Formation of glacial lakes: The IHR is also vulnerable to natural disasters viz., landslide, flash flood and cloudburst, while the impact of climate change increases the intensity and frequency of such disasters. Retreating glaciers contribute to the formation of glacial lakes and increased threats of glacial lake outburst floods (GLOFs). The Chorabari Lake outburst flood, Kedarnath, caused disaster in 2013, and the more recent Chamoli Glacier outburst on 7th Feb., 2021, are the glaring example that resulted in losses of life and property. Such events are expected to increase in IHR due to fragile geology, active tectonics and peculiar hydrometeorology.

Increased frequency of cloud bursts and flash floods: The orographic lifting of air due to unstable inclines resulted in unexpected heavy precipitation in IHR. Many researchers reported occurrence of cloudbursts mainly in monsoonal period around the southern rim of the Himalaya and along the Gangetic plains (Sah and Mazari 2007; Dimri et al. 2017). The frequency of cloud bursts and resultant flash floods is projected to increase due to climate change. It would escalate soil erosion hazards, degradation of agricultural lands, damage to irrigation structures, economy and service sectors, and threats to human life and livestock etc.

Increased frequency of droughts: Droughts are expected to increase, resulting in the decreased number of rainy days and a shrinking of cropping seasons. It would seriously impact agriculture, especially in rainfed areas. More than 60 percent of cultivated lands in IHR are rainfed. Climate change signatures are also visible in the fragile landscape of the Himalaya, causing abnormal floods and droughts (Tewari et al 2017). There is increasing concerns about current and potential climate change impacts that need to be mitigated by adopting resource–conservation technologies (RCTs), conservation agriculture (CA) practices and agro–meteorological analysis of climate–soil–crop relationship.

Increased threats to water security – demand and supply issue: Water security is threatened by increased water demand associated with improved socio-economic conditions in the downstream region. The impacts of climate change are also visible in the IHR, putting enormous stress to the hydrological system (Matthew et al. 2013). Water stress often manifests drought and flood that need to address through systematic management measures.

Challenges

- The information concerning changing climate scenarios in IHR and its implications on water resources and the related threats are yet not thoroughly investigated and understood.
- Research on climate change vis-à-vis its impact on ecosystems (e.g., forests, water, agricultural resources, etc.) is still in the infancy stage in IHR.
- As such, there is a paucity of long-term climate data in the region. There is a lack of reliable micro-level data. The climate data collection network is presently insufficient to meet the requirement of climate change research.
- Current generation climate models and downscaling methodologies have limitations in capturing the observed hydro-climatic variations of the Himalayan river basins (Hasson et al 2019).
- The climatic effects of black carbon on the Himalayan glaciers are not adequately understood, partly due to the large spatio-temporal variability of black carbon in the region (Kopacz et al 2011).
- Each IHR river basin has its characteristic ecological complexity, endowment and water needs, creating diverse governance challenges.
- The assessment of the water flowing in the IHR river basins suffers from great uncertainty (Kattelman 1987).

What needs to be done?

Strengthening resilience and adaptive capacity to climate-related hazards and natural disasters in all countries and integrating climate change measures into national policies, strategies and planning are the two major targets of SDG 13. Understanding the past and potential future of climate change in IHR based on robust scientific analysis is important to better comprehend the region's present and future risks. Evidence-based actions to reduce disaster risk, to mitigate and adapt to climate change, and adopt good governance, are central to ensuring prosperity in the IHR. Challenges and opportunities vary at different levels: micro (watershed and spring-shed), meso (river basin), and macro (regional), and so are the needed actions. Water-related decision-making is influenced by different stakeholders and interest groups across micro, meso and macro scales. At the micro level, informal local institutions are influential decision-makers. There should be greater synergy between the formal state and informal local institutions. At the meso level, the formal state institutions are the critical decision-makers. Their decisions should be evidence-based rather than politics-based, supported by science and local knowledge. At the macro level, different states at the national level (and different nations at the international level) play an important role in decision-making. Trust building among themselves and settling issues and conflicts wisely are important for the interest of humanity. Important researchable, developmental, and policy issues focusing on the mitigation or reduction in the impact of climate change on water resources in IHR are enlisted below:

Researchable and developmental issues

- Systematic data collection to understand the current status of climate and water resources in IHR should form the first step of the action plan. Understanding glaciers and snow cover is vital for water resources planning and management. The climate data collection efforts need to be strengthened and intensified.
- Rescuing a shrunk glacier is rather difficult. Therefore, it is important, to concentrate on mitigating the impact of the glacier melt and prepare at the societal and economic level.
- To address the water crisis caused by the dried-up springs, spring-shed management strategies and conservation measures should be developed by merging scientific and community knowledge. Few studies have established a relationship among precipitation, recharge and spring discharge, but in small and scattered areas with variable results (Negi and Joshi 2004, Sharma et al 2016, Kumar and Sen 2020).
- A workable and realistic management plan for spring-sheds needs hydrogeological and hydrological characterization of catchments and a reliable modelling approach (Kresic and Stevanovic 2010). Additional field investigations of declining springs and further research, about detailed geohydrology and modelling studies are required in spring catchments.

Policy issues

- India needs to reduce its greenhouse gas emissions and move on to renewable energy sources like wind, solar, and ocean waves, in addition to improving forest cover and other means to absorb the country's carbon dioxide emissions. India is committed to reducing its emission intensity (annual greenhouse gas emissions per unit gross domestic product) by up to 35 percent from 2005 levels.
- India's commitment to achieving Net Zero emissions by 2070 is revealed in the 26th Conference of Parties (CoP26) with a five-fold strategy named '*panchamrita*' by focusing on renewable energy and reducing carbon emission (https://pib.gov.in/PressReleasePage.aspx?PRID=1768712).
- Particular attention concerning the IHR needs to be given to the possible use of aquifers in the foothill regions.
- A two-tiered policy approach is required to manage hydrology in IHR. A framework policy for all the IHR river basins, and more specific policies and practices for individual basins within that framework are needed.
- The policy must recognize all constituents of flows (water and its composition, energy content of flows, biodiversity, and sediment budget in all parts of their basins) beyond simple hydraulic quantifications.
- Organized governance at the basin level is required by treating the IHR as a large catchment with land use zones designed basis on water as a product, along with crops, fruits, timber, flowers, minerals etc. The existing reductionist policy fails to address such an aspect of governance of river basins in IHR.
- The policy must integrate water demand and infrastructural development infrastructure for flood mitigation, irrigation, water supply, functioning of ecosystems, pollution control, etc.
- The spatio-temporal heterogeneity has varied impacts from climate change. The IHR also has many implications that require a holistic approach with the involvement of local communities to usher its long-term benefits to upcoming generations. The national mission for sustaining the Himalayan ecosystem also highlighted the importance of community participation in adapting and mitigating the climate change.

CONCLUSION

The Indian Himalayan Region (IHR) is the storehouse of fresh water for millions of people downstream. It stores water in the form of snow and glaciers. The three major rivers (Indus, Ganga and Brahamputra) and mountain springs which originate from melting snow and glaciers and rainfall events in IHR, meet more than half of the water requirement of people in India. However, water resources are under the increased threat of climate change. The precipitation is declining while snow and glacial melt is increasing, leading to loss of water storage, formation of glacial lakes and flash floods, endangering life and property. The challenges of managing water resources in IHR are enormous because of the paucity of databases, poor data-collecting network, uncertainties of assessing river flows, and uncertainties in climate models because of large hydro-climatic variability in the Himalayan region, etc. Nevertheless, India has the potential trained human resources, relevant infrastructure, and political willpower to convert these challenges into opportunities. Since water resource management in mountain ecosystems is a complex issue, it must be tackled by integrating the efforts of all the stakeholders holistically. All efforts must be made to minimize GHGs production to curb global warming and climate change.

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Potential of Laser Levelling Technology for Enhancing Land and Water Productivity in North Western Gangetic Plains

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***Abstract*:** Rice-wheat cropping system is dominating cropping pattern in North Western Gangetic Plains. The effect of Laser Land Levelling on-farm input water use efficiency and crop production was studied. The major constraint in the adoption of this technology is a huge capital investment in laser leveller, so collective effort or custom hiring can help. Also, it has been suggested to raise the knowledge level of the farmers along with more exposure to the extension agencies to enhance the adoption of this technology. Further evaluation of this technology is strongly recommended under different Agro-climatic zone for their large-scale adoption in the whole Indo Gangetic plans in India.

***Keywords*:** Irrigation, Laser land levelling, Precision technology, Rice, Wheat

The Indo Gangetic Plains (IGP) covers an arc from the Swat valley in Pakistan, through the Indian states of Punjab, Haryana, Delhi, Uttar Pradesh, Bihar, West Bengal, parts of Rajasthan, across the Nepal Terai and into Bangladesh, where it characterizes most of the country. IGP is a fertile alluvial plain in South Asia, which is home to an estimated one billion people around seventh portion of the world's population (Bhatt et al 2021). The Plain is bounded by the floodplains of the rivers Indus to the west and Ganges to the east, the Himalayan foothills to the north, and the Deccan plateau to the south (Jain et al 2020). It is approximately 3,000 km from east to west and 250-300 km from north to south. It contains some of the Subcontinent's richest agricultural land (Abrol 1999, Prakash et al 2014). Rice and wheat are the two principal food grains in the region, crops which are grown in sequence on 13.5 million hectares of the Plain and contribute 80 percent of its food production (Jat et al 2006: Parihar et al 2022). It also constitutes 85% of South Asia's Rice-Wheat system and other important crops are maize, sugarcane, and cotton are also cultivated in this region (Gupta et al 2003).

Declining water tables have serious implications by increased pumping costs as farmers have to shift to costly deep tube wells (Tripathi et al 2016, Pandey 2016), where in some areas there are indications of water quality decline due to possible intrusion of brackish water from adjoining saline groundwater regions. In Punjab and Haryana, with the water table falling in 90 percent of the area of the State (Chaudhry and Sachdeva 2020). It is estimated that to meet growing food needs, India will have to produce almost 40 percent more food with almost 10 percent less water by 2025 (FAO 2017). Groundwater depletion is already a huge area of concern, particularly in the NWGP. The total water requirement for the rice-wheat system in the NWGP is estimated at between 1,382 mm and 1,838 mm, 80 percent of which is for irrigation in the rice component (Jat et al 2006). Water withdrawals from aquifers are estimated at 13-17 cubic kilometres per annum, in NWGP which greatly exceeds the recharge level, so that water tables have been falling (Aryal et al 2013).

Indian farmers commonly use flood irrigation in the Indo Gangetic plain (IGP) moreover, the highly irrigated areas like Punjab, where 99% of the water is obtained through tube wells and canals (Brar 2014). Flood irrigation method is commonly adopted in the Indo Gangetic plain (IGP) of India as well as in other countries (Kumar et al 2013, Jat et al 2021). In Northwest India, conventional irrigation is inefficient when flow rates are not enough to achieve rapid irrigation. The inefficiency is due to deep drainage below the root zone. Flood irrigation also causes temporary waterlogging, with adverse effects on crops like wheat, maize, and legumes (Kaur et al 2020). Waterlogging is more prolonged and more severe on heavy textured soils, and on soils used for rice culture because of the well-developed, shallow, hardpan as a result of puddling (Singh et al 2009). This leads to aeration stress in upland crops, especially in wheat (Kukal and Aggarwal 2003). When land is flood irrigated, any degree of undulation in the soil surface can seriously reduce both water and land productivity (Sheikh et al 2022). Hence farmers in the NWGP have traditionally spent considerable time and resources leveling the land, usually by passing a weighted tractor or animal-drawn leveling plank or harrow repeatedly over the dry field (Aryal et al 2015). The field is then irrigated so that high spots can be identified and further levelled. There are clear limits to the degree of inaccuracy that can be achieved by such conventional techniques.

Low efficiency of irrigation and poor recovery of water charges are the major problems associated with agricultural water management in India. In the rice-wheat (RW) system of the Indo-Gangetic Plains (IGP), about 10-25 % of irrigation water is lost due to poor water management and uneven fields. LLL is an alternative land leveling technology that has the primary benefit of a reduction in the loss of irrigation water occurring due to highly undulating land. Therefore, applying LLL rather than traditional land leveling (TLL) can help reduce the use of irrigation water and save energy through reduced duration of irrigation (Jat et al 2011).

To arrest this dangerous trend of groundwater exploitation, there is an urgent need to conserve irrigation water through various on-farm water conservation practices. Land Leveling through Laser Leveller is one such proven technology that is highly useful in the conservation of irrigation water (Pradeep et al 2014). Laser levelling uses high-precision laser technology to level out the undulations in agricultural fields. Laser levelling is an assistive technology, in the sense that it supplements rather than replaces the traditional method of leveling a field. In traditional levelling, a grading implement with a blade is towed behind a tractor over the field. The height of the blade is adjusted manually by the operator so as to achieve a surface that appears undulated to the human eye. The innovation in laser leveling is to use a laser guidance system to position the blade of the grading to implement automatically. Technology can ensure very accurate and precision land leveling to extent of ± 2 cm. Remains other input constant like fertilizer and seed, laser land leveling can increases crop yields by around 11 percent and water saving of around 25 percent (Hung et al. 2022). Studies have also demonstrated that leveling reduces weeds by up to 40 percent and labor time spent weeding by up to 75 percent (Bhatt and Sharma 2009). In the rice field, this technology brings down the water use by 36.19 cm along with the yield improvement of 0.78 t/ha. Further, by the adoption of this technology the Punjab state could achieve 0.99 Mha-m (million-hectare metre) water-saving and can save 583.51 million kilowatt-hour of electricity (Sidhu et al 2010).

Laser levelling technology is widely used in developed countries such as Australia, Japan and the USA, where large-scale irrigated agriculture is practiced (Bhatt and Sharma 2010). This technology spread rapidly to the extent that a survey estimated that by 2012 there were more than 9030 units of laser leveller in operation in the rice-wheat area of the NWGP (Jat 2012).The main objective of this paper to study the benefits, need and challenges in adoption of laser land levelling technology in the NWGP India. Also this study may provide scientific insights for researchers working on laser land levelling technology and its importance for NW India.

MATERIAL AND METHODS

Description of laser leveller: Description of various components of laser land leveller is explained in Table 1 and shown in Figure 1.

Working principle: The fields may be required ploughing

Table 1. Description of various components of laser land leveller

Part name	Description
Laser transmitter	Laser transmitter transmits a laser beam which is intercepted by the laser receiver mounted on the levelling bucket The control panel mounted on the tractor interprets the signal from the receiver and opens or closes the hydraulic control valve which will raise or lower the bucket. It needs to place on higher ground, so laser beam does not get blocked by tractor.
Laser emitter	The laser emitters mounted on a tripod stand placed outside the field and high enough to have unobstructed laser beam travel. The laser emitter unit sends continuous self-levelled laser beam signal with 360° laser reference up to a command radius of 300-400 m (Irsel and Altinbalik2018)
Laser beam receiver	The laser receiver mounted on the scraper (bucket), is an omni-directional (360°) receiver that detects the position of the laser reference plane and transmits it to the control box.
Control box	The control box is to be mounted on the tractor so that the operator can easily access the switches and view the indicator lamps. The control box receives and processes signals from the laser receiver mounted on the bucket to actuate the double acting hydraulic valves.
Hydraulic valve unit	The valve assembly regulates the flow of tractor hydraulic oil to the hydraulic cylinder to raise and lower the scraper blade. The solenoid control valve controls the flow of oil to the hydraulic ram which raises and lowers the bucket.
Drag Bucket	The levelling bucket can be either three-point linkage mounted or pulled by the tractor's drawbar. A 60 hp tractor will pull a 2 m wide x 1 m deep bucket in most soil types. The design specifications forthe bucket should match the available power from the tractor (Maqsood and Khalil2013).
Power unit	It is preferable to have a four-wheel drive tractor than two-wheel drive and the higher the horsepower the faster will be the operation. Power shift transmissions in the tractor are preferred to manual shift transmissions.

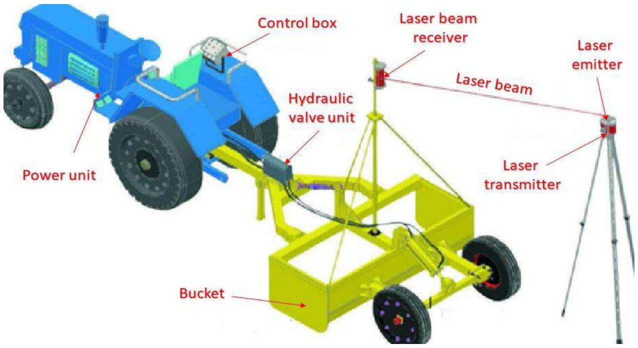

Fig. 1. Components of laser leveler with tractor

before and after land levelling. Depending on the amount of soil that must be cut it may also be necessary to plough during the levelling operation. Laser land levelling technology achieves a flat even surface by using a rotating laser transmitter placed at the side of the field. This controls the degree of cut and fill to be made by the tractor towed laser land leveller. A levelling blade is used on wet fields and a drag bucket on dry fields. As the tractor moves the leveller across the field, the signal from the transmitter is picked up by a receiver mounted on top of the laser land leveller. This is then routed via a control box in the tractor cab which operates hydraulic valves, which raise and lower the bucket or blade so as to level out undulations in the field. The tripod-mounted transmitter in the foreground, at the side of the field and the corresponding receiver is visible above the laser land leveller. The soil can also be seen, piling up within the drag bucket as high spots are eliminated including even the tyre tracks behind the machine.

RESULTS AND DISCUSSION

Adoption of laser leveller in Punjab and Haryana: The work on laser land levelling was started in rice crop initially from 2005-06 from 8 units of machine at PAU, Ludhiana and various farmer's field in farmer's participatory research mode. Few machinery modifications has also been done like hitch system of scraper bucket was modified from its V shape to Y shape which improved it's turning radius by 27 percent for its easy manure-ability in the small fields and hydraulic control system of the scraper bucket was simplified for easy, simplified and leak proof operation and machine working. The main intention was to authenticate the replicated trial's results (in terms of water saving and better yields etc.) with the farmer's managed fields. Then more efforts were made for the popularization of this technology by organizing trainings for farmers, contractors and Govt officials, demonstrations, field days and workshops for all the stake holders. Machinery manufacturers were also enrolled into the potential future growth of the technology to maintain the price & quality simultaneously (Lohan et al 2014). In both states (Punjab and Haryana) number of laser levellers increased and exponential growth curves fitted in each case showing in Figure 3. Increasing trend showed this technology is highly adapted in both states.

Irrigation water conservation in different crops: A significant reduction in total water use in wheat as well as rice was recorded due to precision land leveling compared to traditional land levelling. Kaur et al (2012) emphasized the adoption of laser level technology the paddy cultivation. The study shown that with laser levelling farmers could save irrigation water and energy by 24 percent and water productivity improved by 39 percent (Pradeep et al 2014). It has a great potential for optimizing the water-use efficiency in paddy cultivation without any disturbing and harmful effect on

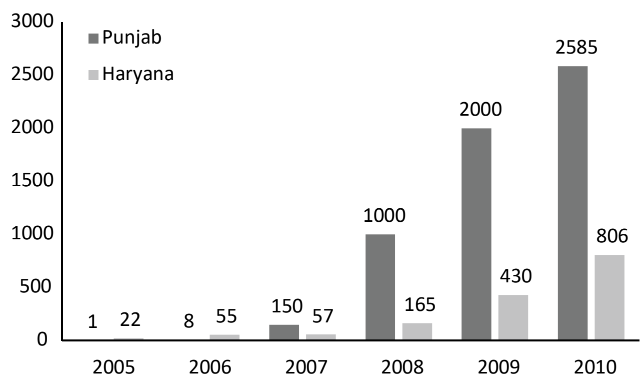

Fig. 2. Year wise availability of laser leveller in Punjab and Haryana

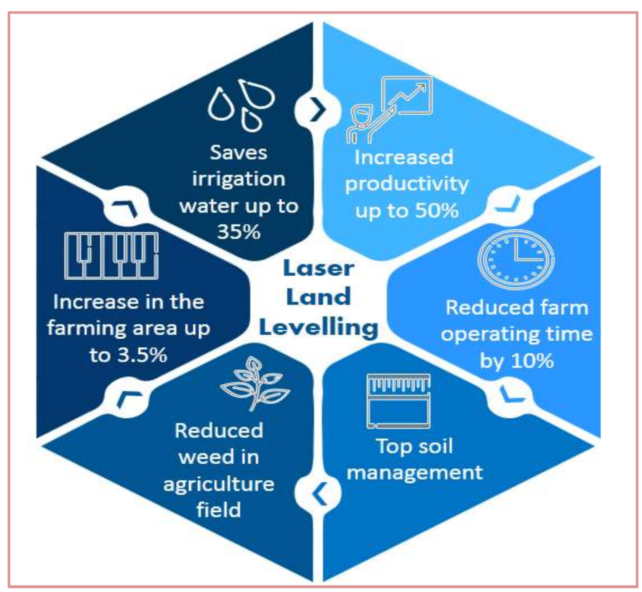

Image courtesy: Parihar et al 2021

Fig. 3. Benefits of laser land levelling

its productivity. On wheat and cottons crops it was observed that about 21 percent of irrigation water may be saved by the adoption of laser levelling technology and also obtained higher yield and profit margins comparatively traditional methods (Shahani et al 2016). In the pearl millet cultivation with laser land levelling technology, It has been observed that it saves 32.18 percent of irrigation water (Ahuja et al 2016). Kanannavar et al (2016) suggested laser levelling technique recorded 63.68 percent more water productivity over traditional levelling, indicating more production with less irrigation water. It was established that laser land levelling technology saves precious water and results in the highest water productivity in groundnut production also. Land levelling with laser leveller is a recent resource conservation technology and has been proven to save water and energy over the conventional methods (Table 2).

Reduced weed emergence: Laser land levelling results in uniform moisture distribution to the entire field and allows uniform crop stand and growth, thus resulting in lesser weed infestation. On the other hand, unlevelled fields frequently exhibit patchy growth. The areas with sparse plant populations are zones of higher weed infestation because weeds are mostly C4 plants and possess the inherent genetic capability to suppress crop growth. Reports on reduction of weed population by laser land leveling indicate beneficial effects in term of reduction in weed population. Also results in enhanced weed management efficiency as removal of less number of weeds manually requires less time. Moreover a 75% reduction of labour and cost of weeding can be achieved through precision land leveling (Rickman 2002). Reduction in weed population in wheat after 30 days of sowing was recorded under precisely levelled fields in comparison to traditional levelled fields (Jat et al 2006). Ahuja et al 2016 studies the weed occurrence in pearl millet cultivation using laser land levelling, it was observed that weed occurrence reduces by 27.81 percent (Table 2).

Crop production: A considerable increase in yield of crops is possible due to laser land leveling. Results shows that a perceptible yield advantage of laser land leveling over traditional leveling. Jat et al. 2003 reported that the wheat production increased from 4.3 to 4.6 t ha^{-1} under laser leveling. The improvement in crop production through laser land leveling was associated with overall improvement in the growth and yield attributing characters of the crop due to better environment for the development of the plants under well-levelled field. In another investigation, Pal et al 2003 reported a significant improvement in growth and yield of upland paddy due to laser land leveling compared to traditional land levelling. Ahuja et al 2016 studied the pearl millet cultivation with laser land levelling. It was reported that pearl millets yield and net profit increased by 52.77 percent and 141.57 percent respectively. The above studies indicate that laser levelling is prominent technology for achieving favourable environmental conditions for the crop establishment which leads to better crop growth and production (Table 2).

Cost of crop production: Rickman (2002) reported that although the initial cost of land leveling is convincingly high,

Table 2. Influence of laser levelling technology over traditional levelling method

Reported authors	Irrigation water conservation	Increased cultivable area	Increased yield	Uptake efficiency of fertilizer	Reduced weed	Reduced irrigation cost/Energy saving	Crop	Location
Sapkal et al 2019	-	-	12%	-	-	-	Paddy	Haryana
Kanannavar et al 2016	63.68%	-	-	-	-	-	Groundnut	Karnataka
Shahani etal 2016	21%	-	-	-	-	-	Wheat and cotton	Sindh, Pakistan.
Ahuja et al 2016	32.18%	-	52.77%	-	27.81	-	Peal millet	Haryana
Aryal et al 2015	10–12%	-	7-9	-	-	-	Wheat	Punjab and Haryana
Kaur et al 2012	39%	-	4%, 42 %	-	-	44 %	Paddy, Sugarcane	Punjab
Jat et al 2011	50%	-	16.6%	Significantly improved	-	-	Wheat	Uttar Pradesh
Sidhu and Vatta 2010	36.19 cm	-	0.78 t/ha.	-	-	-	Paddy	Punjab
Bhatt and Sharma 2009	25-30%	-	-	-	-	-		Punjab
Jat et al 2006	25%	3-6 %	4%	-	-	25%	Paddy	Haryana
Rickman 2002	-	-	24%	-	up to 40	-		Punjab
Pal et al 2003	-	-	7.30 %, 6.10%	-	-	-	Paddy, Wheat	Uttar Pradesh

however a conclusive financial benefit observed from land leveling. The additional cost and benefits of precision land leveling over long period that the irrigation cost was reduced by 44 percent over the conventional practice. It has a great potential for optimizing the water-use efficiency in paddy cultivation without any disturbing and harmful effect on its productivity in long term. The incremental per hectare increase in gross margins of the technology adopters has been to the tune of 3244 Rs/ha (Ahuja et al 2016). The adoption of this technology has reduced irrigation cost by 720 Rs/ha which is about 44 percent over the conventional practice. Also, enhance the cultivable area by of 3-6 per cent this is due to a reduction in bunds and channels in the field. Undoubtably, laser land levelling technology is a proven resource conservation technology which reduces the overall cost of the production. The effect of laser land levelling toward the resource conservation has presented in the Table 2.

Benefits of laser land levelling: Laser land levelling is a recognized technology that has been developed in order to solve some of the problems faced by conventional levelling methods. It is more accurate, and it can be used on a wide variety of soil types, while conventional levelling can only be used on the most suitable soil types. The advantages of laser land levelling over conventional levelling techniques are that it improves crop establishment, reduces weed infestation, and improves uniformity of crop maturity. It also increases cropping intensity because it helps farmers to use their time more efficiently. Laser land levelling is an innovative technique that has been used in India for quite some time now (Suthakar et al 2008). It is a cost-effective and efficient way to prepare the land for irrigation purposes and can be done with less water than conventional levelling techniques. Conventional levelling techniques require a lot of water while laser land levelling needs less. It also increases the size of the field as it eliminates bunds. Some of the key benefits of the laser levelling over conventional levelling method shown in the Figure 3.

Challenges to adopting this technology: In order to understand the poor adoption of this technology several surveys conducted to identify the reasons behind it. Financial constraints and lack of knowledge about the technology are important limiting factors in the adaption of this technology. In other words, cash constraints at time of sowing are known to be a major limitation in adaption of technology. The main factors that are significantly associated with adoption of this machine are farm size and government connections. Also, there are very few subsidised programmes running by state government for promoting laser levelling technology. On the other hand, at least 2 skilled persons required to operate it in the field. Where skilled persons are not readily available in the agricultural field for operating such machineries. Besides, this technology requires more maintenance compare to other traditional levelling techniques and is less efficient in uneven and small sized fields. Most of the manufacturer and dealers are from Punjab and Haryana hence, states namely Madhya Pradesh, Uttar Pradesh, Bihar, Jharkhand and West Bengal have poor availability of dealership at block level (Pradeep et al 2014). Alternatively, adoption of this technology might be low if farmers are misinformed or unaware of benefits of laser land levelling. Studies about of technology adoption provide some evidence of breakdowns in flow of information in some regions where farmers complain that they are underserved by official information channels, such as visits from agricultural extension officers and technology demonstrations.

CONCLUSION

The laser land levelling is very promising technology because it has many advantages over the traditional ploughing methods, such as irrigation water saving, increased cultivation area, reducing the weed problems and increase the crop yield. This method is also more time efficient and requires less labour which makes it seem like an attractive to farmers. The effectiveness of laser land levelling technology is determined by its suitability for different types of crops, adoption of this technology promotes agricultural productivity, energy use efficiency, and water use sustainability. IGP having scope to use and test this technology for resources conservation, where conditions are very different on agroecological and social fronts. The laser level technique is costly for marginal and small farmers. The availability of institutional support and provision of technical knowledge to the farmers needs to be given more attention to the adoption of this technology.

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Monitoring of Landuse /Landcover Changes for the Sub-Basin of Godavari Basin

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Abstract: Land use/land cover mapping and change detection using remote sensing and geographic information system (GIS) technique helps in assessing the land surface information on course scale as well as finer scale. This paper attempts the assessment of land use / land cover changes for the Wainganga Sub-basin which is a part of the Godavari basin. The change detection was done for the last 34 years (1985-2019) and found significant changes during 2005-2019; before that, the changes were not very noticeable. In the last 34 years, the agricultural land was shrunk by 3.20%, while waste cum barren land reduced by 0.20% of the study area. The shrunk part of agricultural land converted into forest, built-up and urban, and water bodies. The built-up expanded and variation in that period was 106% with respect to the base period.

Keywords: Land use/land cover, Remote sensing, GIS, Classification, Change detection, Wainganga Sub-basin

Land use and land cover (LULC) is a significant component to understand the connections of human activities with the environment, therefore, it is necessary to simulate changes (Prakasam 2010). In developing countries, the increasing impact of climate change and LULC changes are likely to be severe due to poor adaptability. The LULC also plays a vital role in meteorology and hydrology, and this is a crucial factor in the environmental alteration process taking place over the globe these days *(*Agaton et al 2016). Historical and pinpoint details of LULC are a supporting tool for the sustainable development program; it provides a better understanding plus solutions to overcome social, economic and environmental problems. LULC and hydrology have a complex association, incorporating an extensive range of surface-vegetation-atmosphere interactions at varying spatial and temporal scales. Hydrological processes like evapotranspiration (ET), groundwater recharge, base flow, and overland flow, consider the LULC as a driver (Garg et al 2017). For watershed planning and management, profound knowledge of the study area, magnitude and the factors which affect the LULC of a watershed is required. Yirsaw et al (2017) observed LULC changes and employed many remote sensing, GIS tools and used imagery to detect and monitor the changes. NDVI was also used to identify land cover mainly determine the vegetation cover (Lunetta et al 2006). McGinn et al (2021) interlinked the changes in LULC with the water resources of Myanmar. Panandiker et al (2020) analyzed the LULC change for Sal watershed Goa and linked it with the future climate change projection to assess the future streamflow. There are two ways to obtain data for LULC classification viz. satellite or aerial images. Satellite imagery is the most popular data source to analyze the alteration in LULC and their quantification because of its higher temporal resolution, a compatible data format for computer processing, and a precise geo-coding system. Remote sensing offers an economical and time-saving way to assess the Earth's information on a large spatial and finer temporal scale. These remote sensing imageries can be accessed any time for the past and existing time (McGinn et al 2021). Chen et al (2003) used Improved Change-Vector Analysis for land cover detection and observed good potential in LULC monitoring over the other methods. Since, real-time studies on LULC changes using remote sensing and GIS have not been done extensively for Wainganga sub-basin (Fig. 1), the present study focuses on identifying the changes in LULC during the last three decades (1985-2019) over the sub-basin.

MATERIAL AND METHODS

The present study classified decadal LULC from 1985 to 2019 in the GIS environment (Fig. 2). For 1985, 1995 and 2005, open-source decadal LULC (Roy et al 2016) was used, while for the LULC map of 2019, Landsat8 satellite imageries were used. The decadal LULC map, as well as LULC of 2019, has a 30m x 30m spatial resolution. The Wainganga sub-basin covers the seven tiles of the above satellite imagery. LULC was classified into five major classes viz. built-up, agricultural land, waste cum barren land, forest and waterbody.

Image pre-processing: LULC change detection essentially requires the pre-processing of a satellite image (El-Kawyet al 2011). In the present study, layer stacking image processing operations were considered to merge all seven satellite imageries.

Supervised classification: The ENVI software was used to process the set of Landsat imageries. Training samples were designated for each of the predetermined LULC classes by demarcating ROI (region of interest) around characteristic sites. The spectral signatures for the respective land cover types recorded by the satellite images were derived by the pixels encircled by this ROI, a spectral signature is considered to be acceptable when misperception among the land covers to be mapped [is]minimal' (Gao and Liu 2010). When obtained spectral signature was acceptable and go for the classification process. In the present study, the supervised maximum likelihood method was used for the classification. Supervised classification generates a thematic raster layer (the classified image) and a distance file. Both the thematic layer and the distance file were used for post-classification thresholding.

Accuracy assessment: Accuracy assessment necessitates that a sufficient quantity of samples per map class be gathered when the classified outcomes are compared with actual ground conditions (Kafi et al 2014). It was obtained by dividing the total number of correctly classified pixels by the total number of reference pixels.

Change detection: The identification of differences in the state of an object or phenomenon by observing it at different times is known as Change detection (Saini et al 2019). It describes the changes in the same image of different times. This type of analysis is helpful to recognize various variations appearing in different classes of land use land cover i.e. increase in land-use area or decrease in land cover.

RESULTS AND DISCUSSION

Land use land cover change detection for Wainganga sub-basin: The five LULC classes were produced, including agricultural land, forest, built-up and urban, waste cum barren land, and waterbodies. Inside the Wainganga basin, major part of the area is used for agricultural activities followed by forest having highest physical appearance. To investigate the changes in the study area, LULC map of 1985, 1995 and 2005 was taken from earth data, while Landsat-8 imageries were used to prepare the LULC of 2019. The Accuracy assessment was done in the ENVI software and the report of LULC 2019 (Table 1). The spatial distribution of different classes over the earth surface in the particular year 1985, 1995, 2005 and 2019 are depicted in Figure 3 to 6.

Decadal Changes in LULC

Changes during 1985-1995: Decadal changes in land cover were detected for the decade 1985-1995 and agricultural land, built-up and Urban, and water bodies are increased in 1995 by 0.53, 0.03 and 0.14%, respectively, of the total study area, while land covered by Forest and Waste cum barren land was decreased (Table 2).

Changes during 1995-2005: Decadal changes in land cover were detected for the decade 1995-2005 and found the maximum changes in agricultural land and the forest area (Table 3). The highest portion of agricultural land was converted into forest land.

Changes during 2005-2019: During 2005-2019 significant changes were observed in the study area. The area covered by built-up and urban, forest and water bodies were

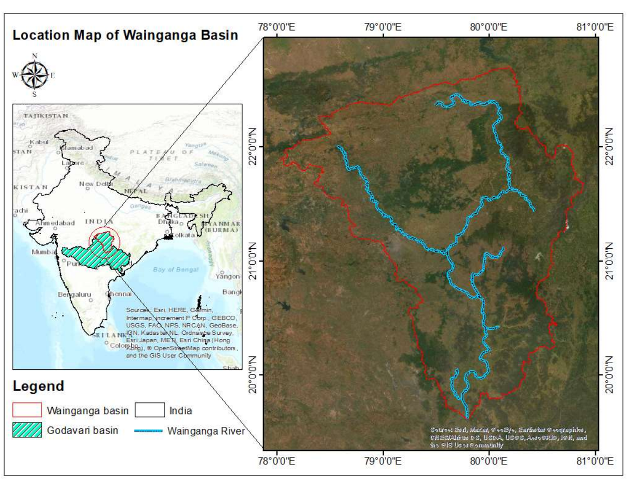

Fig. 1. Location of study area

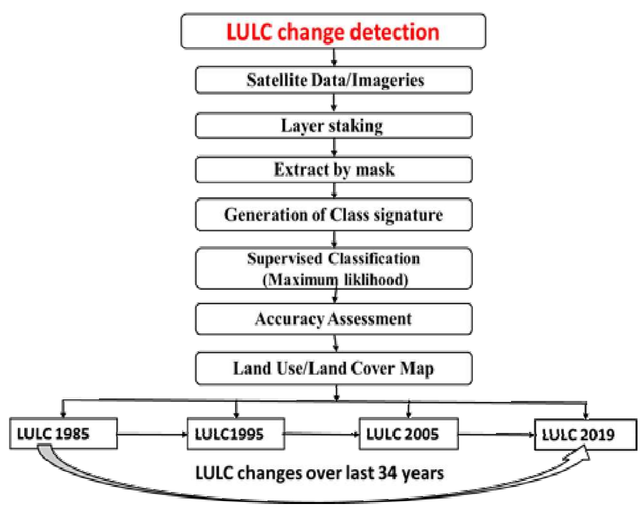

Fig. 2. Flowchart for the LULC change detection methodology

expanded while agricultural land and waste cum barren land were contracted. The agricultural area and waste cum barren land were shrunk by 3.53 and 0.14% of the total area of the basin and in that area, Forest, Water bodies, and built-up and Urban expanded by 2.32, 0.7 and 0.65 % of the total basin area (Table 4).

Variation in LULC during the last 34 years (1985-2019) : The changes in the physical appearance of land that comes under the Wainganga sub-basin in the last three decades (1985-2019), shows the increase in built-up and Urban,

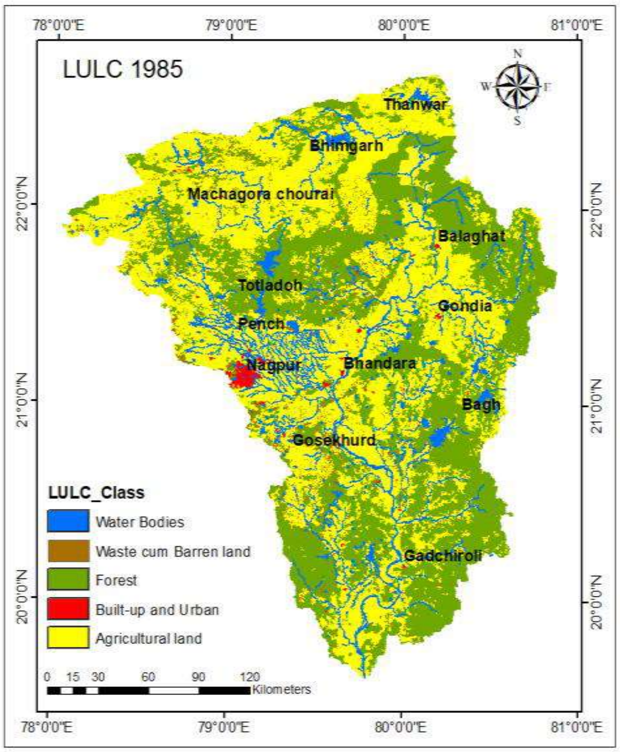

Fig. 3. LULC of the year 1985

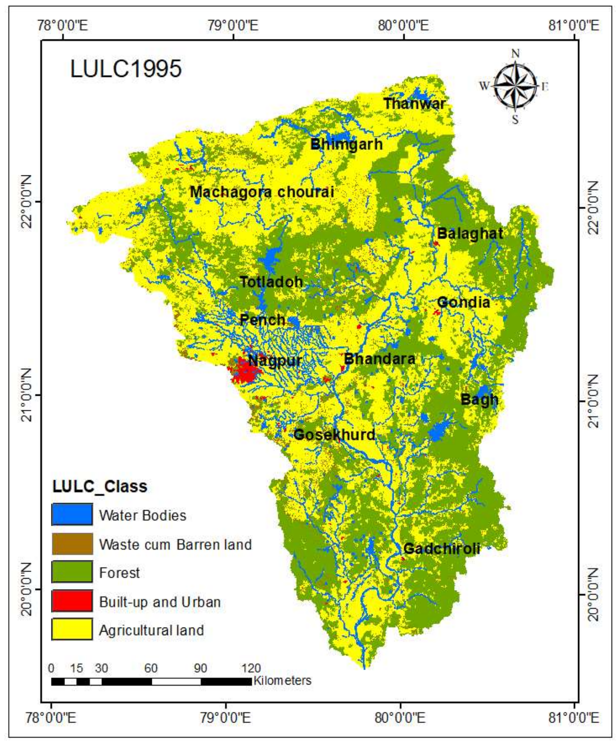

Fig. 4. LULC of the year 1995

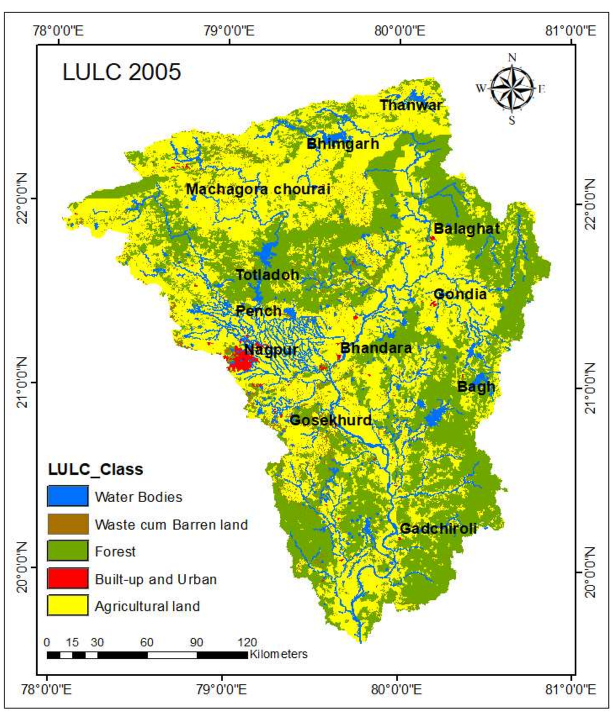

Fig. 5. LULC of year 2005

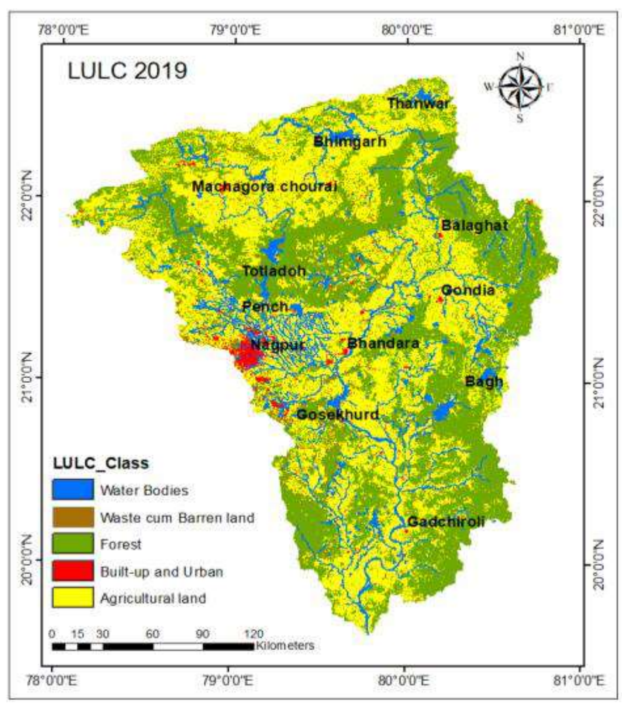

Fig. 6. LULC of the year 2019

Table 1. Accuracy assessment report of LULC 2019

Class	Reference totals	Classified totals	Number correct	Producers accuracy	Users accuracy	Kappa
Agricultural land	58	46	46	79.31%	100.00%	1
Built-up and Urban	4	6	4	100.00%	66.67%	0.66
Forest	54	66	54	100.00%	81.82%	0.67
Waste cum Barren land	2	2	2	100.00%	100.00%	1
Water bodies	4	2	2	50.00%	100.00%	1
	122	122	108	88.52%	89.70%	

Overall Classification Accuracy = 88.52%, Overall Kappa Statistics = 0.81

Table 2. Statistics of LULC changes during 1985-1995

LULC class	Area covered (%)		Changes during 1985-1995	Remark
	1985	1995		
Agricultural land	51.94	52.47	0.53	Increased
Built-up and Urban	0.65	0.68	0.03	Increased
Forest	41.54	40.88	-0.66	Decreased
Waste cum Barren land	3.25	3.21	-0.04	Decreased
Water bodies	2.62	2.76	0.14	Increased

Table 3. LULC changes during 1995-2005

LULC class	Area covered (%)		Changes during 1995-2005	Remark
	1995	2005		
Agricultural land	52.47	52.27	-0.2	Decreased
Built-up and urban	0.68	0.69	0.01	Increased
Forest	40.88	41.04	0.16	Increased
Waste cum barren land	3.21	3.19	-0.02	Decreased
Water bodies	2.76	2.81	0.05	Increased

Table 4. LULC changes during 2005-2019

LULC class	Area covered (%)		Changes during 2005-2019	Remark
	2005	2019		
Agricultural land	52.27	48.74	-3.53	Decreased
Built-up and urban	0.69	1.34	0.65	Increased
Forest	41.04	43.36	2.32	Increased
Waste cum barren land	3.19	3.05	-0.14	Decreased
Water bodies	2.81	3.51	0.70	Increased

Table 5. LULC changes during 1985-2019

LULC class	Area covered (%)		Changes during 1985-2019	Remark
	1985	2019		
Agricultural land	51.94	48.74	-3.20	Decreased
Built-up and urban	0.65	1.34	0.69	Increased
Forest	41.54	43.36	1.82	Increased
Waste cum barren land	3.25	3.05	-0.20	Decreased
Water bodies	2.62	3.51	0.89	Increased

forest and water bodies and shrinkage of agricultural land and waste cum barren land (Table 5). Inside the study area, 3.2% of the agricultural land has been converted into other classes in the last 34 years and the major area was converted into the forest. There was a minute change in Waste cum Barren class, which is only 0.2%. The built-up and urban area expanded by 0.69% of the total area. In 1985, 0.65% of the study area was covered by Built-up and urban areas, while in 2019 was 1.34%. Similarly, water bodies were increased from 2.63 to 3.51% in the last three decades.

From the statistics of decadal LULC, observed that the significant land cover changes occurred in the last decade. The built-up and urban water bodies have increased significantly in the previous decade while agricultural land has converted into forest cover.

CONCLUSION

LULC change analysis indicated significant changes during 2005-2019, before that the changes were not very noticeable. In the last 34 years, the agricultural land was shrunk by 3.20%, while waste cum barren land reduced by 0.20% of the study area. The shrunk part of agricultural land converted into the forest, built-up and urban, and water bodies. The built-up expanded and variation in that period was 106% of the base period. The water bodies also grew. Quantified the changes in land use pattern using remote sensing and GIS techniques- Decadal Land use land cover change detection found the major changes in the agricultural land, which decreased by 3.20% of the total area in the last 34 years.

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Impact Assessment of Heavy Metals Accumulation in Surface Water Bodies in the Adjoining Forest of Shoghi-Shimla-Dhali Bypass of Himachal Pradesh

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Abstract: A study was conducted to investigate the impact of seasonal variation in heavy metals concentration of surface water (streams) sampled by collecting 72 samples during pre-monsoon, monsoon and post-monsoon from four study sites. The heavy metals like Cu, Cd, Cr, Pb, and Ni and As, Hg were estimated using inductively coupled plasma emission spectrometer (ICAP-6300 Duo). The concentrations of all heavy metals were within permissible limit except Hg which was not detected as given by BIS and CPCB. The mean value for heavy metals were Cd (0.011,0.013,0.006 mg/l), Cr (0.037,0.039,0.024 mg/l), Pb (0.022,0.024,0.012 mg/l), Cu (0.035,0.043,0.0024 mg/l), Ni (0.022,0.026,0.017 mg/l) and (0.037, 0.041, 0.028 mg/l) recorded during pre-monsoon, monsoon and post-monsoon. Seasonal variations have significant effect on heavy metal concentration of surface water samples. Road construction material like dirt and debris added heavy metals into adjacent water bodies ultimately degraded the water quality in terms of physico-chemical and biological characteristics.

Keywords: Heavy metal, Road construction, National highways, Seasonal variation

Water is an essential resource for the survival of living organism. The change in composition of water which is available at optimum level for suitable growth of biotic components is referred as seasonal variation in quality of water (Raji et al 2016). The metallic elements with density greater than 5g/m^3 and high atomic weight are termed as heavy metals. These metallic elements generally occur in traces as these are toxic in nature and tend to accumulate hence are commonly called as trace metals. Their presence in surface water at above permissible limit is undesirable. Heavy metals affected biological system even at very low concentration. The particulate and sediment has been adsorbed by heavy metals and not available to biological organisms (Qian et al 2015). Heavy metal contamination in the sediment is a significant problem because of their toxicity, non-degradability and easy bioaccumulation potential in biota and food chain (Sundaray 2014). The heavy metals are inert in the sediment environment and often considered as conservative pollutant and can be released into the water in respect to certain disturbances and pose potential risk (Kumar and Maiti 2015, Zhang et al 2015). The various chemical parameters in natural aquatic system occurred in low concentration and this concentration increases as a result of rapid development and exploitation of natural resources. Road construction activities were considered as a major source of pollutants to aquatic bodies as these pollutants generally originated from soil erosion, diesel oil, construction debris and dirt and added to adjacent water bodies directly or indirectly, leading to physical, chemical and biological degradation of water quality (Abewickrema et al 2013). Water quality has been degraded due to pollutants which are produced during road construction as highway runoff accumulate tremendous amount of suspended solids, metals and hydrocarbons such as oil and polycyclic aromatic hydrocarbons (Mooselu et al 2021). A various organic and inorganic pollutants has been obtained from bitumen which is generally obtained from petroleum, vehicle exhaust, lubricating oil and gasoline leached out in nearby water bodies and affect their habitat (Banerjee et al 2018). Surface water bodies have also been affected by crushed ash. Runoff water more contaminated by pollutants which are emitted by vehicles. The leaching of pollutants in water bodies generally depend upon the pH of environment (Licbinsky et al 2012). The present study characterized the effect of road expansion on the quality of surface water, through the use of techniques such as collection of water sample at different sites along the highway and chemical analysis of heavy metals.

MATERIAL AND METHODS

Study area: A 51 m wide and 27 km long stretch of NH-22 (Shoghi- Shimla- Dhali) bypass come across the Mashobra division of district Shimla, located in the land of snowy mountains, Himachal Pradesh and is extended from latitude 31° 05' 10" - 32° 10' 50" in North to longitude 76° 57' 05" - 70° 07' 45" in East in the Western Himalaya (Table 1). The study area was divided into four sites (Fig. 1). The four sites S_1 (Shunghal), S_2 (Raghanv), S_3 (Majjhar) and S_4 (Dhali) were selected in order to study the effect of highway expansion activity on heavy metal concentration in surface water.

Sample collection: In the year 2018 and 2019, the surface water (streams) samples were collected during pre-monsoon (April and May), monsoon (June, to September) and the post-monsoon (October and November) seasons from each site based upon weather report and laboratory workload. In total there were 12 treatment combinations (4×3) which were replicated three times in randomized block design.

Sample preparation: Samples were collected in 2 litters' plastic bottles. Collection was carried out by careful immersion of sample bottles deep inside the water and sealing with tight fitting corks after collection in order to avoid air bubbles and labelled with the appropriate sample site no. Samples were transferred the refrigerator at 4°C prior to analysis.

Sampling method and analysis: For heavy metal (Cd, Cr, Pb, Cu, As, Ni and Hg) analysis, the samples were preserved at temperature below 4°C but slightly above the freezing point, by adding one ml concentrated nitric acid to avoid microbial utilization. The samples were analysed as per the procedures by APHA (2012). The water samples were first filtered using whatman filter paper (No. 1). The heavy metals were estimated using Inductively Coupled Plasma Emission Spectrometer (ICAP-6300 Duo) and expressed as mg/l.

Interpretation of water quality: The water quality parameters of surface water (streams) studied at different sites of the study area was compared with water standards prescribed by Bureau to Indian Standard (BIS, 2015) and Central Pollution Control Board (CPCB) and was used to discuss the results on effects of highway expansion on water quality.

Statistical analysis: The data recorded were statistically analysed by using OPSTAT.

RESULTS AND DISCUSSION

Spatial and Seasonal Variation in Heavy Metals concentration in Surface Water

Cadmium : The cadmium in surface water bodies lies in between 0.004 to 0.015 mg/l which was within the permissible limits as mentioned by BIS (Table 2). The pooled effect showed a significant seasonal variation with respect to chromium. The average maximum cadmium (0.013 mg/l) concentration was in monsoon, followed by pre monsoon (0.011 mg/l) while the lowest (0.006 mg/l) in post-monsoon season. It might be due to the movement of waste leachates with the runoff enhanced the concentration of cadmium in monsoon. Dan et al (2014) also reported higher cadmium concentration in water in monsoon season. Wearing of old tyres and leakage of lubrication oil was considered as the major source of cadmium (Doamekpor et al 2016).

Chromium : The chromium concentration lies in between the range from 0.022 to 0.041 mg/l in water sources located along the national highway which was within permissible limits of 0.05 mg/l as mentioned by BIS (Table 3). The pooled effect of both the years on distribution of chromium in water bodies followed a decreasing pattern from monsoon > pre-monsoon> post monsoon (0.039, 0.037 and 0.024 mg/l). The chromium concentration enhanced in the surface water bodies in monsoon might be due to weathering of soil and rocks found in earth crust (Akpe et al 2018). The major source of cadmium was brakes and tyres wear (Xue et al 2020). The

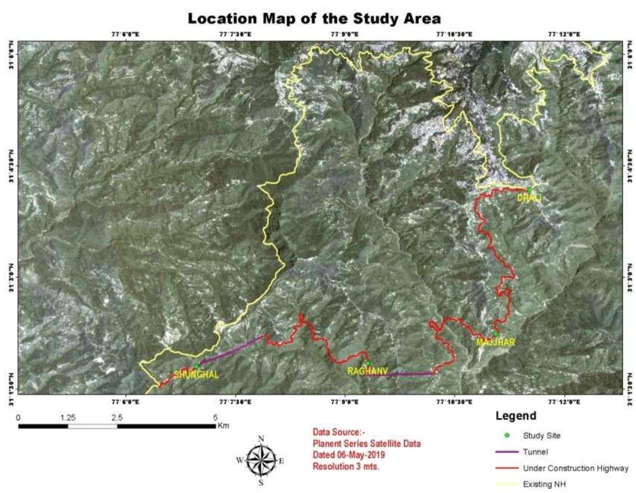

Fig. 1. Location map of the study area

Table 1. Global positioning system of study area

Parameters	Characteristics
Elevation	1493 – 2250 m
Slope range	100 - 300 m (Solan); 300 - 400 m (Shimla)
Soil	Sandy loamy (Solan); Alluvial (Shimla)
Minerals	Sand, Stone in (Solan); Lead, Zinc Dolomites (Shimla)
Temperature	14 -28C (Summer); 1.7 to 14.8C (Winter)
Rainfall	1450 mm in Solan;1253 mm in Shimla
Relative humidity	33 to 91 %
Land use pattern	Open, Barren, Cultural Waste Land
Wind speed	1.7 to 3.8 km/h
Vegetation	Tropical dry deciduous forest and Himalayan Chir pine forests
Global positioning system	31° 05' 10" - 32° 10' 50" N 76° 57' 05" - 70° 07' 45"E

results are in collaboration with the findings of Polkowska et al (2007) where higher concentration of heavy metals was in monsoon seasons.

Lead: The lead concentration range from 0.012 to 0.026 mg/l in water sources along the national highway which was within permissible limits as mentioned by BIS (Table 4). The average highest (0.024 mg/l) of lead content was in monsoon while the lowest (0.012 mg/l) concentration of lead was in post- monsoon. The influence of three way interaction year x season x site was significant. The highest (0.028 mg/ l) lead content was d at Dhali in the monsoon during 2019 while the lowest (0.008 mg/l) concentration of lead was at Raghanv in the post- monsoon during 2018.Generally it is present in low concentration in natural bodies due to its less solubility in water, ultimately it become toxic and difficult to eliminate and affect aquatic environment because of bottom feeding habit of aquatic organisms (Muhammad et al 2014). The increase in monsoon due to dry and wet deposition (Zhang et al 2007) of atmospheric fallouts from traffic emissions and from vehicular exhaust (Valavanidis and Vlachogianni 2010). It aligns with earlier work where traces of heavy metals were detected in water bodies in monsoon (Raji et al 2016). Combustion of leaded gasoline was considered as the major source since it was the most distinctive heavy metal from road traffic pollution (Xue et al 2020).

Copper: The concentration of copper in surface water bodies ranged from 0.023 to 0.046 mg/l which was within the permissible limits (Table 5). The maximum (0.043 mg/l) value of copper content was in monsoon while the lowest (0.024 mg/l) in post-monsoon. Higher concentration in monsoons might be due to weathering of rocks and soil. Akpe et al (2018) also observed higher copper concentration in water in monsoon season. The variation in copper concentration at different water sources in study area showed the maximum copper in surface water bodies at Dhali (0.036 mg/l) while lowest was at Majjhar and Raghanv (0.032 mg/l). The highest

Table 2. Spatial and seasonal variations in cadmium concentration (Cd-mg/l) of surface water at different sites

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.013	0.011	0.004	0.009	0.009	0.012	0.004	0.009	0.011	0.012	0.004	0.009
Raghanv	0.009	0.012	0.006	0.009	0.013	0.013	0.006	0.011	0.011	0.013	0.006	0.010
Majjhar	0.012	0.014	0.008	0.011	0.013	0.012	0.005	0.010	0.012	0.013	0.007	0.011
Dhali	0.009	0.016	0.007	0.011	0.008	0.014	0.007	0.010	0.009	0.015	0.007	0.010
Mean	0.011	0.013	0.006	0.010	0.011	0.013	0.005	0.010	0.011	0.013	0.006	0.010
CD (p=0.05)		Sites		NS		Sites		NS		Sites		NS
		Season		0.002		Season		0.002		Season		0.001
		Site x season		NS		Site x season		NS		Site x season		0.003
		-		-		-		-		Site x season x year		NS

Desirable Limit (mg/l) : 0.01

Table 3. Spatial and Seasonal variations in Chromium concentration (Cr-mg/l) of surface water at different site

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.039	0.041	0.024	0.034	0.036	0.038	0.020	0.031	0.037	0.040	0.022	0.033
Raghanv	0.04	0.039	0.027	0.035	0.032	0.037	0.024	0.031	0.036	0.038	0.025	0.033
Majjhar	0.036	0.042	0.029	0.036	0.033	0.039	0.023	0.032	0.035	0.041	0.026	0.034
Dhali	0.039	0.041	0.020	0.034	0.039	0.037	0.025	0.034	0.039	0.039	0.023	0.034
Mean	0.039	0.041	0.025	0.035	0.035	0.038	0.023	0.032	0.037	0.039	0.024	0.033
CD (p=0.05)		Sites		NS		Sites		NS		Sites		NS
		Season		0.002		Season		0.002		Season		0.001
		Site x season		NS		Site x season		NS		Site x season		NS
		-		-		-		-		Site x season x year		NS

Desirable Limit (mg/l) : 0.05

copper was at Dhali (0.046 mg/l) in monsoon while the lowest (0.023 mg/l) was at Shunghal and Majjhar in the post-monsoon season. The combined effect of seasons x sites x year was significant and maximum was in monsoon at Dhali (0.049 mg/l) during 2019 while the lowest (0.021 mg/ l) copper was noticed in post-monsoon season at Shunghal in 2019 and at Majjhar in 2018. The possible source of copper in surface water might be metal plating, wear of bearing and brake parts (Budai and Clement 2011).

Nickel: The nickel concentration in surface water bodies in the study area varied from 0.014 to 0.028 mg/l (Table 6) which was within the permissible limit of nickel in water is as prescribed by BIS. The pooled effect of both the years indicated that average highest nickel (0.026 mg/l) was in monsoon while lowest (0.017 mg/l) concentration of nickel in post-monsoon. It might be due to strong scouring effect which is called first flush effect (Ghosh and Maiti 2018) in monsoon whereas in pre-monsoon small particles were washed away by the light rainfall. Among different sites highest concentration of nickel (0.023 mg/l) was in Dhali while lowest in Majjhar and Raghanv(0.020 mg/l). The interaction among season and sites was significant and indicated highest concentration in monsoon at Shughal (0.028 mg/l) while the lowest) concentration was in post-monsoon season at Raghanv(0.014 mg/l. The combined effect of year x season x site was found to be significant and revealed that the highest (0.030 mg/l) nickel was at Dhali and Shunghal in the monsoon of the year 2019. However, lowest (0.011 mg/l) concentration was at Raghanv in 2019 and in 2018 at Majjhar during post-monsoon season. The presence of higher nickel concentration may be due to natural as well as anthropogenic causes. Nickel comes from mainly gasoline, lubricating oil, bearing wear and break part wear (Xue et al 2020).

Arsenic: The concentration of arsenic in surface water bodies ranged from 0.026 to 0.041mg/l which was within the

Table 4. Spatial and seasonal variations in lead concentration (Pb-mg/l) of surface water at different site

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.020	0.024	0.011	0.018	0.024	0.025	0.012	0.021	0.021	0.024	0.012	0.019
Raghanv	0.021	0.023	0.008	0.017	0.022	0.026	0.016	0.021	0.022	0.025	0.012	0.019
Majjhar	0.023	0.021	0.011	0.018	0.025	0.026	0.014	0.022	0.024	0.023	0.013	0.020
Dhali	0.019	0.024	0.013	0.019	0.025	0.028	0.012	0.022	0.022	0.026	0.013	0.020
Mean	0.021	0.023	0.011	0.018	0.024	0.026	0.014	0.022	0.022	0.024	0.012	0.020
CD (p=0.05)		Sites		NS		Sites		NS		Sites		NS
		Season		0.002		Season		0.002		Season		0.001
		Site x season		0.003		Site x season		NS		Site x season		NS
		-		-		-		-		Site x season x year		0.004

Desirable Limit (mg/l) : 0.05

Table 5. Spatial and seasonal variations in copper concentration (Cu-mg/l) of surface water at different site

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.038	0.034	0.026	0.033	0.039	0.044	0.021	0.034	0.039	0.039	0.023	0.034
Raghanv	0.033	0.041	0.023	0.033	0.033	0.046	0.029	0.036	0.033	0.044	0.026	0.032
Majjhar	0.029	0.047	0.021	0.032	0.031	0.038	0.024	0.031	0.030	0.042	0.023	0.032
Dhali	0.031	0.042	0.025	0.033	0.041	0.049	0.026	0.038	0.036	0.046	0.025	0.036
Mean	0.033	0.041	0.024	0.033	0.036	0.044	0.025	0.035	0.035	0.043	0.024	0.034
CD (p=0.05)		Sites		NS		Sites		0.003		Sites		0.002
		Season		0.002		Season		0.002		Season		0.002
		Site x season		0.004		Site x season		0.005		Site x season		0.003
		-		-		-		-		Site x season x year		0.004

Desirable Limit (mg/l) : 0.01

permissible limits as prescribed by BIS (Table 7). The data with respect to pooled effect of both the years indicated that seasonally average highest concentration of arsenic (0.041 mg/l) was in monsoon which was statistically at par with (0.037 mg/l) in pre-monsoon while the lowest (0.028 mg/l) arsenic was in post-monsoon season. Higher concentration in monsoons may probably be due to transportation of waste leachates with the runoff of rainwater as also reported by Li and Zhang (2010.) The major source of arsenic in surface water comprises road runoff due to tyre wear and corrosion of radiators (Dixit and Tiwari 2008).

The monsoon season showed the highest concentration for all the heavy metals as compared to other two seasons. In pre-monsoon season, rainwater had less scouring power on particles and the contact time between rain water and pavement sediments was longer before runoff formation. It means that soluble pollutants could dissolve into runoff water in the early stage and non -soluble pollutants were brought into runoff under scouring effect of rain water having high intensity (Wang et al 2017). Concentration of heavy metals in water samples was greater in pre-monsoon than post-monsoon due to lessor inflow of water which decrease the water column to dilute heavy metals during pre-monsoon period (Karunanidhi et al 2022). Spatial variation in heavy metals concentration among all sites might be due to combination of certain factors like rainfall precipitation intensity, duration, vehicular emission and antecedent sunny days (Xue et al 2020). In pooled data, the interaction among sites x season showed non-significant effect for heavy metal Cr, Pb, As whereas Cd, Cu, Ni showed significant effect. The interaction among sites x season x year showed non-significant effect for Cd, Cr, Pb, Cu and Ni showed significant variation. The positive skewness value point towards heavy metals distribution with along right tail was shown by Pb and

Table 6. Spatial and seasonal variations in nickel concentration (Ni-mg/l) of surface water at different site

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.018	0.026	0.013	0.019	0.022	0.030	0.020	0.024	0.020	0.028	0.016	0.021
Raghanv	0.021	0.023	0.018	0.021	0.019	0.029	0.011	0.020	0.020	0.026	0.014	0.020
Majjhar	0.019	0.025	0.011	0.018	0.026	0.022	0.019	0.022	0.023	0.024	0.015	0.020
Dhali	0.021	0.021	0.016	0.020	0.026	0.030	0.025	0.027	0.024	0.026	0.021	0.023
Mean	0.020	0.024	0.014	0.020	0.023	0.028	0.019	0.023	0.022	0.026	0.017	0.021
CD (p=0.05)	Sites			NS	Sites			0.003	Sites			0.002
	Season			0.002	Season			0.002	Season			0.002
	Site x season			0.004	Site x season			0.005	Site x season			0.003
	-			-	-			-	Site x season x year			0.004

Desirable Limit (mg/l) : 0.02

Table 7. Spatial and seasonal variations in arsenic concentration (mg/l) of surface water at different sites

Season/ Site	2018				2019				Pooled			
	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean	Pre-monsoon	Monsoon	Post-monsoon	Mean
Shunghal	0.037	0.039	0.029	0.035	0.038	0.044	0.031	0.038	0.038	0.041	0.030	0.036
Raghanv	0.035	0.041	0.024	0.033	0.040	0.041	0.035	0.039	0.038	0.041	0.029	0.036
Majjhar	0.034	0.037	0.021	0.031	0.039	0.042	0.033	0.038	0.036	0.040	0.027	0.034
Dhali	0.036	0.042	0.025	0.034	0.038	0.045	0.028	0.037	0.037	0.040	0.026	0.036
Mean	0.035	0.040	0.025	0.033	0.039	0.043	0.032	0.038	0.037	0.041	0.028	0.036
CD (p=0.05)	Sites			0.003	Sites			NS	Sites			NS
	Season			0.003	Season			0.002	Season			0.002
	Site x season			NS	Site x season			NS	Site x season			NS
	-			-	-			-	Site x season x year			NS

Desirable Limit (mg/l) : 0.02

Table 8. Distribution of heavy metals in study area

Heavy metals Site	Skewness						Kurtosis					
	Cd	Cr	Pb	Cu	Ni	As	Cd	Cr	Pb	Cu	Ni	As
Shunghal	-0.534568	-0.911979	-0.700254	-0.53307	0.0627613	-0.128919	-1.98253	-1.35803	-1.98026	-1.00664	-0.27258	-0.73721
Raghanv	-0.326751	-0.44806	-1.262313	0.2359016	-0.099053	-1.538635	-2.43484	-1.84181	1.418022	-0.51749	1.158431	2.411223
Majjhar	-1.074734	-0.518567	-0.749483	0.7510213	-0.993199	-1.382434	-0.08048	-0.42045	-1.36798	-0.05982	1.092576	2.464304
Dhali	0.9303202	-1.01693	-0.273994	0.1804542	-0.10472	-0.367416	-1.25371	-1.06453	-2.00553	-1.89909	-0.13036	-1.43951

As in all sites whereas Cr showed positive value at Shunghal and Majjhar. The Cd and Ni showed distribution with a long right tail at Dhali and Shunghal. Inspite of these, Cd and Ni showed negative value of skewness which gives distribution with a significant long left tail at sites Shunghal, Raghanv and Majjhar and Raghanv, Majjhar and Dhali respectively. The Cr had distribution with significant long left tail at Raghanv and Dhali, whereas at Shunghal Cu showed distribution with a significant long left tail. The negative value of Kurtosis observed for heavy metals (Cd, Cr, Cu) at all sites where as Pb and Ni, As were observed with negative value of kurtosis at sites Shunghal, Majjhar, Dhali and Shunghal and Dhali respectively showed flat distribution around study area. The positive value of Kurtosis for heavy metals (Cu and Pb) at site Raghanv showed non-uniform distribution throughout the study area. Apart from these, Ni and As were observed with positive value at site Raghanv and Majjhar showed non–uniform distribution around the respective study areas (Table 8).

CONCLUSION

The heavy metal concentration lies within the permissible limit and does not affect water quality beyond permissible limit. The concentration of heavy metals in surface water samples vary depending upon sediment, pollutant and exhaust gases during construction of roads. Further, the quality of water needs to be monitored regularly in wake of detrimental impacts caused by national highway expansion activities.

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Relative Abundance of Cyanobacteria in Soil of Khasi Hills, Meghalaya and Determination of Antioxidant Potential of some Dominant Species

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Abstract: The relative abundance and antioxidant potential of some dominant cyanobacteria in the selected landuse type of Khasi Hills, Meghalaya were evaluated. A total of 105 species were isolated. Species of the genus belonging to *Oscillatoria* were with relative abundance of 41 to 75%. However, species occurring throughout the year with high relative abundance were *Synechococcus aeruginosus* (77-82%), followed *Oscillatoria limosa* (75%) and *Anabaena spiroides* (72%). The genus of *Oscillatoria* (*O. curviceps* and *O. limosa*) were with high phenolic (77.05 and 73.15µgGAE/ml respectively) and flavonoid contents (65.10 and 47.33µgGE/ml respectively). These can also scavenge DPPH radical and superoxide anion more efficiently than the other species. *Oscillatoria* species which was observed with high relative abundance in this part of the region could be used as a good source of antioxidant.

Keywords: Relative abundance, Antioxidant, Cyanobacteria, *Oscillatoria*, Khasi hills

Soil ecosystems formed the most important non-aqueous habitat for the terrestrial algae. Tropical India provides a favourable environment for the growth of cyanobacteria (Chellappa et al 2004, Geethu and Shamina 2021). Cyanobacteria are the oldest phototrophic microorganisms and play an important role as primary producers and pioneer organisms in soil crust (Budel et al 2016) and improve carbon and nitrogen contents, aggregation and stability of soil and also secretes the exopolysaccharides (Rossi et al 2018). The search for natural antioxidant has gained much interest due to the toxicity of synthetic antioxidant (Andrade et al 2019). Several studies have demonstrated that the amount of carotenoids contributed to the antioxidant capacity of microalgae (Takaichi 2011). Cyanobacteria are known to be a source of bioactive substances with many potential antioxidant compounds (Singh et al 2017, Morone et al 2019). Antioxidants are effective because they can donate their own electrons to Reactive Oxygen Species (ROS) and thereby neutralizing its adverse effects (Kohen and Nyska 2002). Thus the study was conducted in order to isolate the cyanobacteria with high relative abundance which can then be used as antioxidants.

MATERIAL AND METHODS

Collection of samples: Soil was collected from lands under shifting cultivation (1st year, 2nd year and 3rd year), agricultural farmlands (potato/rice field, potato/maize field), forests and plantation (Mawphlang Sacred Grove, pine forest and citrus plantation) from various locations of Khasi hills, Meghalaya. Surface soil and soil near the roots regions were collected randomly from 15 different parts of collection spots (10 m^2) with each collection spot approximately 10 m apart from each other. Soil samples from each collection spots were mixed together to form a composite mixture for the study of cyanobacteria community structure in selected land use type.

Preparation of samples: About 10 g of each soil sample was placed in a flask and diluted 100-fold with distilled water. BG 11 culture media was used in liquid or solidified form with 1.5% agar were used for culture and isolation. All the isolated cyanobacteria were observed under trinocular microscope (Olympus BX41) and identified to the possible lower taxonomic level by consulting standard books and monographs (Desikachary 1959, Philipose 1967, Prescott 1982, John et al 2002). Classification of cyanobacteria was carried out in accordance with Komárek et al (2014) and Guiry and Guiry (2020). The dominant cyanobacteria were grown in controlled condition of temperature at 25±1°C in light intensity of 40 µmol photons m^{-2} s^{-1} for 30 days. Culture experiments were conducted under a regime of 16 hour light/8 hour dark.

Data analysis: Relative abundance of a species was calculated (Dey et al 2010),

Relative abundance = Y/X 100

Where, X = total number of samples collected, Y = number of samples from which soil cyanobacteria was isolated

Preparation of cyanobacteria extract: 1g of air dried material was extracted at room temperature using methanol. It was then filtered through a filter paper (Whatman 1) and evaporated to dryness. The extract was then dissolved in methanol and store in the refrigerator at 20°C.

Analysis of antioxidant compounds and activity: The total phenolic content was determined by following Slinkard and Singleton (1977) using Folin & Ciocalteau reagent and gallic acid as standard whereas total flavonoid was determined by spectrophotometric method given by Quettier-Delue et al (2000). DPPH radical scavenging activity was measured by method described by Brand-Williams et al (1995). The superoxide anion radical scavenging activity was measured following the methods given by Nishikimi et al (1972). The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

RESULTS AND DISCUSSIONS

Distribution and relative abundance of cyanobacteria: Altogether 105 species belonging to 5 orders were observed in soils of different landuse type. Oscillatoriales (38%) were recorded to be dominant order followed by Nostocales, Synechococcales, Chroococcales and Spirulinales (Fig. 1).

Relative abundance was higher in agricultural farmlands followed by forests and plantation and lower in shifting cultivated land. *Synechococcus aeruginosus* was observed in soil of all landuse except in sacred grove. The highest relative abundance was in pine forest (82%) followed by citrus plantation.This could be attributed to the ability of this genus to tolerate stress (Stuart et al 2009). Some species observed with high abundance were *Anabaena spiroides* (72%) followed by *An. variabilis*,*An. oscillaroides* and *An. spiroides*. Some *Scytonema* species such as *Sc. hyalinum*, *Sc. geitleri*and *Sc. schmidtii* were observed in the form of crust (Table 1). Species of *Nostoc*, *Anabaena*, *Cylindrospermum* and *Scytonema* are widespread in Indian soil and were known to contribute abundantly to soil fertility (Nayak and Prasanna, 2007). *Aphanothece castagnei* and *Gloeothece rhodochlamys* were both observed in soils of potato/rice field with a higher abundance (66.6%). Species of *Pseudoanabaena batrachospermum* (52%) were observed in soil of potato/rice field. Species of *Oscillatoria* observed during rice cultivation in potato/rice field were *O. limosa* (75%) followed by *O. curviceps* (65%), *O. prombosidea* (45%), *O. princeps* (42%), *O. agardhii* (41%) (Table 1). Vijayan and Ray (2015) observed the dominance of Oscillatoriales revealed the ecological status of agroecosystem as a result of agricultural practices.

Antioxidant compounds and potential of dominant species: *O. curviceps* has the maximum amount of phenolic (77.05 µgGAE/ml) and flavonoid content (65.10 µgGE/ml). *Gloeothece rhodochlamys* was with lowest amount of phenolic (9.43 µgGE/ml). Kharkongor and Ramanujam (2017) recorded highest amount of phenolic compound could be the reason for a speciesto exhibit the highest DPPH radical and superoxide anion radical scavenging activity. It is already known that the smaller DPPH and Superoxide anion radical scavenging activity at % inhibition IC_{50}, the better is the ability of an algal species to scavenge these radical and anions. Thus *O. curviceps* (156.23 µg/ml) could scavenge DPPH radical more efficiently than *Aphanothece castagnei* (786.89µg/ml). The same could also be accounted for superoxide anion scavenging activity in which *O. curviceps (*213.54µg/ml)can scavenge superoxide anion more efficiently than *Synechococcus aeruginosus* (889.14µg/ml). The antioxidant properties of some selected cyanobacteriain which DPPH radical scavenging activity was highest in *Oscillatoria* sp that could be used as excellent source of anti-oxidant. Guerreiro et al (2020) observed that cyanobacteria from paddy fields have high levels of carotene. Therefore, antioxidant activity could possibly be attributed to the presence of high amount of carotenoids in *Oscillatoria* sp. Martinez and Barbosa (2008) concluded that carotenoids could quench radicals by hydrogen atom transfer or by accepting electrons from radicals. Kharkongor and Ramanujam (2017) observed highest amount of phenolic compound could be the reason for a species to exhibited highest DPPH radical scavenging activity and superoxide anion radical scavenging activity (Table 2).

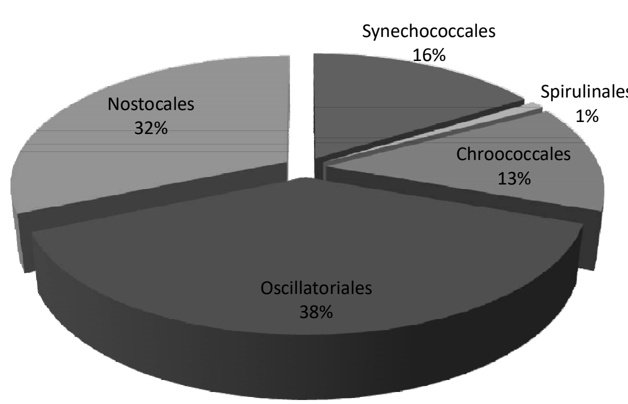

Fig. 1. Distributional pattern of different orders of Cyanobacteria

Table 1. Relative abundance and distribution of Cyanobacteria in soil of Khasi hills, Meghalaya

Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
Phylum - Cyanobacteria Class - Cyanophyceae								
Order - Synechococcales								
Aphanocapsa elachista West & G.S. West	-	-	+ *	-	-	+ *	-	-
A. incerta (Lemmermann) G. Cronberg & Komárek	-	-	-	+ *	-	-	-	-
Leptolyngbya battersii Gomont	-	-	-	-	+ *	-	-	-
L.faveolarum (Gomont) Anagnostidis & Komárek	-	-	+	-	-	-	-	-
L. vincentii Komárek	-	-	-	+ *	-	-	-	-
Merismopedia sp	-	-	-	-	-	-	-	+ *
Pseudoanabaena batrachospermorum (Skuja) Anagnostidis & Komárek	-	-	-	+ **	-	-	-	+ *
P. catenata Lauterborn	-	-	-	-	-	-	-	+
P. galeata Böcher	-	-	-	+ *	+ *	-	-	-
Pseudoanabaena sp	-	-	-	-	-	-	-	+ *
Schizothrix borealis Komárek & Kovacik	-	-	-	-	-	+ *	-	-
S. lateritia Kützing ex Gomont	-	-	-	-	-	+ *	-	-
S. telephoroides Gomont	-	-	-	-	-	-	+ *	-
S. tenuis Woronichin	-	-	-	-	-	-	-	+ *
S. tinctoria Gomont ex Gomont	-	-	-	-	-	-	+ *	-
Synechococcus aeruginosus Nägeli	+ *	+ *	+ *	+ *	+ *	-	+ ***	+ ***
*Sy. elongatus (*Nägeli) Nägeli	-	-	-	+ *	-	-	-	-
Order - Spirulinales								
Spirulina laxissima G.S. West	-	-	-	+ *	-	-	-	-
Order - Chroococcales								
Aphanothece castagnei (Kützing) Rabenhorst	-	-	-	+ ***	-	-	-	-
Ap. densa Silva	-	-	-	-	+ *	-	+ *	-
Chlorogloea novacekii Komárek & Montejano	-	-	-	-	-	+	-	+ **
Chlorogloea sp	-	-	-	-	-	-	-	+ *
Chroococcus minor (Kützing) Nägeli	-	-	-	+ **	-	-	-	+ **
Ch. tenax (Kirchner) Hieronymus	-	-	-	+ *	-	-	-	-
Gloecapsa decorticans (A. Braun) P. Richter	-	-	-	-	-	-	+ *	-
G. alpina Nägeli	-	-	-	-	-	+ *	-	-
G. muralis Kützing	-	-	-	+ **	-	-	-	-
G. rupestris Kützing	-	-	-	-	-	-	-	+
Gloeothece rhodochlamys Skuja	-	-	-	+ ***	-	-	-	-
Gl. tepidariorum (A. Braun) Lagerheim	-	-	-	-	-	+	-	-
Microcystis aeruginosa Kützing (Kützing)	-	-	-	-	+*	-	-	-
M. smithii Komárek & Anagnostidis	-	-	-	+	-	-	-	-
Order - Oscillatoriales								
Geitlerinema splendidum (Greville ex Gomont) Anagnostidis	+	-	-	+	-	-	-	-
Ge. unigranulatum Singh	-	-	-	+	-	-	-	-
Lyngbya dendrobia Brühl & Biswas	-	-	-	+ **	-	-	-	-
L. martensiana Meneghini ex Gomont	-	-	-	+ **	-	-	-	-
L. putealis Montagne ex Gomont	-	-	-	+ *	-	-	-	-
L. semiplena J. Agardh ex Gomont	-	-	-	-	-	+ *	-	-

Cont...

Table 1. Relative abundance and distribution of Cyanobacteria in soil of Khasi hills, Meghalaya

Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
L. shackletonic West & G. S. West	-	-	-	+ *	-	-	-	-
L. taylorii Drouet & Strickland	-	-	-	-	-	+	-	-
Lyngbya sp	-	-	-	-	-	-	+	-
Microcoleus chthonoplastes Thuret ex Gomont	-	-	-	-	-	+ *	-	-
Microcoleus sp	-	-	-	-	-	-	-	+ *
Oscillatoria agardhii Gomont	-	-	-	+ **	-	-	-	-
O. pseudogeminata G. Schmid	+	+ *	-	-	+	-	+	+ *
O. santa Kützing ex Gomont	-	-	-	-	+ *	-	-	-
O. tenuis C. Agardh ex Gomont	-	-	+ *	-	-	-	-	+ *
O. vizagapatensis Rao	-	-	-	+	-	-	-	-
O. curviceps C. Agardh ex Gomont	-	-	+ **	+ ***	+ **	-	+ *	-
O. irrigua Kützing ex Gomont	-	+ *	+ **	+ *	-	-	-	-
O. limnetica Lemmermann	-	-	-	-	-	-	-	+ **
O. limosa C. Agardh ex Gomont	-	-	-	+ ***	+ **	+ *	-	-
O. princeps Vaucher ex Gomont	-	-	-	+ **	-	-	-	-
O. tergestina Kützing	-	-	-	+	-	-	-	-
O. amphibia Agardh ex Gomont	-	-	-	+ *	-	-	-	-
O. anguina Bory ex Gomont	-	-	-	+ *	-	-	-	-
O. brevis Kützing ex Gomont	-	+ **	-	-	-	-	-	-
O. germinata Schwabe ex Gomont	-	-	-	-	-	+	-	+ ***
O. nigra Vaucher ex Gomont	-	-	-	-	-	+	-	-
O. proboscidea Gomont	-	+ *	-	+ **	-	-	-	-
O. rubescens De Candolle ex Gomont	-	-	-	+	-	-	-	+
Oscillatoria sp	-	-	-	+ *	-	-	-	-
Phormidium abronema Skuja	-	-	-	+ *	+ *	-	-	-
P. inundatum Kützing ex Gomont	-	-	-	-	-	-	+ *	-
P. papyraceum Skuja	-	-	-	+ **	-	-	-	-
P. retzii Kützing ex Gomont	-	-	-	-	-	-	+	-
P. tenue Gomont	+	+ *		-	+ **	-	-	-
P. corium Gomont ex Gomont	-	-	-	-	-	-	-	+
Phormidium sp 1	-	-	+	-	-	-	-	-
Phormidium sp 2	-	-	-	+	-	-	-	-
Plectonema sp	-	-	-	-	-	-	-	+
Symploca muscorum Gomont ex Gomont	-	-	-	-	-	+	-	-
Order - Nostocales								
Amorphonostoc pruniforme (Kützing) Elenkin	-	-	-	+	-	-	-	-
Anabaena constricta (Szafer) Geitler	-	-	-	-	-	-	+	-
An. catenula Kützing ex Bornet & Flahault	-	-	-	-	-	+	+	-
An. iyengarii Kharadwaja	-	-	-	+ **	-	-	-	-
An. oryzae F. E. Fritsch	-	-	-	+ **	-	-	-	-

Cont...

Table 1. Relative abundance and distribution of Cyanobacteria in soil of Khasi hills, Meghalaya

Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
An. oscillarioides (Bory ex Bornet) Flahault	-	-	+ **	-	-	+ **	-	-
An. sphearica Bornet & Flahault	-	-	-	+ **	-	-	-	-
An. spiroides Klebahn	-	-	+ **	-	+ **	-	+ ***	+ ***
An. torulosa Lagerheim ex Bornet & Flahault	-	-	-	+	-	-	-	-
An. variabilis Kützing ex Bornet & Flahault	-	-	-	+ *	+ **	-	-	+***
Aulosira sp	-	-	-	+ **	-	-	-	-
Calothrix sp 1	-	-	-	+ *	-	-	-	-
Calothrix sp 2	-	-	-	-	-	-	-	+ *
Cylindrospermum michailovskoense Elenkin	-	-		-	-	+ *	-	-
Cy. muscicola Kützing ex Bornet & Flahault	-	-	-	-	-	+ *	-	-
Nostoc carneum C.Agardh ex Bornet & Flahault	-	-	-	+ **	-	+ **	-	+ *
N. commune Vaucher ex Bornet & Flahault	-	-	-	-	-	+*	-	-
N. linckia Bornet ex Bornet & Flahault	-	-	-	-	-	-	+ *	-
N. muscorum C. Agardh ex Bornet & Flahault	-	-	-	-	+ **	-	-	-
N. padulosum Kützing ex Bornet	-	-	-	+*	-	-	-	-
N. pruniforme Hariot	-	-	-	-	-	-	+ *	-
N. spongiaeformae C. Agardh ex Bornet & Flahaut	-	-	-	+ **	-	-	-	-
Scytonema geitleri Bharadwaja	-	-	-	-	-	-	-	+ **
Sc. hyalinum Gardner	-	-	-	-	-	-	-	+ **
Sc. mirabile Bornet	-	-	-	-	-	+ *	-	-
Sc. schmiditii Gomont	-	-	-	-	-	-	-	+ **
Scytonema sp 1	-	-	-	+ **	-	-	-	-
Scytonema sp 2	-	-	-	-	-	+ *	-	-
Stigonema hormoides Bornet & Flahault	-	-	-	-	-	-	-	+ **
St. mammilosum Agardh ex Gomont	-	-	-	-	-	-	+ **	-
St. ocellatum Thuret ex Bornet & Flahault	-	-	-	-	-	+ *	-	-
Tolypothrix sp	-	-	-	-	-	-	-	+ *
Westiellopsis sp	-	-	-	-	-	+ *	-	-

(SC 1 - 1st year, SC 2 - 2nd year, SC 3 - 3rd year, P/R - Potato/Rice field, P/M - Potato/Maize field, MSG - Mawphlang Sacred Grove, PF - Pine Forest, CP - Citrus Plantation). Relative abundance - (*) indicates 20% to 39%, (**) indicates 40% to 59%, (***) indicates > 60%; ' + ' indicates present, ' -' indicates absent)

Table 2. Antioxidant potential of some dominant cyanobacterial species

Cyanobacteria species	Total phenolics content (µgGAE/ml of extract)	Flavonoid content (µgGE/ml of extract)	DPPH Radical scavenging activity IC_{50} (µg/ml)	Superoxide anion scavenging activity IC_{50} (µg/ml)
Anabaena oscillarioides	23.12±1.67	12.34±0.98	456.34±1.54	543.78±0.34
An. spiroides	24.00±2.12	11.23±1.65	567.56±0.45	678.10±1.08
An. variabilis	24.12±1.87	11.19±1.34	456.21±1.12	657.05±1.87
Aphanothece castagnei	25.26±1.19	15.23±2.34	786.89±4.23	345.54±3.09
Gloeothece rhodochlamys	27.56±1.21	12.43±1.45	689.19±5.67	452.16±8.12
Oscillatoria curviceps	77.05±1.23	65.10±1.89	156.23±9.54	213.54±4.12
O. germinata	54.10±1.45	48.34±3.23	167.65±7.23	256.19±1.23
O. limosa	73.15±3.56	47.33±5.34	172.29±10.21	237.45±9.65
Scytonema geitleri	57.34±5.78	42.76±1.42	598.21±2.65	256.87±1.87
Sc. hyalinum	40.17±2.22	19.45±1.98	456.34±2.98	548.21±1.98
Sc. schmidtii	41.86±3.89	21.24±2.67	324.56±12.09	439.10±8.43
Synechococcus aeruginosus	21.16±4.32	10.28±1.88	764.17±3.12	889.14±1.87

Values are mean ± SE of three replicates

CONCLUSION

All the twelve cyanobacterial species have some antioxidant activity. *O. curviceps* has the maximum amount of phenolic and flavonoid content. This species also scavenged DPPH radical and superoxide anion more efficiently than all the other species. Other species with potentially higher scavenging activity was observed for *O. germinata*, O. *limosa* and *Scytonema geitleri*. The high antioxidant activity along with high relative abundance of these species can be an excellent source of raw materials from this part of the region.

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Community Forest Supports Three Different Morphs and First Record of the 'Banded' Morph of Asiatic Golden Cat (*Catopuma temminckii*) from India

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***Abstract*:** Observing the different pattern of coat colours in cat species attracted the attention of biologist to study the different morphs in their habitats. One such cat with different body morphs i.e., Asiatic golden cat (*Catopuma temminckii*, hereafter AGC) distributed throughout eastern Nepal, Northeast India, Thailand, Myanmar, China, Sumatra and Indonesia covered an elevation range of 0–4000m. This rare and elusive cat is exceptionally polymorphic and so far reported with six different morphs throughout its distribution range. To monitor threatened vertebrate species, we deployed 79 camera traps in different habitat of West Kameng district, Arunachal Pradesh, India which resulted in total efforts of 7313 camera trap nights. In the different camera traps, observed nine photo capture of AGC in five localities of West Kameng with different morphs. Among which, we first reported a new '*banded*' morph of AGC which is different from the earlier observed morphs throughout distribution range. These evidences highlights the importance of community forests as a suitable habitat for various conservation importance and rare carnivore species in West Kameng district.

***Keywords*:** Asiatic golden cat, Camera trapping, Banded morph, *Catopuma temminckii,* Community forest

India represents 15 species of cats, which constitutes around 37% of the global felid diversity. Among the 14 felid genera, India supports nine genera of felid with its wide varieties of habitats (Mukherjee et al 2019). The Asiatic Golden Cat also known as Temmink Cat, is less studied felidae species among the feline family, but recently many by catch evidences has gained interest on this species for their conspicuous pelagic coloration. The AGC distributed in most of Southeast Asia countries which includes eastern part of Nepal to North-eastern India, through eastern Bangladesh, Bhutan, Cambodia, Sumatra, Lao PDR, Thailand, Viet Nam, Myanmar, Malaysia (McCarthy et al 2015). Despite of its wide distribution this species is well studied throughout its range for its ecology, behaviour and taxonomy. The body size of AGC ranges from 73 to 105 cm and weighs from 9 - 16 kg, (Mukherjee et al 2019). Radio collared study in Thailand found traveling range varies between 55 to 9,275 meters with a mean of 1,597 meters (Grassman et al 2005). Photographic evidence depicts that it has a large sphere of activity, moreover, they reportedly are largely diurnal. Chiefly this small-sized cat species is associated with tropical, subtropical moist, mixed evergreen forests, and dry deciduous forests (McCarthy et al 2015). The population of AGC is declining due to its habitat loss, as Southeast Asian forests face the world's fastest regional deforestation (Jutzeler et al 2010), an increase in human population devours the wildlife. Rapidly increase of agricultural land, plantation destroys the natural habitat of *Catopuma temmincki*. AGC has been hunted for its meat and fur in China. Harvesting of large skin has previously come from Jiangxi (Sunquist and Sunquist 2002). Moreover, skin of AGC was collected from Tachileik market of Myanmar, which was for selling (Shepherd 2008). Tiger and leopard bones are used as traditional medicine but declination in the population of these large felines made AGC's bone as a substitute (Wang 1998). Due to these threats AGC is categorized as Near Threatened by the IUCN Red List of Threatened Species and protected under Appendix I of CITES and listed as Schedule I under Wildlife protection Act, 1972 (McCarthy et al 2015). West Kameng has a good forest cover. Global forest watch reported that a good amount of forest cover still remains in West Kameng district although there is a loss of forest cover observed (https://www.globalforestwatch.org). With this context, West Kameng holds a significant amount of habitat for AGC. Locals know very little about AGC presence in this landscape. Locals of this district have a strong dependency on forests, some of them reported their presence when they went to forest for collecting forest products.

MATERIAL AND METHODS

Study area: The district of Arunachal Pradesh, India covers 7422 km2, and 8.86%, of the state's overall geographic area. This landmass lies between 91° 30' to 92° 40' East longitudes and 26° 54' to 28° 01' North latitudes. The district shares two international borders in the north and west with Tibet and Bhutan respectively, furthermore Tawang lies in the north-west border and Assam shares a southern border with this district. A larger portion of it lies in the higher mountainous region, which is made up of a mass of sloppy peaks and valleys. The extent of its altitudinal variations from 115 to 5780 m asl. The Sela, Bomdila, and Chaku ranges are the three main mountain chains in the study area (Kashung et al 2018). The Kameng river, a tributary of Brahmaputra flows through the district which is the major water source of the district. West Kameng is home to five major tribes viz. the Monpa, Miji, Sherdukpen, Aka and Bugun. Monpas and the majority of the population practice Buddhism. This landmass has two protected areas namely Eagle nest Wildlife Sanctuary and Sessa Orchid Wildlife sanctuary for better preservation of its rich biodiversity.

Methods: During a project to enlist threatened vertebrates in the Indian Himalayan landscape, camera traps were deployed in the West Kameng district under Arunachal Pradesh, India. We deployed a total of 79 trail cameras of Ultra-compact SPYPOINT FORCE-11D trail cameras (SPYPOINT, GG Telecom, Canada, QC), SCOUTGUARD (SG562 D), and Browning 940 Defender trail cameras to record the faunal composition in this landscape. Trail cameras were distributed across the various habitat types in the district. Cameras were activated for 24 hrs per day with minimum delay between captures and three rapid shots with time-date stamps. We installed the trail cameras from February 2019 to March 2020 which recorded 7313 trap nights. The relative abundance index (RAI) was calculated. The naïve occupancy and activity patterns from the aggregated camera trap data observed (O'Brien 2011, MacKenzie et al 2006, Ridout and Linkie 2009). The photographs were considered as independent records when the interval between records was more than 30 min (Oliveira et al 2018).

RESULTS AND DISCUSSION

After the data mining, nine independent detections of AGC from five different camera trap locations were observed (Fig. 1). On the basis of photographic evidence, the overall (two camera trap locations were clustered with RP and EN3, so we only show here RP and EN3) calculated RAI was 0.123 and naïve occupancy was 0.06. The activity pattern of this feline species is mostly diurnal, depicting the highest peak after 12:00 PM (Fig. 2). The activity overlap between AGC and its potential competitors was also assessed (Fig. 3). Five potential competitors listed from the camera trap study like *Marten flavigula*, *Pardofelis marmorata*, *Prionailurus bengalensis*, *Paradoxurus hermaphroditus* and *Cuon alpinus.* The highest overlap value i.e. overlap coefficient (Δ) calculated between *Marten flavigula*, *Pardofelis marmorata*, *Cuon alpinus* and AGC . The findings of the present study depicts that the feline species abundance is very low in this area. The present study gives an important note on this species, found two morph and a rare morph of *Catopuma temminckii,* which is first time reported from non-protected

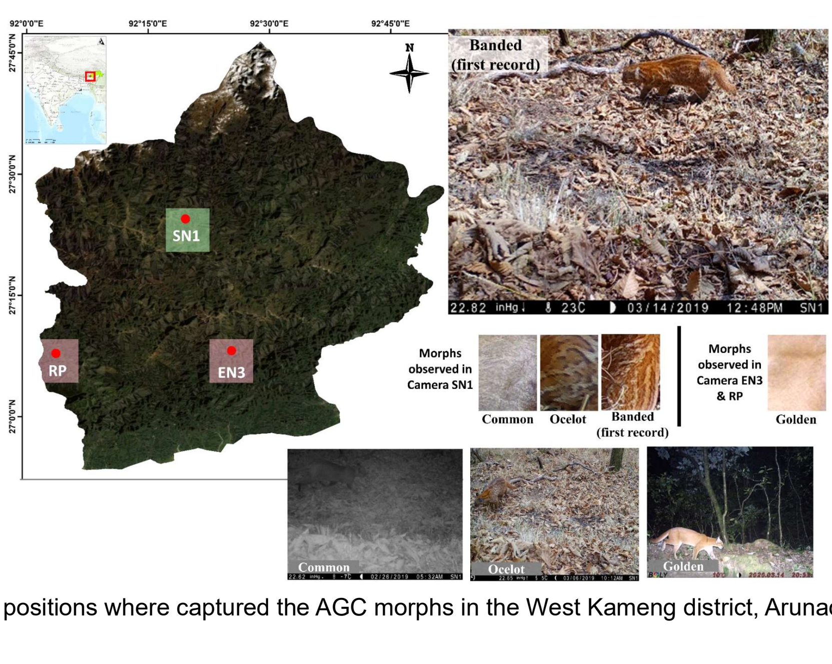

Fig. 1. Camera trap positions where captured the AGC morphs in the West Kameng district, Arunachal Pradesh, India.

area of Arunachal Pradesh (Semnak, Pagma). Along with the common morph, two different rare morphs were recorded in the same camera trap (Camera position: 3030109.9205 N / 437561.3371 E) between 26th February to 14th March 2019. The golden or brown colour morph, which is a common morph recorded on 26th February at 05:32 hrs. On 6th March 2019 at 04:09 hrs, the 'Ocelot' morph use the same trail, its rosette-like pattern illustrates its identity, the same morph was again captured at 10:12 hrs on the same day. On 14th March 2019, the 'banded' morph was captured in the same camera at 12:48 hrs. The three morphs recoded in the same camera trap depict co-occurrence as they use the same locality (Vernes et al 2015). Moreover, the 'banded' morph first record from India. The common Golden morph also recorded from other two places in this district i.e. Eagle nest Wildlife Sanctuary (camera trap name EN3) and Brokpalengchen area (camera trap name RP).

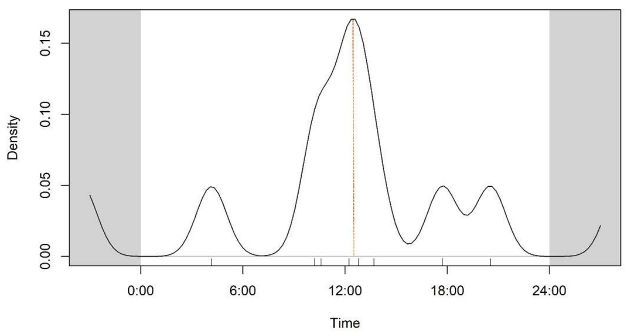

Fig. 2. Activity of the Asiatic golden cat in the study landscape, where the peak activity found at late 12:00 PM, the species showing diurnal activity

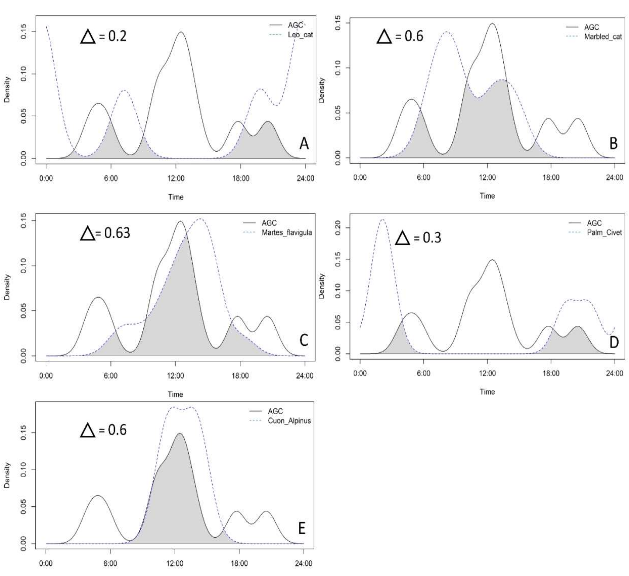

Fig. 3. Depicts the overlap between the AGC and its potential competitor in the study landscape. AGC shows highest overlap between *Marten flavigula*, *Pardofelis marmorata* and *Cuon alpinus*. Where A depicts overlap between AGC and *Prionailurus bengalensis*, B depicts overlap between AGC and *Pardofelis marmorata*, C depicts overlap between AGC and *Marten flavigula*, D depicts overlap between AGC and *Paradoxurus hermaphroditus* and E depicts overlap between AGC and *Cuon alpinus*. Δ refers the overlap coefficients (Ridout and Linkie 2009)

Nijhawan et al (2019) reported six morphs of AGC from Dibang Valley district of Arunachal Pradesh, India. However the banded morph of AGC, has no such evidence from previous records. This morph is first time reported from India. The coat colour is not like other morphs of the species, it has long thick bands which runs from neck to starting of tail, which is not continuous, and the dull fox-red colour bounded by deep fox red colour is the pattern of that individual make difference from all the morph. The bands are different from the 'rosettes' and other pattern. However, this individual also have black tuft on its tip of tail, and the white strip runs from the inner corner of the eyes, which clearly state it is an Asiatic golden cat. The prey of the feline are, birds including pheasants, medium-sized mammals, deer (Grassman 1998, Kawanishi and Sunquist 2008), like Northern Red Muntjac *Muntiacus vaginalis*, Wild Boar *Sus scrofa*, Kalij Pheasant *Lophura leucomelanos*, White-throated Laughingthrush *Garrulax albogularis* also captured in the camera trap, which determines the Asiatic Golden Cat's prey abundance in this area. The scarcity of studies on AGC results in poor data on this felidae. The maximum records came from bycatch camera trap data from biodiversity surveys or large carnivore surveys. The poor data on this felidae may affect its conservation action. Many bycatch studies of the AGC indicate that this species have conspicuous coat pattern and the different morphs also co-occur, which pop up many questions about the rare feline species (Jigme 2011, Vernes et al 2015, Dhendup 2016, Ghose et al 2019, Nijhawan et al 2019). This elusive highly polymorphic cat species was recorded from different altitude ranges in Bhutan which gave a thought that specific morphs selected different altitudes, for their adaptation to dealing with the changing forest types and vegetation cover found at different elevation (Sangay et al 2014). However, the altitudinal records of AGC from the present study does not satisfy the theory, because 'common', 'ocelot', and 'banded' morphs were captured in the same camera station at 2353 meter which suggested the co-occurrence of different morphs at same altitude. AGC is an interesting subject of study because of its pelage colouration, the newly reported 'banded' morph first record from West Kameng, Arunachal Pradesh, as well as the country which gives an irreplaceable question to the science. How many different coat colours do have a single species? This species needs proper study for its long-term viability and reason behind its' coat colour variation. Knowledge sharing among the locals can be important for this elusive feline for protecting its habitat.

AGC is a rare, elusive feline species, difficult to study in the wild. This is the first record of AGC from non-protected areas of West Kameng, which indicates the habitat of the non-protected area are also crucial for wildlife sustainability. This district has only one protected area in terms of faunal conservation i.e. Eagle nest wildlife sanctuary with an area of 218 km^2, so conservation strategies should focus on community level conservation. The fine scale ecological studies on this Near-threatened feline species is recommended for knowing the on-site variables which impact the species occurrence. Fine scale camera trap studies empirical for rare species which also help to evaluate the resource partitioning among the sympatric species. So, further studies can bring the cause of habitat preference of this species which can help for decision making for long term viability of the species.

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Diversity of Epiphytic Fern and Fern Allies in Darjeeling Region of Eastern Himalaya, India

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***Abstract*:** Pteridophytes represent a diverse group of plants which forms an interesting and conspicuous part of the forest ecosystem. In this context, the present study examines the species diversity and composition of epiphytic fern and fern allies in Darjeeling eastern Himalaya. Altogether, 73 species were recorded under 22 genera belonging to 10 families. Polypodiaceae was the most dominant and diverse followed by Pteridaceae. The number of species was plotted against different altitudinal gradients which resulted in hump-shaped species distribution pattern. Maximum number of species richness was recorded from the mid-altitudinal range. Host tree diameter and bark texture had the greatest influence on species diversity. Our finding suggests that bigger host trees having rough bark texture generally sheltered maximum species compared to trees having smooth bark. Additionally, the life form and threat status of fern and fern allies have also been assessed.

Keywords: Ferns, Epiphytes, Diversity, Host tree, Conservation

Pteridophytes or vascular cryptogams also traditionally known as fern and fern allies are the first terrestrial plants that originated during the Silurian period and flourished in the Carboniferous (Anderson 2021). Although angiosperms are the most dominant flora in the present world, earlier studies collated around 13,600 extant species of pteridophytes distributed worldwide encompassing around 1200 species with 70 families and 192 genera in India (Hassler and Swale 2001, Moran 2008, Patil et al 2016, Mukhopadhyay 2018, Krishnan and Rekha 2021). Many of the pteridophytic species can be seen as epiphytes on trees, lithophytes on rocks or hydrophytes (Krishnan and Rekha 2021). Ferns and fern allies have flourished in areas of high topographic complexity and are well adapted in diverse habitats from tropical to sub-alpine climatic zones (Page 1979, Dixit 2000, Chowdhury et al 2016, Anderson 2021). Although fern richness and abundance can be seen in moist and shady forests, they also thrive in dry habitats (Alien and Daniel 2019). The pteridophytes perform several critical ecological functions as indicator plants for habitat loss and fragmentation (Silva et al 2018), improving soil conditions (Walker 1994) and also in removing inaccessible arsenic from mined wastelands (Tu and Ma 2005). Furthermore, they are known to adapt to various disturbances and accumulate toxins from the environment (Meh et al 2010). The pteridophytes are not distributed randomly, rather their distribution is completely related to abiotic variables influencing microhabitat characteristics, such as soil texture and fertility, atmospheric temperature and humidity, precipitation and light intensity (Nobrega et al 2011, Patil et al 2016). Ferns are often used by human beings for several purposes and many earlier works have highlighted their use in ethnobotanical and pharmacological explorations (Singh et al 2001, Gogoi 2002, Chen et al 2005, Singh et al 2008a, 2008b). Their antifungal and antimicrobial properties both from the gametophytes and sporophytes are well documented (Guha et al 2004, 2005, Ganguly et al 2011, Goswami et al 2016). The foliage of ferns in the international florist market is highly valued for its long post-harvest life, low cost, year-round availability and versatile design in form, texture and colour (Safeena 2013, Deepa et al 2017). In addition, with certain exceptions ferns have also been used as food globally (Mannan et al 2008). Several botanical explorations have been carried out in Darjeeling Himalayan region over the past one and a half centuries, however, there is no any concise effort highlighting the distribution and ecology of vascular epiphytes from the region. This paper makes an effort to elaborate the diversity and distribution of epiphytic pteridophytes along different elevational gradient. The present investigation also presents a list of ferns and fern allies that occurs in diverse habitats of the study area. Concurrently, species conservation status and life forms have also been assessed.

MATERIAL AND METHODS

Study area: The present study was conducted in Darjeeling Himalaya- an integral part of the eastern Himalayan ecosystem. Geographically, the area extends between 27°2'9.62"N latitude to 88°15'45"E longitude covering an altitudinal range between 98m to 3636m asl (Fig. 1). The area

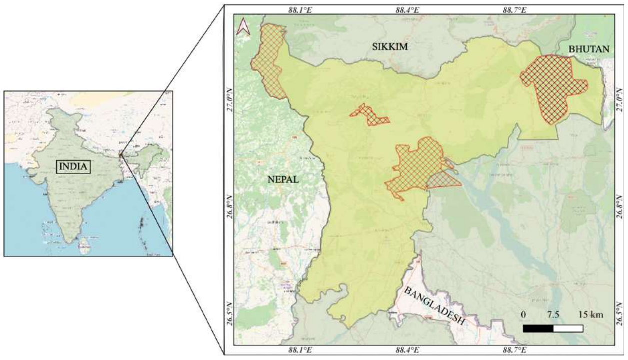

Fig. 1. Study area (Shaded portions indicate protected areas)

remains bordered by Sikkim, Nepal and Bhutan in the north, west and east respectively. Due to the altitudinal variation that ranges from tropical plain to sub-alpine zone, different climatic setup is available in the region which offers a conducive environment for the growth and development of diverse plant species with rich vegetation (Das 2004). The region exhibits a typical monsoon climate, with wet summers and dry winters within four recognized seasons (i) Spring and summer from March to May (ii) Monsoon from June to August (iii) Autumn from September to November and (iv) Winter from December to February (Bhujel 1996). The area consists of a complex terrain system, with varied aspects with an abrupt altitudinal variation that brings about distinct changes in rainfall and precipitation. The temperature varies with a minimum of 2.4°C to a maximum of 9.6°C during winter, 8.3°C to 19.1°C during spring and summer and 12°C to 18°C during autumn season with an average annual precipitation of about 337.3mm. Two National Park and two Wildlife Sanctuaries have been established which occupy a total area of 332.74km^2. Besides these, there are several reserved, un-reserved and social forests in the region.

Field sampling: A field survey was carried out in different forest types of Darjeeling Himalaya keeping in mind the fertile period of fern and fern allies with process of random sampling. Data on the abundance of epiphytic ferns were collected in the field with the assistance of a local tree climber. The reproductive period of the taxa was also noted through field visits in different seasons. Host tree species with >15cm CBH (circumference at breast height) were identified and sampled based on epiphyte abundance and accessibility including bark texture which was classified into smooth and rough (Wyse and Burns 2011). Due to the complex tree architecture encountered in the field, the host trees were divided into two zones (Johansson 1974), the trunk zone covering the area below the first branching till the base and inner crown zone covering the remaining area above the first branching. The epiphytic ferns were classified into different categories holoepiphytes (no contact with the ground), facultative (can grow both on host tree and ground) and accidental (for plants that occasionally grow as epiphytes) (Klein et al 2022). The voucher specimens collected were mounted into herbarium sheets following the conventional methodology (Jain and Rao 1977). The proper identification of the taxa was made following suitable literature (Frazer-Jenkins 2008, Mehra and Bir 2008, Kholia 2010, Frazer-Jenkins et al 2017, 2018, 2021). Herbaria such as Lloyd Botanical Garden and Calcutta University Herbarium (CUH) were also consulted for proper identification. Correct nomenclature was maintained following World Flora Online (WFO 2022) and Global Biodiversity Information Facility (GBIF 2022). The conservation assessment of the taxa was determined following the online data source Threat Search (BGCI 2022). The Herbarium exsiccates were deposited at Calcutta University Herbarium (CUH) for future study. The location and altitude of the study sites were recorded by a global positioning system (GPS; Garmin Terex H) and the map was produced using QGIS version 3.20 (QGIS 2022).

RESULTS AND DISCUSSION

The topography and great altitudinal variation of the region facilitated the luxuriant growth of pteridophytes. A total of 73 species under 22 genera belonging to 10 families in different habitats within the study area were recorded. Polypodiaceae was the most diverse and abundant family with 42 species representing 58%, followed by Aspleniaceae (7 spp.; 10%) and Pteridaceae (6 spp.; 8%). Three families Davalliaceae, Hymenophyllaceae and Lycopodiaceae were represented by 4 species (5%) each. Dryopteridaceae and Oleandraceae had two species (3%) each while Hypodematiaceae and Nephrolepidaceae were monospecific (1%) (Fig. 2A). Amongst the genera, *Lepisorus* had the maximum number of species (8 species) followed by *Asplenium* (7 species), *Goniophlebium*, *Pyrrosia*, and *Loxogramme* (each 6 species) while *Vittaria* was represented by 5 species (Fig. 2B). Concerning life forms, holophytes (56 spp.) were dominant representing 77% followed by facultative species (16 spp.) which represents 22% whereas single species grew as epiphytes accidentally. All the pteridophytic taxa showed an ecological variation from lithophytes growing on rocks to epiphytes on the host tree trunk except *Lepisorus loriformis* and *Huperzia squarrosa* which were truly epiphytic. Majority of the pteridophytic species were herbaceous and perennial in nature. Similarly, the fern allies were represented by a single pendulous genus with 3 species *Huperzia squarrosa*, *H. hamiltonii* and *H. pulcherrima*. Furthermore, late monsoon to mid-autumn was observed to be the most fertile period for the taxa under study (Table 1). An earlier study on fern and fern allies from Western Ghats identified many epiphytes common to the fern and fern allies of Darjeeling Himalayas and some of them have also been identified in this communication too.

Distribution within the host tree: The morphological and physiological characteristics such as bark roughness, canopy soil chemistry and branch inclination including tree architecture have an impact on epiphytic community development (Ganguly and Mukhopadhyay 2008, Wang et al 2016). The forests in the study area were composed of dense multi-layered canopy with dominant host tree species like *Duabanga grandiflora, Callicarpa vestita, Tetrameles nudiflora, Schima wallichii*, *Lagerstroemia parviflora, Shorea robusta, Mallotus repandus, Bridelia retusa*, *Diploknema butyracea, Wrightia sikkimensis, Syzygium cumini, Castanopsis indica, Engelhardia spicata, Ficus neriifolia, Magnolia campbellii*, *Lithocarpus pachyphyllus*, *L. fenestratus*, *Alnus nepalensis, Quercus lamellosa, Saurauia nepaulensis, Ficus auriculata*, *Ostodes paniculata*, *Symplocos glomerata*, etc distributed in different vegetation zones. CBH of the host tree ranged from 20 to 400cm. Maximum numbers of species were found in the host tree having large CBH with rough bark as compared to the host tree with smaller CBH having smooth bark texture. This reflects a positive relationship between phorophyte size and abundance of epiphytes which supports the findings of other studies (Brown et al 2015). The availability of enough time and space for colonization and higher diversity of microhabitats are the main reason for more epiphytic species on large trees rather than on smaller trees (Neider et al 2001, Flores-Palacios and Garcia-Franco 2004, Wang et al 2017). In addition to that, the distribution and development of epiphytic fern and fern allies were affected by host bark traits, trees with coarse bark supported maximum species as they retain moisture for longer and seedling recruitment (Wyse and Burns 2011) while smoother bark sheltered only a few species. Previous studies highlight that epiphytes are related to crown height and orientation of the host tree in terms of climatic variables (Trembley and Castro 2009, Ganguly and Mukhopadhyay 2012a, Zhao et al 2015). The number of species significantly decreases from the trunk zone to the inner crown zone of all the host trees. About 88% of fern and fern allies were distributed in the trunk zone while only 12% occurred towards the inner crown zone (Fig. 3). The decrease in species number from the trunk zone to the inner crown zone may be due to an increase in ultraviolet radiation, decrease in humidity and increase in photon flux density along with the increasing canopy height of the hosts, lack of adaptation for high water stress in vertical canopy branches (Patino et al 1999, Mantovani 1999, Wang 2016). Host tree species such as *Saurauia nepaulensis, Ficus neriifolia, Engelhardia spicata, Duabanga grandiflora* and *Lithocarpus pachyphyllus* with maximum branching sheltered the majority of species and the distribution was contiguous. Other host trees such as *Macaranga denticulata, Schima wallichii* and *Bridelia retusa* with thin branching sheltered comparatively lesser species.

Species richness pattern along elevational gradient: The study suggests that pteridophytic species are morphologically more flexible which allows them to grow across the entire altitudinal gradient. The observed number of species was plotted against the altitudinal ranges which reflected a significant and positive correlation ($R^2=0.618$). The results showed a hump-shaped distribution pattern while the higher altitude gives a monotonically decreasing trend. Maximum species richness was found between 1850-2300m with steep increase up to 2300m, while it decreased gradually beyond that altitude. In the lowest altitudinal band upto 500m, species such as *Asplenium nidus, Drynaria quercifolia, Microsorum punctatum, Pyrrosia lanceolata* and *P. nuda* were most dominant. Some species like *Asplenium yoshinagae*, *A. planicaule*, *Arthromeris wallichiana, Drynaria mollis, Goniophlebium amoenum, Lepisorus sordidus,*

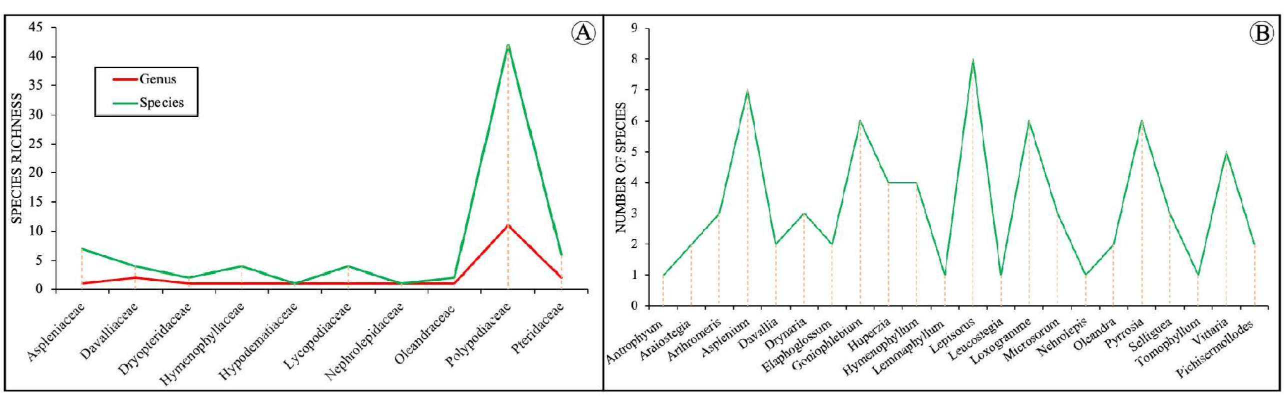

Fig. 2. A. Species richness under different families B. Number of species under each genus

Table 1. Status of epiphytic fern and fern allies in Darjeeling Himalaya

Taxa	Life form	Elevation (m)	Fertile period	Distribution	Conservation status
Aspleniaceae					
Asplenium ensiforme Wall. ex Hook. & Grev.	Hol	1800-2800	June-Sep	TZ	NT
A. laciniatum D.Don	Fac	1400-2100	Aug-Nov	TZ	NT
A. nidus L.	Fac	150-1500	Aug-Nov	ICZ	NT
A. phyllitidis D.Don	Hol	600-1600	Jul-Dec	TZ	LC
A. planicaule Wall. ex Mett.	Fac	1200-2400	Aug-Oct	ICZ	LC
A. tenuifolium D.Don	Fac	1200-2400	Aug-Dec	TZ	NT
A. yoshinagae Makino	Fac	1200-2400	Jun-Nov	TZ	LC
Davalliaceae					
Araiostegia dareiformis (Hook.) Copel.	Fac	2100- 2400	Jul-Oct	TZ	Unknown
A. multidentata (Wall. ex Hook.) Copel.	Hol	1400-2100	Jul-Dec	TZ	Unknown
Davallia bullata Wall.	Fac	900-1800	Jul-Oct	TZ	Unknown
D. pulchra D.Don	Fac	1600-2700	Jul-Nov	TZ	NT
Dryopteridaceae					
Elaphoglossum marginatum T.Moore	Hol	1400-2200	May-Jul	TZ	NT
E. stelligerum (Wall. ex Baker) T.Moore ex Salomon	Acc	500-1700	Jun-Aug	TZ	NT
Hymenophyllaceae					
Hymenophyllum badium Hook. &Grev.	Hol	2100-3000	Aug-Nov	TZ	Unknown
H. exsertum Wall. ex Hook.	Hol	900-1400	Aug-Nov	TZ	NT
H. simonsianum Hook.	Hol	1000-2400	Aug-Nov	TZ	NT
H. tenellum D.Don	Hol	500-1700	Aug-Nov	TZ	NT
Hypodematiaceae					
Leucostegia truncata (D.Don) Fraser-Jenk.	Hol	500-2200	Jul-Sep	TZ	NT
Lycopodiaceae					
Huperzia hamiltonii (Spreng.) Trevis.	Hol	2000-3200	Jul-Oct	TZ	EN
H. phlegmaria (L.) Rothm.	Hol	950-1450	Jul-Sep	TZ	EN
H. pulcherrima (Wall. ex Hook. & Grev.) Pic.Serm.	Hol	1400-2600	Jul-Oct	TZ	VU
H. squarrosa (G.Forst.) Trevis.	Hol	500-1600	Jun-Sep	ICZ	EN
Nephrolepidaceae					
Nephrolepis cordifolia (L.) C.Presl	Fac	500-2000	Aug-Nov	TZ	NT
Oleandraceae					
Oleandra pistillaris (Sw.) C.Chr.	Fac	1200-1700	Aug-Oct	TZ	DD
O. wallichii (Hook.) C.Presl	Fac	1100-3000	Aug-Oct	TZ	NT
Polypodiaceae					
Arthromeris himalovata Fraser-Jenk. & Kandel	Hol	2400-2700	Jun-Nov	TZ	NT
A. lehmannii (Mett.) Ching	Hol	1800-2700	Jun-Nov	TZ	NT
A. wallichiana (Spreng.) Ching	Hol	900-2700	Aug-Dec	TZ	NT
Drynaria mollis Bedd.	Hol	1500-2800	Jun-Aug	TZ	NT
D. propinqua (Wall. ex Mett.) J.Sm.	Hol	1600-2400	May-Oct	TZ	NT
D. quercifolia (L.) J.Sm.	Hol	400-1000	Jul-Dec	TZ	NT
Goniophlebium amoenum (Wall. ex Mett.) Bedd.	Hol	100-1600	Aug-Nov	TZ	NT
G. hendersonii (Atk.) Bedd.	Hol	1800-3000	Jul-Sep	TZ	Unknown
G. lachnopum (Wall. ex Hook.) Bedd.	Hol	1500-2400	Jun-Oct	TZ	VU

Cont...

Table 1. Status of epiphytic fern and fern allies in Darjeeling Himalaya

Taxa	Life form	Elevation (m)	Fertile period	Distribution	Conservation status
G. argutum (Wall. ex Hook.) Bedd.	Hol	1200-2200	Sep-Nov	TZ	NT
G. microrhizoma (C.B. Clarke ex Baker) Clarke ex Bedd.	Hol	1200-2400	Jul-Oct	TZ	EN
G. subamoenum (C.B. Clarke) Bedd.	Fac	1000-2400	Jun-Nov	TZ	VU
Lemmaphyllum rostratum (Bedd.) Tagawa	Hol	1200-2000	Mar-Jul	ICZ	NT
Lepisorus contortus Ching	Hol	2200-2600	Aug-Nov	TZ	NT
L. loriformis Ching	Hol	2000-3000	Jul-Sep	TZ	NT
L. mehrae Fraser-Jenk.	Hol	1500-2400	Jul-Nov	TZ	NT
L. normalis (D.Don) C.F.Zhao, R.Wei & X.C.Zhang	Hol	900-2600	Aug-Nov	TZ	NT
L. nudus Ching	Hol	1500-2400	Oct-Feb	TZ	NT
L. scolopendrium (Ching) Mehra & Bir	Hol	1400-2800	Jul-Sep	TZ	NT
L. sordidus (C.Chr.) Ching	Hol	1200-1400	Jul-Sep	TZ	VU
L. sublinearis Ching	Hol	1800-2400	Jul-Sep	TZ	NT
Loxogramme chinensis Ching	Hol	1000-1700	Sep-Nov	TZ	NT
L. cuspidata (Zenker) M.G.Price	Hol	1400-1900	Jun-Sep	TZ	DD
L. grammitoides (Baker) C. Chr.	Hol	800-1200	Jul-Nov	TZ	NT
L. involuta (D.Don) C.Presl	Hol	800-2200	Aug-Oct	TZ	NT
L. lanceolata (Sw.) C. Presl	Hol	1500-2400	Aug-Dec	TZ	NT
L. porcata M.G.Price	Hol	850-1200	Aug-Nov	TZ	NT
Microsorum cuspidatum (D.Don) Tagawa	Fac	1000-1850	Aug-Dec	TZ	VU
M. membranaceum (D.Don) Ching	Fac	500-2600	Aug-Dec	TZ	NT
M. punctatum (L.) Copel.	Fac	300-750	Aug-Nov	TZ	NT
Pichisermollodes ebenipes (Hook.) Fraser-Jenk.	Hol	300-1000	Jul-Sep	TZ	Unknown
P. stewartii (Bedd.) Fraser-Jenk.	Hol	1200-2000	July-oct	TZ	Unknown
Pyrrosia costata (Wall. ex C.Presl) Tagawa & K.Iwats.	Hol	1300-2200	Jul-Dec	ICZ	NT
P. heteractis (Mett. ex Kuhn) Ching	Fac	300-2000	Jul-Oct	TZ	NT
P. lanceolata (L.) Farw.	Hol	300-1800	Jul-Oct	ICZ	NT
P. mannii (Giesenh.) Ching	Hol	700-1400	Jul-Oct	TZ	VU
P. nuda (Giesenh.) Ching	Hol	400-1500	Jul-oct	ICZ	NT
P. porosa (C.Presl) Hovenkamp	Hol	1400-2200	May-Oct	TZ	DD
Selliguea griffithiana (Hook.) Fraser-Jenk.	Hol	1600-2800	Jun-Nov	TZ	Unknown
S. hastata (Thunb.) Fraser-Jenk.	Hol	1500-2500	Jun-Sep	TZ	NT
S. oxyloba (Wall. ex Kunze) Fraser-Jenk.	Hol	1200-2800	Jun-Oct	TZ	NT
Tomophyllum donianum (Spreng.) Fraser-Jenk. & Parris	Hol	1200-2530	Aug-Nov	TZ	NT
Pteridaceae					
Antrophyum coriaceum (D.Don) Wall. ex T. moore	Hol	600-1500	Aug-Dec	TZ	VU
Vittaria elongata Sw.	Hol	1400-2800	Jul-Oct	ICZ	LC
V. flexuosa Fee	Hol	1600-2500	Jul-Oct	ICZ	LC
V. himalayensis Ching	Hol	400-1500	Jul-Oct	TZ	Unknown
V. ophiopogonoides Ching	Hol	1350-3000	Aug-Nov	TZ	Unknown
V. sikkimensis Kuhn	Hol	1600-2500	Jul-Oct	TZ	Unknown

Abbreviations used: Hol-Holoepiphytes, Fac-Facultative epiphyte, Acc-Accidental epiphyte; TZ-Trunk zone, ICZ-Inner crown zone; NT-Not threatened, DD-Data deficient, LC-Least concern, VU-Vulnerable, EN- Endangered

Microsorum cuspidatum, *Huperzia pulcherrima*, *Lepisorus mehrae*, *Loxogramme cuspidata, Microsorum membranaceum, Pyrrosia costata*, *P. mannii*, *Vittaria elongata* and *V. flexuosa* were predominant at mid elevational ranges. *Arthromeris himalovata*, *A. lehmannii*, *Davallia pulchra, Goniophlebium hendersonii, Huperzia hamiltonii*, *Lepisorus loriformis, Oleandra wallichii, Selliguea griffithiana* and *Vittaria ophiopogonoides* were common pteridophytic epiphytes from higher altitudes (2500-3000 m) (Fig. 4). The study reveals that the mid-altitudinal zone that falls within the temperate forest represented higher species richness as the climatic condition of this zone makes it conducive for plant species to flourish (Ganguly and Mukhopadhyay 2012a, Zotz 2016, Dormann et al 2020). It may also be due to the large canopy cover which prevents penetration of light that makes the most favourable conditions for both fern and fern allies. In line with previous findings (Rezende et al 2015, Malik and Nautiyal 2016, Sinha et al 2018, Bhat et al 2020), our results showed a decreased number of species in the higher altitudinal zone. Extremely low temperature, short period of the growing season and geographical barriers (Chawla et al 2008, Rosa-Manzano et al 2019, Schroter and Obenhuber 2022), stunted forest vegetation near the treeline (Kromer et al 2005), decreased soil fertility (Halbritter 2018), uneven topography, and an increase in steepness and lesser top soil depth at high altitudes (Timsina et al 2021) could be the reason for the reduction in species richness towards higher altitudinal regions.

Conservation assessment: Considering the database for a conservation assessment of plants, the majority of the species have been categorized as not threatened (NT) representing 59% (Fig. 5). Further, three species (4%) were data deficient (*Loxogramme cuspidata, Oleandra pistillaris* and *Pyrrosia porosa*). Some of the species that were least concerned (7%), *Asplenium phyllitidis, A. planicaule*, *A. yoshinagae*, *Vittaria elongata* and *V. flexuosa*. Taxa like *Antrophyum coriaceum, Goniophlebium lachnopum*, *G. subamoenum*, *Huperzia pulcherrima*, *Lepisorus sordidus, Microsorum cuspidatum* and *Pyrrosia mannii* were

Fig. 3. Photo collage of fern and fern allies A. *Lemmaphyllum rostratum,* B. *Lepisorus sublinearis,* C. *Lepisorus scolopendrium,* D. *Pyrrosia nuda,* E. *Asplenium ensiforme,* F. *Pyrrosia costata,* G. *Goniophlebium amoenum,* H. *Selliguea griffithiana,* I. *Loxogramme involuta,* J. *Pyrrosia heteractis,* K. *Lepisorus normalis,* L. *Elaphoglossum stelligerum,* M. *Huperzia pulcherrima,* N. *Pyrrosia mannii*

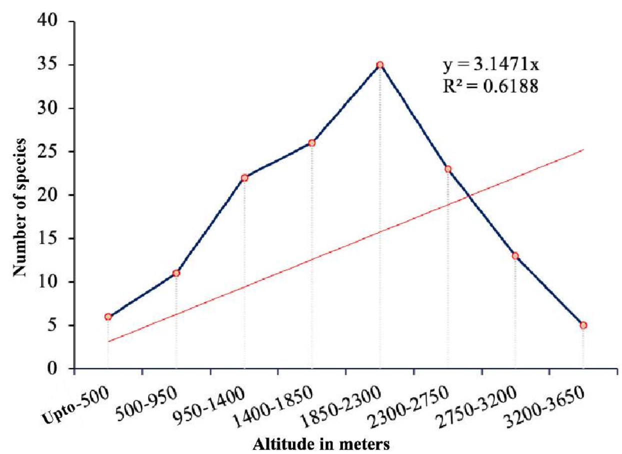

Fig. 4. Distribution along an elevational gradient

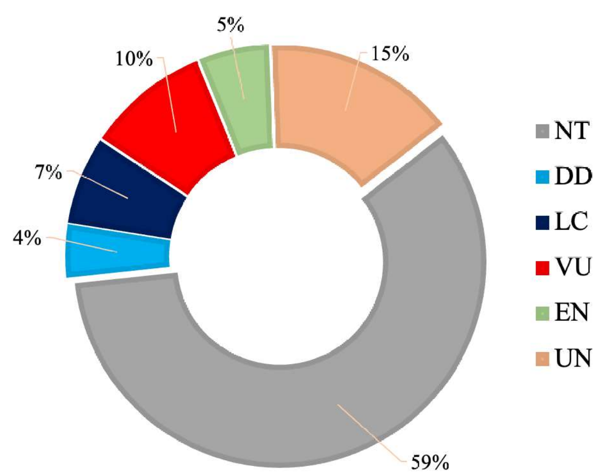

Fig. 5. Threat status of fern and fern allies

considered as vulnerable (10%). Concurrently, four species representing 5% of the taxa were categorized as endangered (*Goniophlebium microrhizoma*, *Huperzia hamiltonii*, *H. phlegmaria* and *H. squarrosa*). However, the threat status of the remaining 11 species is still unknown. Although a reasonably good number of taxa have been observed in the study, the plants are still under serious threat. Almost all forests are affected in the hilly region as the human population and habitat modification have been increasing at an alarming rate (Ganguly and Mukhopadhyay 2012b). Still many tribes and local people depend upon herbal medicines for their primary health care services (Mownika et al 2021), collection of plants for ethnobotanical practices and their commercial aspects by local inhabitants is also the reasons for the decline in the population of species at certain habitats (Uniyal and Shiva 2005, Thakur and Sidhu 2017). Some ethnomedicinal plants have also been identified that are widely used. For example, the rhizome of *Drynaria quercifolia* is used as an antibacterial, anti-inflammatory also treats typhoid, cough, dyspepsia and phthisis (Raha et al 2020, Ojha 2021), rhizome of *Nephrolepis cordifolia* is used against chest congestion, rheumatism and cough (Luitel et al 2014), the rhizome of *Oleandra wallichii* is reported to be used by aged as rejuvenating (Benniamin 2011, Malla 2018). Additionally, *Lepisorus mehrae* treats back pain, stomach problem and fever (Pradhan 2020) and *Loxogramme involuta* treats cuts and wounds (Malla 2018). Although the majority of these species (not threatened) were locally common, a large number of taxa have been exposed to anthropogenic threats regularly. Lack of inadequate conservation of taxa in the region has been identified. The rich diversity of the area is threatened due to deforestation, expansion of agriculture and many other developmental activities. Hence, it is noteworthy to focus on such species for future study and conservation approaches which will subsequently conserve the rich and diverse flora of the region.

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Effect of Water Quality on Zooplankton in Mangrove Areas of Munroe Island, Kerala

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Abstract: The present study was directed to evaluate the influence of water quality variables on selected zooplankton groups in the Munroe Island. Ten sampling stations were selected and zooplankton samples were collected with WP net (200 µm). Fourteen water quality parameters were considered for the analysis to recognize their influences on zooplankton community. The pH ranged from 6.90 to 7.53.The level of solids, conductivity, salinity, total alkalinity and total hardness was high in Pattamthuruthu when compared to other stations. Palliyanthuruthu registered a minimum DO.The BOD and COD varied from 0.17 to 0.876, 9 to 19 respectively. The Pezhumthuruthu shows maximum abundance of zooplankton (72745No/m^3) and minimum at Kidapram I (8002No/m^3). Among the zooplankton communities, copepoda showed maximum abundance (32740No/m^3) and chaetognatha minimum (3No/m^3).The water quality variables such as total hardness, total alkalinity, salinity, temperature, electrical conductivity and total dissolved solids tend to increase towards the sampling stations adjacent to the Ashtamudi estuary. The variation in zooplankton abundance and hydrological features clearly corroborate the influence of Ashtamudi estuary and similarly the influence of Kallada River on Villimangalam , Kidapram II and Kidapram I was also reflected during the study period. The zooplankton abundance in Munroe Island was significantly influenced by the variations in water quality due to various natural as well as anthropogenic stresses.

Keywords: Water quality, Zooplankton, Anthropogenic activities, Munroe Island

The Munroe Island is the combination of eight small islands, located at the confluence of two water bodies - Ashtamudi Lake and Kallada River. Due to its rapid settlement, the island is known as 'sinking island' (Jha and Sahib 2016). At present the ecological condition of Munroe Island is under risk, as it is facing land erosion. Regrettably, the land erosion occurred mostly due to the destruction of mangrove ecosystems. The fluctuations in the environment of the island might be the result of global warming and it may be the effect of the tsunami in 2004 (Nair et al 2020). Mangrove ecosystems are the treasurable pool of aquatic fauna and flora (Deng et al 2021) and provide ideal breeding and feeding grounds for a wide range of organisms and also they can provide natural defenses against natural calamities and can develop specific arrangements for flood protection and control coastal erosion (Joseph et al 2019). Mangroves are generally recognized as important ecosystems that offer a variety of economic as well as ecological benefits to adjacent communities (Goldberg et al 2020). Globally, mangroves have been seriously affected by natural as well as anthropogenic degradation (Kolli et al 2022). Zooplankton comprises suspended fauna in water-bodies having limited locomotion and is the key components of the food web of aquatic ecosystems (Krztoń and Kosiba 2020) and have strong relations with their environment throughout the lifespan and they reflect quick variations in their populations according to disturbance (Malik et al 2020). These groups are the frequently used indicator fauna for the monitoring of coastal ecosystems due to their various characteristic community structures (Stankovic et al 2014). Disappointingly, aquatic ecosystems are extremely affected through various types of point-source pollutants (Dani et al 2020). Considerable work on zooplankton in the Indian coast has been done from different parts (Biju et al 2016, Thirunavukkarasu et al 2020, Venkataramana et al 2021, Saravanakumar et al 2021). However, the scattered reports on the mangrove environments of Kerala are available (Vidyasagaran and Madhusoodanan 2014, Pillai and Harilal 2018, Surya and Hari 2018). However, the present effort is a first case approach to study the zooplankton abundance in the Munroe Island. The present study aimed to assess the zooplankton abundance during the monsoon period, and also to investigate the influence of water quality on zooplankton abundance.

MATERIAL AND METHODS

Study area and sampling framework: The Munroe Island is a backwater island village situated in the southern part of

Kerala. The Munroe Island lies between 8.9952° N, 76.6105° E and encompasses an area of 13.4 sq. km. The Island is named in honor of John Munroe, former Resident Colonel of the princely state of Travancore. Munroe Island is a tourist destination of Kerala and also this area is characterized with the presence of different types of mangroves. The Island is primarily used for fishing, recreational and aquaculture activities. Currently, Munroe Island is under the threats of continuous flooding and soil erosion. Location map of sampling sites is depicted in (Fig. 1). General characteristics of the sampling sites are depicted in (Table 1).

Environmental variables: The water samples were taken in 1-liter bottles from the ten sites (Fig. 1) of the Munroe Island at a depth of 15-30 cm from the surface during the monsoon season (June to September), 2021. In this study, 14 environmental variables (temperature, turbidity, pH, conductivity, TDS, dissolved oxygen, BOD, COD, hardness, alkalinity, salinity, nitrate, phosphate and silicate) of water were selected. The analytical experimental procedures followed for the analysis of the environmental variables followed APHA (2012).

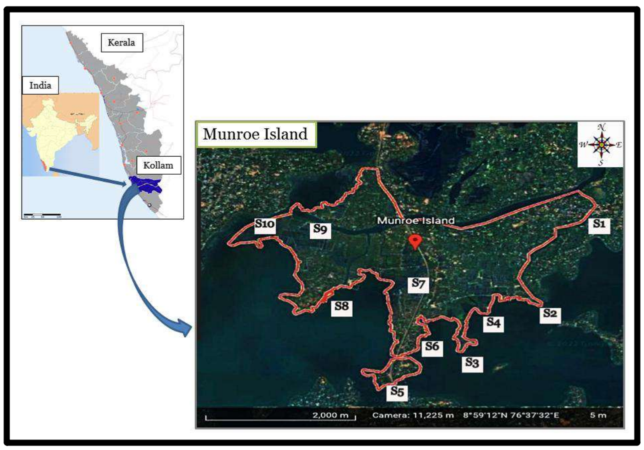


Fig. 1. Location map of study area in Munroe Island, Kerala

Response variables: Zooplankton samples were collected nocturnally at ten sites in the Munroe Island and was collected before dawn from surface waters using a Working Party (WP) net (mesh size 200 µm, mouth area 0.6 m^2) fitted with a flow meter to estimate the volume of water filtered. The net was hauled for 10 minutes at the surface using a small boat at a speed of approximately 2 knots (Fraser 1966). Samples were preserved in 5% formaldehyde. In the laboratory, zooplankton samples were identified by using standard identification procedures (Santhanam et al 2018). Abundance data were expressed as number per cubic meter (No /m^3).

Statistical analysis: Pearson correlation (r) was applied to examine the association between various environmental variables (Gacesa et al 2022). The canonical correspondence analysis was applied to detect the relationship between the environmental variables and response variables (Androniceanu et al 2020). The statistical software PAST (Version 4.04) was used for the present investigation.

RESULTS AND DISCUSSION

Environmental variables: During the study period, the water temperature was in the range of 21.20 - 28.45 °C in the study period. The pH and dissolved oxygen in the range of 6.90 - 7.53 and 5.64 - 7.40 mg/l respectively (Table 2). Dissolved oxygen is an important parameter as it can directly indicate the ability of an aquatic system for the sustenance of life (Li et al 2017). The highest dissolved oxygen level at S_1 was due to the photosynthetic activity of aquatic plants and significant turbulence in this area. Reduction in dissolved oxygen could be due to the presence of contaminants that affect the water quality extensively (Zhi et al 2021). The biological oxygen demand (BOD) was lowest at station S_1 (0.17 mg/l) and highest at station S_6 (0.87 mg/l) and chemical oxygen demand (COD) was in the range 9 - 19 mg/l (Table 3).

Table 1. Details of the sampling stations

Sites	Site code	Anthropogenic interferences	Latitude	Longitude
Villimangalam	S_1	Fishing, boating, bridge construction & resorts.	9.0002°	76.6187°
Nenmeny	S_2	Fishing, boating,& resorts	8.9869°	76.6225°
Palliyanthuruthu	S_3	Fishing,& boating.	8.9823°	76.6215°
Neetumthuruthu	S_4	Fishing, &boating.	8.9768°	76.6184°
Pezhumthuruthu	S_5	Fishing, boating, bridge construction & resorts.	8.9777°	76.6073°
Pattamthuruthu	S_6	Fishing, &boating	8.5849°	76.3625°
Kandramkani	S_7	Fishing, boating, &bridge construction	8.9759°	76.6082°
Peringalam	S_8	Fishing, boating, &bridge construction	8.5912°	76.3615°
Kidapram II	S_9	Fishing, boating, &bridge construction	8.9954°	76.6042°
Kidapram I	S_{10}	Fishing, boating, &bridge construction	8.5956°	76.3619°

BOD and COD were comparatively low because of the dilution due to the monsoonal rainfall. Total hardness was in the range 450 (S_1) - 3550 mg/l (S_7), may be due to the influence of the Ashtamudi estuary or it may found due to various anthropogenic activities (Chithra et al 2022). The total alkalinity ranges between 23 and 61 mg/l and varies based on the fluctuation in the pollution level.

The total alkalinity play significant role in estimating the ability of an aquatic system to neutralize acidic contamination from rainfall or wastewater (Priyadharshini and Abraham 2020). The electrical conductivity was in the range of 397.35 – 2848 µS/cm. Electrical conductivity is suitable to identify the quality of waterbodies, and generally high EC values specifies a high level of contamination (Kur et al 2019). The total dissolved solid (TDS) content of water samples ranged between 230 - 1676.3 mg/l. The recorded values of salinity were between 1.77 and 9.73 ppt. The steady increase in TDS, EC and salinity were due to the saltwater intrusion from Ashtamudi estuary through different interconnected channels (Chithra et al 2022). Nitrate was in the range of 0.185 - 0.644. The quantity and form of nitrogen in the water can strongly related to dissolved oxygen content (Zheng et al 2019). Phosphate concentration in water samples varies from 0.12 -0.21mg/l.

The present concentration of phosphate poses no threat to aquatic life. Silicate concentration ranged between 3.83 and 7.65 mg/l. Dissolved silicate is an important nutrient in brackish water. The major source of dissolved silicate in the present examination may be due to the influx of water from land drainage (Dani et al 2021) and also added by the Kallada River through several channels.

Zooplankton abundance: The zooplankton was maximum at S_5 (72745No/m^3) followed by S_6 , S_3 , S_4, and S_8 (Fig. 2). Among the zooplankton communities, copepoda was maximum (32740 No/m^3) and chaetognatha showed minimum (3 No/m^3). Water quality variables such as dissolved oxygen, salinity and temperature play a pivotal role in influencing zooplankton abundance. The higher abundance of copepoda may be due to their quick

Table 2. Water quality parameters of Munroe Island

Stations	Temperature (^{0}C)	Turbidity (NTU)	EC	TDS	pH	DO (mg/l)	Salinity (ppt)
S1	26.25 ± 0.07	7.34 ± 0.12	397.35 ± 1.48	230 ± 0.21	6.90 ± 0.08	7.40 ± 0.71	1.77 ± 0.11
S2	25.55 ± 0.49	7.79 ± 0.34	2030.20 ± 24.75	999.5 ± 1.86	7.30 ± 0.02	6.79 ± 0.14	4.07 ± 0.06
S3	26.45 ± 0.49	6.67 ± 0.49	2051.00 ± 46.67	1184.5 ± 19.84	7.18 ± 0.04	5.64 ± 0.93	6.61 ± 0.16
S4	28.45 ± 0.07	8.10 ± 0.06	2228.50 ± 23.33	1290.6 ± 10.77	7.36 ± 0.07	6.64 ± 0.49	7.17 ± 0.03
S5	24.45 ± 0.49	8.58 ± 0.36	2495.00 ± 1.41	1473.9 ± 38.66	7.20 ± 0.07	6.82 ± 0.18	7.35 ± 0.21
S6	24.30 ± 0.14	9.35± 0.11	2848.00 ± 5.66	1676.3 ± 31.35	7.37 ± 0.11	6.80 ± 0.15	9.73 ± 0.26
S7	24.35 ± 0.35	7.61 ± 0.16	1241.00 ± 16.97	744.6 ± 20.71	7.53 ± 0.09	6.99 ± 0.13	7.63 ± 0.18
S8	27.30 ± 0.28	8.29 ± 0.17	1068.00 ± 25.46	612.15 ± 4.38	7.26 ± 0.07	6.73 ± 0.36	8.71 ± 0.26
S9	21.20 ± 0.14	8.81 ± 0.07	933.55 ± 19.30	537.07 ± 4.99	7.46 ± 0.07	6.94 ± 0.49	3.32 ± 0.30
S10	24.65 ± 0.35	7.55 ± 0.10	565.30 ± 30.97	320.15 ± 7.03	6.95 ± 0.06	6.62 ± 0.46	3.10 ± 0.09

Table 3. Water quality parameters of Munroe Island

Stations	BOD (mg/l)	COD (mg/l)	Hardness (mg/l)	Alkalinity (mg/l)	NO_3- (mg/l)	PO_4^{3-}(mg/l)	SiO_2 (mg/l)
S1	0.17 ± 0.16	9 ± 1.41	450.00 ± 70.71	23.00 ± 1.41	0.401 ± 0.014	0.22 ± 0.05	7.21 ± 0.34
S2	0.41 ± 0.06	11 ± 1.41	3100.00 ± 141.42	53.00 ± 1.41	0.185 ± 0.005	0.30 ± 0.04	4.63 ± 0.70
S3	0.25 ± 0.40	13 ± 1.41	2350.00 ± 141.42	37.00 ± 1.41	0.644 ± 0.029	0.34 ± 0.10	5.13 ± 0.70
S4	0.78 ± 0.29	15 ± 2.83	3350.00 ± 70.71	47.00 ± 1.41	0.306 ± 0.021	0.29 ± 0.04	4.96 ± 0.36
S5	0.41 ± 0.06	14 ± 1.41	2650.00 ± 70.71	45.00 ± 2.83	0.245± 0.058	0.32 ± 0.04	3.93± 0.32
S6	0.87 ± 0.16	14 ± 2.83	3300.00 ± 141.42	61.00 ± 1.41	0.306 ± 0.021	0.27 ± 0.07	3.83 ± 0.42
S7	0.39 ± 0.09	13 ± 1.41	3550.00 ± 70.71	56.00 ± 2.83	0.385 ± 0.041	0.15 ± 0.01	4.82 ± 1.14
S8	0.45 ± 0.13	19 ± 1.41	1350.00 ± 70.71	34.00 ± 2.83	0.345 ± 0.041	0.22 ±0.02	5.46 ± 0.49
S9	0.67 ± 0.44	13 ±2.83	1425.00± 35.36	29.00 ± 1.41	0.378 ± 0.048	0.29 ± 0.17	5.01 ± 0.29
S10	0.60 ± 0.21	11 ± 1.41	1100.00 ± 141.42	26.00 ± 2.83	0.298 ± 0.032	0.25 ± 0.03	7.65 ± 0.68

reproductive quality, egg production, and the ability to tolerate environmental variations (Brennan et al 2022).

Canonical correspondence analysis: The relationships between zooplankton abundance and water quality variables were explored by CCA ordination (Turhan and Gokçe 2022). The first canonical axis accounted for 72.65% (Eigenvalue, 0.031) and the second for 21.1% (Eigenvalue, 0.009) of the variance. The CCA model confirmed that water quality variables such as DO, NO_3-, PO_4^{3-}, SiO_2, positively correlated to zooplankton groups such as Amphipoda and Decapoda in the first axis. The conductivity, TDS, pH, hardness, alkalinity, salinity, and BOD showed negative correlation with zooplankton groups such as chaetognatha, copepoda (Eigenvalue, -0.55), and luciferidae, in the first axis (Fig. 4). In the second canonical axis, environmental variables, temperature, turbidity, BOD (Eigenvalue, 0.76), COD, salinity (Eigenvalue, 2.63), PO_4^{3-} (Eigenvalue, 1.12), and SiO_2 (Eigenvalue, 0.62), and zooplankton group copepoda were positively correlated whereas Conductivity, TDS, pH, hardness, alkalinity, DO, and NO_3- (Eigenvalue, -0.99) and zooplankton groups such as chaetognatha, amphipoda, decapoda, and luciferidae in the second axis (Fig. 4). All the aforementioned hydrographical variables revealed a strong relation to zooplankton abundance.

The ordination of the response variables by CCA showed that the patterns were significantly associated to the spatial variation observed in the Munroe Island and indicated clear separation of zooplankton groups based on their environmental requirements. Zooplankton groups such as amphipoda and decapoda showed the peak of its abundance where dissolved oxygen level was high (Granata et al 2020, Purushothaman et al 2021). The CCA also revealed the abundance of the remaining groups in habitats with high conductivity, TDS, alkalinity, hardness and salinity concentration, implying were able to tolerate habitats with such environmental conditions (Furst et al 2019, El-Metwally et al 2022). The obtained CCA results are very much similar to the indication of correlation coefficient results (Table 5). This analysis discloses the influence of water quality variations in Munroe Island.

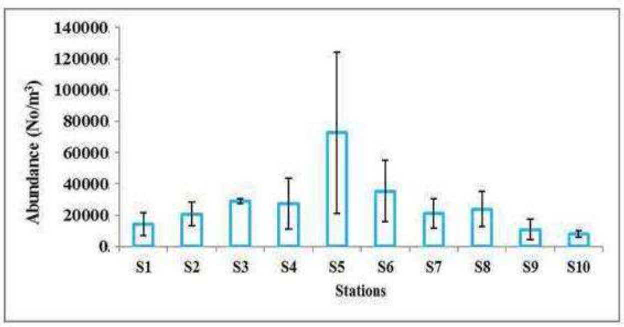
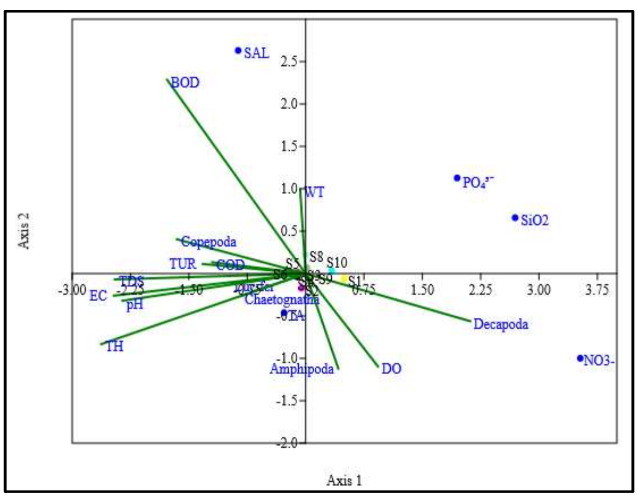


Fig. 2. Zooplankton abundance (No/m^3) in study sites of Munroe Island



Abbreviations: Z1: Chaetognatha, Z2: Amphipoda, Z3: Decapoda, Z4: Copepoda, Z5: Luciferidae, WT: water temperature, DO: dissolved oxygen, EC: electrical conductivity, TDS: total dissolved solids, TH: total hardness, TA: total alkalinity, SAL: salinity, BOD; biological oxygen demand, and COD: chemical oxygen demand

Fig. 3. Canonical correspondence analysis triplot with zooplankton community and environmental variables

Table 4. Canonical correspondence analysis

Variables	Axis 1	Axis 2
Chaetognatha	-0.276	-0.088
Copepoda	-0.551	0.135
Amphipoda	0.142	-0.373
Decapoda	0.705	-0.187
Luciferidae	-0.321	-0.041
Temperature	-0.024	0.331
Turbidity	-0.442	0.038
Conductivity	-0.821	-0.087
TDS	-0.817	-0.023
pH	-0.785	-0.107
Dissolved oxygen	0.312	-0.365
BOD	-0.593	0.762
COD	-0.412	0.044
Total hardness	-0.876	-0.277
Total alkalinity	-0.277	-0.462
Salinity	-0.867	2.631
NO_3-	3.532	-0.999
PO_4^{3-}	1.948	1.128
SiO_2	2.693	0.659

Table 5. Pearson correlation coefficient matrix analyzedvariables of Munroe Island ($p < 0.05$)

Variables	Z1	Z2	Z3	Z4	Z5	WT	TUR	EC	TDS	pH	DO	BOD	COD	TH	TA	SAL	NO_3-	PO_4^{3-}	SiO_2
Z1	1																		
Z2	0.14	1																	
Z3	0.62	0.23	1																
Z4	0.49	0.36	0.69	1															
Z5	0.05	0.02	0.91	0.55	1														
WT	0.75	0.83	0.29	0.94	0.85	1													
TUR	0.21	0.35	0.82	0.19	0.79	0.24	1												
EC	0.24	0.41	0.56	0.09	0.14	0.71	0.28	1											
TDS	0.29	0.46	0.39	0.10	0.16	0.73	0.25	0.30	1										
pH	0.94	0.77	0.96	0.04	0.82	0.43	0.19	0.24	0.22	1									
DO	-0.07	0.62	0.70	-0.34	-0.45	-0.46	-0.27	-0.89	-0.79	-0.63	1								
BOD	0.85	0.32	0.45	0.03	0.79	0.43	0.23	0.41	0.35	0.19	-0.84	1							
COD	0.09	0.98	0.97	0.25	0.49	0.60	0.01	0.28	0.25	0.19	-0.95	0.35	1						
TH	0.50	0.23	0.63	0.21	0.65	0.81	0.53	0.31	0.41	0.03	-0.58	0.55	0.31	1					
TA	0.72	0.24	0.58	0.32	0.70	0.93	0.30	0.41	0.31	0.04	-0.88	0.55	0.30	0.40	1				
SAL	0.84	0.09	0.11	0.15	0.73	0.51	0.25	0.04	0.32	0.11	-0.43	0.40	0.31	0.05	0.03	1			
NO_3-	0.56	0.90	0.64	0.15	0.74	0.60	0.19	0.39	0.38	0.06	0.26	0.12	0.40	0.13	0.21	0.10	1		
PO_4^{3-}	0.89	0.47	0.64	0.33	0.80	0.16	0.16	0.23	0.21	0.40	0.49	0.92	0.32	0.04	0.05	0.36	0.49	1	
SiO_2	0.30	0.06	0.78	0.02	0.23	0.66	0.11	0.40	0.30	0.41	0.59	0.20	0.37	0.40	0.40	0.02	0.27	0.02	1

Values in bold are different from 0 with a significance level alpha=0.05

CONCLUSION

The water quality variables influencing zooplankton abundance fluctuated markedly with the spatial scales in Munroe Island of Kerala which were affected by various human interference. The higher concentrations of conductivity, TDS, hardness, alkalinity and salinity were considerably contributed by the Ashtamudi estuary. Results from CCA indicated, the clear separation of zooplankton groups based on the changes occurred in the water quality in spatial scale. This first case approach provides new insights for the conservation policies of Munroe Island under various anthropogenic influences.

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Growth and Instability Analysis of Shrimps Farming in India and Karnataka

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Abstract: The present study was undertaken to analyze the area and production trends of shrimps farming in India and Karnataka. This study was primarily based on secondary data and was analysed using statistical tools like Compound Annual Growth Rate (CAGR) and Instability Indies such as Coefficient of Variation (CV) and Cuddy Della Valle II (CDV-II). During the study period, the area and production of tiger and scampi shrimps in India were trending downward whereas the area and production of white shrimps in India during the same period showed an upward tendency year over year. The same pattern was seen in Karnataka as well. The C.V. of white shrimps was more compared to other types of shrimps in India and Karnataka. CDV-II instability index showed that white shrimp's area and production fluctuated the most followed by Scampi and tiger shrimps. Environmental issues have always been the point of debate in shrimp farming which was the main hindrance to the increased area under shrimp farming. Hence, the policy maker must look upon the issues and allow the development of shrimp farming in an environment-friendly and sustainable manner.

Keywords: Compound Annual Growth Rate (CAGR), Coefficient of Variation (CV), Cuddy-Della Instability Index (CDII)

Aquaculture sector contributes around 1 per cent to India's Gross Domestic Product (GDP) and over 5 per cent to the agricultural GDP (Anonymous 2021a). Brackishwater aquaculture, the farming of shrimps, shellfishes and finfishes along the coastal line of the country and in inland saline areas is a vibrant farming sector, under the aquaculture umbrella. Brackish water aquaculture sector is dominated by shrimp farming is the economic engine of Indian aquaculture when considering the significant contribution of this sector to food production, employment generation and economic benefits. Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu. (CIBA) defined the freshwater aquaculture as "the farming of Brakishwater organism, in estuaries, coastline, backwater, lagoons and mangroves. etc. involving interventions in the rearing process to enhance production including shrimps, sea bass, grey mullet mud crabs etc. is called freshwater aquaculture. It is also called coastal aquaculture. These areas are commonly located at estuaries, coastlines, backwater, lagoons and mangroves. There are about 3.9 million ha of estuaries and 0.5 million ha of coastal mangrove areas available in the country. The estimated brackish water area suitable for undertaking shrimp cultivation in India is around 11.90 million ha which is spread over nine states and four Union Territories out of which 1.23 million ha area already under shrimps farming which is only 12.96 per cent of the potential area. Hence, India has a lot of potential in shrimp farming (Marine Product Export Development Authority, Kochi, Kerala (MPEDA 2021). Shrimps farming is an aquaculture business that exists in a brackish water environment, producing shrimps for human consumption. In India, mainly three types of shrimps are commonly reared which are tiger shrimps (*Penaeus monodon*), white shrimps (*Litopenaeus vennamei*) and scampi shrimps (*Macrobrachium rosenbergii).* Commercial Shrimps farming started in India during the 1900's with the production of tiger and scampi shrimps whereas white shrimp production started in 2009 as a result aquaculture business grew quickly.

Shrimps are an important source of many components such as proteins, vitamins, minerals and omega 3-fatty acids which will help us to protect from heart and brain related problems. The total shrimp production in the world in 2020-21 was 22.50 Million tonnes. Ecuador is the world's top shrimp producer, with 30 per cent (6.72 Million tonnes) of total shrimp production, followed by India with 24 per cent (5.38 Million tonnes). When it comes to shrimp importers, the United States is the largest importer of shrimps which is accounting for nearly 30 per cent (7.00 Million tonnes) of the total exports of India (Anonymous 2021b). The total area under shrimp culture in India is 1,66,722.51 ha with a production of 8,43,633 tonnes (Anonymous 2021c). The total shrimp production in India has decreased from 8,51,664 tonnes in 2019-20 to 7,56,651 tonnes in 2020-21, owing to the COVID-19 pandemic and related lockdown, as well as continuing production concerns due to diseases, particularly white spot diseases.(https://aquaasiapac.com/2021/07/31/indias-farmed-shrimp-in-2020-a-white-paper/)

West Bengal has the highest area and production of shrimps, followed by Odisha and Andhra Pradesh. The total area under shrimp culture in Karnataka is 3145.39 ha, with a production of 3185.84 tonnes; among the total shrimps, the area under tiger shrimp culture is 2175 ha, with an output of 1050 tonnes. The area under white shrimps is around 970.39 hectares, with a yield of 2185.84 tonnes. In Karnataka, the area under scampi shrimps is insignificant (https://mpeda.gov.in/?page_id=651). Shrimp production touched more than 7.0 lakh tonnes in 2021-22 of which 87 per cent is exported to the United States of America, China, Japan, European Union and South East Asia earning a robust foreign exchange to the tune of more than Rs. 35,000 crores (Anonymous 2021c). Shrimp farming is gaining importance in recent years due to the increase in the demand in the international market as well as in the domestic market too. In this backdrop, the present study aims at exploring the and magnitude of shrimp farming in India and Karnataka

MATERIAL AND METHODS

The secondary data were collected to fulfil the study's objectives of 2008-09 to 2019-20, from the Statistical Offices of Karnataka, Handbook of Fisheries Statistics, MPEDA Website and other published sources to analyse the growth and instability analysis of shrimp area and production in the state as well as in the country.

Compound annual growth rate: For computing compound annual growth rates of area, production of different types of shrimps, the exponential function of the following form was used.

$$Y_t = a.b^t.e^u (1)$$

Where, Y = Dependent variable (area or production shrimps) , a = Intercept term

b = Regression coefficient , t = Time period, e^u = error term

The equation (1) was transformed into log linear form and written as;

$$\log Y = \log a + t \log b + U_t \text{ ....(2)}$$

Equation (2) was estimated by using Ordinary Least Squares (OLS) technique.

Compound growth rate (g) was then computed

$$g = (b – 1)\times 100 \text{ .... (3)}$$

Where, g = Compound growth rate in per cent per annum , b = Antilog of log b

The standard error of the growth rate was estimated and tested for its significance with 't' test statistic.

Instability analysis: In order to study stability in different types of shrimps with respect to area, and production, co-efficient of variation was estimated using the expression given below.

$$\text{C.V.} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Cuddy and Della Instability Index (CD II): The formula suggested by Cuddy and Della (1978) was used to compute the degree of variation and trend as follow:

$$\text{Instability index} = \frac{\text{Standard Deviation}(\sigma)}{\sqrt{1 - R^2}\text{Mean}(\overline{x})} \times 100$$

RESULTS AND DISCUSSION

Extent and magnitude of shrimp farming in India and Karnataka: The area and production of tiger shrimps in India was showed decreasing over the years from 2008-09 to 2019-20 (Table 1). India's average tiger shrimp area, production from the past twelve years was 81.64 Lakh ha and 82.23 Lakh tons with a negative compound annual growth rate of 6.97 per cent and 8.04 per cent respectively. Similarly, the area and production of scampi shrimps also showed decreasing trend over the years. India's average scampi shrimp area, production over the past twelve years was 8.40 Lakh ha and 6.89 Lakh tons with the compound annual growth rate of -1.87 per cent and 2.56 per cent, respectively. However, the area and production of white shrimps in India significantly increasing over the years from 2008-09 to 2019-20. India's mean white shrimp area, production from the past twelve years was 46.40 Lakh ha and 3039.34 Lakh tons with the compound annual growth rate of 104.76 per cent and 121.43 per cent, respectively. The total area and production of total shrimps in India were significantly increasing over the year in the study period. Its average total area, production from the past twelve years was 136.45 lakh ha and 398.48 Lakh tonnes with the growth rate of 2.93 per cent and 22.62 per cent respectively. The co-efficient of variation of tiger shrimps, white shrimps, and scampi shrimps and total shrimps area was 28.26., 82.54., 44.40., and 11.71 per cent respectively. The coefficient of variation of tiger shrimps, white shrimps, scampi shrimps and total shrimps production were 35.62., 85.00., 44.81., and 57.58 respectively.

The Cuddy and Della Valle-II instability index was used to compute the degree of variation around the trend. The white shrimp's area as well as production fluctuated the most followed by scampi shrimps and tiger shrimps. Cuddy and Valle Instability Index of white shrimps area was highest i.e. 47.62 followed by scampi area and tiger shrimps. Similarly, Cuddy and Valle Instability Index of white shrimps production was highest i.e. 52.25 followed by scampi shrimp, tiger shrimps and total shrimps (13.17). The area and production of tiger shrimps in Karnataka was showed decreasing trend over the years from 2009-10 to 2019-20 (Table 2).

Table 1. Extent and magnitude of shrimp farming in India (2008-09 to 2019-20)

Year	Tiger shrimps		White shrimps		Scampi shrimps		Total	
	Area ('000 ha)	Production ('000 tons)	Area ('000 ha)	Production ('000 tons)	Area ('000 ha)	Production ('000 tons)	Area ('000 ha)	Production ('000 tons)
2008-09	108789	75996	-	-	18421	12806	127210	88802.79
2009-10	102259	95919	283	1731	8154	6568	110696	104218
2010-11	113853	118575	2931	18247	5511	3721	122295	140543
2011-12	114370	135466	7837	80717	6244	4269	128451	220452
2012-13	93110	123302	22715	147516	3432	3625	119257	274443.9
2013-14	72177	76798	57267	250507	9175	3545	138619	330850
2014-15	71400	73155	50240	353413	9307	7989	130947	434557
2015-16	68846	81452	59116	406018	12706	10152	140668	497622
2016-17	58851	58163	87252	501297	6151	3377	152254	562837
2017-18	59099	57691	93496	622327	7121	9983	159716	690001
2018-19	58359	54902	75494	618678	7129	7222	140982	680802
2019-20	58653	35437	100206	711674	7520	9540	166379	756651
MEAN	81647.17	82238.10	46403.16	309343.83	8405.91	6899.75	136456.16	398481.64
S.D.	23078.20	29295.19	38305.33	262959.71	3732.79	3092.35	15991.48	239657.88
CAGR (%)	-6.97***	-8.04***	104.76***	121.43***	-1.87*	2.56 *	2.93***	22.62**
C.V. (%)	28.26	35.62	82.54	85.00	44.40	44.81	11.71	60.14
R^2	0.87	0.60	0.66	0.62	0.72	0.83	0.74	0.95
Cuddy Della Valle II	32.35	22.37	47.62	52.25	43.82	44.03	5.96	13.17

Note: 1. * Significant at 10 per cent level of significance
2. ** Significant at 5 per cent level of significance
3. *** Significant at 1 per cent level of significance
4. S.D. Standard Deviation
5. C.V. = Co-efficient of Variation
6. R^2= Co-efficient of Discrimination

Source: MPEDA, Govt. of India, 2009-2020

Table 2. Extent and magnitude of shrimp farming in Karnataka (2009-10 to 2019-20)

Year	Tiger shrimps		White shrimps		Total	
	Area ('000 ha)	Production ('000 tons)	Area ('000 ha)	Production ('000 tons)	Area ('000 ha)	Production ('000 tons)
2009-10	1484	1581	01	01	1485	1582
2010-11	1715	2090	03	01	1716	2091
2011-12	650	609	72	232	722	841
2012-13	240	180	154	484	394	664
2013-14	94	56	157	517	251	573
2014-15	688.6	498.7	124.7	623.2	813.4	1121.9
2015-16	1948	682	333	1045	2281	1727
2016-17	735	635	405	1457	1140	2092
2017-18	302	59	399	1465	701	1524
2018-19	690	94	219	918	909	1012
2019-20	590	34	539.97	1195.1	1129.97	1229.1
MEAN	830.6	592.60	218.69	721.66	1049.30	1314.27
S.D.	585.27	642.54	169.66	508.74	567.01	506.87
CAGR (%)	-4.68*	-26.31**	73.85***	90.95***	0.305**	0.92**
C.V. (%)	70.46	108.42	77.57	70.49	54.03	38.56
R^2	0.73	0.73	0.65	0.61	0.83	0.84
Cuddy Della Valle II	`69.35	76.24	45.57	43.86	54.03	38.47

See Table 1 for details

Karnataka's average tiger shrimp area, production from the past eleven years was 0.83 Lakh ha and 0.53 Lakh tons with a negative compound annual growth rate of - 4.68 and - 26.31 per cent, respectively. Similarly, the area and production of white shrimp in Karnataka was significantly increasing over the years from 2009-10 to 2019-20. Karnataka's average white shrimp's area, production over the past eleven years was 0.218 Lakh ha and 0.721 Lakh tons with the compound annual growth rate of 73.85 per cent and 90.95 per cent respectively. The area and production of total shrimps in Karnataka was significantly increasing over the year in the study period. Its average total area, production from the past eleven years was 1.04 lakh ha and 1.31 lakh tonnes with CAGR of 0.3 per cent and approximately one per cent, respectively. Scampi Shrimps area and production in Karnataka were negligible during the study period. The co-efficient of variation explains the variability in both area and production of shrimps in Karnataka. The co-efficient of variation of tiger shrimps, white shrimps and total shrimps area was 70.46, 77.57, and 54.03 per cent respectively. Similarly, co-efficient of variation of the shrimp's production was 108.42, 70.49, and 38.56 respectively (Guledagudda et al 2020). The Cuddy and Della Valle instability index was used to compute the degree of variation around the trend. The index showed that tiger shrimp's area as well as production fluctuated the most as compared to white shrimps in Karnataka. The Cuddy & Valle Instability Index of tiger shrimps area was 69.35 followed by total shrimps, white shrimps. The, tiger shrimps production the instability values was 76.24 followed by white shrimps (43.86), total shrimps (38.47) (Kumar et al 2021). The results indicated that the area and production of tiger shrimps and scampi shrimps in India as well as in Karnataka showed decreasing trend over the study period which was associated with a negative Compound Annual Growth Rate (CAGR) which was mainly due to the Number of the shrimps life cycle in a year is less which is almost two crop a year, poor quality of fingerlings, non-availability of good quality fingerlings, less number of hatcheries specifically for the rearing of tiger and scampi shrimps, the productivity of tiger and scampi shrimps is less, susceptible to many diseases such as white spot syndrome viruses, gregarine diseases, lack of demand in the international market. The white shrimps in India and Karnataka showed an increasing trend during the study period with positive CAGR which was mainly due to the number of cycles in a year is more which is almost three crops a year, the productivity of white shrimps or Exotic Shrimps was more. Improvements in production and farming technologies, introduction of genetically improved species, very high demand in the international market, more number of modern types of hatcheries specifically meant for the exotic shrimps which are controlled by aquaculture authority of India.

CONCLUSIONS

The white shrimps/ exotic shrimps area and production were increasing over the study period due to the high demand in the international market. The domestic shrimps like tiger and scampi shrimps area and production showing a decreasing trend. Hence, the policy maker should provide enough emphasis on shrimp farming by providing them credit and other extension facilities and should not neglect tiger shrimps and scampi shrimps farming because of its their low yield. The promotion of this farming helps to improve rural livelihood which is one of the major goals of sustainable aquaculture.

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Assessment of Some Morphometric and Meristic Characters of *Schizopyge niger* from Two Lacustrine Populations of Kashmir, India

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Abstract: Morphometric studies were conducted using twenty quantitative body parameters of a Schizothoracid species *Schizopyge niger* collected from two lakes of Kashmir *viz*. Dal and Manasbal. 240 samples of the fish were studied for morphometric and meristic characters employing local fishing gears. Twenty conventional morphometric characters examined in the present study, showed high co-efficient of determination(R^2) values ranging from 0.70 to 0.92 for Dal lake and 0.53 to 0.93 for Manasbal lake signifying that the traits were highly correlated to each other. Out of eight meristic characters examined, three characters *viz.* Pectoral fin rays, Caudal fin rays and Pelvic fin rays were significantly different

Keywords: Schizothorax, Dal, Manasbal, Snowtrout, Ael gaad

The valley of Kashmir is endowed with vast potential of aquatic resources in terms of upland rivers, streams, high and low altitude natural lakes. The biological productivity of these aquatic resources is incredibly high. These habitats comprise essentially of indigenous and exotic fish species. The water within the water bodies of Kashmir is cold, crystal clear, has high oxygen content and also temperate climate provides exceptional habitats for a peculiar type of fish fauna. The Dal lake, situated in the northeast of Srinagar is one of the most beautiful lakes in India and the second largest lake in Jammu and Kashmir (Rather and Nazir 2015). The lake comprises of five basins viz.; Hazratbal, Nishat, Nehrupark, Nigeen and Brarinambal. Each basin maintains its individual character but these basins vary in their morphometry, water quality and biodiversity (Abubakr and Kundangar 2005). The lake is shallow with saucer shaped basin and has an open drainage i.e., regular inflow and outflow of water takes place (The lake is probably of fluvial origin, formed from the oxbows of river Jhelum. The total open water surface of the Dal lake is only 11.4 km^2 and the rest is under floating gardens most of which have now settled permanently (Qadri and Yousuf 2008). Fish like *Schizothorax curvifrons*, *Schizopyge niger*, *Schizothorax esocinus, Crossocheilus diplochilus*, *Carassius carassius*, *Puntius conchonius*, *Gambusia holbrooki, Botia birdi, Cyprinus carpio* var. *communis*, *Cyprinus carpio* var. *specularis* have been encountered from Dal lake, Kashmir (Ahmed et al 2017).

The Manasbal lake is situated between the districts of Ganderbal and Bandipora of Kashmir valley. The lake is encircled by three villages *viz.* Jarokabal, Kondabal and Sumbal. This is the solitary lake in Kashmir that develops summer stratification and is categorised as warm monomictic lake (Dad et al 2008).The lake covers an area of 2.81 km^2 and its volume and maximum depth are 0.0128 km^3 and 13 m respectively. It is primarily fed by precipitation (rain and snow fall) and springs. The lake water discharges into the Jhelum River through a regulated outflow channel (Dar et al 2015). Seven commercially important fish species namely, *Schizothorax esocinus, S. curvifrons, S. niger, S. plagiostomus, Tryplophysa sp., Cyprinus carpio* (*C. carpio* var*. communis* and *C. carpio* var. *specularis* and *Ctenopharyngodon idella* contribute to the commercial fisheries of this lake (Mehraj et al 2016). The exotics contribute to a great extent to the total fish production in the lake as in other lakes of the Kashmir valley. However, the endemic schizothoracids are fast diminishing as these fishes cannot thrive well in polluted water (Bhat and Yousuf 2004, Balkhi 2007).

Schizopyge niger locally known as 'Ael gad'**,** inhabit both lentic as well as lotic water bodies of Kashmir. The fish is characterised by elongate, fusiform body with a short, blunt and slightly prognathous upper jaw. Barbels are shorter than eyes, sides with small blackish dots are present and scales are very small. *S. niger* differs from all the other

Schizothoracines in the combination of thick lower lip folds and few gill rakers. The fish population has declined to a large extent due to multiple factors especially habitat destruction, overfishing, competition for food and breeding grounds from exotic carps, water pollution etc (Mir and Channa 2010, Bhat et al 2010).

The long-term isolation of populations and interbreeding can lead to morphometric variations within populations, and this morphometric variation can provide a basis for population differentiation. As morphometric and meristic characters have key importance in the systematic classification of fish, the present study has been conducted to observe the phenotypic variation among lacustrine populations of this species.

MATERIAL AND METHODS

A total of 240 samples of *S. niger* were collected from two lakes of Kashmir namely Dal and Manasbal. Dal lake is known as the “Jewel of Kashmir”. This is the second biggest lake of Jammu and Kashmir. The Dal having catchments of about 316 km^2 and is situated between geographical coordinates of 34.1106° N, 74.8683° E. Manasbal Lake, the urban valley lake has its own unique identity due to its natural view which attracts the tourists and is situated between geographical coordinates of 34.2561° N, 74.6763° E. 120 samples of *S. niger* were collected from Dal and 120 samples of *S. niger* were collected from Manasabal and examined for evaluation of body parameters from the period of 15 March 2021 to 15 March 2022. The samples were collected with the help of local fishers and carried to the laboratory containing ice packs. The samples were then thoroughly cleaned and dried. Examination of morphometric and meristic characters were carried out by following the methods given by Lagler et al (1962) and Laevastu (1965), Morphometric measurements were made using digital callipers. In the present study, 20 morphometric and 8 meristic characters of each fish sample were studied. Total length (TL) and all other measurements were taken in millimetres. Meristic counts were analysed following the conventional method as described by Hubbs and Lagler (1958). All the meristic counts were set up against incoming light direction in the room with the help of needle and small pins for easy counting. Statistical analysis was carried out with the use of MS Excel and SPSS software.

RESULTS AND DISCUSSION

Morphmetry: Measurements of numerous morphometric traits of *S. niger*, are described in Table 1. Out of two sampling sites, caudal fin length displayed highest coefficient of variation (19.48%) and pre anal length displayed lowest

Table 1. Descriptive statistics of morphometric traits of *S. niger* (Pooled)

Morphometric measurement	Mean ±SE	CV%
Total Length (TL)	209.61±1.60	11.87
Standard Length (SL)	173.43±1.40	12.52
Fork Length (FL)	191.45±1.50	12.16
Head Length (HL)	37±0.33	13.81
Pre-Orbital Length (ProL)	14.36±0.14	15.17
Post-Orbital Length (PoOL)	26.8±0.30	17.40
Eye Diameter (ED)	11.00±0.09	13.32
Pre-Dorsal Length (PDL)	94.28±0.68	11.30
Pre-Pectoral Length (PPcL)	42.93±0.31	11.30
Pre-Pelvic Length (PPvL)	113.64±0.51	12.41
Pre-Anal Length (PAL)	141.79±0.95	10.40
Dorsal Fin Length (DFL)	32.72±0.27	13.02
Pectoral Fin Length (PCL)	32.53±0.45	21.89
Pelvic Fin Length (PVL)	30.75±0.32	16.34
Anal Fin Length (AFL)	28.15±0.26	14.51
Caudal Fin Length (CFL)	31.05±0.39	19.48
Body Depth (BD)	36.61±0.38	16.31
Caudal Depth (CD)	30.07±0.30	15.26
Dorsal Fin Base (DFB)	21.24±0.20	14.65
Anal Fin Base (AFB)	13.82±0.14	16.59

Table 2. Correlation between various morphometric traits of *S. niger* for Dal (Srinagar)

Morphometric characters	y=a+bx	R^2
Total length and standard length	6.70+0.79x	0.82
Total length and fork length	6.86+0.88x	0.88
Total length and head length	0.87+0.18x	0.77
Total length and pre orbital length	1.56+0.07x	0.80
Total length and post orbital length	10.46+0.17x	0.92
Total length and eye diameter	0.34+0.05x	0.81
Total length and pre dorsal length	18.08+0.36x	0.71
Total length and pre pectoral length	7.82+0.16x	0.72
Total length and pre pelvic length	30.66+0.31x	0.78
Total length and pre anal length	36.87+0.50x	0.71
Total length and dorsal fin length	1.86+0.14x	0.73
Total length and pectoral fin length	18.18+0.24x	0.71
Total length and pelvic fin length	7.64+0.18x	0.83
Total length and anal fin length	2.57+0.14x	0.80
Total length and caudal fin length	13.70+0.21x	0.77
Total length and body depth	7.18+0.20x	0.76
Total length and caudal depth	5.26+0.17x	0.83
Total length and dorsal fin base	1.28+0.10x	0.74
Total length and anal fin base	2.46+0.07x	0.70

coefficient of variation (10.40%). Coefficient of determination (R^2) values fluctuating from 0.70 t0 0.92 for Dal and from 0.53 to 0.93 for Manasbal, implying that the traits were highly interrelated to each other.

Meristics: For meristics, Z test, number of pectoral fin rays, caudal fin rays and pelvic fin rays were significantly ($p<0.01$) different amongst fish from these stocks and variance existed in other meristic traits (Lateral line scales: Z = 2.42, $p>0.05$; Scales above the lateral line: Z= 0.50, $p>0.05$; Scales below the lateral line: Z= 0.91, $p>0.05$; Dorsal fin rays: Z= 0.71, $p>0.05$; Pectoral fin rays: Z =5.2, $p<0.01$; Caudal fin rays: Z= 6.4, $p<0.01$; Pelvic fin rays: Z=6.2, $p<0.01$; Anal fin rays: Z=0.4, $p>0.05$.

In the present study, numerous morphometric traits compared exhibited high coefficient of corrections (r) values, which specify that the morphometric characters investigated are extremely correlated to each other. Akyol and Kinacigil (2001) establish that discriminate examination of seven morphometric characteristics in mature specimens of grey mullets demonstrated the fact that *Liza aurata* and *Liza saliens* were alike in forms Research work done on morphological characteristics of *Cirrhinus reba*, *Gudusia chapra, Channa punctatus* have observed the similar result which was in agreement with our present findings (Lashari et al (2004), Narejo (2010) and Dars et al (2012).

Shah et al (2011) examined the morphometry of farmed female rainbow trout in Kashmir and testified great level of interdependence amongst the fourteen morphometric characters studied. Bhat et al (2016) documented similar results in *Cyprinus* sp. Out of eighteen characters in relation

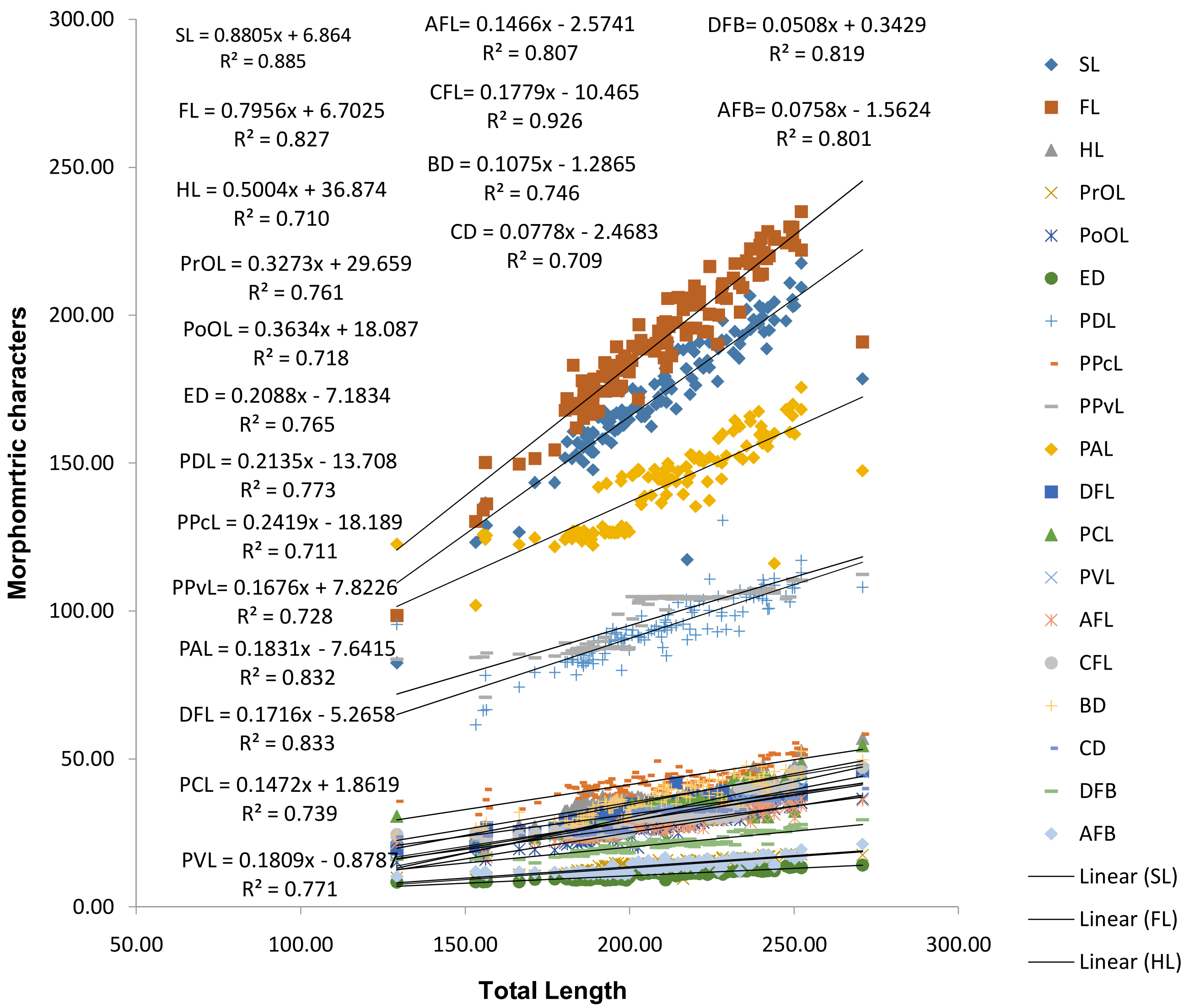

Fig. 1. Logarithmic relationship of different morphometric traits of *S. niger* from Dal

Table 3. Correlation between various morphometric traits of *S. niger* for Manasbal (Ganderbal)

Morphometric characters	y=a+bx	R^2
Total length and standard length	11.42+0.78x	0.80
Total length and fork length	11.12+0.85x	0.81
Total length and head length	1.06+0.15x	0.71
Total length and pre orbital length	3.55+0.05x	0.86
Total length and post orbital length	12.23+0.15	0.92
Total length and eye diameter	5.09+0.03x	0.83
Total length and pre dorsal length	37.20+0.28x	0.58
Total length and pre pectoral length	39.70+0.04x	0.84
Total length and pre pelvic length	29.04+0.32x	0.67
Total length and pre anal length	43.33+0.47x	0.54
Total length and dorsal fin length	29.62+0.04x	0.86
Total length and pectoral fin length	5.56+0.12x	0.53
Total length and pelvic fin length	7.71+0.04x	0.88
Total length and anal fin length	13.52+0.06x	0.89
Total length and caudal fin length	24.81+0.04x	0.80
Total length and body depth	12.07+0.10x	0.80
Total length and caudal depth	9.84+0.07x	0.92
Total length and dorsal fin base	3.04+0.05x	0.72
Total length and anal fin base	0.02+0.06x	0.89

to total fish length, ten characters showed high values of correlation coefficient and eight characters showed moderate correlation coefficient. Qadri et al (2017) observed high coefficient of correction (r) values for various morphometric traits with standard length showing highest degree of correlation (R^2=0.88) with total length in *S. curvifrons*. The results of the present investigation for meristics revealed significant differences in three out of eight meristic charcteristics. Hossain et al (2010) recorded substantial

Table 4. Representing Z test performed on various meristic traits of two lacustrine populations

Meristic characters	Z value	P value
LL vs LL	2.42	>0.05
SALL vs SALL	0.50	>0.05
SBLL vs SBLL	0.91	>0.05
DFR vs DFR	0.71	>0.05
PCFR vs PCFR	5.2	<0.01
CFR vs CFR	6.4	<0.01
PLFR vs PLFR	6.2	<0.01
AFR vs AFR	0.4	>0.05

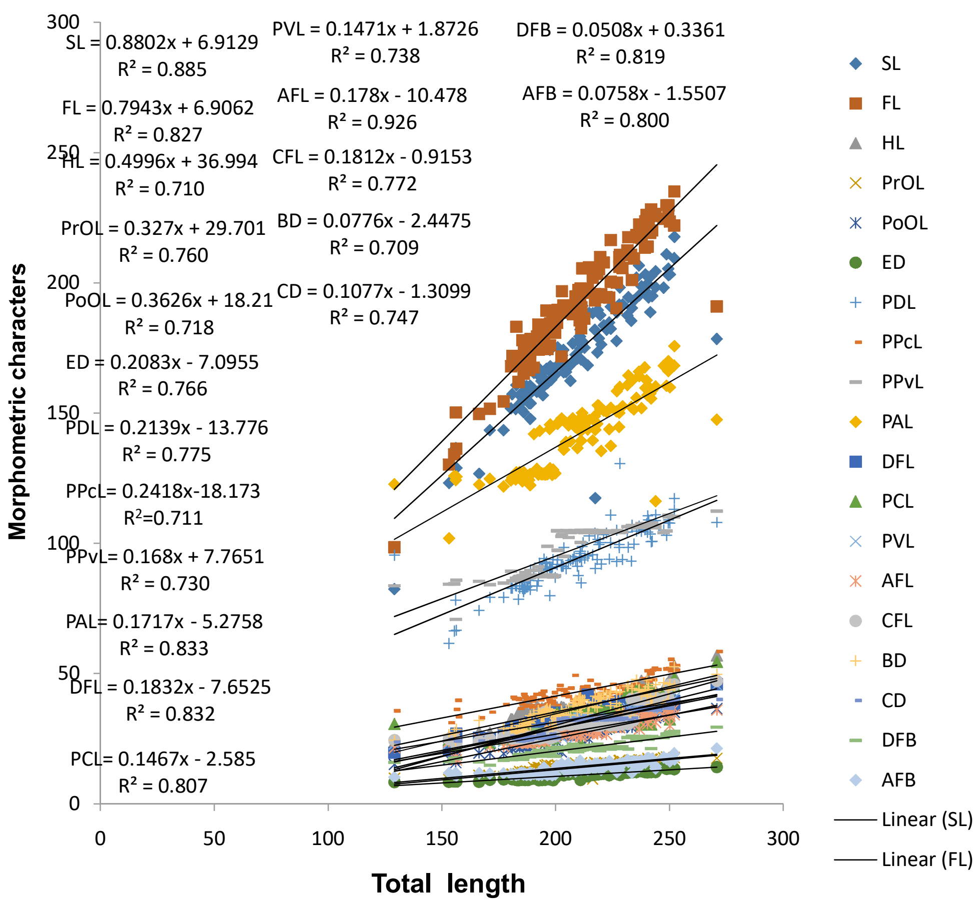

Fig. 2. Logarithmic relationship of different morphometric traits of *S. niger* from Manasbal

differences in two of 9 meristic counts in threatened carp, *Labeo calbasu* two isolated rivers, the Jamuna and the Halda and a hatchery in Bangladesh. Ample facts were shown to accept the statement that morphometry can distinguish between different species of fish and different populations

CONCLUSION

The populations of *S. niger* exhibit dissimilarity in morphometric and meristic traits in the studied locations. Twenty conventional morphometric characters examined in the present study, showed high co-efficient of determination(R^2) values ranging from 0.70 to 0.92 for Dal lake and 0.53 to 0.93 for Manasbal lake signifying that the traits were highly correlated to each other. Out of eight meristic characters examined, three characters (Pectoral fin rays, Caudal fin rays, Pelvic fin rays) were significantly different However, a detailed study involving the molecular genetics and environmental aspects may further confirm the present findings explicitly. In order to have better conservational policy and restocking programs, further studies are recommended on determining other possible populations of this species in other regions of valley.

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Potential Fishing Zones Persistence along Southern Tamil Nadu: A Case Study

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Abstract: Indian National Centre Ocean Information Services (INCOIS) develops potential fishing zones (PFZs) advisory maps for the coastal/marine fishing community using satellite technology to forecast fish shoal aggregation. Two hundred and sixty (260 nos) PFZ advisory maps for Southern Tamil Nadu between November 2015 and October 2017 were analysed to determine the possible persistence of the Spatio-temporal relationship with location and depth with PFZ hits. For ease of understanding, the region's fishing areas were divided into seven zones (one-degree grid) and further subdivided into 16 grids from Gopalapattinam of Pudukkottai to Neerodi of Kanyakumari waters, Tamil Nadu. The analyses indicate that the PFZ's area's persistence was more along Thoothukudi waters (Zone 4; 43.21%) and Kanyakumari (Zone 7; 28.43%), with 581 and 585 hits, respectively. The Chi-square test for goodness of fit (χ^2 = 1320.36) proved a significant difference among the total number of zone-wise hits. The results of bathymetry grid plotting hoped that a maximum number of PFZ hits were in the nearshore (<50 m; 41.5%), followed by the mid-continental shelf (50 to 200 m; 29.7%) and the continental slope (>200 m; 28.8%) regions. The total number of hits was more during the monsoon season (549 hits), followed by pre-monsoon (307 hits), post-monsoon (297 hits), and the least was during the summer season (92 hits). The Chi-square test for independence of attributes (χ^2 = 22.47) of season-wise and region-wise data also concludes that there is a significant association between region and season of occurrences of PFZ hits.

Keywords: Bathymetry, Gulf of Mannar, Geo-coordinates, Palk Bay, PFZ

The Indian marine fishery resources are open-access fishery harvested by diverse fishing gears and crafts. Therefore, managing the limited life below the water was difficult (Sathianandan 2013). Indian marine fish production exhibited different developmental stages and leaps to 3.56 mmt in 2019. Tamil Nadu leads the marine fish production (7.75 lakh tonnes) maritime states (CMFRI 2020), with a coastline of 1076 km scattered and 13 coastal districts. It comprises the Coromandel Coast (357 km), Palk Bay (294 km), Gulf of Mannar (365 km) and the West coast between Kanyakumari and Neerody (60 km). The State has a fisherman population of 1.05 million from 608 marine fishing villages, of which 0.20 million are engaged in fishing (CMFRI 2012). Given the fishermen's welfare and future developmental plans for increased production, several organizations are strengthening the fishing community's livelihood. Besides the decrease in coastal fishery resources, fishers are venturing into deep water, increasing the scouting time, which many fishing trips may not be economically feasible (Sinha et al 2017). However, marine fish stocks' wild fluctuations were significantly affected by the changes in ocean conditions and studying this will help the changes in ocean conditions. Analyzing this will formulate a suitable fishery management plan for marine resources (INCOIS 2020). Therefore, it is necessary to understand the oceanological and environmental parameters that influence fish stocks and their distribution. At this juncture, INCOIS develops PFZ advisory maps based on Sea Surface Temperature (SST) and Chlorophyll over the Arabian Sea and Bay of Bengal retrieved from thermal infrared channels of NOAA-AVHRR and optical bands in IRS-P4 OCM/MODIS Aqua data (MSSRF 2020). Various electronic and social communication modes were used to disseminate the PFZs information along the Indian coastline and Island regimes (INCOIS 2020). PFZ advisories were found most valuable to traditional, motorized. Small mechanized sector fishers engaged in pelagic fishing activities such as ring seining, gill netting, etc. The fishers utilized the advisory maps, which revealed that the maps significantly reduced the scouting time and fuel consumption and effort (Victor 2012). PFZ data has been validated by conducting fishing trips in PFZ and non-PFZ regions along with coastal areas of India (Nammalwar et al 2013, George et al 2014). The possible trends of PFZs based on the frequency of their occurrences have been utilized by various authors, viz Kerala coast (Kripa et al 2014), Maharashtra coast (Kamei et al 2014); Goa coast (Sreekanth et al 2016); North Andhra Pradesh coast (Edward et al 2019, Edward et al 2020). Studies have been conducted on the northern Tamil Nadu coast (Nammalwar et al 2013); however, no studies have been undertaken along the Southern Tamil Nadu coast. Hence, the current research attempts to conclude possible trends of PFZs along the Southern Tamil Nadu coast and analyze the frequency of its occurrences based on depths to help the fishermen recognize the fishing zones quickly and carry out fishing economically along the regions.

MATERIAL AND METHODS

The PFZ advisories received via email for Southern Tamil Nadu, India, from INCOIS, Hyderabad, for two years, from November 2015 to October 2017, were collected and used to analyze its frequency occurrences. The study site area was Southern Tamil Nadu viz Gopalapattinam of Pudukkottai (Lat. 09°00' to 10°00' and Long. 79°00' to 79°45') to Neerodi of Kanyakumari waters (Lat. 07°00' to 08°00' and Long. 77°00' to 78°00'). Every 1 x 1-degree grid falling into this area and connected with the fishing area was named zones starting from 1 to 7 from top to bottom (Fig. 1a). Each 1 x 1-degree grid is further divided into 16 smaller grids, starting from A to P, top to bottom and left to right (Fig. 1b). Details of each zone are given in Table 1, and the respective base maps are shown in Figures 1 a & b. A base map of INCOIS advisories with zones and smaller grids was overlaid in the PFZ advisory chart, and the PFZ lines (Special Oceanic Processes, SOPs indicated as curved line marking on the advisory chart) coinciding with the grids were recorded. Each occurrence of PFZ lines in a grid was taken as a hit. The number of PFZ hits on each grid was recorded for two years and taken for further plotting. The data were analyzed depth-wise, viz., up to 50 m - Near Shore (NS), 50 to 200 m - Mid Continental Shelf (MCS) and above 200 m - Continental Slope (CS) and region-wise for the frequency of its occurrences. Likewise, the raw data was segregated season-wise, viz., March to May as summer, June to August as pre-monsoon, September to November as Monsoon and December to February as post-monsoon seasons, based on the climate of this area (Ranjith et al 2018). The number of PFZ hits on each grid was added season-wise and taken for further plotting. The PFZ hit data for depth, season, area, and rainfall were statistically analyzed with SPSS software (Version 16.0) for Pearson's Correlation, Chi-square test for goodness of fit and Chi-Square test for independence of attributes. Colour-based hit charts for different depth zones and seasons were prepared based on the number of hits in each grid (Very high: > 30, High: 21-30, Medium: 11-20, Low: 1-10) using open-source software QGIS (Version 2.4.0) by incorporating required depth contour lines (50 m and 200 m)

Table 1. PFZ hits zone and its subgrids and region-wise geocoordinates along Southern Tamil Nadu

Region	Zone	Position		Sub- grids
Rameswaram waters*	1	Lat Long	09°00' to 10°00' 79°00' to 79°45'	9
	2	Lat Long	09°00' to 09°45' 78°15' to 79°00'	5
Thoothukudi waters**	3	Lat Long	08°24' to 09°00' 79°00' to 79°26'	5
	4	Lat Long	08°00' to 09°00' 78°00' to 79°00'	16
Kanyakumari waters**	5	Lat Long	08°00' to 08°21' 77°00' to 78°00'	6
	6	Lat Long	07°00' to 08°00' 78°00' to 78°48'	13
	7	Lat Long	07°00' to 08°00' 77°00' to 78°00'	16

Overlapping coastal regions: *Pudukottai waters & **Tirunelveli waters

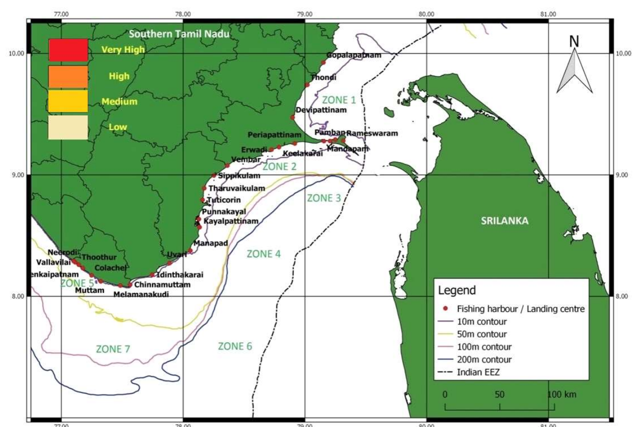

Fig. 1a. A base map of the Southern Tamil Nadu coast with different zones

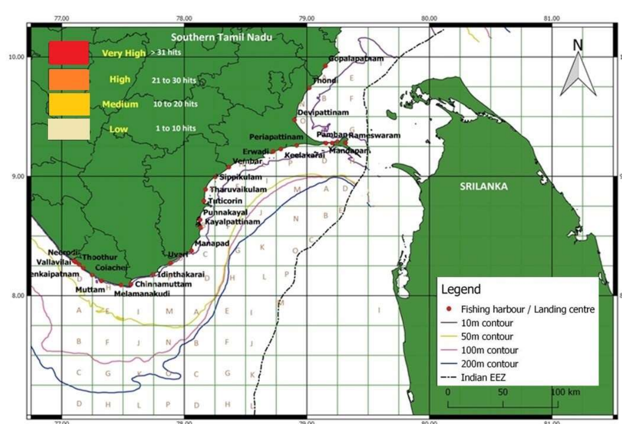

Fig. 1b. A base map of the Southern Tamil Nadu coast with different zones with sub-grids

in the study area. The monthly rainfall data for the study period were extracted from the Customized Rainfall Information System (CRIS) of the India Meteorological Department

RESULTS AND DISCUSSION

Zones and region wise: A detailed investigation of 260 PFZ advisory maps of Southern Tamil Nadu revealed their spatiotemporal relationship with geo-coordinates & depth and the persistence of the PFZ hits. The analyses indicate that PFZ hits (SOPs indicated as curved line marking on the advisory chart) were more between 8 to 9° N Latitude and 78 to 79° E Longitude of Thoothukudi waters, followed by between 7 to 8° N Latitude and 77 to 78° E Longitude of Kanyakumari waters. PFZ advisories were high from August to December (ranging between 12 to 18 nos per month), followed by January & February with 10 & 7 nos. PFZ hits were more between 8 to 9° N Latitude and 78 to 79° E Longitude of Thoothukudi waters, followed by between 7 to 8° N Latitude and 77 to 78° E Longitude of Kanyakumari. The trend is the same when we go through the number of grids with high and very high-frequency hits. Out of these zones, 3 and 4 come under Thoothukudi waters, and parts of Zones 5, 6 and 7 comes under Kanyakumari waters. Moreover, zone 1 and 2 comes under the Palk Bay waters. The PFZ hit data were plotted according to the different zones and smaller grids. Table 2 depicts the region/zone-wise number of PFZ hits and their percentage. The Chi-square test for goodness of fit ($\chi2$ = 1320.36) also proved that there is a significant difference ($P<0.01$) among the total number of hits zone-wise, and it is not the same. As per Table 2, Zone 4 from Thoothukudi waters has the maximum number of PFZ hits (538), followed by Zone 7 (354) and 6 (196) from Kanyakumari waters. Kamei et al. (2014) opined that this might be due to the uniqueness of the particular zones' topographical locations.

Depth wise: The results of bathymetry grid plotting hoped that the maximum number of PFZ hits were in the nearshore (<50 m; 41.5%), followed by the mid-continental shelf (50 to 200 m; 29.7%) and the continental slope (>200 m; 28.8%) regions. When comparing the PFZ hit frequencies among different depth zones and regions (Table 3) nearshore (NS) area, which is less than 50 m depth, shows the maximum number of PFZ hits (517), which is about 41.5% of the total PFZ hits, followed by MCS (369) and CS (359) with 29.7 and 28.8% hits, respectively. The Chi-square test for independence of attributes (χ^2 = 123.15) of depth-wise and region-wise data also concludes that there is a significant association between a region and depth of occurrences of PFZ hits. From the colour-based hit chart (Figs. 2, 3 & 4), a definite trend in depth-wise PFZ hits has been depicted. Starting with more number hits along NS regions, there is a steady decrease in the frequency of hits as depth increases, leading to a reduction in hits' frequency. An earlier study along the southwest coast (Kripa et al 2014) found the NS region to be the most frequently occurring zone, followed by MCS and CS. The authors have described the effect of river flow and discharge in the NS regions on more PFZ than in outlying areas. But, Edward et al (2020) reported the higher PFZ hits in the north Andhra Pradesh coast's deeper waters and attributed the reasons to low saline pools and freshwater plumes away from the coast. The present study area's relatively high productivity may be due to river Thamirabarani down south and its influence on nearshore waters.

The region-wise analysis shows an increasing trend in PFZ hits frequency from Rameswaram to Kanyakumari waters (Table 3 and 4). The reason being during NEMS, the East India Coastal Current (EICC) in the western Bay of Bengal flows equatorward (from higher to lower latitudes), and the main flow turns around Sri Lanka and transports low saline waters into the Arabian Sea, which makes the Kanyakumari waters more productive (Mukherjee et al 2014). Rameswaram waters have the lowest (79) PFZ hits of the three regions studied, and the more productive area is Kanyakumari waters, with the highest number of PFZ hits (585), followed by Thoothukudi waters (581). In all three regions, nearshore waters are more productive when compared to MCS and CS. Season-wise analysis for all the respective areas also showed a similar cyclic pattern in the occurrence of PFZ during different seasons, with the lowest and peak during summer and monsoon, respectively.

Season wise: The total number of hits was more during monsoon season (37.1 %), followed by pre-monsoon (26.7 %), post-monsoon (26.1%), and the least was during the summer (10.1%) season (Figs. 5, 6, 7 & 8). The zone-wise PFZ hit data for different seasons are depicted in Table 4. The Chi-square test for independence of attributes (χ^2 = 22.47) of

Table 2. PFZ hits and their persistence in per cent contribution

Region	Zone	Hits*	% Hits
Rameswaram waters	1	57	4.58
	2	22	1.77
Thoothukudi waters	3	43	3.45
	4	538	43.21
Kanyakumari waters	5	35	2.81
	6	196	15.74
	7	354	28.43
Total		1245	100

*Chi-square test for goodness of fit, $\chi2$ = 1320.36, significant – $P<0.01$

season-wise and region-wise data also concludes that it is a significant association between region and season of occurrences of PFZ hits. The plotting of PFZ advisories and lines revealed that the total number of hits was more during the monsoon season (549), followed by pre-monsoon (307), post-monsoon (297), and the least during the summer

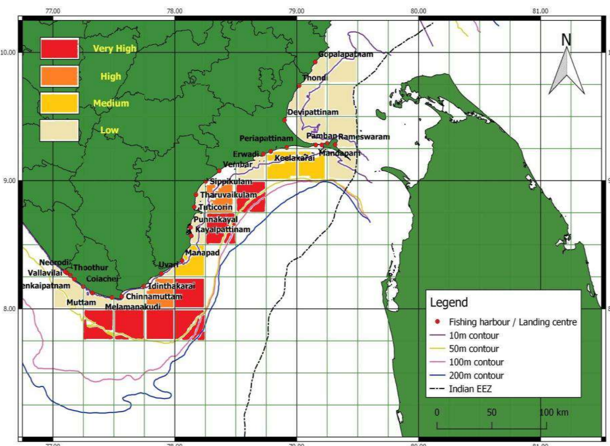

Fig. 2. Colour based hit chart for Near Shore (NS, <50 m) areas

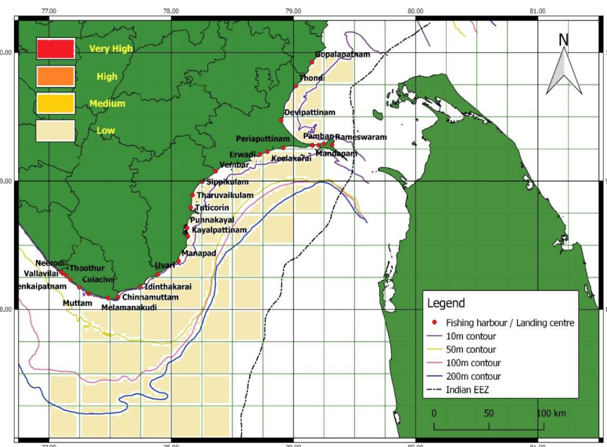

Fig. 5. Colour based hit chart for the summer season

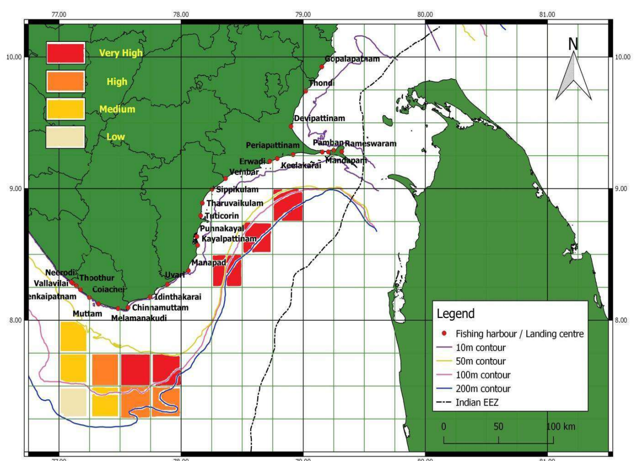

Fig. 3. Colour based hit chart for Mid Continental Shelf (MCS, 50-200 m)

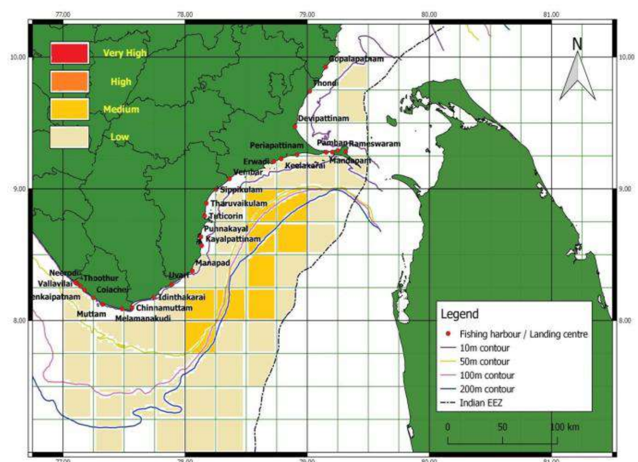

Fig. 6. Colour based hit chart for the pre-monsoon season

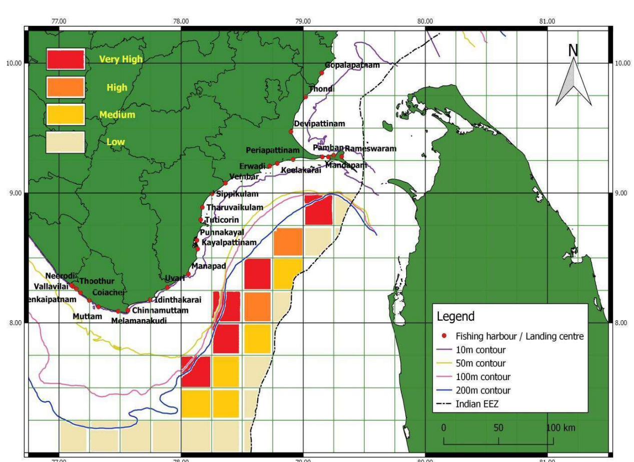

Fig. 4. Colour based hit chart for Continental Slope (CS, >200 m)

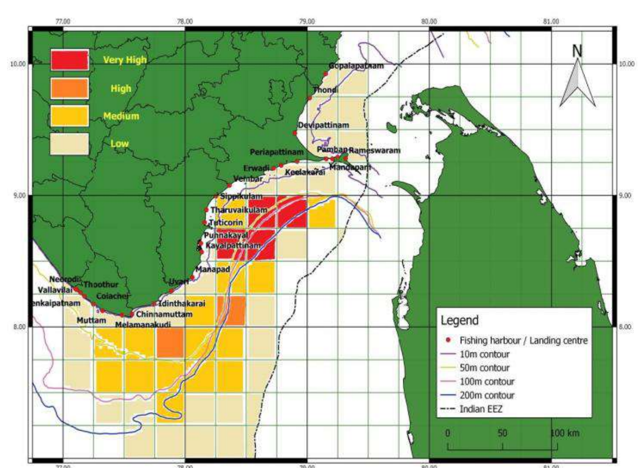

Fig. 7. Colour based hit chart for the monsoon season

Table 3. PFZ hits region-wise and depth-wise

Region	Depth zones*			Total
	< 50 m Nearshore	50 – 200 m Mid continental shelf	>200 m Continental slope	
Rameswaram waters	79	0	0	79
Thoothukudi waters	221	169	191	581
Kanyakumari waters	217	200	168	585
Total	517	369	359	1245

*Chi-square test for independence of attributes, $\chi2$ = 123.15, significant – P<0.01

Table 4. PFZ hits region wise and season wise

Region	Seasons*				Total
	Summer	Pre-Monsoon	Monsoon	Post-Monsoon	
Rameswaram waters	9	14	33	23	79
Thoothukudi waters	35	166	269	111	581
Kanyakumari waters	48	127	247	163	585
Total	92	307	549	297	1245

*Chi-square test for independence of attributes, χ^2 = 22.47, significant – P<0.01

season. The pattern of PFZ was cyclic during different seasons, with the lowest during summer followed by the increment in the number of hits during pre-monsoon and reaching a peak during Monsoon. Further, it decreases during post-monsoon, and the cycle of rotation continues. Pearson's Correlation (r = 0.97) also proved that there is a significant correlation between season-wise rainfall (mm) and PFZ hits in the study area (Fig. 9). This peak in the number of PFZ hits during monsoon may be due to the maximum rainfall in this season in the study area. Edward et al (2019) also observed a similar peak in monsoon seasons along the northern Andhra Pradesh coast due to the freshwater influx during Northeast Monsoon. Persistence in the PFZ's area was more along zone 4 of Thoothukudi waters due to the Thamirabarani River's discharge at Punnakayal estuary onset Northeast Monsoon (NEMS) creating more SOPs (Mukherjee et al 2014).

The data were analyzed region-wise, viz., Rameswaram waters, Thoothukudi waters, and Kanyakumari waters, for their frequency (Table 2). The region-wise analysis shows an increasing trend in PFZ hits' frequency as we move towards Kanyakumari waters. Rameswaram waters have the lowest (69) PFZ hits of the three regions studied. The relatively low productivity of nearshore waters may be due to the absence of significant rivers in the study area. Even though there is a river Thamirabarani south of the study area, its influence on nearshore waters is less. The present study area gets a considerable freshwater influx during the southwest monsoon. These offshore currents and low saline pools away from the coast support the present study for the occurrence of high-

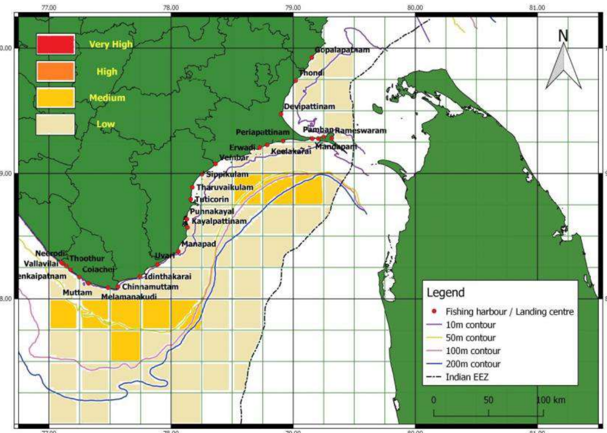

Fig. 8. Colour based hit chart for the post-monsoon season

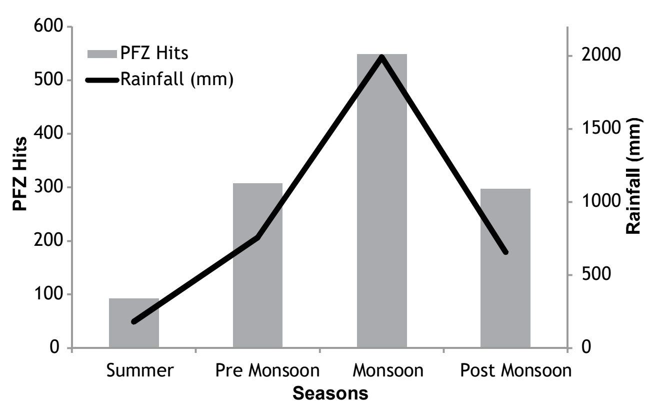

Fig. 9. Season wise rainfall (mm) and PFZ hits (r=0.97, significant, P<0.05)

frequency hit zones, mainly in the Mid Continental Shelf and Continental Slope depth zones. In the Andhra Pradesh Coast, Vinayachandran and Kurian (2007) confirm that freshwater plumes were located at a distance of 100 km away from the coast, supporting the similar occurrence of PFZ hits. Kripa et al (2014) state that relatively high river discharges in the area and high nutrient content in the releases because of high mangrove afforestation are likely causes of the persistent occurrence of PFZs in the Southeastern Arabian Sea of Kerala.

CONCLUSION

The present study reveals that the river Thamirabarani favours higher productivity in nearshore waters than in deeper areas. Season-wise and depth-wise analysis in the PFZ hits occurrence pattern was more in the coastal waters for all the regions. Thus, INCOIS, the remotely sensed data on PFZ advisories, is a helpful tool for enhancing fishermen's fish catch. The PFZ hits validation information provides baseline data for correlating the PFZs with marine fishery resources.

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Histopathological Effects of Some Heavy Metals and Environmental Factors on Brain of Common Carp *Cyprinus carpio,* Reared in cages and Wild Fish in Euphrates River, Babil, Iraq

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Abstract: The current study was conducted to study the concentrations of some heavy metals (iron, zinc, and copper) in sediment, water temperature, pH, salinity, T.D.S,O_2,bod5, and histological effects on the brain of cage fish and wild fish Cyprinus carpioin the Euphrates River from October of 2018 until November of 2019. Three sites were selected on the Euphrates River in the middle of Iraq first site was Abu Luka in Musayyib near the thermal power station second site was Al Saddah near the cement factory third site was in the village of Al-Husayn. Results showed that in the first and second sites the concentration of heavy metal in sediments and fish brains was greater than in the third site, In general, cage fish at the first and second sites were higher than wild fish with their contents with heavy metal because they were held close to the sources of pollution. It was also noticed that the heavy metal concentration of both fish and sediment takes the following order according to the seasons of the year (summer > spring > autumn > winter), so they have a positive correlation with both the temperature and bod5 and inversely correlation with the pH, salinity and T.D.S and O2, The results of histological examination of the brain showed cellular degenerative changes in telencephalon brain and metencephalon brain tissue. The pathological damage was characterized by the presence of vacuolation between neurons in regions of the telencephalon brain and metencephalon brain near the stratum per ventricular layer with the presence of inflammatory cell infiltration and presence necrosis of neurons and glial cells in visceral tissue. The concentration of zinc was below the international determinants of World Health Organization and Canadian guidelines as well as the Environmental Protection Agency. But was higher than Food and Agriculture Organization Standards. The value of copper was higher than the World Health Organization's international specifications, the Food and Agriculture Organization, and Canadian specifications, but it is within the limits allowed by the US Protection Agency.

Keywords: *Cyprinus carpio*, Babil, Heavy metal ecological, Histopathology, Fish Cages, Euphrates River, Iraq

Heavy metal pollution is a source of great concern due to its harmful effects on the environment, animals, and human health (Yarur et al 2019). In the past few decades, heavy metals have been polluted in aquatic environments in abundance due to increasing urbanization (Carolin et al 2017). Heavy elements are metallic elements that have a relatively high density and are toxic even if they are in low concentrations in the bodies of living organisms. (Tiimub et al 2013). (Díaz et al 2018) mention Aquatic ecosystems are more sensitive to heavy metals than terrestrial ecosystems. Heavy metals are non-degradable and can easily accumulate in living organisms, including fish (Strungaru et al 2018)There are several ways to accumulate heavy metals in fish through direct absorption from water and food (Shesterin 2010). Each of the heavy elements has physical properties, so it accumulates in certain parts of the fish body tissues more than the rest of the other body tissues (Jon et al 2006, Uysal et al 2008)

Moreover, heavy metals entering fish through the gills have a chance to accumulate in various body systems and excessive amounts of them can reach a level toxic to humans (Kumar et al 2007), can cause hepatotoxicity, or To the kidneys (nephrotoxicity), to the central nervous system (neurotoxicity), or to the genes (genotoxicity) (Sharma et al 2014, Gupta et al 2015).

It was mentioned by (Su et al 2013) that histological biomarkers are used consistently in most toxicological studies because they indicate the general health of fish and are important indicators of environmental pollution (Omar et al 2013). As the concentrations of heavy metals in the muscles are often used as an indicator for assessing the pollution of the aquatic environment in the long term and evidence of the health risks of fish consumption by humans (Islam et al 2015).

Babil Governorate is considered a center for fish farming in Iraq and due to the presence of many industrial facilities that dump their waste untreated into the Euphrates River, which leads to increased levels of pollution and negative damage to fish. Therefore, the study aims to Measure some of the physical, chemical, and physiological environmental factors of the specific sites on the Euphrates River, measuring the concentration of heavy metals in the sediments of the studied sites on the Euphrates River. Studying the histological changes in the brain of fishes from floating cages and the river from which they were caught due to the presence of pollutants.

MATERIAL AND METHODS

Description of the Study Area: The present study was conducted on groups of some floating cages on the Euphrates River in Hillafirst group in Al-Musayyib Floating cages in the Abu Luka region, north of Hilla and located within the coordinates N32˚50'22.02" E44˚16'17.731"It consists of 40 floating cages with dimensions 4x4x2 meters for each cage, the distance between the base of the cage and the river bed 2-3 meters depending on the distance or location of the cage from the beach. The river is about 110 m wide and 6 m deep. The density of agriculture is 78 fish/m^3 or around 2500 fish per cage (Fig. 1). The second group in Sadat Al-Hindi Floating cages in the Al-Sadah area, north of the city of Hilla and is located within the coordinates N32 ˚ 44'19.169" E44 ˚16'5.19"It consists of 70 floating cages with dimensions 4x4x2 meters for each cage, the distance between the base of the cage and the riverbed 2-3 meters depending on the distance or location of the cage from the beach. The river is about 100 m wide and 6 m deep. The density of agriculture is 75- 50 fish/m^3 or around 2500 - 1500 fish per cage, and floating feed is used to feed the fish. The third group in the village of Al-Husayn Floating cages located in the village of Al-Husayn, south of the city of Hilla, located within the coordinates N32˚23'20.767" E44˚23'34.922 "It consists of 20 floating cages with dimensions 3x3x2 meters for each cage, They are floating cages that represent a control station because they are located relatively far from the sources of industrial pollution and on another branch of the river, That they perform all Ministry of Agriculture and Environment requirements. The depth between the base of the cage and the bottom of the river is 3 meters. The width is 65 m and the depth are 10 m. The culture density is 44 fish / m3 and the floating feed is used for feeding fish.

Sample collection: Water samples were obtained from the stations on a monthly basis from the center of the river in October 2018 and November 2019 Sediment samples from the sites studied were collected from the area next to the cages, They were placed and kept in a refrigerated box until they reached the laboratory, then held in the freezer until the measurements had been made.

Samples of wild fish were obtained using a cast net. The caught fish were placed in plastic containers of 30 liters and filled with river water. For a period of three hours, they were transported under refrigeration to the physiological laboratory at the College of Veterinary Medicine, in Al-Qasim green University, tissue extraction (brain) For the purpose of estimating heavy metals. Since it was preserved under high cooling in the laboratory and reduced with formalin at a concentration of 10 % for histopathology, fish that appeared in good health were selected with an average weight of 1900-2000 g. Some environmental properties of water were field-measured using a multi-meter and included water temperature ©, pH, salinity (mg /l), and T.D.S. O2 (mg / L) The BOD5 procedure described in (Rice et al 2012) was used to approximate the bio-requirement for oxygen by calculating the (primary) dissolved oxygen in the water sample. The samples were incubated for 5 days at a temperature of 20 0 C with dark Winkler bottles in a dark place. From the following equation, then extract the essential oxygen requirement:

BOD5 = Amount of dissolved oxygen (primary) – amount of final dissolved oxygen (after incubation)

Trace metals extraction: After removing the solid parts from them, the sediment samples were dried at 80°C for 48 hours, milled using a Swiss-made Blender model electric mill, and then passed through a 0.4-micron-sieve to get minutes of less than 63 micros. The process (Moopam 1983) was used to remove heavy metals from sediments and digest the aforementioned fish samples using the digestion procedure until a flame-atomic absorption spectrophotometer equipped with a cathode lamp for each element, Affiliated to the Central Laboratory, College of Agriculture, University of Basra.

Histological technique: Samples of the brain were obtained, and then the histological technique described by (Luna 1968) was being used to extract parts of the tissue. Which was conducted in Histopathology for postgraduate studies Laboratory of the Poultry and Fish Diseases department / College of Veterinary Medicine, University of Baghdad, using a tissue processor developed by histo-line laboratories company.

Statistical analysis: The experiment was designed according to the completely randomized design (CRD) and were analysed to Duncan's multiple range tests.

RESULTS AND DISCUSSION

Chemical and Physical Factors

Temp: water temperature levels ranged from its lowest (12.33°C) in site (3) during the winter season of 2019 to the highest (29.167°C) in the summer season of 2019 at site (1) and as seen in the table (1). The results showed there were significant differences between the seasons of the study ($p<0.05$), No major variations in water temperature between sites have been observed ($p<0.05$). These results are in agreement with many studies conducted on the Euphrates River in central of Iraq, including Jubouri (Al-Jubouri 2019). In the current study, and through statistical analyzes, it was found that there is a direct relationship between temperature and heavy metal, as shown in Table 1. The reason for this may be that the temp has an important effect on the chemical reactions of the aquatic environment because it has the ability to directly effect on metabolic activities of organisms and that this was consistent with several studies on the Euphrates River in the city of Hilla, including (Hammoud et al 2017) and (Samer Saleem Alshkarchy Amjed K. Raesen 2020), where they also indicated that there is a direct correlation between temperature and the concentration of heavy metal

PH: Table 1 shows the seasonal changes in pH values during the study period, as the lowest value was (6,066) at site 2 during the summer of 2019 and the highest value was (8,23) at site 1 during the winter season of 2019. The statistical analysis showed substantial variations across the seasons of study ($p < 0.05$). The reason for the differences in the pH value during the seasons of the year may be due to the abundance of aquatic plants and phytoplankton, which leads to an increase in the effectiveness of photosynthesis and then leads to the consumption of carbon dioxide in the water and raises the pH values and this has been confirmed by several studies. Local ones (Kassim et al 1997, Abowei 2010). Or perhaps the cause of this dilution in the water as a result of increased precipitation and washing the soils adjacent to the river (Al-Mousawi et al 1994). the results of the current study showed that the pH value ranged between (6.66-8.23) throughout the study period in all Locations The water of the Euphrates River tends to be light alkaline, and the narrow ranges of pH values are due to the Euphrates having a buffer capacity that resists changes in pH values and this has been confirmed by several studies, including (Hassan et al 2010).

Salinity: The lowest salinity value was reported during the summer at site 3 (0.513), while the highest value was (0.845) at the site (1) during the 2019 winter. The results showed that during each fall season there were significant differences between the study sites, where the first site was higher than the site (3). The first and second sites were greatly superior to the third site for the summer season. It was observed in the same way, by the winter season's statistical analysis. On the other hand, we note that the major seasonal variations have exceeded winter over the rest of the year, followed by spring, autumn, and summer, respectively, as seen in Table 1. It was observed from the results that salinity values increased as we went south towards the second site, due to the large flows of industrial and agricultural wastes to the river. The results of the current study showed an increase in salinity values during the fall and winter seasons, and this may be due to the increase in the entry of dissolved ions by washing soil with rainwater into the river. These results are in agreement with several local studies on the Euphrates, including (Bernet et al 2001, Hassan et al 2010) or perhaps the high salinity values are attributed to the mixing processes and the rise of materials from the deep layers to the surface layers. This is what was indicated by (Al-Fatlawi 2005) in a study he conducted on the Euphrates River. Water too. In addition to that, the solubility of heavy metal in the water increases as the salinity values decrease (Phillips et al 1978).

TDS: The value of total dissolved solids ranged between (401 mg/liter) the lowest value during the summer season at site (3) and (660 mg/liter) the highest value during winter season at site (1) as showed in table (1). Statistical analyses showed that there were variations between the sample groups at a relevant level ($p < 0.05$). The highest values of dissolved solids were observed in the winter season, and no significant differences were noted within the study sites, followed by spring and autumn and then summer, as it seemed that the lowest value in all of the above seasons was the third site where there was a significant difference from the first and second sites; which did not show a significant difference Between them The high values of total dissolved

Table 1. Seasonal changes in the chemical and physical properties of water in the study sites

Location Test	Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Temp c	14.667c	20.8b	29.167a	18.867b	14.33c	20.133b	27.733a	17.967b	12.33c	20.467b	28.967a	18.833b
PH	8.23a	7.3cd	6.2e	7.1d	7.96ab	7.4bcd	6.066e	7.33cd	7.8abc	7.3cd	6.1e	7.033d
Salinity ‰	0.845a	0.8096bc	0.540f	0.758d	0.832a	0.8098bc	0.537f	0.743de	0.826ab	0.802c	0.513g	0.732e
T.D.S mg/l	660.167a	632.5bc	427.667f	592.5d	650a	632.667bc	419.667f	581.167ed	645.5ab	627.167c	401.33g	572.167e
O_2 mg/l	7.7667ab	6.993cd	5.6e	7cd	7.803ab	7.22bc	5.966e	7cd	8.133a	7.33bc	6.533d	7.1cd
BOD_5 mg/l	1.286ef	2.2667c	4.42a	2.45c	1.2ef	2.1667c	4.16a	2.41c	0.8033f	1.5433de	3.0967b	1.8833cd

solid in the winter season are attributed to the rains when they fall, especially in densely populated cities and industrial areas. In a study, it carried out on the Shatt al-Hilla, where he mentioned that dissolved solids are directly proportional to the temperature, and in the same context he mentioned (Jawad 2012), in an environmental study he conducted on the Euphrates River explained the reason of dissolved solids increase when the amount of water in the stream increases Due to the increase in contact with rocks, he mentioned that there is a relationship between salinity and the concentration of dissolved solids, as the higher values of the dissolved solids recorded correspond to the higher values of salinity.

Dissolved oxygen O_2: During the study period, the lowest concentration of dissolved oxygen was recorded and was (5.6) mg /l on site (1) during the summer season of 2019, while the highest concentration was (8.133) mg /l on site (3) in January 2019, as shown in Table 1. The statistical study showed that the third site in the summer season outperformed the first and second sites, which showed no significant difference between them at a significant level ($p<0,05$), although it was not observed that there was a significant difference between the sites in each spring, winter, and autumn season. As for the significant differences between the seasons, the results of the statistical analysis confirm that there is a significant difference between them, where the winter, followed by spring and autumn, respectively, is below the significant level of ($p <0.05$) The reason for the high values of dissolved oxygen in the winter is due to the decrease in temperature, which leads to an increase in the solubility of oxygen, and this is confirmed by the correlation between oxygen and temperature, while the decrease in the con of dissolved oxygen in the summer may be attributed to the high temperature and the lack of operation Photosynthesis in addition to the river's effect on the excreta of the river and that the process of its decomposition requires the consumption of dissolved oxygen. This is confirmed by the correlation between oxygen and the vital oxygen requirement. The results of the study agreed with the results obtained by (Al-Hilli 2019) in a study he conducted on the Euphrates River. In the city of Samawah to study the structures of the fish community and some environmental and health influences, On the other hand, the lowest value of dissolved oxygen in his study was in the spring, while in the current study it was in the summer The reason for the difference may be due to the different sampling sites and collection time.

BOD5: During the winter season of 2019 at a site (3) the lowest value was registered (0.8033) mg /l and the highest value (4.42) mg/l in the summer season of 2019 at a site (1) in Table 1. The statistical analysis showed that there were significant differences between the semesters of the study, as the summer season was high in the value of bod to the rest of the study seasons, followed by the fall and the spring, which did not indicate a significant difference between the first and second sites. As for the third site, there have been significant differences in all seasons. These results were in agreement with(Al-Rubaie et al 2011)in a study he conducted on the Diyala River to study the effect of organic pollution. On some types of fish and some bottom crustaceans. The values of the BOD in the Euphrates water did not exceed the limits permitted by the international determinants of (3-6) mg / liter, due to the ability of the Euphrates water to self-purify and the great dilution of pollutants due to the large cross-sectional area and continuous good aeration(Al-Sultani 2011, Khudairi 2014).

Heavy Metals in Sediment

Zinc: The study results showed that the lowest value of zinc was 34,204 µg/g in site (3) during the autumn season, with the highest value being 83,307 µg/g in site (2) during the summer season, as seen in Table 2 statistical analysis revealed that there were significant differences between season as well as between the study sites ($p<0.05$). Where the zinc recorded the lowest value at the third site, which, as previously mentioned, contains a licensed cage farm. The highest value was at the second and first sites, although it was not found that there was a significant difference between them, although it was noted that there was a small variation in numbers between them. As for the seasons of the year in which the research was done, the results were found the current analysis showed an increase in the value of zinc during the summer season; followed by spring, then winter, and autumn. Table (2) By comparing these concentrations of the current study for each site and for all seasons of the year, we note that the first site shows that the zinc value increased in the summer and spring and decreased in the winter, followed by the autumn season, with a significant difference indicated by statistical analysis results below a significant level ($p<0.05$). No significant differences between the seasons of the year were noticed for the second site. On the other hand, the third site shows there is a significant difference in the value of the zinc, where the summer exceeded the autumn. As for the winter, the difference with the autumn was not important, but the spring did not notice a change from the season Summer and winter for the third site. As shown in Table 2.

Copper: The concentration of copper in the sediments ranged between 18.14-47.863µg/g, and the lowest value was recorded in autumn at a site (3), while the highest value was recorded in the summer season at the site (2), Table 2. And it was observed that there are significant differences between the months and between the study sites ($p<0.05$) by statistical analysis. There was no significant difference between summer and spring, and the autumn and winter results came afterward, and no significant difference was found between them. On the other hand, site (2) shows an increase in the value of Cu relative to the rest of the study sites and it was found that while there were no significant differences for the same site during the various seasons of the year, it was found that the summer season has outperformed numerically the rest of the seasons of the year and did not show significant differences between it and the rest The seasons of the year are below the significant level ($p <0.05$). The value of copper element differed in the third site, so the autumn was at the lowest value, and there was no significant difference between the remaining three seasons, Table (2). This was revealed by statistical analysis ($p <0.05$).

Iron: The results of the statistical analysis of the iron indicate the dominance of the second site during the summer season, where its value was (342.787) µg/g, followed by the spring season and, finally, the winter season and the autumn season, which revealed no significant difference between them ($p < 0.05$). It had no significant difference from the second site as with the first site, but the summer season was at the highest value, followed by (spring, winter, and autumn) respectively. The third site had the lowest value in the autumn season when it reached (241.42) µg/g and increased significantly in the winter season where it was (254.167) µg/g, and the highest value came in the summer and spring, with no significant difference between them (269.7), 262.8) µg/g, respectively($p < 0.05$) Table 2.

This percentage was in agreement with (Al-Dehaimi 2010), where it indicated that the main reason for the pollution of the Euphrates River water with heavy metal is due to industrial sources ' disposal of waste without treatment. The results of the statistical analysis indicated that third station was the least with the values of the concentration of heavy metals studied and the reason may be due to That is to its distance from the sources of pollution and factory waste. In the same context,(Al-Lahibi et al 2016).(Al-Khafaji et al 2011) found in a study he conducted on the Euphrates River in the city of Nasiriyah to estimate the heavy metal in sediments and common carp, the high concentration of heavy metal in sediments compared to the tissues of the studied fish. Regulating the absorption and consumption of heavy metal and indicating that high concentration of iron and zinc in fish tissues is due to the high concentration of these minerals in the sediments. These results are in agreement with international studies, including (Ben Salem et al 2014), which indicated that the concentrations of heavy metals in sediments are higher than those measured in water and fish samples. The reason for this is that the sediments act as a sink for heavy metal metals, or the reason may be due to the tendency of most suspended particles to bind with minerals, forming complexes containing more complex particles and then being deposited in the sediments and gradually accumulating and this is confirmed by (Al-Khafaji et al 2011), where it was mentioned that sediments are the final basin for many plankton in the water column, including heavy metals whose final fate is sediments, so they are naturally present if their source is from nature or by human activities industrial and agricultural waste and sewage.

The results in the current study also revealed a seasonal difference in the concentration of heavy metals in sediments in the Euphrates River in the study area over the course of a year, where the highest concentration of heavy metals studied was more in the summer and spring seasons than in the winter and autumn. These results differed with (Al-Khafaji et al 2013), where noted that the lowest value of heavy metal is in the spring and the highest value in the winter season. The reason for this difference between the current study and its study may be due to the location of the study conducted by the current study.

On the other hand, the results of the current study agree with several studies conducted on the Euphrates, including (Salah et al 2012, Farhood 2015) where they mentioned the high concentration of heavy metals in the spring and their decrease in the winter. The reason for this is that the high temperature works on increased evaporation of water from the river, and thus the concentration of metals increases. The seasonal fluctuations of heavy metals in the ecosystem are affected by some of the physical characteristics of the water such as temperature and salinity as well as the chemical

Table 2. Seasonal changes in the concentrations of heavy metal in sediment for the studied areas µg/g

Location/ Element	Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Zn	72.909bc	77.78abc	80.235a	71.773c	77.462abc	83.307a	82.82a	79.836ab	38.36ed	37.54ed	41.69d	34.204e
Cu	35.942e	39.797ed	43.84bc	38.286de	35.773cd	45.353ab	47.863a	41.217e	18.86gf	20.81gf	22.267f	18.14g
Fe	294.053f	314.84cd	333.533ab	300.106ef	306.968def	323.167bc	342.78a	309.01de	254.1h	262.8gh	269.7g	241.42i

* Means with the same letter are not significantly different in same row

properties of the water including pH and dissolved oxygen levels (Wong et al 2001). Several international studies also confirmed this reasoning, where (Rajeshkumar et al 2018) stated when noticing the high concentration of heavy metals in summer compared to winter, this could be due to increased water evaporation and less precipitation, which led to an increase in the concentration of heavy metals, and in the same context, an illness (Duman et al 2012). The reason for this is that the heavy metal concentration is lower in winter and autumn compared to spring and summer due to increased monsoon precipitation, which may lead to dilution of heavy metals throughout the rainy seasons.

Heavy Metals in the Fish Brain

Zn in the fish brain: Table 3 shows the rates of zinc concentrations µg / g dry weight of the brain of wild fish and cage fish from the three study sites on the Euphrates River during the study period. It was found through statistical analysis that significant differences exist between the three study sites and between cage fish and wild fish, as well as between Seasons ($p < 0.05$). Cage fish in each of the first and second sites had the highest concentration of zinc component in the brain, ranging between (48.201-33.113) and (50.932-43.294), respectively, compared to wild fish in the same two sites where the values ranged between (36.639-25.465) and (34.309-23.713 respectively). As for the third site, the wild fish at concentrations ranged between (22.319-15.736) values higher than the cage fish in which the zinc ranged from (14.453-10.653). The summer season showed an increase in the value of the zinc component in fish brains with a concentration (of 50.932 In the second location of cage fish, followed by spring, then autumn and winter, respectively, as the lowest value of zinc was reached in the third site for cage fish in the winter season (10.635). Regarding the summer, the highest value of cage fish was in the second site, followed by the first site, and then wild fish in each of the first and second sites, which did not notice a significant difference between them, followed by wild fish from the third site, and then cage fish in the third site. Then came the spring season, the results of which showed that the second site of cages had the highest value in each of the first sites, followed by the second site, after which the wild fish in each of the first and second sites, in which there was no moral difference between them, then the third site was fishing, followed by the third site cages. The highest value recorded in the fall was in the second site for fish, cages cultured in cages, followed by the first site also for fish farming in cages, and then wild fish in each of the first and second sites after the lowest value. The third site was for wild fish, followed by fish farmed in cages for the same site. We note that the winter season took the following arrangement pattern: cage fish in the second site> cage fish in the first site> wild fish in the first site> wild fish in the second site> wild fish in the third site>fish cultured in cages in the third site.

Cu in Fish brain: Table 3 shows the mean concentrations of copper µg/ g dry weight of the brain of wild fish and fish cultured in cages from the three study sites on the Euphrates River during the study period, it was found through statistical analysis that significant differences exist (between the three study sites and between cage fish and wild fish. Also between seasons of the year) ($p < 0.05$). The highest value of copper (7.647 µg / g) in summer was for cage fish in the second site and the lowest value was (1.837 µg / g) for cage fish in the third site. The copper element took the following arrangement pattern in the fall and winter seasons: cage fish for the first and second sites> wild fish in the first and second sites> wild fish in the third site> cage fish in the third site. It is noted from the results of the current study that the highest value of the copper was in the summer and spring seasons, the second site for cage fish, where it was (7.6473 and 7.4687) respectively, followed by cage fish in the second site, then cage fish in the first site, followed by wild fish in each of the first and second sites for both. The seasons, while the third site, wild fish were higher than cage fish, for both seasons (summer and spring)

Fe in fish brin: Table 3 shows the rates of iron concentrations (µg/ g) dry weight of the brain of wild fish and

Table 3. Seasonal changes in the concentrations of heavy metal in brain of *Cyprinus carpio* for the studied areas µg/g

Sit		Site 1 (Abu Luka)				Site 2 (Al-Sadah)				Site 3 (village of Al-Husayn)			
Metal	Fish	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Zn	Wild	25.47fg	30.53e	36.64d	30.68e	23.71fg	31.21e	34.31de	26.46f	15.74ghi	17.42h	22.32g	17.05hi
	Cage	33.11de	42.37c	48.20ab	35.90d	43.29c	48.45ab	50.93a	45.21bc	10.65k	13.22jki	14.45jkh	11.92ik
Cu	Wild	3.78efg	4.52d	4.88d	3.81ef	3.72efg	4.29ed	4.82d	3.73efg	2.65hi	3.35fg	3.79efg	2.55hi
	Cage	5.68c	5.86c	6.74b	5.78c	5.65c	7.47a	7.65a	6.03c	1.84 j	2.57hi	3.06gh	1.99ij
Fe	Wild	266.9fg	280.19de	286.34bcd	268.48fg	265.07fg	275.13ef	288.78bcd	261.83g	216.6j	233.4i	242.86h	214.06j
	Cage	284.2cde	296.46ab	295.39ab	287.15bcd	282.88cde	293.40bc	304.41a	281.15de	167.1k	185.2k	188.46k	166.34k

*Means with the same letter are not significantly different in same Metal cage fish in the three study sites on the Euphrates River during the study period. It was found through statistical analysis that significant differences exist between the three study sites and between cage fish and wild fish as well as Between seasons of the year ($p < 0.05$), the highest value reached 304,409 µg/ g for cage fish in the second site in the summer and the lowest value was 166,341 for cage fish in the third site for the autumn season, where it was found that there is a superiority in the concentration of iron in cage fish in both The first and second sites were followed by the wild fish in both sites. As for the third site, the results were different. The wild fish exceeded the value of the iron accumulated in the brain than the cage fish. You, the results of the comparison between the seasons, were the highest summer values for cage fish in the second and first sites, followed by the wild fish in the first and second sites, and the wild fish in the third site were the highest 242,856 of fish in cages of the same site with a value of 188,456 and the results of the spring season were results of fall were the highest in the value of cage fish in the first site, then cage fish in the second site, followed by wild fish in each of the first and second sites, which had no significant difference between them, and finally the third site in which wild fish surpassed the cage fish. The results of the winter season showed that the cage fish in the first and second sites were of the highest values, followed by the wild fish of the same two sites and finally the wild fish of the third site, and then the cage fish of the same site. The results showed that the accumulation in the brain differed according to the mineral studied, the season of the year and the location of the fish. The reason for this is that differences in the accumulation of heavy metals in fish brain are affected by some factors depending on the metabolism of the element by the fish. The results showed an increase in the value of heavy metals in the summer, followed by spring and then autumn, and winter comes with the lowest value for the concentrations of heavy metals, and the reason for this may be due to the increase in water temperature in the summer, which has a positive effect on the biological activity of freshwater fish, which led to an increase in the metabolic activities of fish. In this season, increased intake and accumulation of heavy metals in the brain. These results are consistent with what was found by (Farhood 2015) in a study he conducted on the Euphrates River near the city of Souk Al-Shuyoukh, where indicated a higher concentration of heavy metals in the summer compared to the winter season, and he explained that higher temperatures increase metabolic activities and fish appetite. An increase in the level of metabolism, which is directly related to the concentration of heavy metals, and international studies also noted that, including (Ben Salem et al 2014) where he indicated that the highest value of heavy metals is in the summer and the lowest value of the concentration of heavy metals in the winter season, explaining this as above. On the other hand (Düşükcan et al 2014) stated in a study to estimate heavy metals in *Luciobarbus xanthopterus* fish that the level of heavy metal accumulation is arranged according to the seasons as follows: Summer> Autumn> Winter> Spring. While the results of this study were as follows: Summer> Spring>Fall> Winter, and the reason for this may be due to the different environmental conditions. the reason may be that the temperature changes the activity of the enzymes responsible for this process and affects the rate of fish absorption of heavy metals results showed that the order of the concentration of heavy metals is iron> zinc> copper. The high concentrations of iron in the brain of the studied fish, which the results showed, as expected, that iron is one of the components of hemoglobin responsible for binding and transporting oxygen in the body. The reason for this may be due to Iron ions precipitate in the form of ferric hydroxide (Fe OH), which is a form of iron that is weakly soluble in water (Tkatcheva et al 2004). Its presence in high concentrations in the environment in which the fish live or to good ventilation, which leads to its mixing in the water. On the other hand (Farhood et al 2020) stated that the concentration of zinc was higher than iron, and the reason for this was attributed to the absorption of the zinc component necessary for metabolism of living organisms in addition to that it works to protect the aquatic environment from the toxic effects of cadmium. The difference from the current study for the difference in the site from which the fish were collected or the different sizes and ages.

Several international studies reported that the accumulation of the high iron component in the organs of them (Öztürk et al 2009, Ben Salem et al 2014) explained the reason for this to the fact that iron is one of the components of hemoglobin responsible for binding and transporting oxygen in the body. It was higher than copper in the brain of fish, and the reason for this was due to the importance of this element in the organisms that live in the aquatic environment, as it is necessary to perpetuate cells, as it enters into the synthesis of several enzymes as an enzyme companion and this is also confirmed (Al-Khafaji et al 2013) and mentioned the same reason Previous and in the same context, (Khudairi 2014)indicated that the zinc component outperformed the copper component per month for the regular carp in its study on the Euphrates River in the city of Hilla.

Copper was the least concentrated in fish brain, and the storage in the tissues of the studied fish was within the limits of homogeneous organization. Perhaps this is due to the fact that copper is an essential element necessary for the hemoglobin, through its entry into some glycoprotein enzymes involved in The production of melanin and catecholamine (which is responsible for the absorption of iron by converting it into ferric to ferrous and transporting it, as well as in detoxification (Ben Salem et al 2014). Or the reason for this may be due, as mentioned (Canli et al 2003) that exposure of fish to heavy metals such as Cu may activate the synthesis of metal-binding proteins that quarantine copper by cadmium and zinc, without producing Toxic effects to fish are considered to be the ability to produce metal-binding proteins (metallothioneins) and their function to bind metals as mechanisms that carry heavy element concentrations (Wagner et al 2003)where metallothioneins belong to a family of low-molecular proteins rich in cysteine involved in the regulation of basic minerals Cu and Zn and eliminating the toxic effects of them (Amiard et al 2006)and this has been confirmed by several local studies, including (Al-Sultani 2011, Khudairi 2014)

Histological changes for the brain: The macroscopic examination showed the absence of macroscopic pathological changes on the brain of common carp fish during the period of study and for all sites in general in terms of size, color, and homogeneity of the brain with no presence of any macroscopic pathological lesions of little importance.

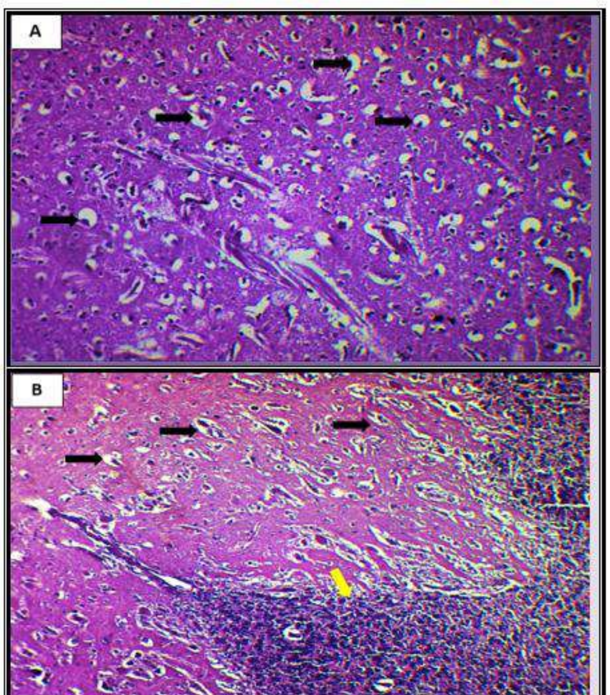

Figure 1: Photomicrograph of brain of common carp fish. A& B: x400. H& E

A& B/ Note the degenerative changes in telencephalon and metencephalon parenchyma of brain. The lesion was characterized by presence of neural vacuolation (black arrows) of telencephalon and metencephalon areas or near the stratum periventricular area (yellow arrow) with infiltration of inflammatory cells.

Results of microscopy of wild fish tissues and the atmosphere of cellular degenerative changes in the telencephalon and metencephalon showed gaps between neurons in regions of the telencephalon and metencephalon near the stratum layer periventricular with infiltration of inflammatory cells and as shown in figure (1), which shows a histological section of the brain of wild common carp during the study period, while cage fish in both the first and second sites showed the presence of neurons cells and cells. Glia in the visceral tissue in the regions of the telencephalon and metencephalon, with gaps between neurons (neural vacuolation), infiltration is also observed in inflammatory cells, as shown in Figure 2, which shows a histological section of the brain of carp fish It is common to be cultured in cages during the study period.

The tissue damage lesion was characterized by the presence of gaps between neurons in telencephalon and the metencephalon near the periventricular layer, with the presence of infiltration of cells and these results were in agreement Al-Bairuty et al (2013) where reported changes in the neuronal bodies of telencephalon and thickening in mesencephalon layers. Bose et al (2013) also reported that the main histological changes observed in the brain of fish

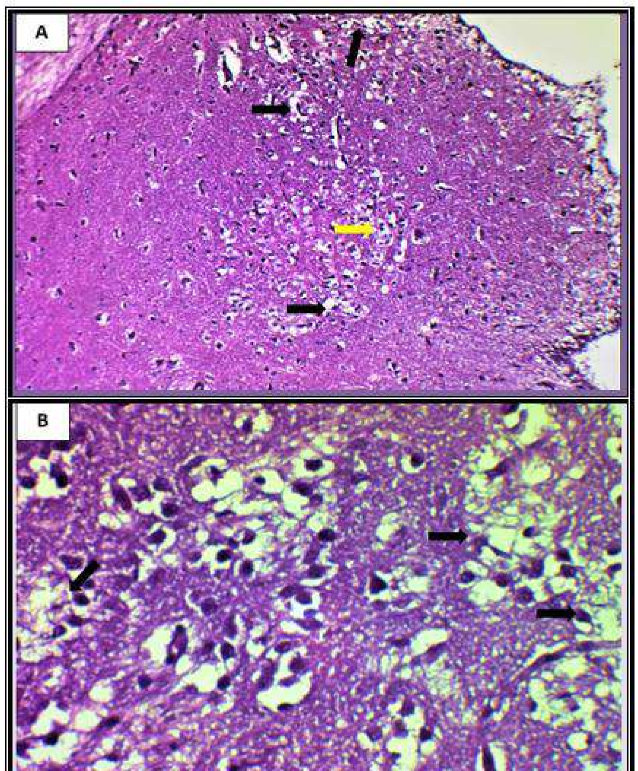

Figure 2: Photomicrograph of the brain of common carp fish. A: x200. H& E. B: x400. H& E.

A &B/ The necrosis of neurons and glia cells were observed in the parenchyma of telencephalon or metencephalon-formed spaces (black arrows), also infiltration of inflammatory cells was observed (yellow arrows).

treated with heavy metals break down into the granular and molecular degeneration of the granular and molecular layer cellular damage in the interior and posterior of brain, degeneration of the nerve cells, vacuolization of the brain cells. The brain has revealed general congestion and meningeal vasodilation along with infiltration of mononuclear cells.

CONCLUSIONS

The presence of an effect of environmental factors on accumulation of heavy metal inside the brain of fish and in sediments, so the H.M had a direct correlation with each of the water temperature and the BOD and an inverse correlation with the pH, salinity, dissolved solids and dissolved oxygen. There is a seasonal variation in the concentrations of the studied metals in sediments. The Euphrates River, as it was high in the summer and low in the winter, and higher concentrations were recorded at Site2, and the lowest concentration of metals was at Site3. The accumulation of heavy metals in polluted areas is in the brain of cage fishes higher from the brain of wild fish and in regions free of heavy metal contamination, the accumulation of heavy metals in the brain of wild fish is higher than the brain of cage fish. The highest element accumulated in the brain of fish and sediments is iron, followed by zinc, then copper.

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Effect of Forest Fires on Soil Carbon Dynamics in Different Land Uses under NW Himalayas

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Abstract: Fires are regarded as features of forest disruption and renovation. The study was conducted to assess the fires effect on soil organic carbon (SOC) and microbial biomass carbon (SMB-C) in four land uses *viz.* forest land, grassland, agricultural land and non-fire site (control). PVC core were used for sampling soils at 0-5, 5-10 and 10-15 cm depths. SOC levels were found to be reduced post fire in all sites. Significant reductions were observed in SMB-C accumulations. However, SOC and SMB-C contents increased again with passage of time in all the sites. Grasslands samples, attained highest values of SOC (1.34 %) followed by 1.29 per cent in agricultural land at 10-15 cm depth. The maximum SMB-C of 172.20 μg C/g soil was in forest land at 0-5 cm depth and later was found to be decreased with increase in depth. Higher rates of carbon and microbial biomass accumulations were observed during the spring and rainy seasons. Therefore, prescribed burning of surface litter at right time could be a superior approach to avoid the fatal loss caused by wildfires and for better re-germination of plant species.

Keywords: Microbial biomass carbon, Forest fire, Organic carbon, Soils, Land uses, Prescribed burning

Severities of forest fires impart significant effect on soil microbial biomass content, therefore high severity of these fires lead to higher rate of reduced microbial biomass over low severity fires (Holden et al 2016, Girona-Garcia et al 2018). Fires do not cause threat only to forest wealth but alter the entire ecology of fauna and flora present in the region. In general, microbial biomass represents the status of numerous microbial communities thriving in the soil responsible for nutrient transformations and maintaining fertility of soil (Mataix-Solera et al 2009, Manral et al 2020). Disturbances caused by fires control the plant community succession and intra competition, ecophysiology and native species genetics, nutrients and rate of erosion and pest behaviour (Brown and Smith 2000). Fires lead to removal of carbon and essential nutrients from the soil pool and therefore a big reason for the reduction of microbial biomass of soil as carbon is utilized as food source by microbes (Zhou et al 2018). Topography of the region is also responsible for varying impacts of forest fires on biomass, land features like valley position, slope gradient, ridge exposure are important (Mabuhay et al 2006). Higher temperatures caused by fires for instance >50°C lead to death of microflora which were heat sensitive, further increase (around 70°C) directly affected the crops and vegetation (Shakesby and Doerr 2006). Fire being a mineralizing agent, plays a vital role in nutrient transformations, therefore, acts as the important soil health indicator. It boosts the nutrient accessibility to flora and fauna for better multiplication and higher activity rates (Hyodo et al 2013). Duration and intensity of forest fires in region also alter the vegetation and landscape of the region and therefore affect the biomass content (Lucas-Borja et al 2019). In some regions, accumulation of heavy fuels in sub surface layers of soil can increase the temperature beyond 500 °C in fire incidence (Neary et al 2005).

Soil microorganisms play various functional roles *i.e.* sources and sink of essential nutrients, maintain soil structure, acts as nutrient transformations catalyst and profoundly establish mutualistic relationships with roots responsible for plant fitness (Van Der Heijden et al 2008; Schmidt et al 2014). Soil microbes are very sensitive to global change including temperature variations (Allison and Treseder 2008; Frey et al 2008), soil nutrients availability (Allison et al 2008, Demoling et al 2008, Allison et al 2010) and moisture availability (Salamanca et al 2003, Hawkes et al 2011). Microbial biomass is a sensitive indicator of soil and thus used to determine the impact of forest fire on soil (Sadeghifar et al 2020, Singh et al 2021). Soil microbial biomass carbon, microbial biomass nitrogen and enzymatic activities are found to alter the post forest fire. In general, fire effects on soils are of two kinds, direct effects *i.e.* burning of soil organic matter, whereas, indirect effects deals with change in other components of ecosystems (Dooley et al 2012, Weber et al 2014). Various intracellular and extracellular enzymes present in microbes are responsible for nutrient transformations. Enzymes are regarded as sensitive indicators for quantifying ecological changes and also plays important role in releasing the essential nutrients and therefore improves availability to plant communities (Sadeghifar et al 2020). In oxidation of soil organic matter (SOM), intracellular enzyme *i.e.* dehydrogenase, plays a vital role in the initial phase by transferring the H^+ & electrons from substrates to acceptors (through co-enzymes). Several research studies showed positive correlation of acid phosphatase activities (ACP) with microbial biomass as ACP activities were reduced after the fire (Hyodo et al 2013, Sadeghifar et al 2020). Proportions of microbial biomass carbon, microbial biomass nitrogen and organic carbon in soils are very important parameters for assessing soil fertility and productivity (Li et al 2016).

In Himachal Pradesh, around 66.52 per cent of total area is occupied by forests. More precisely, out of total geographical area of 55,673 km^2 of state, around 1, 25,885 ha area is covered under Chir-pine forest (Anonymous 2019). In the Himalayan mountainous region of Solan, Himachal Pradesh, biomass burning in the sub-tropical areas, especially in the chir pine forests is a common phenomenon which occurs regularly each year due to which soil composition of the forest is prone to change. Pine (*Pinus roxburghii*) is regarded as the principal species of Himalayan subtropical forest (Zhou et al 2018). Prescribed fires/controlled fires have been commonly practiced in the moist seasons and farmers are performing same from many years. The approach already showed its superiority by lowering the accumulation of heavy fuels in sub surface soils, improving stand quality and recharging soil nutrient pool. Fire resulted in reduction of microbial abundance by an average of 33.2% and fungal abundance by an average of 47.6% (Dooley and Treseder 2012). Prescribed burning lead to minimize the severity of wildfires and its extent, and therefore, facilitated the emergence and germination of desired forest species in the region (Shubham et al 2021). Transformation of essential elements during fire combustion can affect the cycling and availability of nutrients. The combustion of organic matter during fire releases appreciable quantities of available nutrients and thus can be reliable nutrients source for re-growth of forest species. The mineral ash can also influence the soil properties (pH) and microbial activities responsible for nutrient turnover (Deluca et al 2006). Impact of forest fires on the soil microbial biomass carbon has received very less scientific attention in the region till date. Therefore, conducted a field experiment under sub-tropical pine forest areas of Solan, India situated in NW Himalayan region.

MATERIAL AND METHODS

The research investigation was carried out in forest areas of Solan, falls in II^{nd} Agro climatic zone *viz.,* Sub-temperate and Sub-humid zone of Himachal Pradesh, India during 2019. The experimental sites were located at 1196 amsl, with mean annual temperature of 17.4°C with annual rainfall of 1100 mm was recorded during study period. Due to rugged and mountainous topography the climatic conditions in the district vary from place to place. Winter rains were scanty and mostly received during the months of January to March. For the experimentation, four different land uses *viz.* forest land, grassland, agricultural land and non-fire site (control) were selected. All the selected sites were situated at an elevation of 1275 amsl (average). On longitude and latitude basis the study area was situated at 30°51'24" N to 77°09'58"E and all the land uses were nearly adjoining. The minimum distance of 2 km was kept between fire sites and non fire sites for obtaining high accuracy in results. Plot size of 200m×500m was chosen for each land use. In each land use, 3 parallel lines were aligned separating each other by 15 meters, in each line 10 PVC cores were arranged positioned at every 5 meters. PVC cores taken had dimensions of 5 cm diameter and 5, 10 & 15 cm lengths, perforated by holes and driven into the soil and finally capped on top. The collected soil samples were air dried, crushed, and passed through a 2-mm sieve. Soil samples were divided into two parts for the physicochemical and nutrient analysis, and microbial properties. The soil samples were stored at 4 °C until microbial activities were analyzed.

Soil samples were drawn from three depths *i.e.* 0-5 cm, 5-10 cm & 10-15 cm for determining soil physico-chemical properties, organic carbon and microbial biomass carbon analysis. Initial samples were drawn from near the PVC core, while the further monthly successive samples were collected from the cores with least disturbance. One set of samples were drawn from the sites that were burnt by controlled fire, while for the second set samples were taken from non fire region as control treatment. Experimental design taken for the study was completely randomized block design to maintain heterogeneity in results.

Soil organic carbon content of the soils was determined by the wet digestion method (Walkley and Black 1934). For estimation of microbial biomass and soil basal respiration, the soil moisture content was adjusted to 60% and incubated for a week to estimate microbial biomass. Soil microbial biomass carbon (SMB-C) content was determined by fumigation-extraction method given by Vance et al (1987). Soil pH and electrical conductivity were determined as 1:2 Soil: water suspension, measured with digital pH meter (Jackson 2005). Bulk density and particle density was calculated using pycnometer method. Organic carbon of soil was determined by Wet digestion method (Walkley and Black

1934). Soil available N (kg ha^{-1}) was determined by alkaline potassium permanganate method (Subbiah and Asija 1956). Soil available phosphorus (kg ha^{-1}) was determined by Stannous chloride reduced ammonium molybdate method (Olsen et al 1954) and estimated on Spectronic-20D^{+} at 660 nm. Soil available potassium (kg ha^{-1}) was determined by neutral normal ammonium acetate method (Merwin and Peech 1951) and estimated with flame photometer (Table 1).

Statistical analysis: The data generated from the present investigation were subjected to statistical analysis using the statistical package SPSS (16.0) and Microsoft Excel.

RESULTS AND DISCUSSION

Soil characteristics: Soils of the experimental sites were categorized as *Typic Eutrochrept* on sub group level as per USDA soil taxonomy. Soil texture in different sites varies from sandy loam to sandy clay loam. Particle density of the soils varied between 2.32 to 2.35 g cm^{-3}, while bulk density ranged between 1.33 to 1.36 g cm^{-3}. Higher range of soil porosity could be due to presence of sufficient organic matter and higher proportion of fine soil fraction which might have increased the surface area. Available soil nitrogen was in the range of 173.18 as lowest in forest land to 390.28 kg ha^{-1} as highest in the agricultural land use. While soil available phosphorus varied as lowest of 32.59 kg ha^{-1} to highest of 46.90 kg ha^{-1} in agricultural land. Soil available K content ranged between 272.68 kg ha^{-1} in forest land to 431.88 kg ha^{-1} in agricultural site (Table 1). Continuous application of NPK fertilizers in agricultural fields might have increased the soil NPK levels in this land use.

Soil microbial biomass carbon (SMB-C): The inducing fires remarkably reduced the microbial biomass carbon in the land uses to great extent. In forest land, initial SMB-C values was 170 µg C/g soil which was later reduced to 152.44, 137.19 & 121.19 µg C/g soil with a decrease of 10.70, 19.63 & 29.01 per cent in 0-5, 5-10 and 10-15 cm sampling depths, respectively (Table 2). Similarly, in grassland, the per cent decrements were 19.41, 25.24 & 32.95 per cent. In agricultural land fire lowered the SMB-C by 28.19, 35.22 and 37.80 per cent over the pre-fire levels. The un-burnt sites, slight reduction of 1.69, 9.18 and 22.02 per cent. Data showed that highest SMB-C content was recorded in 0-5 cm depth, the microbial biomass carbon content was reduced with increase in soil depth. Amongst the different land uses, highest value of 172.20 µg C/g soil was recorded in forest land (September) and have significant statistical difference over rest of the sites. Monthly variations of SMB-C in post-fire showed a secular variations trend as presented in Figure 1, 2, 3 and 4 in different sampling depths of land uses. The climate had also an direct effect on the SMB-C build up, as higher rates of biomass carbon had been generated during mild winters and rainy period as compared to peak summer months *i.e.* April, May and June. Effect of forest fires have found to have a short time effect on microbial biomass after then they regenerate, multiply and sustain their populations in soil.

Soil organic carbon (SOC %): The forest fire caused a great reduction in SOC under fire induced land uses (Table 3). Amongst the different land uses, maximum of SOC values were recorded in grassland over the rest sites. Comparative results on sampling depths showed soil sampled initially post fire at 0-5 cm, SOC values of 1.09, 1.19, 1.16 and 1.06 per cent were recorded in forest, grassland, agricultural, non fire site, respectively. Later, the SOC content showed secular variations trend with passage of time (on monthly basis) (Fig. 1). Overall highest values of SOC *i.e.* 1.34 per cent was in the grassland (August and October) followed by 1.29 per cent in agricultural land and 1.25 per cent in non fire site. However,

Table 1. Initial physico-chemical properties of soils

Parameters	Land uses			
	Forest Land	Grassland	Agricultural Land	Unburnt site (Control)
Particle density (g cm^{-3})	2.32	2.33	2.35	2.32
Bulk density (g cm^{-3})	1.33	1.34	1.36	1.33
Porosity (%)	42.70	42.48	42.12	42.67
pH (1:2)	5.93	6.05	6.44	6.57
Electrical conductivity (dS m^{-1})	0.22	0.24	0.27	0.25
Soil organic carbon (%)	1.41	1.49	1.40	1.38
Available N (kg ha^{-1})	173.18	179.54	390.28	291.29
Available P (kg ha^{-1})	32.59	35.65	46.90	41.54
Available K (kg ha^{-1})	272.68	319.54	431.88	339.52
SMB-C (µg C/g soil)	170.71	163.38	159.36	157.91

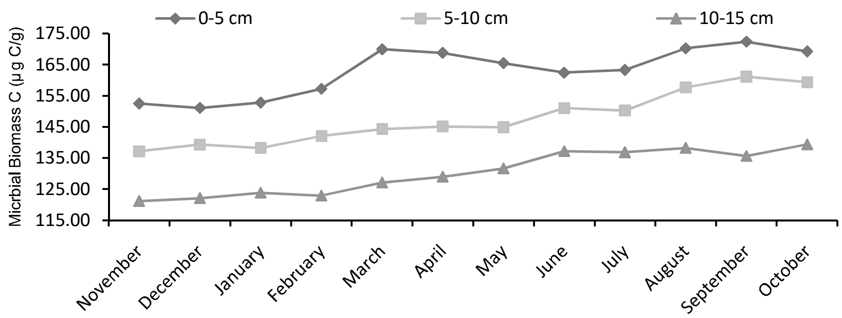

Fig. 1. Effect of forest fire on monthly variations in soil microbial biomass carbon (µg C/g) in different samping depths under forest based landuse

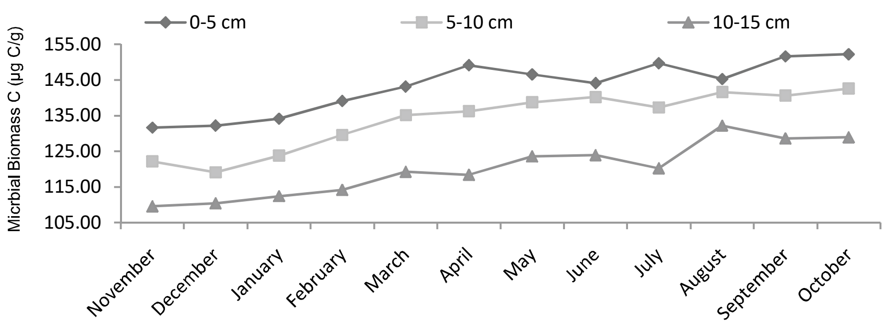

Fig. 2. Effect of forest fire on monthly variations in soil microbial biomass carbon (µg C/g) under different samping depths in grassland based landuse

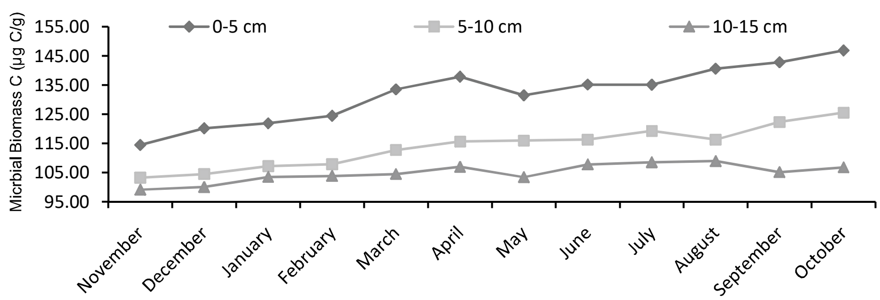

Fig. 3. Effect of forest fire on montly variations in soil microbial biomass carbon (µg C/g) under different samping depths in agricultural based landuse

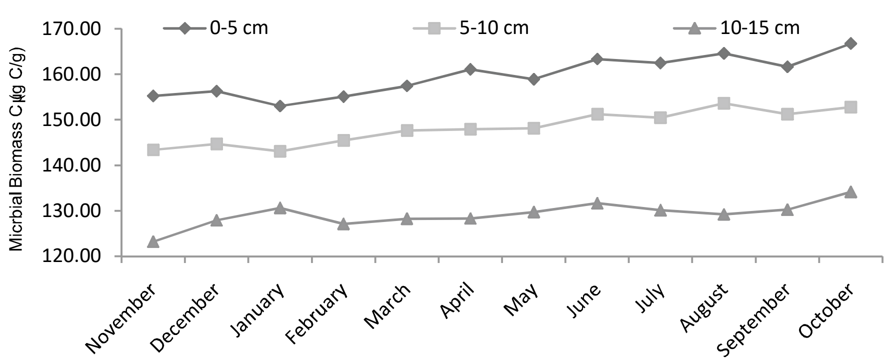

Fig. 4. Effect of forest fire on montly variations in soil microbial biomass carbon (µg C/g) under different sampling depths in unburnt site (Control)

Table 2. Effect of forest fire on soil microbial biomass carbon content (SMB-C) of soil

		Soil microbial biomass C(µg C/g soil)													
Land uses	Depth of sampling	November	December	January	February	March	April	May	June	July	August	September	October	CV (%)	C.D. (0.05)
Forest Land	0-5 cm	152.44	151.06	152.76	157.22	169.90	168.78	165.43	162.41	163.26	170.20	172.34	169.20	0.74	2.08
	5-10 cm	137.19	139.34	138.20	142.04	144.26	145.12	144.88	151.03	150.22	157.64	161.10	159.34	0.59	1.49
	10-15 cm	121.19	122.08	123.86	122.97	127.13	128.99	131.64	137.19	136.83	138.22	135.67	139.42	1.18	2.62
Grassland	0-5 cm	131.67	132.19	134.17	139.09	143.14	149.12	146.56	144.12	149.66	145.24	151.66	152.19	0.77	1.89
	5-10 cm	122.14	119.07	123.77	129.63	135.12	136.21	138.74	140.19	137.25	141.60	140.60	142.56	0.89	2.03
	10-15 cm	109.54	110.37	112.43	114.15	119.22	118.38	123.54	123.88	120.19	132.23	128.54	128.92	0.70	1.44
Agricultural land	0-5 cm	114.44	120.14	121.90	124.44	133.56	137.88	131.44	135.22	135.09	140.66	142.85	146.90	0.72	1.61
	5-10 cm	103.23	104.46	107.21	107.89	112.73	115.62	115.99	116.32	119.26	116.27	122.44	125.57	1.07	2.07
	10-15 cm	99.12	100.03	103.48	103.81	104.52	106.94	103.39	107.76	108.54	108.92	105.17	106.84	1.12	1.99
Unburnt site	0-5 cm	155.23	156.34	153.06	155.14	157.48	161.10	158.93	163.40	162.50	164.60	161.70	166.77	0.69	1.89
	5-10 cm	143.40	144.67	143.03	145.47	147.62	147.91	148.16	151.22	150.49	153.64	151.22	152.80	0.74	1.86
	10-15 cm	123.14	127.90	130.60	127.09	128.20	128.30	129.70	131.60	130.10	129.20	130.20	134.12	0.90	1.98

Table 3. Effect of forest fire on organic carbon content (SOC) of soil

		Soil microbial biomass C(µg C/g soil)													
Land uses	Depth of sampling	November	December	January	February	March	April	May	June	July	August	September	October	CV (%)	C.D. (0.05)
Forest Land	0-5 cm	1.09	1.12	1.12	1.13	1.14	1.1	1.12	1.12	1.13	1.16	1.15	1.16	1.54	0.03
	5-10 cm	1.13	1.12	1.16	1.18	1.19	1.18	1.21	1.2	1.22	1.22	1.21	1.22	1.96	0.03
	10-15 cm	1.14	1.17	1.17	1.21	1.22	1.24	1.22	1.27	1.29	1.3	1.33	1.37	1.79	0.03
Grassland	0-5 cm	1.19	1.22	1.24	1.28	1.3	1.29	1.28	1.32	1.33	1.34	1.33	1.34	1.17	0.03
	5-10 cm	1.16	1.21	1.23	1.22	1.25	1.26	1.24	1.25	1.27	1.29	1.32	1.31	1.25	0.03
	10-15 cm	1.23	1.22	1.25	1.27	1.27	1.28	1.31	1.34	1.36	1.3	1.41	1.44	1.39	0.03
Agricultural land	0-5 cm	1.16	1.15	1.18	1.19	1.19	1.22	1.24	1.24	1.23	1.26	1.29	1.29	1.23	0.02
	5-10 cm	1.2	1.22	1.25	1.24	1.25	1.28	1.26	1.3	1.29	1.33	1.31	1.32	1.33	0.03
	10-15 cm	1.22	1.21	1.24	1.24	1.27	1.28	1.32	1.31	1.3	1.36	1.36	1.35	1.76	0.03
Unburnt Site	0-5 cm	1.06	1.07	1.10	1.14	1.11	1.12	1.14	1.17	1.21	1.25	1.21	1.25	1.41	0.02
	5-10 cm	1.11	1.10	1.13	1.16	1.15	1.17	1.20	1.23	1.22	1.24	1.26	1.28	1.49	0.03
	10-15 cm	1.15	1.17	1.21	1.21	1.23	1.25	1.28	1.30	1.31	1.32	1.35	1.36	1.14	0.02

the least build up in SOC was in forest area with 1.16 per cent organic carbon. Similarly, in sampling depths of 5-10 cm & 10-15 cm, similar trends were observed (Fig. 5, 6 and 7). Amongst all the sampling depths, overall maximum organic carbon content was in 10-15 cm depth, with maximum of 1.44 per cent in grassland (October) which was 6.67 and 5.88 per cent higher than values recorded in October under agricultural and un burnt sites But here, forest area improved the SOC content by 1.48 per cent over agricultural land. On comparison of pre-fire and post fires effect on SOC, very slight change was recorded in un-burnt sites as compared to fire induced ones. It is also have been found that post fire, the soil have showed regeneration of plant species and thereby improved soil organic carbon content.

Forest fires are regarded as one of the utmost elements of climate change which influences the different soil microbial communities to a great extent as these microbes plays a vital role in carbon dynamics of terrestrial ecosystems. Fires cause a great reduction to microbial populations thriving in soils (Dooley and Treseder 2012, Holden and Treseder 2013, Garcia et al 2018). Burning destroys the different microbes which ultimately lead to reduced microbial biomass.

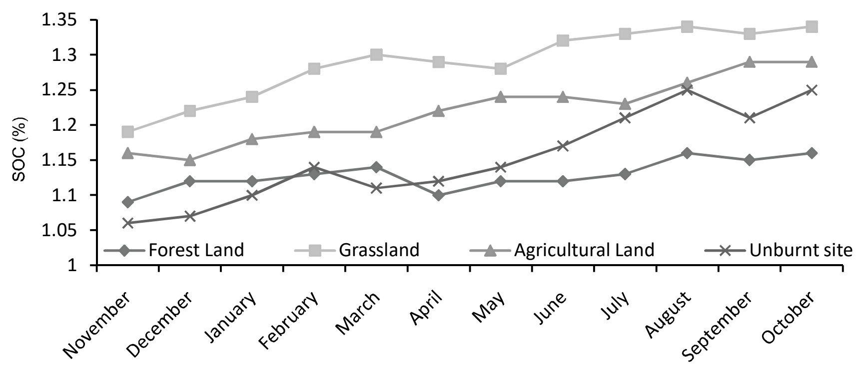

Fig. 5. Effect of forest fire on soil oragnic carbon content (SOC) (Sampled at 0-5 cm depth) under different land uses

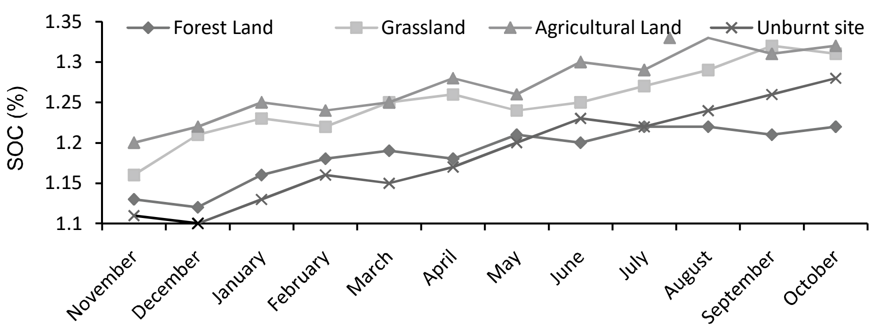

Fig. 6. Effect of forest fire on soil oragnic carbon content (SOC) (Sampled at 5-10 cm depth) under different land uses

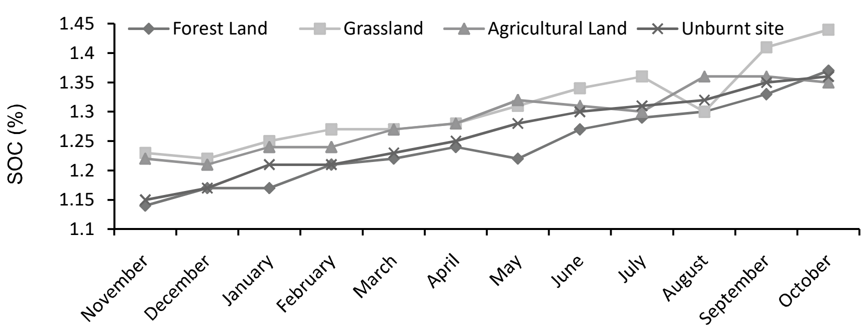

Fig. 7. Effect of forest fire on soil oragnic carbon content (SOC) (Sampled at 10-15 cm depth) under different land uses

Alterations in the nutrient supplies caused by the death of plant species can be a rationale for lower rates of biomass carbon (Mabuhay et al 2003, Hart et al 2005, Smith et al 2008, Shubham et al 2021, Singh et al 2021). Higher soil organic carbon content in grassland could be due to attributed to addition of greater litter as input, which might have provided a substantial carbon to the soil pool for microbial utilization. The results were in line with the findings of Palese et al (2004), Knelman et al (2015), Sadeghifar et al 2020). Forest fires increased the soil temperature to great extent and therefore the soil organic carbon content was reduced. But with the passage of time the microbial biomass carbon and SOC attained their pre fire levels in soil. Higher rates of microbial biomass carbon in mild winters and spring over harsh summers could be attributed to reason that most of the mineralizing microbes favours 25-35°C, beyond this their activity and multiplication restricts. The results were in agreement with the findings of Guerrero et al (2005) and Mabuhay et al (2006). The percentage of organic carbon was found to be decreased at a faster rate immediately after fire incidence and this decrease in organic carbon could be due to decline in the microbial C pool. The results were in line with the findings of Kara and Bolat (2009).

CONCLUSION

Incidences of forest fires are more endemic across the Himalayan region; therefore it becomes very important to study how these fires affect the ecosystem and their capabilities of responding and recovering. The forest fire had imparted a significant effect on reduced microbial biomass carbon. The soil organic carbon content was reduced after the fire incidence but carbon accumulation in soil returned to its pre fire levels with the passage of time. Highest levels of organic carbon were at 10-15 cm depths. Soil microbial biomass carbon contents were higher in forest land (0-5 cm sampling depth). Forest fire has a significant impact on microbial properties and soil enzymatic activity along soil physico-chemical properties. Soil biological properties were more sensitive on surface heating by fires than physico-chemical properties as it provide lethal temperatures for microbes occurring below 100 °C. Effect of fire on organic carbon and microbial biomass carbon was highly dependent on the intensity of the fire, soil moisture content and type of the burned materials. Effect of fires on soil properties and biomass carbon were highly variable and no generalized statements can be suggested for most of the fire-induced changes on soil organic carbon and biomass carbon content. Therefore, prescribed burning of floor litter at right time can be a good choice for avoiding wild fires incidences in region and for better plant regeneration and maximum stockpile of organic carbon in soil pool.

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Effect of Meteorological Parameters on Population Dynamics of Sucking Pest and its Natural Enemy in Brinjal

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Abstract: To assess the effect of meteorological parameters on seasonal population dynamics of whitefly, jassid and LBB on brinjal, experiments were conducted at College of Agriculture, Ganjbasoda during winter season (2016-2018) and rainy season (2018-2020) with NS302 variety. The activity of these insects were initiated in 44th standard meteorological week (SMW) with 30.16, 19.12 and 3.40individuals / plant and peak population of whitefly with 37.84 individuals / plant on 12th SMW, jassid found maximum with 25.36individuals / plant with 11th SMW however population of LBB get maximum (5.14 / plant) in 10th SMW respectively and remained active up to 13rdSMWduring winter season although seasonal activity of these insects were commencement from 27th SMW with 2.5, 1.29 and 3.40 individuals / plant and reached at maximum with 36.17 and 5.78 of whitefly as well as LBB on 42nd SMW whereas population of jassid was found higher (14.99) in 41st SWM and its ended up to 46thSMW during rainy season. The correlation coefficient between whitefly, jassid and LBB with population and meteorological parameters *viz.* maximum temperature (r=0.786*,r=0.782*,r=0.4738) minimum temperature (r=0.85*,r=0.852*, r=0.528*), wind speed (r=0.691*,r=0.451*) and BSS (r=0.801*,r=0.801*,r=0.433*) were strongly significant positive impact except wind speed in case of jassid whereas significant negative correlation with relative humidity (RH) in white fly and jassid (r=-0.512*, r=-0.509*). The Correlation analysis between whitefly, jassid and LBB population and weather parameters revealed that significant positive correlation with maximum temperature(r=0.569*, r=0.540) except jassid (r=0.256) and significant negative correlation with minimum temperature(r=-0.831*) only white fly however rainfall(r=-0.643*r=-0.569*) and BSS (r=-0.656*, r=-0.479*) excluding jassid.

Keywords: Brinjal, Meteorological parameters, Whitefly, Jassid, Lady Bird Beetle

The brinjal or eggplant (*Solanum melongena* L.) is one of the most common and key vegetable crops grown in India and other parts of the world. It is originated from India and second largest producer of brinjal after china. In India, it is cultivated mainly in West Bengal, Orissa, Bihar, Gujarat and Madhya Pradesh states (Garg et al 2018). In Madhya Pradesh, it is cultivated in 0.40 lakh hectare an annual production of 1.016 lakh tones and a productivity of 24.97 MT (Metric Tonn) per hectare(Shaikh and Patel 2012). The fruits contain approximately, 92.0 per cent moisture, 6.0 per cent carbohydrate, 1.0 per cent proteins, 0.3 per cent fats and some minerals. They are fairly good source of calcium, phosphorus, iron and vitamin B. Brinjal crop suffers severely due to infestation of various insect pests, which reduces its fruit yield and quality. In India, the crop is damaged by more than 30 insect pests' right from germination to harvesting (Raghupaty et al 1997). Shoot and fruit borer is most destructive and ubiquitous but whitefly, *Bemisia tabaci* Gennadius and Jassid, *Amrasca biguttula biguttulla*(Ishida) are also an important sucking pest that causes a considerable damage to the brinjal plants (Garg et al 2018). The losses caused by these pests were estimated to range from, 70-92 per cent in the fruit yield (Rosaiah, 2001). Weather plays an important role for determining the geographical distribution and periodic abundance of the insect pests. Among the weather factors; temperature, rainfall, relative humidity plays the crucial role in insect life (Seni and Naik 2018). For this purpose, an attempt was made to know the role of abiotic factors on the population buildup of sucking pest (White fly & Jassid and natural enemy (lady bird beetle (C*occinella septumpuntata*) on brinjal during winter (2016-2018) and rain*y* season (2018-2020), respectively.

MATERIAL AND METHODS

The experiment was laid out at instructional farm of College of Agriculture, Ganjbasoda district Vidisha (MP) India during winter season of 2016-17 & 2017-18 and rain*y* season 2018-19 & 2019-20. The College is situated at 23^{0}85' N latitude, 77^{0}92' E longitude in Ganjbasoda block of Vidisha district at an altitude of 416 m above MSL. The NS302 variety was transplanted during winter and rain*y* season in a plot size 4.5 m x6 m with 60x45 cm plant spacing. All cultural practices were followed from time to time to raise the crop successfully as per package of practices prescribed for the region except plant protection. The crop was regularly monitored after transplanting till final harvest for the seasonal population dynamics of whitefly and Jassid. The observations of the population of whitefly, and Jassid were be recorded on six compound leaves (3 middle + 3 lower) of ten randomly selected tagged plants. Numbers of insects (nymph and adult) were recorded in the morning time and cumulative population of whitefly and Jassid per plant was calculated. The observation of predator population of insect pest, lady bird beetle (LBB) recorded on whole plant basis. Theses populations were converted in mean population by averaging them. Weekly weather data on temperature, relative humidity (RH), rainfall, wind speed (km/h) and bright sunshine hours (BSS) were obtained from college of Agriculture, GanjBabsoda. The mean population data obtained from various Standard Meteorological Week (SMW) were subjected to simple correlation analysis with meteorological parameters maximum and minimum temperature, relative humidity (RH), rainfall, wind speed (km/h) and bright sunshine hours (BSS). Data was analyzed statistically by SPSS software and simple correlation and linear regression was worked out between population of sucking pest and natural enemy and weather parameters (maximum and minimum RH, rainfall, wind speed and BSS) by Karl Pearson's Coefficient of correlation and regression at 5% level of significance.

RESULTS AND DISCUSSION

Seasonal Population Dynamics of Sucking Pest and Natural Enemy

Winter season: The seasonal population dynamics of whitefly, Jassid and LBB on brinjal crop during winter season of was initiated in 44th SMW with 30.16, 19.12 and 3.40 individuals / plant, respectively. Thereafter the activity of whitefly population was gradually increase and reached at peak with 37.84 individuals / plant on 12th SMW however population of jassid found maximum with 25.36 individuals / plant with 11th SMW and the population of LBB get maximum in number with 5.14 per plant during 10th SMW respectively. When the favourable weather parameters were occurred, during these periods maximum temperatures, minimum temperatures, rainfall, wind speeds, relative humidity and BSS were 35.72^{0}C, 19.57^{0}C, 0.00mm, 5.57km/h, 22.64% & 12.09 respectively, 33.22^{0}C, 16.58^{0}C, 0.00mm, 6.22km/h, 19.93% and 11.76, 32.00^{0}C, 17.36^{0}C, 0.00mm, 6.86, 25.50 and 11.49 respectively. After that it was gradually declined as increased age of crop and remained active up to 13rd SMW with 30.17, 20.03 and 2.71 individuals/plant (Fig. 1 and 2). The present finding are more or less supported with result reported by Kumar et al (2016) found that maximum population of White fly, jassid and LLB was recorded during 10th, 12th and 11thSMW.

Rainy season: The seasonal activity of whitefly, Jassid and LBB on brinjal crop during rainy season was commencement from 27th SMW with 2.5, 1.29 and 3.40 individuals / plant. and reached at maximum with 36.17 and 5.78 of whitefly as well as LBB on 42nd SMW whereas population of jassid was found higher with 14.99individuals / plan in 41st SWM, during these period favorable parameters *i.e.* maximum temperatures, minimum temperatures, rainfalls, wind speeds, relative humidity and BSS were 31.78^{0}C, 19.93^{0}C, 1.00mm, 14.86km/h, 49% and 11.33 respectively and 32.50^{0}C, 20.14^{0}C, 11mm, 12.35km/h, 59.64% and 11.43 respectively. After that it was gradually declined and ended up to 46thSMW with 3.21, 1.19 and 1.2 individuals per plant (Fig. 2 and 4). The almost similar finding had reported by Mohapatra (2008) he indicated that peak whitefly population recorded in44th SMW whereas peak Jassid population was observed in 41st SMW

Correlation and regression analysis: Effect of weather parameters on seasonal population dynamics of whitefly, jassid and LBB was analyzed in term of correlation and regression in winter and rainy season pooled data and presented in Table 1 and 2.

Winter season: The correlation coefficient between whitefly population and meteorological parameters *viz.* maximum, (r=0.786*) minimum temperature (r=0.85*), windspeed (r=0.691*) and BSS (r=0.801*) were strongly significant positive impact on population of whitefly. The rainfall (r=-0.1) was non-significant negative impact on population fluctuation while relative humidity (r=-0.512*) was significantly negative correlation with population of whitefly. Step wise regression analysis showed that the maximum and minimum temperature, BSS, relative humidity and wind speed hours were significantly contributed 61.78%, 73.06%, 62.44% 26.25% and 13.13%, variation of whitefly population fluctuation. The present finding are in concordance with the results of Horowitz et al (1984) and Horowitz (1986) also reported that the atmospheric humidity, temperature and rainfall influence the population dynamics of whitefly. Gerling et al (1986) stated that extreme relative humidity, both high and low, were unfavorable conditions for survivable of immature stages of whitefly. They were spatio-temporal (factors influence the population such as natural enemies, climatic and habitats) variations in the area. Echlkraut and Cardona (1989) found that dry conditions were more favourable for whitefly than high precipitation. Similarly, study by Rafiq et al (2008) reported that whiteflies developed rapidly in warm weather and population cane buildup quickly in situations where natural enemies are destroyed and

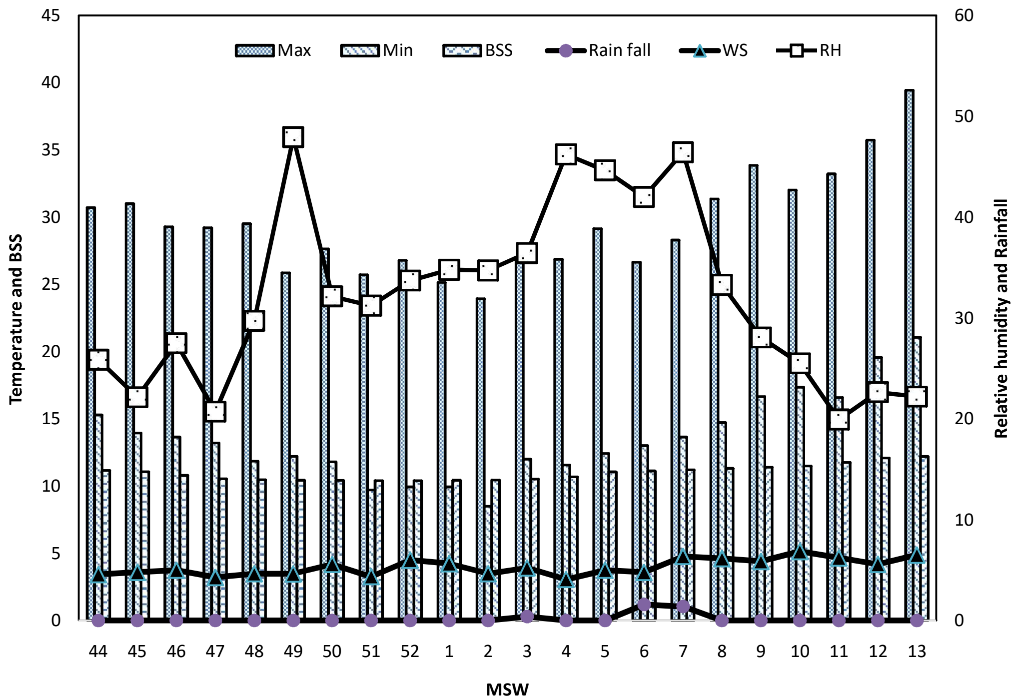

Fig. 1. Weather conditions during winter season brinjal crop study period and their effect on avearge population of sucking pest and their natural enemy during crop growing period 2018-2020

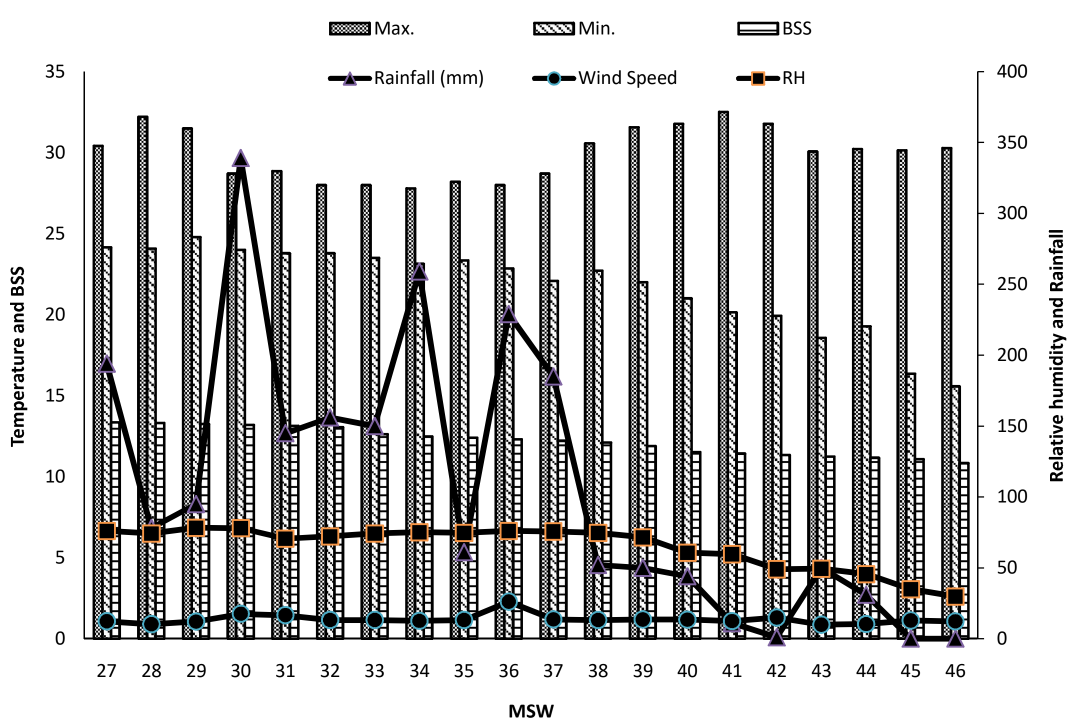

Fig. 2. Weather conditions during rainy season brinjal crop study period and their eggect on average population of sucking pest and their natural enemy during crop period of 2016-2018

weather is favourable. Heavy and prolonged periods of rain can substantially reduce population of whiteflies. Khan (2019) reported that a strong positive and linear relationship was observed between population of whitefly with average temperature and average relative humidity but he contradicted and present result reported that negative correlation with relative humidity. The present results are lineup with result reported by Kumar et al (2016) found that correlation coefficient between white fly population and other abiotic parameters like temperature, relative humidity, rainfall and wind speed was non-significant. The relative humidity (maximum and minimum), rainfall and wind speed have got negative impact on population flare-up of white fly while the temperature (maximum and minimum) has got non-significantly positive impact on the population fluctuation.

A significant positive and linear relationship between jassid population and meteorological parameters like maximum (r=0.782*), minimum temperature(r=0.852*) and BSS (r=0.801*). The other abiotic factors *i.e.* wind speed (r=0.392) showed positive impact on jassid population whereas rainfall (r=-0.047) was non-significant negative impression on population fluctuation while relative humidity(r=-0.509*) was significant negative correlation with population of leafhopper. The regression analysis exhibited that the maximum and minimum temperature, BSS and relative humidity were significantly contributed 61.27%,

Table 1. Correlation between sucking pest and natural enemy population in brinjal with meteorological parameters

Meteorological parameters	Correlation coefficient (r)					
	Winter season (2016-2018)			Rainy season (2018-2020)		
	Whitefly	Jassid	Lady Bird Beetle	Whitefly	Jassid	Lady Bird Beetle
Maximum temperature	0.786*	0.782*	0.473*	0.569*	0.256	0.540*
Minimum temperature	0.85*	0.852*	0.528*	-0.831*	0.015	-0.278
Rainfall (mm)	-0.1	-0.047	-0.030	-0.643*	-0.385	-0.569*
Wind Speed (km/hrs)	0.695*	0.392	0.451*	-0.240	0.010	-0.149
Relative humidity (%)	-0.512*	-0.509*	-0.352	-0.384	0.117	0.310
Sun Shine (hrs)	0.790*	0.801*	0.433*	-0.656*	-0.322	-0.479*

*Significant ($p<0.05$)

Table 2. Regression analysis of sucking pest and their natural enemy population with different weather factors on brinjal

Insects	Variables*	R^2	
		Winter season (2016-2018)	Rainy season (2018-2020)
Bemisia tabaci	Maximum temperature	0.6178 (61.78%)	0.3242 (32.42%)
	Minimum temperature	0.7306 (73.06%)	0.1726 (17.26%)
	Rainfall (mm)	0.0074 (0.74%)	0.4132 (41.32%)
	Wind Speed (km/hrs)	0.1313 (13.13%)	0.0747 (7.47%)
	Relative humidity (%)	0.2625(26.25%)	0.1475 (14.75%)
	Bright sun shine (hrs)	0.6244 (62.44%)	0.4304 .04%)
A. biguttulla bituttulla	Maximum temperature	0.6127 (61.27%)	0.0654 (6.54%)
	Minimum temperature	0.726 (72.60%)	0.0002 (0.02%)
	Rainfall (mm)	0.0023 (0.23%)	0.1478 (14.78)
	Wind speed (km/hrs)	0.1539 (15.39%)	0.0003 (0.03%)
	Relative humidity (%)	0.2594 (25.94%)	0.0138 (1.38%)
	Bright sun shine (hrs)	0.6421 (64.21%)	0.1039 (10.39%)
Coccinellaseptum punctata	Maximum temperature	0.2788 (27.88%)	0.2908 (29.08%)
	Minimum temperature	0.2233 (22.33%)	0.0773 (7.73%)
	Rainfall (mm)	0.001 (0.10%)	0.3233 (32.33%)
	Wind speed (km/hrs)	0.1255 (12.55%)	0.0221 (2.21%)
	Relative humidity (%)	0.1239(12.39%)	0.0963 (9.63%)
	Bright sun shine (hrs)	0.1877(18.77%)	0.2292 (22.92%)

72.60%, 64.21 and 25.94% Jassid population variability. The present results are conformity with Mahamood et al (1990) also observed that among the various environmental factors, the only significant factors that influence the density of Jassid was maximum and minimum temperature. The present findings are accordance with Kumar et al (2016) indicated that correlation coefficient between Jassid and temperature (maximum and minimum) alongwith relative humidity (maximum and minimum) was significant. The temperature (maximum and minimum) has got significantly positive impact on the fluctuating population of jassid.

The significant positive correlation was found between LBB population and maximum (r=0.473*), minimum temperature (r=0.528*), wind speed (r=0.451*) and BSS (r=0.433*). Neither relative humidity (r=-0.352) nor rainfall (r=-0.030) had significant influence on LBB population. This indicates that activity of LBB population increase with the rise of temperature, wind speed and BSS and decrease with the rise of relative humidity and rainfall during winter season.The regression coefficient revealed that the maximum and minimum temperature, BSS and wind speed were contributed 27.88%, 22.33%, 18.77% and 12.55% significantly variation in LBB population fluctuation. The present observations are conformity with findings of Khan (2019).

Rainy season*:* Correlation coefficient between whitefly population and weather parameters revealed that whitefly population had significant positive correlation with maximum temperature(r=0.569*) and significant negative correlation with minimum temperature(r=-0.831*), rainfall(r=-0.643*), and BSS (r=-0.656*) while wind speed (r=-0.240) and relative humidity (r=-0.384) has non-significant negative correlation. The significant contribution of regression coefficient discovered that the BSS, rainfall, maximum, minimum temperature and relative humidity were 43.04%, 41.32%, 32.42%, 17.26% and 14.75% variation in whitefly population fluctuation, On the other hand wind speed showed non-significant deviation in whitefly population buildup. The present findings are supported by Zia *etal*.(2013) they found that whitefly population showed that rainfall and relative humidity were negatively correlated while temperature was positively correlated with whitefly population.

The Correlation coefficient between jassid population and weather parameters expressed thatjassid population had non -significant positive correlation with maximum (r=0.256), minimum temperature(r=0.015), relative humidity(r=0.117) and wind speed (r=0.010) although rainfall (r=-0.385) and BSS (r=-0.322) had non-significant negative correlation. It suggested that the number of jassid population was less dependent on weather parameters except rainfall and BSS. The linear regression showed that the rainfall and BSS contributed 14.78% and 10.39% variation in jassid population. The other abiotic factors *viz*. Temperature, wind speed and relative humidity expressed very less impact on jassid population buildup. The present findings are refuted with result of Patel and Radadia (2018) found that jassid population showed significant positive correlation with maximum temperature, evaporation and sunshine hours whereas, significant negative correlation with wind speed and rainy days. In case of linear regression he indicated that upto 42.5 percent (R2= 0.425) on the population of jassid. Further, Prasad et al (2008) showed the multiple linear regression analysis indicated that the total influence of all the weather parameters was high and significant, it was upto 51.78 percent (R2=0.5178) on the population of leafhoppers.

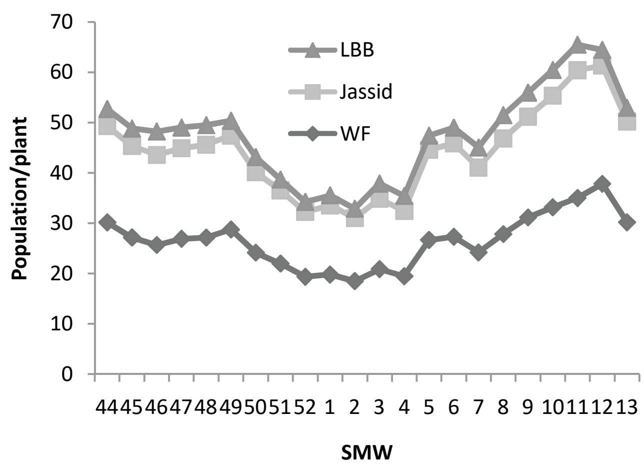

Fig. 3. Influence of weather parameters on sucking pest and their natural enemy during winter season

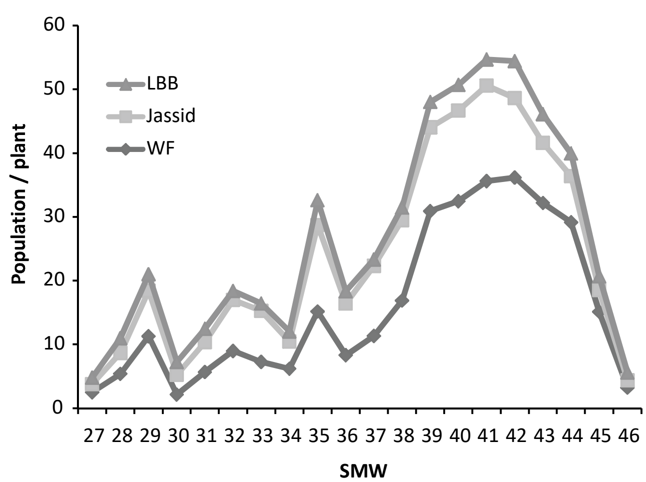

Fig. 4. Influence of weather parameters on population of sucking pest and their natural enemy during rainy season

Hameed et al (2014) noted the multi variable regression model along with coefficient of determination between weather factors and cotton insects clearly showed that highly significant linear relationship was observed between the maximum temperature and jassid population having 10.8 to 48.0 percent role and These results are less supported to present observations.

The correlation coefficient between LBB population and maximum temperature (r=0.540*) was found significantly positive whereas relative humidity was observed non-significant positive (r=0310).On the other hand the correlation coefficient between minimum temperature (r=-0.278) and wind speed (r=-0.149) was recorded non-significant negative correlation while bright BSS (r=-0.479*) and rainfall (r=-0.569*) were calculated significant negative correlation. This indicates that activity of LBB population increase with the rise of maximum temperature and decrease with the rise of rainfall and BSS. Neither the relative humidity nor minimum temperature with wind speed had significant influence on LBB population. The regression coefficient expressed that the rainfall, maximum temperature and BSS added 32.33%, 29.08% and 22.92% variation in LBB population instability. The similar results with meteorological parameters viz., wind speed and rainfall while temperature, relative humidity and BSS was disagree with results reported by Patel and Radadia (2018) he observed that the population of LBB showed highly significant negative association with minimum and average temperature, morning, evening and average relative humidity, wind speed, rainfall and rainy days while highly significant positive association with sunshine hours.

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Development and Optimization of Process Variables of Protein Enriched Rice Based RTE Food Products

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Abstract: The effect of barrel temperature (100, 120 and 140°C), feed moisture content (12, 14 and 16%) and screw speed (300, 350 and 400 rpm) on extruded product physical, functional and textural characteristics were investigated and optimized using response surface methodology (RSM) following Box-Behnken design. A standardized feed mix of Rice: Ragi: Defatted groundnut: Defatted soybean: Bengal gram (60:10:10:10:10) were used for the development of extruded food product. The extrusion conditions of 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed gave an optimized product with a desirability of 0.837. The respective optimized response values identified were Expansion ratio- 3.18, Bulk density- 0.658 g/cm^3, Water absorption index -5.61 g/g, Water solubility index- 30.24 %, Hardness -24.21 N, Fracturability -10.94 N and Crispness- 55.09 N respectively. The optimized extruded food has 14.31% protein, so that 100g extruded product developed at optimized condition will contribute 35.86% of recommended daily allowance for protein in case of boys, 34% for girls, 23.85% for adult men and 26.01% for adult women, respectively.

Keywords: Extrusion, Protein Enrichment, Texture, Optimization, Rice

Extrusion cooking is a well-known method for developing ready to eat (RTE) snack foods from cereals and plant protein food stuff, perceives evoked substantial curiosity and attraction over the last 30 years. In extrusion processing, pre-conditioned raw materials are allowed to pass under a set of processing conditions through a narrow-shaped hole (die) at a controlled rate to attain different products (Ajitha and Jha 2017). Extrusion is one of the commonly adopted processing techniques by agro-food industries which employ unit operations such as mixing, forming, texturing and cooking to develop a novel food product (Singh et al 2007). In modern food industries sensory evaluation of such RTE food products may be done by the application of fuzzy logic (Babu and Rajesh 2021).

Most of the extruded food products are prepared from starchy materials such as rice, corn and wheat flours but these are limited in essential amino acid content with inadequate nutritional value. Even though, the base component needs to be certain starch abundant materials which become gelatinized at the time of extrusion process. The extrudate physical properties such as expansion ratio and bulk density and textural properties such as hardness and crispness are largely affected by starch sources used in extrusion cooking. The extrudates based on rice starches comprising 20% and 30% amylose showed maximum expansion ratio (Pandiselvam et al 2018). The nutritional deficiency of snack foods can be corrected by using composite flour by selecting suitable raw materials containing high amount of carbohydrate, protein, fiber etc. Cereal based food products are ample in carbohydrates and energy but they are insufficient in terms of protein (Balasubramanian et al 2012).

Rice flour has turned an excellent component in the extrusion industries because of its specific attributes like bland taste, appealing white color, hypo allergenicity, and easiness for digestion (Kadan et al 2003). Addition of legume flour imparts a positive influence on levels of protein and dietary fiber of starch based extruded foods. For the manufacture of nutritious rich extruded snacks, proportion of starch fortification varied in accordance with the type of each material. Legumes have been characterized to impart excellent expansion and are considered as highly suitable for the manufacture of low-calorie and high-nutritional snacks. Finger millet also called ragi which is abundant in carbohydrate, protein, calcium, dietary fiber, iron, minerals and less fat. It is a fundamental food for those people having metabolic diseases such as obesity and diabetes (Mathanghi and Sudha 2012). Defatted groundnut cake flour extensively used in the preparation of variety of food products. It is also fortified with cereal flour to yield products with exceptional flavor, texture and color. Due to its unique nutritional properties it has been used in the diets of people suffering from cardio vascular disease, celiac disease and malnutrition. The fat present in soybean is removed and the remaining products is known as defatted soy flour, that can be used to develop high protein, low fat diet food and due to its unique nutritional profile it serve as promising protein source for the future (Singh et al 2008). Bengal gram or Chickpea has predominant amount of most of the essential amino acids except sulfur containing types and it also a very good source of vitamins such as riboflavin, niacin, thiamin and vitamin A precursor called β- carotene. The minerals such as calcium, magnesium, phosphorous and potassium also present in chickpea. Chickpea has numerous positive health effects and in mixing with other cereals and pulses, it might have beneficial impact on certain serious human diseases such as type 2 diabetes, digestive diseases, cardiovascular diseases and some cancer (Jukantil et al 2012). Protein energy malnutrition is a serious health problem especially in children because of changes in eating pattern and life style. The demand for extruded snack product is expanding at a phenomenal rate so that a protein enriched nutritionally balanced extruded snacks can be made by blending legumes such as Defatted groundnut, Defatted soybean and Bengal gram with rice and ragi. Because of this, the present study is concentrated on optimizing the extrusion process condition by using response surface methodology to achieve a desired extruded food product.

MATERIAL AND METHODS

Raw materials: The raw materials utilized for the production of RTE extruded food products are rice (Jaya variety), ragi, defatted groundnut, defatted soybean and Bengal gram. Jaya variety of rice was obtained from Regional Agriculture Research Station (RARS), Pattambi and other raw materials were procured from the local markets in Kuttippuram, Kerala. All the flours then sieved manually using ISS 85 mesh in order to get uniform particle size.

Blending of flours: The standardization of the feed composition of RTE foods were done during the preliminary evaluation in order to develop extrudate with superior expansion ratio and lesser bulk density. Ten feed compositions were prepared by mixing these raw materials in different proportions and extruded at 120°C barrel temperature, 14% feed moisture content (wet basis) and 350 rpm screw speed. The extrudates developed from the feed composition Rice: Ragi: Defatted groundnut: Defatted soybean: Bengal gram in the ratio 60:10:10:10:10 showed superior expansion ratio and lesser bulk density. Hence it was selected for further experiments.

Extruder and processing conditions: A co-rotating Twin screw extruder (Basic Technology Private, Ltd., Kolkata, India) with an L/D ratio 16:1 and die diameter of 3mm were used for the development protein enriched extruded foods. Feed moisture content ranging from 12 to16% (wet basis) with three variation levels 12, 14 and 16%, barrel temperature ranging between 120 to 140°C with three variation levels 120,130 and 140°C and screw speed ranging from 300 to 400 rpm with three variation levels 300,350 and 400 rpm were selected based on the literature (Hagenimana et al 2006, Garber et al 2006) and preliminary studies. The products obtained were dried for one hour in a mechanical dryer at 40°C temperature and then packed in poly ethylene pouches, sealed and stored for further studies.

Proximate composition: The proximate composition of standardized feed mix before extrusion and optimized extruded product was determined by standard method of the AOAC (2005)- moisture, water activity (Aqua lab water activity meter -Aqua lab, Decagon device Inc., Pullman (Wa), USA), crude protein (AOAC, 2005, 920.86), fat (AOAC 2005, 920.85) and the carbohydrate were determined. The carbohydrate content was analyzed by the Anthrone method (Hedge and Hofreiter 1962), dietary fiber was determined by the method of Ranganna (1986) and the energy content was determined by the formula given by Ekanayake et al (1999).

Effect of Extrusion Condition on Extrudate Properties

Expansion ratio: The expansion ratio (ER) of extrudate is the ratio of diameter of extrudate to the diameter of die hole (3 mm). Ten extruded samples were randomly selected and mean diameter was measured with a vernier caliper (Fan et al 1996).

$$\text{Expansion ratio} = \frac{\text{Diameter of extruded product (mm)}}{\text{Diameter of die hole (mm)}} \quad (1)$$

Bulk density: The bulk density (BD) of the protein enriched extrudates was determined using the method described by Chinnaswamy and Bhattacharya (1986).

$$\text{Bulk density (g/cm}^3) = \frac{4\text{m}}{\pi d^2 L} \quad (2)$$

Where, m is the mass (g) of the extruded sample, L is the length (cm) of extrudate and d is diameter (cm) of the extruded sample.

Water absorption index (WAI) and water solubility index (WSI): WAI and WSI were calculated by the method explained by Anderson (1982). The extrudates were milled and sieved in ISS 90 mesh in order to get uniform particle size. 1g of sample was weighed and shifted into a centrifuge tube and 10ml distilled water was added. Using a test tube shaker the centrifuge tube with sample was shaken for 15 minutes. After that the samples was centrifuged at 4000 rpm for 15 minutes. The supernatant was transferred into petri dish for finding its solid content by keeping the petri dish in an oven at 100°C for 2-3 hr and final weight of the petri dish was noted. The weight of the wet sediment was also recorded and

WAI and WSI were determined by using the following equations;

$$\text{Water absorption index (g/g)} = \frac{\text{Weight of wet sediment}}{\text{Intial weight of dry solids taken}} \quad (3)$$

$$\text{Water solubility index (\%)} = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of dry solids}} \times 100 \quad (4)$$

Texture analysis: Textural properties of RTE expanded products were analyzed using a Texture Analyzer (TA.XT texture analyzer, Stable micro systems Ltd). The textural properties such as hardness (HD) and fracturability (FR) were determined by using penetration test. Crispiness (CR) was determined by using shear test. In the penetration test, the 5 mm cylinder probe was used to pierce into the extrudate test sample and the force required attaining a specific piercing depth or the depth of piercing in a particular time, under definite conditions, was estimated and indicated as hardness. The TA setting for penetration test includes mode: Measure Force in Compression, Pre Text speed: 1mm/s, Option: Return to Start, Distance (compression): 4 mm, Post Test speed: 10 mm/s, Data Acquisition Rate: 400 pps and Test speed: 1 mm/s. Kramer shear cell five-blade probe was used, with test speed of 1 mm/s for determining crispness. Adequate amount of extruded products was used to cover the bottom of the cell, without overlapping, and the test was performed until the probe had completed its travel. The peak force obtained (in newton's) was noted.

Optimization of process parameters: The process parameters such as barrel temperature (°C), feed moisture content (% w.b) and screw speed (rpm) were optimized using Box-Behnken design using Response Surface Methodology.

RESULTS AND DISCUSSION

Experimental design: The effect of process parameters such as barrel temperature (°C), feed moisture content (% wb) and screw speed (rpm) on physical, functional and textural characteristics of extrudates were investigated. The experiments were designed based on Box-Behnken design using Response Surface Methodology with three factors at three levels (-1, 0 and +1). The number of experiments (N) or runs in the Box-Behnken design is obtained from the equation N= 2 k (k-1) +C_0 (where k is the number of factors and C_0 is the number of central points). In the present investigation there were 18 experiments with 6 central points. The coded (±1 and 0) and natural value of the independent variable with design matrix is illustrated in Table 1. A second order polynomial was used for three factor design which is given as

$$Y=b_0+b_1A+b_2B+b_3C+b_{11}A^2+b_{22}B^2+b_{33}C^2+b_{12}AB+b_{13}AC+b_{23}BC \quad (5)$$

Where, Y is the predicted response, b_0, b_1, b_2 and b_3 are linear terms, b_{11}, b_{22} and b_{33} represent square terms, b_{12}, b_{13} and b_{23} are interaction terms and A, B and C are the process parameters. The responses evaluated were ER, BD, WAI, WSI, HD, FR and CR. The ANOVA was performed by using Design Expert 7.0.0 (State-Ease, Inc., Minneapolis) to identify the significance at 0.01%, 0.1%, 1%, and 5% for the linear, interaction, and quadratic effects of the independent parameters and goodness of fit. The regression coefficients estimated for the actual values are indicated in Table 2. The quality parameters such as ER and BD are one of the important characteristics of expanded RTE food products. Low BD and high ER are most desirable factors for consumer acceptance (Ajitha and Jha 2017). Apart from these properties, low HD and FR and high CR are also suitable for an extruded food product. Considering these characteristics as prime importance, the optimization of the extrusion process parameters was conducted and recorded.

Proximate analysis: The proximate composition of standardized feed mix before extrusion and optimized extruded product were determined. The protein content of the extruded product is lower than that of the raw feed mix (Table 3). It may be due to hot extrusion process. The reason for this decrease may be due to the denaturation of protein at higher temperature. Low amount of protein was also due to the loss of nitrogen during extrusion due to the development of isopeptide bonds with instantaneous discharge of ammonia (Kasprzak and Rzedzicki 2008, Jhoe et al 2009). Fat content also reduced after extrusion. The possible reason may be due to the burning of fat at high extrusion process temperatures. This variation in fat content during extrusion was occurred by the development of starch-lipid and protein-lipid networks (Singh et al 2007). Carbohydrate was also decreased during extrusion. This possibly owing to the degradation of starch at higher temperatures. The dietary fiber content is increased after extrusion. It may due to increment in soluble dietary fiber due to disturbance in covalent and non-covalent bonds presented in the carbohydrate and protein moieties causing smaller and more number of soluble molecular fragments in addition to the development of resistant starch and 'enzyme-resistant indigestible glucans' created by transglycosidation. (Rashid et al 2015).

Extrudate Properties

Expansion ratio: The effects of process parameters on ER of the extrudates are interpreted in Table 1. The regression coefficients of dependent variables are given in Table 2. ER varied between 2.42 to 3.21. Figure 1(a) and (b) showed the effect of process parameters on expansion ratio. The reason for expansion at higher barrel temperature can be attributed to the starch gelatinization and strengthening of structure (Ainsworth et al 2007). The ER decreased from 3.21 to 2.42

Table 1. Box –behnken experimental design with independent variables and dependent variables of protein enriched extruded food

Run	Independent variables			Dependent variables						
	Temperature (°C)	Moisture content (%w.b)	Screw speed (rpm)	ER	BD (g/cm^3)	WAI (g/g)	WSI (%)	HR (N)	FR (N)	CR (N)
1	130	14	350	2.77	0.775	4.32	23.12	26.57	13.52	54.12
2	140	14	300	3.06	0.681	5.52	27.67	25.15	12.77	53.17
3	120	12	350	2.61	0.821	3.75	20.38	30.16	17.08	48.51
4	140	14	400	3.10	0.673	5.37	29.14	24.77	11.82	54.82
5	130	16	400	2.69	0.752	4.12	25.43	28.15	15.00	51.95
6	140	16	350	2.98	0.663	5.40	28.02	24.31	11.24	54.22
7	140	12	350	3.21	0.652	5.61	30.25	23.52	10.30	55.72
8	130	14	350	2.78	0.776	4.22	23.22	27.85	14.82	53.18
9	130	12	300	2.72	0.762	4.85	24.15	28.45	15.98	51.13
10	130	14	350	2.79	0.769	4.25	23.24	27.52	14.53	53.42
11	130	12	400	2.82	0.745	4.58	26.75	27.12	14.12	52.48
12	120	14	400	2.59	0.835	3.40	19.52	31.20	18.62	47.45
13	130	14	350	2.78	0.765	4.39	22.35	27.32	14.31	53.67
14	130	14	350	2.79	0.764	4.36	22.24	27.41	14.44	53.53
15	120	14	300	2.52	0.841	3.58	18.30	32.99	19.43	46.37
16	130	14	350	2.87	0.765	4.20	22.21	26.44	13.41	54.29
17	120	16	350	2.42	0.858	3.62	15.68	33.86	20.54	45.23
18	130	16	300	2.60	0.783	4.70	21.52	29.12	16.41	50.18

ER- Expansion ratio, BD- Bulk density, WAI- Water absorption index, WSAI- Water solubility index, HD- Hardness, FR- Fracturability, CR- Crispness

Table 2. Regression coefficients of the fitted second order polynomial for dependent variables

Coefficients	Regression coefficients						
	ER	BD (g/cm^3)	WAI (g/g)	WSI (%)	HD (N)	FR (N)	CR (N)
Intercept	2.80	0.77	4.29	22.73	27.19	14.17	53.71
A	0.28****	-0.086****	0.94****	5.15****	-3.86****	-3.69****	3.80****
B	-0.084***	9.500E-003*	-0.12*	-1.36**	0.72**	0.71*	-0.78**
C	0.039*	-7.750E-003*	-0.15**	1.15**	-0.56*	-0.63*	0.73**
AB	-9.250E-003	-6.500E-003	-0.020	0.62	-0.63	-0.63	0.45
AC	-7.500E-003	-5.000E-004	7.500E-003	0.062	0.35	-0.035	0.14
BC	-2.500E-003	-3.500E-003	-0.078	0.33	0.090	0.11	0.11
A^2	0.060*	-0.012	0.11	0.024	0.60	0.45	-1.88***
B^2	-0.050*	-8.750E-003	0.20**	0.83	0.28	0.17	-0.90*
C^2	-0.037	2.500E-004*	0.072	0.90	0.74*	1.04*	-1.37**

Significance at ****(P < .0001), ***(P < .001), **(P < .01), *(P < .05)

Table 3. Proximate composition of standardized feed mix and optimized extrudate

Material	Moisture content (%)	Water activity	Protein (%)	Carbohydrate (%)	Fat (%)	Dietary fibre (%)	Energy content (kJ/100 g)
Standardized feed mix before extrusion	8.95	0.562	16.01	71.54	3.51	8.61	1594.41
Optimized extruded product	4.39	0.430	14.31	68.60	2.20	9.49	1467.54

as the moisture content of feed increased from 12 to 16% (w.b). The increase in feed moisture content can decline the elasticity of the mass by plasticizing the melt and, consequently, lowering gelatinization, decline the expansion and raises the density (Korkerd et al 2016). The process parameter screw speed also showed a positive influence on expansion ratio of the extrudates. Elevated screw speeds may be likely to reduce melt viscosity of the mixture enhancing the elasticity of the dough, causing increased expansion and depletion in the density of the extrudate (Ding et al 2006).

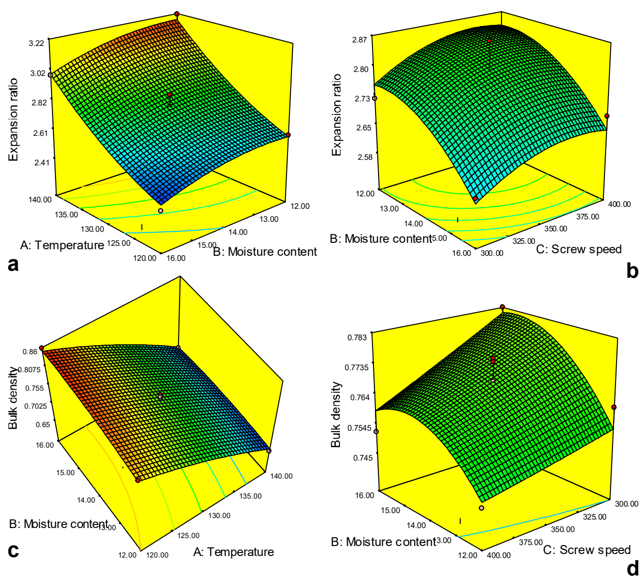

Fig. 1. Response surface diagram illustrating the effect of process parameters on Expansion ratio (a and b) and Bulk density (c and d)

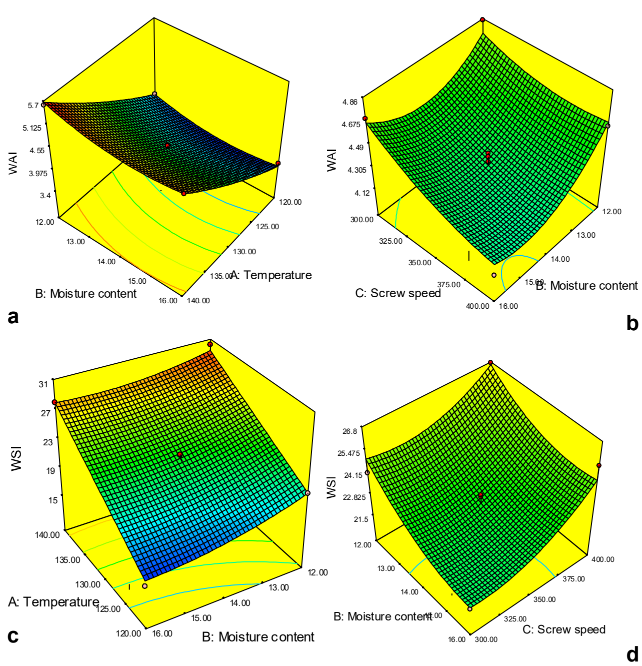

Fig. 2. Response surface diagram illustrating the effect of process parameters on WAI (a and b) and WSI (c and d)

Bulk density: The BD of extrudates decreased from 0.858 to 0.652 g/cm^3 as the barrel temperature increased from 120 to 140°C (Table 1). The 3D graphs (Figure 1(c) and (d)) representing the response surface are also indicates the effect of process parameters on BD. The viscosity of plasticized mass inside the extruder decreases with increase in barrel temperature and it would enhance the bubble enlargement throughout extrusion cooking and thus lowering the density. The higher screw speeds may reduce the melt viscosity of the mix which causes an increase in elasticity of the dough and thus producing extrudates with lower density.

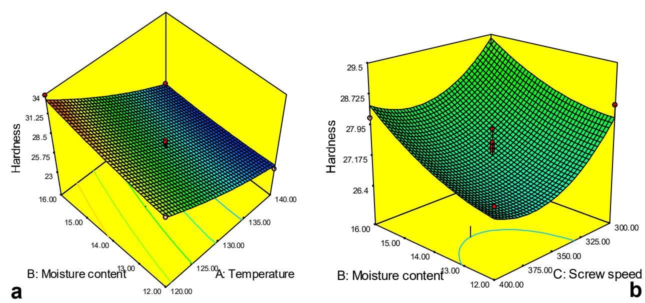

Fig. 3. Response surface diagram illustrating the effect of process parameters on HR

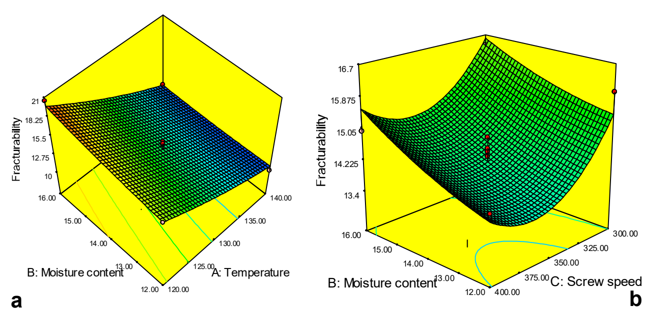

Fig. 4. Response surface diagram illustrating the effect of process parameters on FR

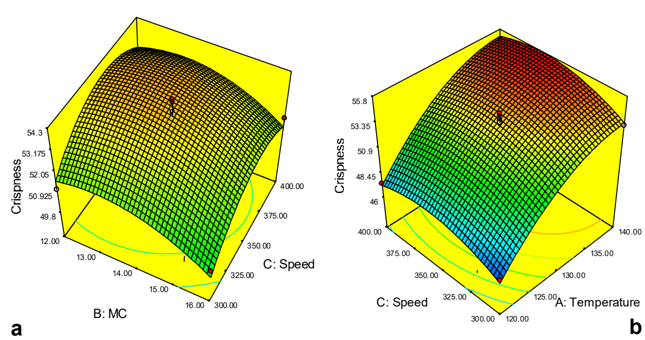

Fig. 5. Response surface diagram illustrating the effect of process parameters on CR

The process parameter moisture content had a negative influence on BD. An increase feed moisture content during extrusion process may lower the elasticity of the dough through plasticization of the melt, resulting in decreased specific mechanical energy and thereby lesser gelatinization, reducing the expansion and an increase in the density of extrudate (Ding et al 2006).

Water absorption index: The water absorption index (WAI) of extrudates samples changed from 3.40 to 5.61 g/g. WAI of extrudates increased significantly with an increase in barrel temperature (Table 1, Fig. 2a). As temperature rises, the starch got damaged because of gelatinization and extrusion process. The elevated temperature produced dextrinization, which create additional hydrophilic space and therefore increased WAI. WAI showed a decreasing pattern with moisture content (Figure 2b)). It may due to the fact that at higher moisture situations cause lesser shear degradation of starch throughout extrusion (Anastase et al 2006). Screw speed also found to have a negative effect on WAI of the extrudates (Fig. 2 b). This is due to the fact that high input of thermal energy due to high residence time i.e., at low screw speeds may cause enhanced level of starch degradation and increased WAI (Yagcı et al 2008).

Water solubility index: The increasing trend was noticed in WSI with temperature (Table 1, Fig. 2c and d). It may be due to the fact that elevated temperature imparts additional soluble constituents leads to increment in WSI (Ding et al 2005). Screw speed also showed an increasing trend in WSI. The increase in screw speed causes increment in specific mechanical energy which resulting the breakdown of macromolecules and thus causing rise in soluble constituents after extrusion cooking. The higher mechanical shear enhanced breakdown of macromolecules to micro molecules with elevated solubility (Dogan and Karwe 2003). But the process parameter moisture content had an inverse effect on WSI. This trend was created by lesser shear disintegration of the starch at the time of extrusion at elevated moisture contents causing lower WSI (Pardhi et al 2017).

Textural properties: For extruded snacks, low values for hardness, factorability and high value of crispness are desirable. The 3D graphs representing the response surface are depicted in Figure 3, 4 and 5. The regression coefficients of dependent variables are given in Table 2. Both HR and FR showed a negative relationship with extrusion temperature but CR had a positive effect. An increase in temperature increased the degree of superheating of water in the extruder. This enhances the bubble development and also decreased melt viscosity, which in turn results reduction in density and hardness of extrudate and produce crispy extrudates. Screw speed also showed a negative trend with both HR and FR but positive trend with CR. It may be related to higher expansion and lower density of extrudates at higher screw speed (Ding et al 2005, Sharma et al 2016). But the process parameter feed moisture content had a positive effect on both HD and FR and negative effect on CR. The reason may be due to plasticizing characteristics of starch containing ingredients causing viscosity reduction and the mechanical energy liberation in the extruder and thus producing the product somewhat denser and suppress the bubble development (Chiu et al 2013).

Optimization of extrusion process variables: The independent variables were optimized numerically by using statistical software Design Expert- (Ver- 7.7.0. -Stat-Ease Inc.). For the optimization of process parameters ER, WAI, WSI and CR values were kept maximum and simultaneously BD, HR and FR values were kept minimum. From the numerical optimization process, it was observed that 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed gave an optimized product with a desirability of 0.837. The resultant optimized response values identified were ER- 3.18, BD- 0.658 g/cm^3, WAI -5.61 g/g, WSI- 30.24 %, HD -24.21 N, FR -10.94 N and CR- 55.09 N. The extrusion process was conducted based on the optimized conditions and the proximate composition of optimized product were recorded (Table 3).

CONCLUSION

Designed experiments applying Box-Behnken method effectively showed the impact of independent variables such as barrel temperature, feed moisture content and screw speed on the response variables including physical, functional and textural properties of extrudates developed from protein enriched formulation. The optimized extrusion process conditions were found as 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed, respectively. The optimized extruded product has 14.31% protein, so that 100 g extruded product developed at optimized condition will contribute 35.86, 34, 23.85 and 26.01% of RDA of protein in boys, girls, adult men and women, respectively.

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Productivity and Carbon Storage Potential of different Land Use Systems in Leh Region of Himalayan Cold Desert

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Abstract: Agroforestry is a sustainable land use system which has the potential to contribute to the improvement of rural livelihood and at the same time helps in fighting against global warming by sequestration of atmospheric carbon. Therefore in the present study, an attempt was made to estimate the total biomass production and carbon storage potential of seven different land use systems viz. agriculture, horticulture, agrisilviculture, agrihortisilviculture, silvipasture, hortisilvipasture and agrihorticulture in Leh region of Himalayan cold desert in the year 2017-2018. The hortisilvipasture land use system recorded maximum total biomass production (30.88 t ha^{-1}) and total carbon stock (15.44 t ha^{-1}) whereas, the minimum total biomass production (8.76 t ha^{-1}) and total carbon stock (4.38 t ha^{-1}) was in agriculture land use system.

Keywords: Biomass, Carbon, Cold desert, Himalayas and land use

Land is the most important natural resource which embodies soil, water and associated flora and fauna involving total ecosystem. The term 'land use' refers to the human activity or economic function associated with a specific piece of land whereas the 'land cover' relates to the type of feature present on the surface of the earth. The land use/land cover is the result of permanent adjustment between the constraining properties of land and the socio-economic attributes whereas the land utilization type defines the technical details about cultivation/form details. Information on land use or land cover allows a better understanding of the land utilization aspects like cropping patterns, fallow lands, forests, pasture lands, wastelands and surface water-bodies which are vital for development planning (Lillesand and Kiefer 2000). The Himalayas is a vast mountain system covering partly/fully 8 countries i.e. Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal and Pakistan. It consists of various kinds of land uses, like forest (Government forest, Village forest, and Municipal forest), cultivated and fallow land, non-cultivable land and alpine pastures. North western Himalayas is basically an agro-ecosystem, where 90 per cent of its total population lives in villages whose economy is dependent on agriculture, horticulture and animal husbandry. In India, cold desert comes under the trans Himalayan zone which is approximately 1,03,11,300 hectares of area (Gupta and Arora 2016). These arid areas are not benefited by the south east monsoon because they lie in the rain shadow area of the Himalayan mountain system. A cold desert ecosystem refers to an area where the climate has characteristics of great extremes of being hot and cold combined with excessive dryness. Temperature in these tracts normally ranges from -45°C during winters to +35°C in summers. In addition, scanty rainfall, massive snowfall, high wind velocity, sparse vegetation, high UV radiation, and extremely xeric conditions are the common features of this region (Devi and Thakur 2011). The soil of cold desert is generally grey and light, characterized by low fertility status coupled with poor water retention capacity and scanty plant cover. In absence of any substantial leaching of minerals from the soil, the bases are continuously added to the soil complex, thereby rendering the pH values on alkaline side (Tundup et al 2018). However, due to huge variations in the geology of the region, the nature and composition of the soil varies. Summers (June to September) the major growth period for the plants, is quite short and because of these plants require more time to establish themselves in such harsh areas (Tiwari and Kapoor 2013). Ladakh in erstwhile state of Jammu and Kashmir and Lahaul and Spiti in Himachal Pradesh form the cold desert. In India, major part of cold desert in the country is confined to Ladakh with approximately 82,665 sq. Km area and 37,555 sq. Km of this area is under the illegal occupation of Pakistan and China (Butola et al 2012). Hence area with India is 45,110 sq. Km, representing 87.40 per cent of total cold arid region. This region is enclosed by the Ladakh and Karakoram range in the north and Zanskar mountain and the Great Himalaya in the south. Land based economy of this region consists of agriculture and allied sectors. The region is ecologically fragile and subsistence agriculture is the backbone of local livelihoods. Agroforestry in this region has a potential to provide options for improvement in livelihood through simultaneous production of food, fodder and firewood (Salve and Bhardwaj 2020). Agroforestry is one of the important terrestrial carbon sequestration systems that help in mitigation of the impact of climate change (Tiwari 2000).

MATERIAL AND METHODS

The present study was carried out in two blocks i.e. Khaltsi and Saspol block of Leh district of erstwhile state of Jammu and Kashmir (now Union Territory of Ladakh). The region is enclosed by the Ladakh and Karakoram ranges in the north and Zanskar mountain and The Great Himalayas in the south. The average altitude of the region is 3200 m amsl. Precipitation is very low and mainly occurs in the form of snow in the winter months . The study sites were selected through multi-stage random sampling technique. Leh district consisted of 16 blocks and out of all the blocks, Khaltsi and Saspol block were selected for the study. In the first stage, two panchayats from each block were selected. In the second stage, from each panchayat, two villages were selected. In the 3rd stage, from each village, nine households were selected in such a way that three farmers were represented by small, medium and large category according to their land holding. Thus, from each block 36 households were selected and a total of 72 households were selected from both the blocks for the study. There were seven land use pattern (Table 3). The experiment was randomised block design with replication (villages).

Table 1. Locality factors of the study area

Latitude	34˚10" N
Longitude	77˚35" E
Altitude	2900-3500 m
Climate type	Dry temperate
Soil texture	Coarse and sandy

Experimental Methodology

Biomass production: The tree productivity refers to increase in tree biomass per unit area. Measurement of tree diameter and volume was made to estimate the standing biomass. For this, five sample plots of 30 × 10 m^2 were laid in each land use system. The tree species from different quadrates were recorded in the intercropping system and sole tree system.

Volume estimation of stem: The diameter of trees was measured for each existing land use systems in each quadrat for computation of standing volume of different tree species. The data were collected for trees from all the quadrats of different land use systems. The different volume equations were used to compute the volume of the different tree species (FSI, 1996). Above ground biomass (AGB): In order to estimate the tree biomass, the volume of individual trees in each sampling quadrat was multiplied with its specific gravity (SG) and stem biomass (SB) was derived. Later, the stem biomass was multiplied with the biomass expansion factor (BEF) to derive above ground biomass. BEF is the value to take into account the biomass of the other aboveground components of trees (leaves, twigs and branches).

AGB = SB × BEF

Stem volume of different tree species were calculated from the volume equations and specific gravity of respective tree species were taken from literature.

SB = volume × specific gravity

Where,

SB = Stem Biomass

BEF = Biomass Expansion Factor

Below ground biomass of trees (BGB): Below ground biomass of trees was calculated by multiplying above ground biomass (AGB) with root: shoot of tree species (IPCC 1996).

BGB = AGB × Root: Shoot

Table 2. Multi-stage random sampling method for choosing the study sites

Block	Panchayat	Village	Farmer (9 farmers from each village according to their land holding)
Khaltsi	Khaltsi	i. Khaltsi ii. Skindiyang	• Three marginal farmer(<1 hectare) • Three small farmer (1-2 hectare) • Three medium farmer (2-5 hectare)
	Tingmosgang	i. Tingmosgang ii. Nurla	• Three marginal farmer(<1 hectare) • Three small farmer (1-2 hectare) • Three medium farmer (2-5 hectare)
Saspol	Saspol	i. Saspol ii. Saspochey	• Three marginal farmer(<1 hectare) • Three small farmer (1-2 hectare) • Three medium farmer (2-5 hectare)
	Gera	i. Gera ii. Alchi	• Three marginal farmer(<1 hectare) • Three small farmer (1-2 hectare) • Three medium farmer (2-5 hectare)

Herbs

Above ground biomass: A herb is a plant whose stem is tender and height is usually not more than one meter. For estimation of herb biomass, quadrats of 50×50 cm^2 size were laid out in the different land use systems in the study sites. All the herbs from quadrats were harvested and oven dried at 65±5°C to the constant weight.

Agriculture Crops: Above ground biomass: Crop biomass was estimated using 1×1 m^2 plots. All the crop plants within the border of quadrats were harvested and collected samples were weighed, sub sampled (separated into root and shoot) and oven dried at 65±5°C to a constant weight.

Below ground biomass of herbs and crops: Below ground biomass of crops and grasses (herbage) was determined by removing crops and grasses with roots carefully. Soil was separated by tapping and roots were washed and segregated species wise. Roots of each species were stored in paper bags and then were dried in oven at 65±5°C to a constant weight.

Carbon stock: Biomass of trees, herbage and crops was converted into biomass carbon by multiplying total biomass with a factor of 0.50 (IPCC 1996).

Carbon Stock = Total biomass × 0.50

RESULT AND DISCUSSION

The total biomass and carbon stock of different land use systems followed the order as hortisilvipasture > agrihortisilviculture > silvipasture > agrisilviculture > agrihorticulture > horticulture > agriculture. Hortisilvipasture land use system recorded maximum total biomass production (30.88 t ha^{-1}) and total carbon stock (15.44 t ha^{-1}) (Table 3). The minimum total biomass production (8.76 t ha^{-1}) and total carbon stock (4.38 t ha^{-1}) was in agriculture land use system. The above ground and below ground biomass in general followed the same trend as that of total biomass and total

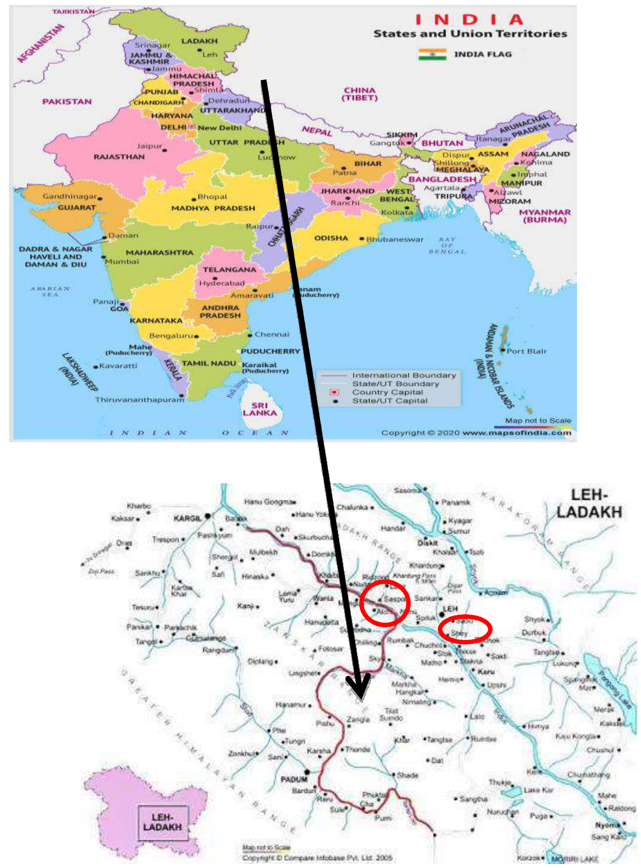

Fig. 1. Location map of the study area

Table 3. Above ground biomass, below ground biomass, total biomass and total carbon stock of various land use systems of the study area

Land use systems	Aboveground biomass (t ha^{-1})		Mean	Belowground biomass (t ha^{-1})		Mean	Total biomass (t ha^{-1})		Mean	Total carbon stock (t ha^{-1})		Mean
	Khaltsi Block	Saspol Block		Khaltsi Block	Saspol Block		Khaltsi Block	Saspol Block		Khaltsi Block	Saspol Block	
Agriculture	6.88	6.16	6.52	2.36	2.14	2.25	9.24	8.29	8.76	4.62	4.15	4.38
Horticulture	11.15	9.99	10.57	6.70	6.11	6.41	17.85	16.10	16.97	8.92	8.05	8.49
Agrisilviculture	17.72	16.50	17.11	6.28	5.79	6.03	23.10	22.29	23.14	11.10	11.15	11.57
Agrihortisilviculture	19.77	18.46	19.11	8.41	7.69	8.05	28.17	26.15	27.16	14.09	13.08	13.58
Silvipasture	17.44	16.39	16.91	9.62	9.41	9.51	27.06	25.79	26.43	13.53	12.90	13.21
Hortisilvipasture	20.73	19.17	19.95	11.22	10.65	10.93	31.95	29.81	30.88	15.98	14.91	15.44
Agrihorticulture	16.76	15.53	16.15	7.14	6.81	6.97	23.90	22.34	23.12	11.95	11.17	11.56
Mean	15.78	14.60		7.39	6.94		23.17	21.54		11.58	10.77	
C.D. (p=0.05)	System	0.78		System	0.47		System	0.80		System	0.40	
	Block	0.42		Block	0.25		Block	0.43		Block	0.21	
	(S X B)	NS		(S X B)	NS		(S X B)	NS		(S X B)	NS	

carbon stock of different land use systems. Further, it is evident that different land use systems had significant effect on biomass production and carbon stock. It is influenced by the age of the components (annual or perennial), type of crop, structure of system, nature and number of woody components etc. The higher biomass production in hortisilvipasture land use system can be because of the higher density of trees as compare to other land uses. The hortisilvipasture land use system consisted of fruit and fodder trees and grasses. Many workers have studied the biomass production and carbon stock of different land use systems and found similar trends. Montagini and Porras (1998) revealed that the mixed species have a greater capacity to produce high levels of biomass because of the difference in carbon fixing rates by species. Deshmukh (1998) reported that management practices also affect the biomass production of trees grown under different agroforestry systems. The higher biomass production in hortisilvipasture land use system can be because of the higher density of trees as compared to other land uses.

CONCLUSION

The total biomass and carbon stock accumulation was significantly variable under different land use systems and it can be concluded that for a cold desert like Ladakh, hortisilvipasture is the type of land use system which accumulates maximum biomass and stores maximum carbon is recommended for better land use management.

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Effect of Seed Priming on Germination and Nursery Establishment of *Woodfordia fruticosa* (L.) Kurz (*Dhawai*)

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Abstract: *Woodfordia fruticosa* (L.) Kurz, (*Dhawai*) is a traditional medicinal shrub widely present in India and South East Asian countries. The natural population of this species is very sporadic because of its lesser germination in normal conditions and is now further declining alarmingly due to unscientific harvesting, which requires urgent conservation action for diversifying agro ecosystem and doubling farmers income. Hence, ensuring quality planting materials is must before introducing into farming systems. The viability of the freshly collected seeds of *Dhawai* was 100 % which decreased with an increase in storage duration. All the priming agents had a positive effect on the enhancement of germination and its parameters. Among all the priming treatments, 500 ppm of GA_3gave the highest germination (94.0 %) with the highest SVI and SVI-mass values of 132.54 and 16.17, respectively. Priming the seeds of *Dhawai* with different priming treatments improved seedling growth and survival. With 500 ppm GA3, seedlings had the longest shoot length (4.48 cm), the longest root length (2.16 cm), the widest collar diameter (0.23 mm), and the heaviest dry weight (6.60 mg). Seeds primed with 500 ppm GA_3 and 0.25 % Proline produced seedlings with the highest number of leaves (6.60). Priming with 4 mM glutamine resulted in the highest survival of the *Dhawai* seedlings. Though there is a high germination and nursery establishment were recorded in both GA_3 and Glutamine seed priming treatments, 24 hours refrigeration treatment can be recommended for easy, effective and eco-friendly mode of quality planting material production in Terai region of West Bengal. In contrast, priming treatments namely longer duration of hot air ovenation, higher concentration of acids, chemicals, hormones and other botanical priming agents deteriorated germination and nursery establishment compared to non-primed seeds.

Keywords: *Dhawai*, Medicinal plant, Seed priming, Germination, Nursery establishment

Woodfordia fruticosa (L.) Kurz, commonly known as *Dhawai* or Fire Flamed Bush (family Lythraceae), is a traditional medicinal shrub widely present in India and South East Asian countries, growing gregariously at higher altitudes of about 1500 m (Uday et al 2014). It is frost-hardy, a good coppicer, and is not grazed, so it grows naturally in large numbers in disturbed and other open areas; it is also a soil binder and an effective nurse for tree species such as *Shorea robusta* (Dinesha et al 2021a). The plant, especially the flowers, possess valuable pharmacological properties because of isolated compounds like tannins, flavonoids, glycosides, sterols, and polyphenols, which have high global commercial demand (Uday et al 2014, Mathew et al 2018). It is also a dye and gum-yielding species used in the perfume, leather, and textile industries and believed to be superior for woollen and silk fabrics (Dinesha et al 2021b). Furthermore, because of its ability to adapt to low-fertile, degraded, and disturbed soils, this species has been identified as a potentially valuable plant genetic resource for improving the marginal smallholder farming systems of tropical regions through soil and moisture conservation. The viability of the stored seeds at normal room conditions declines rapidly from 100 percent to a meagre one percent within a year, while heavy rains and other climatic conditions also affect the viability of seeds (Dinesha et al 2021a). Moreover, seedling survival of the species is also very low under normal conditions. Unfortunately, there have been very few studies on the species' germination and nursery establishment. In sterilized coir-pith compost medium, seeds germinated in 15–20 days with 70% survival (Mathew et al 2018). Transplanting seedlings into polybags containing a 4:1 potting mixture of soil and coir-pith compost improves their chances of survival, growth, development, and field survival (Mathew et al 2018). Seed germination can also be achieved when the seeds are sown in sandy soil during June and October, with 73.33 % survival in the field with polybag seedlings (Shankar and Rawat 2013). The natural population of this species is very sporadic and is now further declining alarmingly, which requires urgent conservation action, especially through introducing them into farming systems through mass cultivation (Mathew et al 2018). However, to achieve this objective, ensuring quality planting materials is a must, which will not only conserve these species *in situ* but also aid in farm diversification while providing an added source of income enhancement for the growers due to their high pharmaceutical demands. Thus, to achieve the objective of conserving this species through mass cultivation, rapid establishment of the species is necessary by ensuring the availability of quality planting materials with rapid and uniform seedling growth, which is possible through seed priming (Farooq et al 2006). Seed priming is a seed treatment that involves controlled hydration to allow metabolic events before germination but is insufficient to allow radical protrusion through the seed coat (Farooq et al 2006). Seed priming treatments such as hydro-priming, chemo-priming, hormonal-priming, amino acid-priming, thermo-priming, botanical-priming, and bio-priming have been used to accelerate uniform germination, disease free seedling growth, better establishment, and improved yield in most of the crops under both normal and stress conditions (Devkota et al 2013, Ghadge 2018). Therefore, the current study was carried out to standardize the seed priming protocol of *Dhawai* for enhancing germination, seedling vigor, and nursery establishment in the Terai zone of West Bengal.

MATERIAL AND METHODS

Experimental site: The present study was carried out in both the laboratory and nursery at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India from May 2019 to May 2021. The geographical location of the study area is 26° 23' 45.8" north latitude and 89° 23' 16.7" east longitude at 43 m above mean sea level, which falls under the Terai region of West Bengal. The Terai region is a heavy rainfall area with an annual average of 2000-3500 mm and average temperatures varies between 18°C (January) and 33°C (August). The relative humidity of the region ranges from 55 % to 90 % and overall, the area is warm and humid. The soil of the nursery/experiment site was moist and sandy to sandy loam with an acidic reaction, low in organic carbon, medium in available nitrogen and phosphorus, and high in available potash (Dinesha et al 2021a).

Freshly harvested seeds of *Dhawai* were collected from the mother stock maintained in the nursery, Department of Forestry, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar. The seeds were cleaned and shade dried to an ideal moisture level, and then used for the study. The characteristic features of the seed material selected for the study are documented (Plate 1). This gregarious shrub flowers profusely during the summer months, and tiny brown seeds are produced abundantly (Shankar and Rawat 2013).

Seed viability (Tetrazolium chloride test): Seeds collected 3, 6, 12, 24 and 36 months earlier stored in air tight glass bottles at room temperature were subjected to tetrazolium chloride (TTZ) test to assess the seed viability (Plate 1).

Control treatment: Seeds not primed with any priming agents, i.e., normal seeds, were taken as controls and were sown directly in the growing medium. All of the experiments in this study used Soilrite®, a commercial germination medium made up of 75 % peat moss and 25 % perlite. A separate control was taken if the duration between the priming treatments was one month or more, or else the same control was considered.

Priming treatments: The experiment was laid out in a completely randomized design (CRD) and replicated three times, with 50 seeds in each replication. The seeds in every treatment were tested for standard germination by keeping the seeds in petri plates over soil rite at room temperature (~25°C). Untreated seeds were taken as control. After the standardization of hydro-priming, the best hydro-priming duration was used for all other priming treatments. The different priming procedures with varying treatments (exposures/concentrations) were considered a sub-experiment, from which the significantly better treatment(s) were finally compared with each other along with the control. The primed seeds were kept in petri dishes with germination media for one month under normal laboratory conditions. The treatment details are given below.

Hydro-priming: *Dhawai* seeds were not directly soaked in water due to their tininess, but were instead kept in muslin cloth bags and then soaked in normal water (the volume of water was five times the seeds' weight) for 6, 12, 24, and 36 hours. The soaked seeds were then shade dried separately to gain their original weight (Plate 2).

Chemo-priming: The chemo-primers namely KNO_3, $CaCl_2$, H_3BO_3, and H_2SO_4 were used to prepare desired concentrations of 0.5, 1.0, and 2.0 %.

Hormonal priming: Hormonal concentrations of 100, 200 and 300 ppm salicylic acid (SA) and 100, 250 and 500 ppm gibberellic acid (GA_3) were prepared (Xie et al 2006).

Amino acid priming: Ascorbate 250 mg and 500 mg were dissolved separately in one litre of distilled water. L-glutamine at 2 mM and 4 mM concentrations were prepared separately by dissolving 29.2 mg and 58.4 mg, respectively, in 100 ml of distilled water (Ali et al 2013). Proline at 0.25 % and 0.50 % concentrations were prepared separately by dissolving 250 mg and 500 mg in 100 ml of distilled water.

Thermo-priming: The seeds were thermo-primed for desired duration as per above treatments. In low temperature priming (3-5°C), the moist seeds were placed in a refrigerator for 6-, 12- and 24-hours duration. In high temperature priming (40°C), the seeds were soaked in a beaker with water for 1, 3 and 6 hours.

Botanical priming: Priming with moringa (*Moringa oleifera*), neem (*Azadirachta indica*), papaya (*Carica Papaya*) and pongamia (*Pongamia pinnata*) was done with their extracts (Ghadge 2018). In addition to these primers, mixture of flowers, stem and root extract of *Dhawai* in equal proportion was also used. The fresh leaves/flowers/stem/roots of the concerned species were collected separately and dried under shade. The shade dried plant parts were powdered separately using electric grinder and one, three and five grams of the powder was dissolved in 100 ml of distilled water to make the desired concentration of one, three and five per cent separately (Ghadge 2018).

Bio-priming: The liquid biofertilizers such as *Azotobacter* (AZB) and Phosphobacteria (PSB) were collected from the Plant Pathology Department of the University. The concentrations (10, 15 and 20 %) of these biofertilizers were prepared (Gowthamy et al 2017).

Evaluation of nursery performance of seedlings of *Dhawai* after seed priming: Seedlings of significantly better performed protocols of hydro-, chemo-, hormonal, amino acid, thermo-, botanical and bio-priming treatments were compared with each other along with control (i.e., seedlings from no priming treatment) for their performance under nursery condition for two months. Germinated primed seeds after growing on the petri dishes with Soilrite (growing media) under normal laboratory conditions for one month were transferred to the nursery in the root trainers containing three parts soil and one-part FYM as growing media. Entirely fifteen treatments each with 20 seedlings including control were evaluated with CRD replicated thrice.

Observations: Germination per cent (GP) was calculated by using International Seed Testing Association guidelines (Anonymous 2010) as proportion of seeds germinated from the total number of seeds treated and expressed as per cent. Germination speed (GS) was estimated following formula given by Czabator (1962). $GS = n_1/d_1 + n_2/d_2 + n_3/d_3 + \ldots\ldots$; where, n = number of germinated seeds, d= number of days. Mean daily germination (MDG) was estimated as total number of germinated seeds divided by total number of days taken for germination. Peak value (PV) was estimated as highest number of seeds germinated in a given day divided by the number of days at this peak value. Germination value (GV) was estimated (Czabator 1962). $GV = PV \times MDG$, where PV is peak value and MDG is mean daily germination. Seedling vigour index (SVI) was estimated by multiplying the standard germination (%) with an average sum of shoot length (cm) and root length (cm) on the 30^{th} day of germination. SVI-mass was estimated by multiplying the standard germination (%) with mean seedling dry weight (mg) on the 30^{th} day of germination. Survived seedlings were counted after two months of transplanting in the nursery and expressed as per cent of the total seedlings transplanted in the nursery. Collar diameter was measured by using digital varnier caliper. Number of leaves were counted and recorded. Shoot and root length were measured using a graded scale. Seedling dry weight (SDW) of randomly selected 10 seedlings from each treatment was quantified by using an electronic weighing balance after desiccating them in hot air oven at 60°C for 48 hours.

Statistics analysis: The IBM SPSS version 2020 was used to perform the statistical analysis using Duncan's multiple range test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed viability: Seed viability of *Dhawai* of different storage duration reveals gradual decline of seed viability with increase in storage duration. The viability of the freshly collected seeds of *Dhawai* was 100 % which decreased with time. Viability of *Dhawai* reduced drastically and was completely lost after six months of storage. Decline in the endogenous levels of gibberellins was considered as the limiting factor for the maintenance of viability and/or germination of seeds. Similarly, viability of *Dhawai* seeds stored at normal room conditions was found to lose completely within one year of storage (Bhagat et al 1992).

Effect of seed priming on *Dhawai* for enhancing germination and seedling vigour- Hydro-priming: Hydro-priming significantly improved germination of *Dhawai* and its various parameters over control (Table 1, Plate 2). Hydro-priming the *Dhawai* seeds for 24 hours gave the highest germination of 76 %, earliest initiation of germination (IG; five days) and completion of germination (CG; 17 days), highest germination speed (GS; 3.90), mean daily germination (MDG; 2.25), peak value (PV; 0.5), germination value (GV; 1.13) and maximum growth of the seedlings as indicated by total seedling length (TSL; 1.35 cm), total seedling weight (TSW; 0.17 mg), seedling vigour index (SVI; 102.21) and seedling vigour index-mass (SVI-mass; 12.9). Therefore, all other priming treatments in the present study was done soaking the seeds in the respective solution of the primer for 24 hours. The germination of control (non-primed) seeds was least with only 21 % and so least were the IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass also. Increasing the duration of hydro-priming from six hours to 24 hours significantly increased the germination but further increasing the duration of priming over 24 hours significantly decreased germination. Germination of hydro-primed seeds for 6-24 hours increased by 45-55 % compared to control while, hydro-priming for 36 hours increased germination by only 18 % compared to control. Similarly, IG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass of the hydro-primed seeds also improved significantly over the non-primed seeds but CG of primed and non-primed seeds was statistically similar though the duration was relatively longer for non-primed seeds (Table 1). The IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass also gradually improved though not always significantly with increasing duration of hydro-priming from six hours to 24 hours. However, further increase of priming duration to 36 hours decreased (not always significantly) these parameters but still remaining better than control.

Priming the seed with water loosens the seed coat resulting in better imbibition of water and oxygen which increased the metabolic activities resulting into quicker, higher and uniform germination including faster seedling growth (Moghanibashi et al 2012, Dastanpoor et al 2013, Ghadge 2018). Hydro-priming treatments not only improved the germination rate and time but also enhanced the seedling vigour as indicated by higher values of germination, seedling length and weight. The present results were consistent with earlier studies where improved germination and its parameters were also observed following hydro-priming of 24 hours (Moghanibashi et al 2012, Dastanpoor et al 2013). Extending priming duration over 24 hours to 36 hours deteriorated germination and its parameters which was probably due to saturation of the seeds with water reducing oxygen thus restricting growth of emerging radical and plumule (Assefa 2008). Hydro-priming involve soaking the seeds followed by drying back before radical emergence and extending soaking time up to 36 hours might had initiated radical emergence in the soaked seeds. Drying back the seeds after radical emergence might have killed the radical reducing germination (Assefa 2008).

Chemo-priming: All the chemo-primers with varied concentration (0.5-2.0 %) significantly increased germination and improved various germination parameters of *Dhawai* over control except sulphuric acid at 1.0 or 2.0 % (Table 2). Sulphuric acid however, at 0.5 % concentration significantly increased germination (38.0 %) over control. Although potassium nitrate, calcium chloride and boric acid at the given concentrations including 0.5 % sulphuric acid significantly increased germination over control but couldn't improve germination and various germination parameters (Table 2) similar to 6-24 hours of hydro-priming (66-76 %, Table 1). Further, it was observed that increasing concentration of these chemo-primers over 0.5 % significantly decreased germination and the various germination parameters. This indicates corrosive and toxic effect of the chemo-primers at concentration over 0.5 % which might have destroyed the emerging radical and plumule, reducing germination and thus also reducing the entire studied germination parameters (Noor-un-Nisa et al 2013). The chemo-primers at various concentrations were also used in earlier studies on field crops including medicinal plants also gave comparable results to this present study (Farooq et al 2006). Among all the chemo-priming treatments, 0.5 % of H_3BO_3 and KNO_3 were found best. Similarly, 0.2-3.0 % KNO_3 in earlier studies was reported to increase the activity of total amylase and proteases in germinating seeds resulting improvement in proteins, free amino acid and soluble sugars during germination which significantly improved germination and its parameters (Noor-un-Nisa et al 2013, Vineeta et al 2018). Priming with 0.01-2.0 % H_3BO_3 was reported to enhance seed performances due to its role in rejuvenation and the buildup of nucleic acid and membranes, enhanced synthesis of protein, and improved antioxidant system (Noor-un-Nisa et al 2013).

Hormonal priming: Hormonal-priming significantly increased germination and improved various germination parameters of *Dhawai* over control (Table 3; Plate 3). However, salicylic acid (SA) was not that effective like GA_3, instead reduced germination and germination parameters when its concentration was increased from 100 to 300 ppm in contrast to increasing concentration of GA_3 (100-500 ppm). SA increased germination by 2-10 % whereas, GA_3 increased

Table 1. Effect of hydro-priming on germination and its parameters of *Dhawai*

D	IG	CG	GP	GS	MDG	PV	GV	TSL	TSW	SVI	SVIm
0	7.5^a	19.0^a	21.0^d	0.92^d	0.56^d	0.21^b	0.12^b	1.26^c	0.13^b	26.4^d	2.72^d
6	5.5^b	19.5^a	70.0^{ab}	3.34^b	1.80^b	0.48^a	0.86^a	1.32^{ab}	0.17^a	92.1^{ab}	11.60^{ab}
12	6.0^b	18.5^a	66.0^b	3.00^b	1.78^b	0.40^{ab}	0.72^a	1.31^{ab}	0.15^{ab}	86.1^b	9.90^b
24	5.0^b	17.0^a	76.0^a	3.90^a	2.25^a	0.50^a	1.13^a	1.35^a	0.17^a	102.2^a	12.90^a
36	6.0^b	18.5^a	39.0^c	1.84^c	1.05^c	0.27^b	0.28^b	1.28^{bc}	0.15^{ab}	49.8^c	5.64^c
M	6.00	18.5	54.40	2.60	1.49	0.37	0.62	1.30	0.15	71.32	8.54
Sig.	0.00*	NS	0.00*	0.00*	0.00*	0.04*	0.01*	0.02*	0.05*	0.00*	0.00*

D- duration in hours (zero hour is control); M- mean; IG- Initiation of germination; CG- Completion of germination; GP- Germination per cent; GS- Germination speed; MDG- Mean daily germination; PV- Peak value; GV- Germination value; TSL- Total seedling length (cm); TSW- Total seedling weight (mg); SVI- Seedling vigour Index; SVIm- Seedling vigour Index-mass; S- Significant (α= 0.05); NS-Non significant germination by 59-72 % over control (22 %) with similar trend in increment of germination parameters. Hormonal treatments however, effectively reduced the initiation (4-5 days; Table 3) and completion of germination period 15-16 days as compared to 4.5-7.0 days for IG and 16-19.5 days for CG by hydro- and chemo-priming (Table 1 & 2). IG was earliest as 4.0 days with 100, 250 and 500 ppm of GA_3 priming while, extended up to 5.5 days with 100 ppm of Salicylic acid priming as compared to 7.5 days with control. Similarly, CG was recorded earliest at 15.0 days with 100, 200 and 300 ppm SA priming while, a bit extended up to 16.0 days with 100 and 250 ppm GA_3 priming as compared to 19 days with control. Maximum GS and MDG were recorded in 500 ppm of GA_3 which is at par with 100 ppm and 250 ppm GA_3 while, minimum values were in control. Both PV and GV also followed similar trend (Table 3). TSL varied from 1.41 cm to 1.26 cm with a mean of 1.33 cm. TSW varied from 0.14 mg to 0.18 mg with a mean of 0.16 mg. Similarly, both SVI and SVI-mass followed similar trend (Table 3).

Among the hormonal-priming, 500 ppm of GA_3 was found the best priming treatment for improving germination and its various parameters. This may be attributed to the key role of gibberellins in the break of dormancy and in the control of reserve hydrolysis on which the growing embryo depends (Lovegrove and Hooley 2000). The faster emergence of seeds primed with GA_3 might be due to its stimulation effect in the formation of enzymes during early phases of germination which helped for a fast radicle protrusion and hypocotyl elongation to penetrate the soil up (Salisbury and Ross 1992). In contrast, SA exhibited the reverse trend as it is a

Table 2. Effect of chemo-priming on germination and its parameters of *Dhawai*

Treatments	Conc.	IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	21.0^{g}	1.26^{f}	0.13^{d}	2.72^{f}
KNO_3	0.5 %	5.0^{de}	16.0^{b}	0.17^{abc}	1.36^{ab}	0.17^{abc}	9.59^{a}
	1.0 %	5.5^{cde}	16.5^{b}	0.17^{abc}	1.30^{cdef}	0.17^{abc}	6.07^{de}
	2.0 %	6.0^{bcd}	17.5^{ab}	0.18^{a}	1.39^{a}	0.18^{a}	7.57^{c}
$CaCl_2$	0.5 %	5.0^{de}	17.0^{ab}	0.15^{bcd}	1.29^{def}	0.15^{bcd}	7.75^{bc}
	1.0 %	5.0^{de}	17.0^{ab}	0.17^{ab}	1.32^{bcde}	0.17^{ab}	7.03^{cd}
	2.0 %	4.5^{e}	17.0^{ab}	0.16^{abc}	1.33^{bcd}	0.16^{abc}	7.44^{c}
H_3BO_3	0.5 %	5.0^{de}	17.0^{ab}	0.15^{cd}	1.28^{def}	0.15^{cd}	8.70^{ab}
	1.0 %	5.5^{cde}	18.0^{ab}	0.15^{bcd}	1.31^{cdef}	0.15^{bcd}	7.20^{c}
	2.0 %	5.0^{de}	17.5^{ab}	0.17^{ab}	1.35^{abc}	0.17^{ab}	7.82^{bc}
H_2SO_4	0.5 %	5.5^{cde}	17.0^{ab}	0.15^{bcd}	1.28^{ef}	0.15^{bcd}	5.57^{e}
	1.0 %	7.0^{ab}	17.5^{ab}	0.13^{d}	1.27^{ef}	0.13^{d}	2.74^{f}
	2.0 %	6.5^{abc}	17.0^{ab}	0.16^{abc}	1.31^{bcdef}	0.16^{abc}	2.59^{f}
Mean		5.62	17.23	41.15	1.31	0.15	6.37
Sig.		0.00*	0.29	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details

Table 3. Effect of hormonal priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5^{a}	19.0^{b}	22.0^{d}	0.73^{e}	0.33^{d}	0.24^{d}	27.72^{d}	2.97^{d}
	100 ppm	5.5^{a}	15.0^{b}	32.0^{c}	1.07^{c}	0.32^{d}	0.34^{d}	41.64^{c}	4.97^{c}
SA	200 ppm	5.0^{a}	15.0^{b}	29.0^{c}	0.97^{cd}	0.36^{d}	0.34^{d}	39.72^{c}	4.83^{c}
	300 ppm	5.0^{a}	15.0^{b}	24.0^{d}	0.80^{de}	0.40^{d}	0.32^{d}	31.24^{cd}	3.76^{cd}
	100 ppm	4.0^{b}	16.0^{a}	85.0^{b}	2.66^{b}	1.31^{b}	3.48^{b}	118.14^{b}	14.45^{a}
GA_3	250 ppm	4.0^{b}	16.0^{a}	81.0^{b}	2.53^{b}	0.81^{c}	2.03^{c}	105.28^{c}	12.15^{b}
	500 ppm	4.0^{b}	15.5^{ab}	94.0^{a}	3.04^{a}	1.57^{a}	4.77^{a}	132.54^{a}	16.17^{a}
Mean		4.64	15.36	52.43	1.68	0.73	1.65	70.90	8.47
Sig.		0.00*	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details; SA- Salicylic acid; GA_3- Gibberellic acid germination inhibitor and at high concentration is toxic to the germinating seeds (Zhang et al 2004, Xie et al 2006). During seed germination, GA is synthesized in embryos and secreted into aleurone layers (Lovegrove and Hooley 2000). The production of a-amylases in aleurone layers is believed to be essential for seed germination which is tightly regulated by gibberellic acid (GA), abscisic acid (ABA) and SA (Lovegrove and Hooley 2000, Zhang et al 2004, Xie et al 2006). In aleurone cells, GA is perceived by receptors that promote the expression of α- amylases but antagonizes with germination inhibitors like ABA and SA that block the production and expression of α-amylases and abscisic-acid-inducible WRKY gene suppressing seed germination (Lovegrove and Hooley 2000, Xie et al 2006). The germination was promoted due to exogenous application of GA as it increased the proportion of GA in the seed system

Plate 1. Collection of *Dhawai* seed for seed viability test

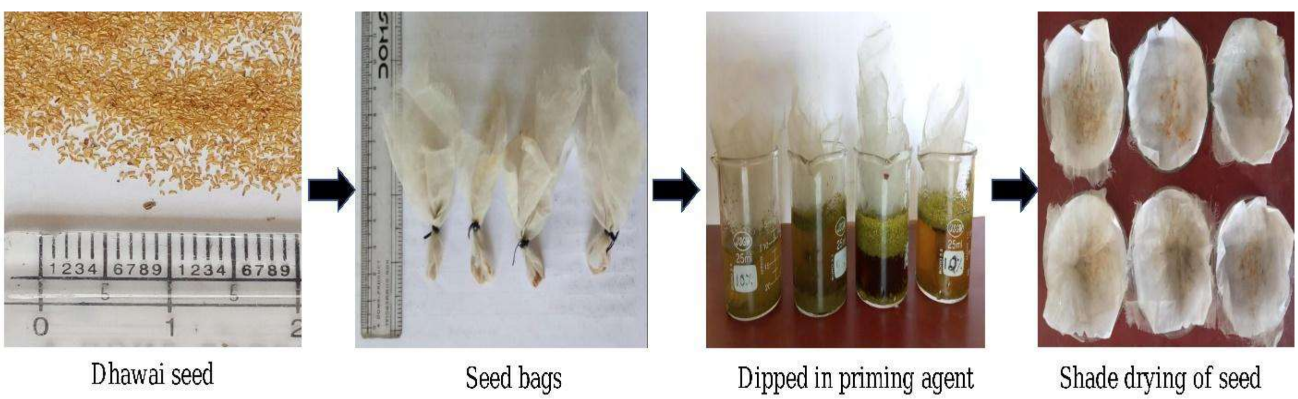

Plate 2. *Dhawai* seed priming process

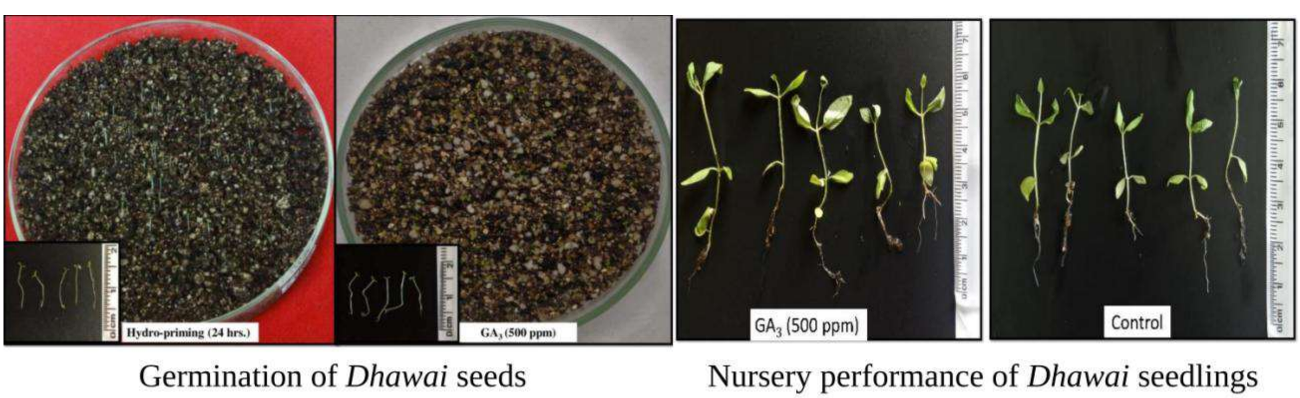

Plate 3. Germination of seeds and nursery performance of *Dhawai* seedlings

promoting germination. The exogenous application of SA even at low concentration decreased the proportion of endogenous GA with antagonizing effects of inhibiting α-amylase and WRKY gene suppressing seed germination.

Amino acid priming: Amino acid priming significantly improved the germination and germination parameters over control (Table 4). Priming with Ascorbate, Glutamine and Proline significantly improved germination by 25-29 %, 36-66 % and 34-58 %, respectively over control (22 %). Increasing the concentration of Glutamine from 2 mM to 4 mM significantly increased germination by 30 % but increasing Proline from 0.25 % to 0.50 % significantly decreased germination by 24 % while, increasing Ascorbate from 250 mg l^{-1} to 500 mg l^{-1} also decreased germination but the decrease was non-significant. Among the amino acid priming treatments, Glutamine 4 mM gave significantly higher germination (88.0 %) than all other amino acid priming treatments. Amino acid priming also significantly reduced the days to IG (3-4 days) and CG (13-15 days) over control by 3.5-4.5 days and 4-6 days, respectively (Table 4) which is two days and 4-4.5 days earlier, respectively than hydro-priming (Table 1); 1.5-3 days and three days earlier than chemo-priming (Table 2) and 1-1.5 days and 1-2 days, respectively than hormonal priming (Table 3). However, IG and CG exhibited a mixed influence on increasing concentration of amino acids. Similar trend on other germination parameter was also observed with amino acid priming as was observed with germination (Table 4). Maximum GS and MDG were recorded in 4 mM of Glutamine which is statistically at par with 0.25 % of Proline while, minimum values were in control (Table 4). Similarly other germination parameters also followed similar trend i.e. maximum values with 4 mM Glutamine priming while, minimum values with control (Table 4).

Among the amino acid priming, 4 mM Glutamine priming was found the best primer for improving germination and its various parameters. Providing Glutamine exogenously might have supplemented the Glutamine synthetase activity, aiding nitrogen metabolism and acted as substrate for protein synthesis. Thus, increasing Glutamine increased germination by increasing the availability of nitrogen and glutamate leads to ammonium release in an oxidative deamination reaction catalyzed by glutamate dehydrogenase (Mifflin and Habash 2002). Proline increased germination over control in very less concentration of 0.25 % which was reported due to its influence on production of protein and sugar in the germinating seeds (Ali et al 2013). Increasing the concentration of Proline and Ascorbate significantly decreased the germination due to toxic effects of these primers at higher concentrations to the germinating seeds (Yang et al 2012). Earlier studies with field crops exhibited excellent germination with amino acid priming both under stress and normal conditions which was attributed to their influence on production of protein, seed sugar, oil, fiber content, moisture content, and ash (Yang et al 2012, Ali et al 2013). Increased germination especially during stress was found with amino acid priming due to increased cell division, cell wall expansion, and other developmental processes. This is because amino acids are key substance in the network of plant antioxidants, including glutathione and enzymatic antioxidants that detoxify H_2O_2 to counteract oxygen radicals produced by the Mehler reaction and photorespiration during stress (Ali et al 2013). *Dhawai* seeds being inherently micro-biotic in nature with very fine seed coat were reported with very less germination under normal natural and control conditions (Mathew et al 2018, Dinesha et al 2021a). The micro-biotic nature with very fine seed coat might normally render the seeds to stress under normal germinating conditions restricting the seeds to germinate. Priming with amino acids might have played a role of stress busters for the *Dhawai* seed aiding its germination.

Thermo-priming: Priming *Dhawai* seeds with varying

Table 4. Effect of amino acid priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5[a]	19.0[a]	22.0[e]	1.52[e]	0.33[b]	0.51[d]	27.72[e]	2.86[e]
Ascorbate	250 mg l^{-1}	4.0[b]	13.0[c]	51.0[cd]	3.93[c]	1.07[a]	4.17[b]	68.32[cd]	7.90[cd]
	500 mg l^{-1}	4.0[b]	14.5[b]	47.0[d]	3.14[d]	0.94[a]	2.94[c]	65.08[d]	7.64[d]
Glutamine	2 mM	3.0[c]	13.5[bc]	58.0[c]	4.31[c]	0.93[a]	4.03[b]	79.70[c]	8.83[c]
	4 mM	3.0[c]	14.0[bc]	88.0[a]	6.29[a]	1.00[a]	6.29[a]	125.37[a]	14.74[a]
Proline	0.25 %	3.0[c]	15.0[b]	80.0[b]	5.34[b]	0.41[b]	2.20[c]	105.34[b]	12.07[b]
	0.50 %	3.5[bc]	13.5[bc]	56.0[c]	4.00[c]	0.86[a]	3.45[c]	78.44[c]	8.99[c]
Mean		3.64	14.07	57.43	4.07	0.79	3.37	78.57	9.00
Sig.		0.00*	0.02*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details

duration of high and low temperature significantly influenced germination, IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass (Table 5). Refrigerating (3-5ºC) the seeds increased germination by 52-67 % over control (22 %). Increasing the duration of refrigeration also increased germination. Germination recorded with six hours of refrigeration was 74 % which increased significantly by 11 % with increase in duration to 12 hours. Further increase in duration of refrigeration to 24 hours non-significantly increased germination by 4 %. Hot air ovenating the seeds for one hour also increased germination by 46 % over control (22 %). In contrast to refrigeration, increasing the duration of hot air priming (40ºC) by keeping the seeds in hot air oven from one hour to three hours significantly reduced the germination by 31 % and further increasing the duration to six hours reduced germination by 10 % below control. Micro-biotic nature of the *Dhawai* seeds with very thin seed coat made it prone to longer duration of hot air ovenation (Baskin and Baskin 2004). Exposing these seeds with longer duration of hot air ovenation might have damaged the seed coat injuring or killing embryos including endosperm resulting in lower germination (McDonnell et al 2012). Among the thermo-priming treatments, 24 hours refrigeration was found the best for germination of *Dhawai* followed by 12 hours refrigeration. Low temperature was reported to enhance germination and various parameters due to influence membrane permeability regulating the movement of gibberellins toward their activity places while, also increasing the soluble protein content in the germinating seed (Salisbury and Ross 1992, Lovegrove and Hooley 2000).

Botanical priming: Priming of *Dhawai* seeds with different concentrations of botanical or plant extracts significantly influenced germination and various germination parameters (Table 6). All the plant extracts used significantly increased germination over control (20 %) by 19-25 % for moringa extract, 9-21 % for neem extract, 2-66 % for papaya extract, 22-51 % for pongamia extract and 37-61 % for *Dhawai* extract. Increasing the concentration of all these extracts from one to five per cent significantly decreased the germination of *Dhawai* except moringa and neem extracts with which mixed response of germination was observed. Lowest concentration of one per cent used for papaya and *Dhawai* extract gave 86 % and 81 % germination, respectively with significant difference between the two treatments. Further increase in concentration to three and five per cent reduced germination more in papaya as compared to *Dhawai* though the reductions were significant in both the cases. Increasing concentration from one to three per cent reduced germination by 59 % with papaya extracts and 11 % with *Dhawai* extract while, from one to five per cent the reductions were 64 % and 24 %, respectively though the reductions were lesser from three to five per cent with 5 % and 13 %, respectively as compared to increasing concentration from one to three per cent. Next to one per cent papaya and *Dhawai* extract priming, another better priming treatment found was one per cent pongamia extracts with 71 % germination. The influence of all these botanical extracts priming on the germination parameters followed the same trend as was observed with germination (Table 6).

Among the botanical-priming treatments, one per cent papaya extracts priming gave best results. The extracts were prepared from young leaves which were reported to contain alkaloids which might have increased germination due to enhanced enzymatic and other metabolic activities of the germinating seeds (Devkota et al 2013, Ghadge 2018). Priming the seeds with plant extracts prior to germination was reported to enhance seed metabolism helping faster germination and increasing seedling vigour (Devkota et al 2013). These plant origin natural priming agents are cheap, safe, eco-friendly over synthetic priming chemicals with stimulatory effect on germination and seedling growth (Devkota et al 2013, Ghadge 2018).

Bio-priming: Bio-priming the seeds with different concentrations of *Azotobacter* (AZB) and Phosphobacteria (PSB) significantly influenced germination and various

Table 5. Effect of thermo-priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5[a]	19.0[a]	22.0[e]	0.73[e]	0.33[de]	0.24[de]	27.72[e]	2.97[e]
	6 hours	5.0[b]	16.0[b]	74.0[b]	2.28[b]	0.71[c]	1.62[c]	97.72[b]	12.23[b]
Refrigeration	12 hours	5.0[b]	16.0[b]	85.0[a]	2.66[a]	0.88[b]	2.33[b]	111.76[a]	14.24[a]
	24 hours	5.0[b]	17.0[ab]	89.0[a]	2.62[a]	1.07[a]	2.79[a]	118.38[a]	13.80[ab]
Hot air ovenated at 40ºC	1 hour	5.0[b]	17.0[ab]	68.0[c]	2.00[c]	0.86[b]	1.71[c]	87.06[c]	9.87[c]
	3 hours	5.0[b]	16.5[ab]	37.0[d]	1.13[d]	0.38[d]	0.43[d]	47.74[d]	5.65[d]
	6 hours	7.5[a]	17.0[ab]	12.0[f]	0.35[f]	0.25[e]	0.09[e]	15.24[f]	1.68[e]
Mean		5.36	16.36	55.29	1.68	0.64	1.31	72.23	8.63

See Table 1 for germination parameter details

germination parameters of *Dhawai* (Table 7). Both AZB and PSB priming treatments significantly increased germination over control (20 %) by 30-59 % for AZB and 19-44 % for PSB. Increasing the concentration of these bio-primers from 10 to 25 % significantly increased the germination by 20-29 %. The germination achieved with 20 % AZB priming (79 %) was comparable to germination achieved with 24 hours hydro-priming (Table 1); 100, 250 & 500 ppm GA_3 priming (Table 3); 4 mM Glutamine & 0.25 % Proline priming (Table 4); 12- & 24-hours refrigeration (Table 5) and 1.0 % papaya and *Dhawai* extracts priming (Table 6). The influence of both AZB and PSB bio-priming treatments on the germination parameters followed the same trend as was observed with germination (Table 7). Both the AZB and PSB significantly improved germination and its parameters. The possible mechanism of these plant growth promoting bacteria on the germination process is that these useful bacteria can excrete phytohormones such as auxins and gibberellins along with vitamins thereby improving seed germination and early development (Gowthamy et al 2017, Vasava et al 2018). Besides, during metabolism the bacteria excrete organic acids like citric acid and malic acid as well; thus, helping nutrient uptake at a later stage of growth (Gowthamy et al 2017, Vasava et al 2018).

Table 6. Effect of botanical priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	20.0^{i}	1.26^{c}	0.13^{c}	2.62^{i}
Moringa	1 %	5.0^{bc}	16.0^{bc}	42.0^{ef}	1.33^{bc}	0.16^{ab}	6.61^{efg}
	3 %	5.5^{b}	16.5^{ab}	39.0^{fg}	1.28^{bc}	0.15^{bc}	5.65^{g}
	5 %	5.0^{bc}	16.5^{ab}	45.0^{e}	1.34^{ab}	0.17^{ab}	7.32^{ef}
Neem	1 %	4.5^{cd}	15.5^{bc}	36.0^{g}	1.31^{bc}	0.16^{ab}	5.73^{g}
	3 %	5.0^{bc}	15.0^{c}	41.0^{f}	1.29^{bc}	0.16^{ab}	6.22^{fg}
	5 %	6.0^{a}	16.0^{abc}	29.0^{h}	1.27^{c}	0.13^{c}	3.76^{hi}
Papaya	1 %	4.0^{d}	16.0^{abc}	86.0^{a}	1.39^{a}	0.18^{a}	14.79^{a}
	3 %	5.0^{bc}	17.0^{a}	27.0^{h}	1.31^{bc}	0.16^{ab}	4.12^{h}
	5 %	5.5^{ab}	16.5^{ab}	22.0^{i}	1.31^{bc}	0.16^{ab}	3.46^{hi}
Pongamia	1 %	5.5^{ab}	16.0^{abc}	71.0^{c}	1.29^{bc}	0.15^{abc}	10.36^{cd}
	3 %	5.5^{ab}	17.0^{a}	54.0^{d}	1.29^{bc}	0.15^{bc}	7.83^{e}
	5 %	5.0^{bc}	17.0^{a}	42.0^{ef}	1.30^{bc}	0.15^{abc}	6.30^{fg}
Dhawai	1 %	4.0^{d}	16.0^{abc}	81.0^{b}	1.32^{bc}	0.17^{ab}	13.37^{b}
	3 %	4.0^{d}	16.0^{abc}	70.0^{c}	1.33^{bc}	0.16^{ab}	11.03^{c}
	5 %	5.0^{bc}	15.5^{bc}	57.0^{d}	1.35^{ab}	0.17^{ab}	9.70^{d}
Mean		5.03	16.22	47.63	1.31	0.16	7.43
Sig.		0.00*	0.03*	0.00*	0.03*	0.01*	0.00*

See Table 1 for germination parameter details

Table 7. Effect of bio-priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	20.0^{f}	1.27^{c}	0.13^{b}	2.53^{d}
AZB	10 %	6.0^{ab}	13.5^{c}	50.0^{cd}	1.33^{a}	0.16^{a}	8.13^{b}
	15 %	5.0^{c}	15.0^{b}	53.0^{c}	1.28^{bc}	0.14^{ab}	7.64^{b}
	20 %	4.0^{d}	15.0^{b}	79.0^{a}	1.34^{a}	0.16^{a}	12.78^{a}
PSB	10 %	5.5^{b}	14.0^{bc}	44.0^{de}	1.31^{ab}	0.16^{a}	6.89^{bc}
	15 %	5.0^{c}	15.0^{b}	39.0^{e}	1.26^{c}	0.15^{ab}	5.75^{c}
	20 %	4.0^{d}	15.0^{b}	64.0^{b}	1.27^{c}	0.13^{b}	8.32^{b}
Mean		5.14	14.64	49.71	1.29	0.15	7.43
Sig.		0.00*	0.00*	0.00*	0.00*	0.02*	0.00*

See Table 1 for germination parameter details

Comparison of priming treatments in *Dhawai:* The different priming treatments in *Dhawai* were compared with the best two treatments in each category of primers with respect to germination achieved (Fig. 1). Among all the priming treatment of *Dhawai*, 500 ppm of GA_3 gave highest germination of 94.0 % with highest SVI and SVI- mass of 132.54 and 16.17, respectively (Fig. 1).

Nursery performance of seedlings of *Dhawai* : Priming the seeds of *Dhawai* with different priming treatments significantly influenced the seedling shoot length, collar diameter and number of leaves and but dry weight, root length and survival showed insignificant variation among the treatments (Fig. 2, Plate 3). Not all the priming treatments improved these parameters over control. Shoot length was longest when the seeds were primed with 500 ppm of GA_3 and shortest was recorded with control. Priming the seeds with 500 ppm GA_3 produced seedlings with longest roots and seedlings with shortest roots were recorded with control. Seeds primed with 500 ppm of GA_3 also produced seedlings with widest collar diameter of 0.23 mm while, 24 hours hydro-

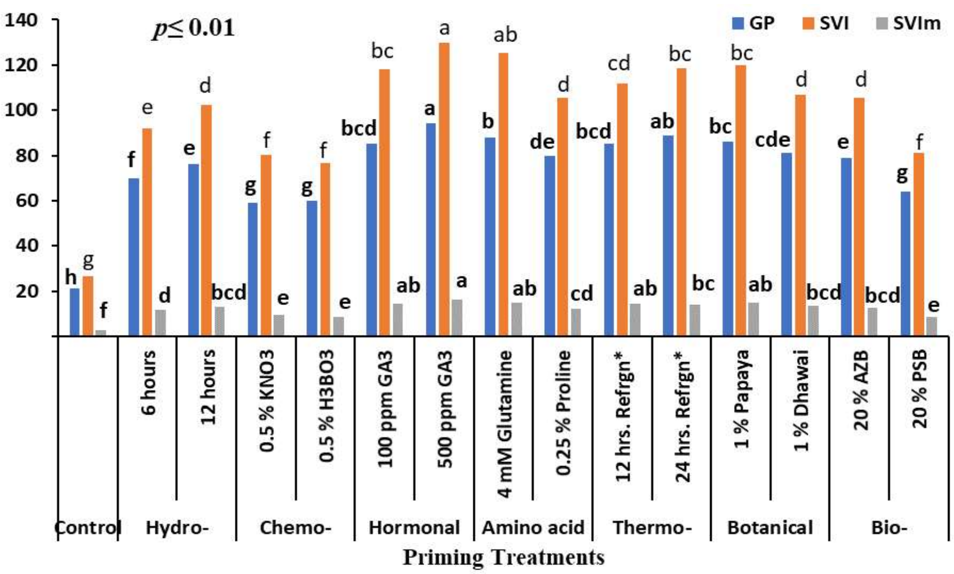

Fig. 1. Germination of *Dhawai* with best two priming treatments

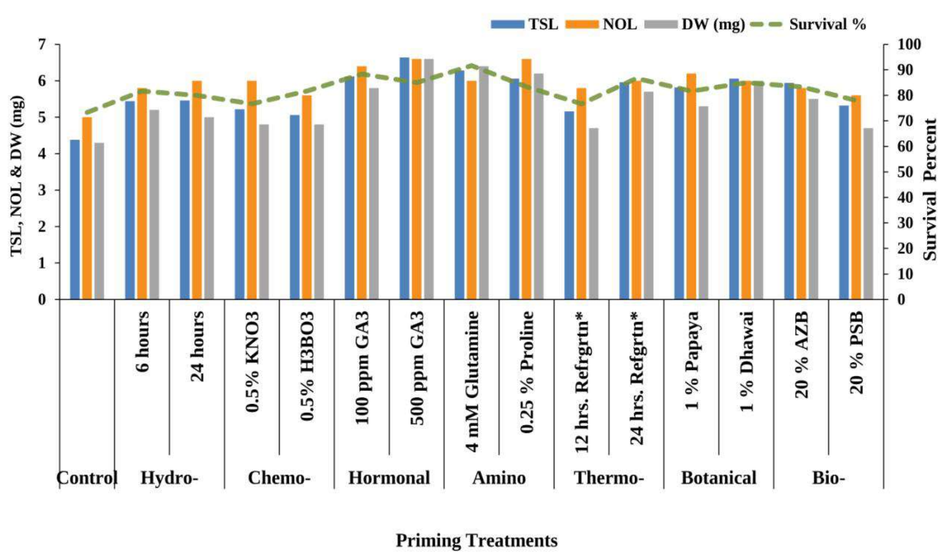

Fig. 2. Nursery performance of seedlings of *Dhawai*

priming, 0.5 % H_3BO_3 and control produced seedlings with narrowest collar diameter of 0.14 mm. Seeds primed with 500 ppm GA_3 and 0.25 % Proline produced seedlings with highest number of leaves (6.60) and lowest number of leaves (5.00) was recorded with control (Fig. 2). Dry weight of the seedlings was heaviest (6.60 mg) when the seeds were primed with 500 ppm of GA_3 and lightest (4.30 mg) was recorded with control (Fig. 2). Survival of the seedlings with these priming treatments was 73.33-91.67 % with a mean of 82.22 %. Priming treatments 4mM Glutamine resulted into highest survival of the seedlings and lowest survival was observed with control (Fig. 2). Among various priming treatments, 500 ppm of GA_3 was found the best priming treatment for improving growth parameters under nursery establishment study. GA_3 primed seeds recorded significantly higher seedling growth parameters as compared to control. This may be attributed to the key role of gibberellins in germination as growth regulator which are involved both in the break of dormancy and in the control of reserve hydrolysis on which the growing embryo depends (Lovegrove and Hooley 2000). The faster emergence of seeds primed with GA_3 might be due to its stimulation effect in the formation of enzymes during early phases of germination which helped for a fast radicle protrusion and hypocotyl elongation to penetrate the soil up (Salisbury and Ross 1992). These were further promoted due to exogenous application of GA as it increased the proportion of GA in the seed system promoting seedling growth and development. Similar influence of GA_3 on seedling growth of some field crops were also reported in earlier studies (Salisbury and Ross 1992, Lovegrove and Hooley 2000). In contrast, maximum survival was recorded with 4 mM Glutamine followed by 500 ppm GA_3 and 1 % *Dhawai*. Providing Glutamine exogenously might have supplemented the Glutamine synthetase activity, aiding nitrogen metabolism and acted as substrate for protein synthesis. Glutamate also leads to ammonium release in an oxidative deamination reaction catalyzed by glutamate dehydrogenase (Mifflin and Habash 2002).

Earlier studies with field crops exhibited excellent results with glutamine priming both under stress and normal conditions which was attributed to their influence on production of protein, seed sugar, oil, fiber content, moisture content, and ash (Ali et al 2013). Increased survival especially during stress was found with glutamine priming due to increased cell division, cell wall expansion, and other developmental processes. The micro-biotic nature with very fine seed coat might normally render the seeds to stress under normal germinating conditions restricting the seeds to germinating and seedling growth. Applying glutamine might have played a role of stress busters for the *Dhawai* seed aiding its germination, seedling growth and survival. Nevertheless, priming with 1 % *Dhawai* improved seedling survival; this might be due to synergetic effect of plant on its own species to germination, growth and survival. Similarly, improved germination and survival with *Dhawai* botanical extract seed treatment was also reported earlier (Devkota et al 2013).

CONCLUSION

The seed priming agents had positive effect in enhancement of germination and its parameters. Among all the priming treatments of *Dhawai*, it was found that priming with 500 ppm of GA_3 was the best followed by 4mM Glutamine and 24 hours refrigeration. Even though there is a high germination, seedling growth and establishment were recorded in both GA_3 and Glutamine seed priming treatments, 24 hours refrigeration treatment can be recommended for easy, effective and eco-friendly mode of quality planting material production in Terai region of West Bengal. In contrast, priming treatments such as the longer duration of hot air ovenation, higher concentration of acids, chemicals, hormones and other botanical priming agents deteriorated germination, seedling vigour and nursery establishment compared to non-primed seeds. However, further studies on seed biology, types of seed dormancy, genetic behaviour and pharmacological investigations can be undertaken along with studies on effect of priming on stresses (biotic and abiotic), seed storage, pests, diseases and yield parameter.

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Farmers Risk Perception, Management Strategies and Effectiveness: Evidences from Coconut Farmers of Kerala, India

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Abstract: This paper explains the perceptions of farmers regarding various risk events and conditions causing it, various risk management strategies being adopted by farmers and the effectiveness of these strategies; with the help of primary data collected from 273 coconut farmers of Kerala. Risk management strategies like membership in farmer collective (Producer company), farm diversification and value addition were considered in the study. Efficacy of these chosen risk management strategies were assessed and confirmed with the help of ordered logistic regression analysis. Strategies adopted by farmers were found to be effective in general, and were found to have varying degrees of effectiveness. The study emphasized on the need to have proper mechanism to ensure steady supply of inputs at reasonable prices. With adequate government support, producer companies can emerge as important organization for the betterment of coconut farmers. Guidance and support for farm diversification and value addition may help farmers of perennial crops to cope with income risk and thus to improve their income and livelihood.

Keywords: Coconut, Diversification, Producer company, Risk management, Value-addition

Coconut is an important plantation crop, which is grown in more than 90 countries. India is one among the leading coconut producing countries across the globe. For crops like coconut, adaptability to different risky situations is very less as it is a perennial crop. Farmers can't change the crop if the market price/ climate/ policy is not favorable. This makes the farmers more vulnerable compared to those who are doing cultivation of short-duration crops. Apart from that, owing to limited product diversification and value addition, income from coconut framing is mostly tied to coconut oil prices only– which is often susceptible to the pressure from availability of cheaper sources of oil seeds (MoA 2008, Jayasekhar et al 2016). Kerala is the leading coconut producing state in the country. This small state account for around 37 percent of area and around 31 percent of production of coconut in the country. In Kerala, coconut dominates in the total area under cultivation, and it accounts for nearly 40 percent of net sown area in the state. Among all crops it is the second largest contributor to the state income. Thus, addressing the risk scenario of coconut farming is vital to help the farmers in the state. By keeping this in mind, the present study attempts to provide empirical evidence of occurrence of various risk events as shown by farmers' perception, and the effectiveness of major risk management strategies adopted.

MATERIAL AND METHODS

Present study was conducted in two major coconut producing districts, Calicut and Malappuram districts based on higher area under coconut and also by considering the presence of coconut producer companies, which may be able to help farmers in risk management. Three villages from each district were selected after considering presence of adequate number of both members and non-members of Producer Company. Respondents were selected from these villages after satisfying multiple criteria – like member or non-member of Producer Company, irrigating and not irrigating, etc. Primary data were collected through personal interview method from a total of 273 coconut farmers using a structured pre-tested interview schedule.

Analytical Tools

Farmers' perception of risk: Farmers' perception of risks (reflected through incidence of risk events) was analyzed using percentage and tabular analysis. Farmers were asked about the occurrence and intensity of various risk events during past 5 years. The response percentages were presented as never, low, medium and high. Major risks like a) Production risk encompassing drought, rainfall inadequacy, rainfall untimeliness, pest/ disease attack, lack of technology, lack of technical know-how, inadequacy of inputs, untimeliness of inputs, lack of capital, high temperature, b) Price risk arising from: lack of marketing information, input price hike, failure of institutions, import policies, over supply in the market, substitute products, and change in food habits and; c) Income risk due to price policy of the government, volatility in input prices, subsidy policies, fluctuations in production and damage of products were considered for the study.

Efficacy of various risk management strategies in managing risks: Major risk management strategies generally adopted by the farmers were identified after discussion with farmers and agricultural professionals in the region. Major three risk management strategies, viz. membership in producer company, farm diversification and selling of value-added products were considered for the analysis. Ordered logistic regression, as used in Oksuzler (2008) was used to study the efficacy of these selected risk management strategies being adopted by farmers. Dependent variable was the trend in income from coconut farming over the past five years in presence of risks. Both adopters and non-adopter farmers of the various risk management strategies were asked about their income trend over the years in presence of risks in farming. Then it was checked whether participation/ adoption of these strategies is helping farmers in managing income risk. Yang (2010) had used binary logistic regression in his study for a similar purpose. Since the response come beyond binary in the present study context, and because this was having a specific order, we used an ordered logistic regression model. The empirical model used in the study is:

$$INC = \beta_0 + \beta_1 PCM + \beta_2 VA + \beta_3 DF + \beta_4 IG + \beta_5 AGE + \beta_6 EDU + \beta_7 MME + \varepsilon$$

Where, INC = Income change over years (1: increasing, 2: no change, 3: decreasing), PCM = Producer Company membership (1 for member, 0 otherwise), VA = Value addition (1: Always, 2: Very often, 3: Cautious, 4: Seldom, 5: Never), DF = Diversification of the farm (1: Always, 2: Very often, 3: Cautious, 4: Seldom, 5: Never), IG = Income group (I for APL, 0 for BPL), AGE = Age of the farmer in years, EDU = Years of education of the farmer, MME = Mass media exposure score. Marginal effects were worked out for meaningful interpretation by means of predicting the effect of use of a particular risk management strategy on the probability of helping farmers to be in increasing income status over the years.

RESULTS AND DISCUSSION

Socio-economic characteristics of the sample farmers: Socio-economic profile of respondent farmers is mentioned in Table 1.

Farmers' Perception of Risks in Farming

Farmers' perception of production risk: Lack of financial capital was perceived as the most frequently occurring risk aspect by most coconut farmers. 24 percent farmers opined that this problem affect them most frequently. High temperature and drought were the other major issues that affect coconut farmers most frequently. This will negatively affect nut production in a great extent, and many times premature nuts will fall down and thus, much yield loss will be there as a result of high temperature and drought. Rainfall inadequacy and untimeliness of rainfall were perceived as a low to medium level risk by most of the coconut farmers. Since coconut is a perennial crop and a sturdy palm, it may not be much visible to the farmers the ill-effects caused by rainfall anomalies. As the nut production takes place throughout the year, obviously rainfall anomalies will have its impact on yield. Also, the effect of such a negative rainfall anomaly might not be felt immediately, but after a few months only.

Farmers' perception of price risk: Nearly 33 percent coconut farmers reported input price hike as high risk. Though output price is increasing, it is not catching up the rate of increase in input prices and thus resulting in income loss to the farmers. Oversupply in the market was perceived as another frequent issue by 32 percent coconut farmers. As mostly farmers sell through the local traders, over supply create less

Table 1. Socio-economic characteristics of the sample farmers

Characteristics		Number
Sample farmers (No.)		273
Average age (years)		56.93
Average education (years)		8.60
Average household size (No.)		4.44
Average number of trees (No.)		49.92
Irrigation status (%)	Irrigating	38.83
	Not irrigating	61.17
Income groups (%)	BPL	33.33
	APL	66.67
Farm income trend for past 5 years (%)	Increasing	68.50
	No change	21.61
	Decreasing	9.89
Producer company membership (%)	Member	46.89
	Non-member	53.11
Value addition of products (%)	Always	35.53
	Very often	4.40
	Cautious	13.92
	Seldom	12.82
	Never	33.33
Diversification of farm (%)	Always	9.52
	Very often	20.88
	Cautious	7.69
	Seldom	21.98
	Never	39.93

demand and less price for the product in many occasions. Though import policies of government have a higher impact on coconut prices, only 12.36 percent farmers perceived this as a frequent risk aspect. Factors like availability of substitute products in the market and change in food habits of the consumers were not felt as a frequent issue by the farmers. Most of the farmers reported that they never faced this issue. Though farmers didn't perceive substitute products and change in food habits as a major risk phenomenon, cheap oil sources like palm oil is a major competitor for coconut oil. Coconut price is tightly linked with coconut oil price. Also, other oils like sun flower oil is also getting acceptance slowly.

Farmers' perception of income risk: Among the various factors that affect income change of farmers (Table 4), subsidy policies by government was the major factor reported by most of the farmers (29.09 percent) as a major issue. This was followed by volatility in input prices (23.27 percent), price policy by the government (18.55 percent) and fluctuations in production (17.09 percent). Farmers were complaining about the shooting up of fertilizer prices and the less amount of support they receive through schemes. Procurement prices were low earlier, but now it increased. But they face lot of difficulties in getting the money through government procurement. Damage of products was not seen as a major issue by most of the farmers. It was affecting most farmers in a low to medium extent only.

Table 2. Farmers' perception of occurrence of production risk

Risks	Respondents affected (%)			
	Never	Low	Medium	High
Drought	20.00	34.91	32.73	12.36
Rainfall inadequacy	4.36	49.82	39.64	6.18
Rainfall untimeliness	8.00	68.00	22.18	1.82
Pest/ disease attack	35.64	37.82	15.27	11.27
Lack of technology	50.91	32.73	14.91	1.45
Lack of technical know how	54.91	29.82	12.73	2.55
Inadequacy of inputs	43.27	40.36	11.27	5.09
Untimeliness of inputs	50.18	36.36	9.45	4.00
Lack of capital	67.27	2.55	5.82	24.36
High temperature	55.64	10.91	21.09	12.36

Table 3. Farmers' perception of occurrence of price risk

Risks	Respondents affected (%)			
	Never	Low	Medium	High
Lack of marketing information	41.09	43.64	11.27	4.00
Input price hike	7.27	25.45	34.18	33.09
Failure of institutions	64.73	12.00	16.36	6.91
Import policies	54.55	17.45	15.64	12.36
Over-supply in the market	49.45	9.09	9.09	32.36
Substitute products	76.36	18.91	4.73	0.00
Change in food habits	72.36	21.45	6.18	0.00

Table 4. Farmers' perception of occurrence of income risk

Risks	Respondents affected (%)			
	Never	Low	Medium	High
Price policy of the Government	44.00	17.09	20.36	18.55
Volatility in input prices	14.18	32.00	30.55	23.27
Subsidy policies	29.82	13.82	27.27	29.09
Fluctuations in production	7.27	35.64	40.00	17.09
Damage of products	49.82	35.27	10.91	4.00

Efficacy of risk management strategies: The ordered logistic regression indicate that the risk management strategies adopted by the farmers viz, membership in producer company, value addition of products and farm diversification positively influences increasing income over the years. This indicates they were effective in managing income risk faced by the farmers (Table 6),

Table 6. Efficacy risk management strategies adopted: Ordered logistic regression

Variable		Coefficient	Std. Error
Producer company membership		-0.862**	0.354
Value addition of products	Always	-2.224***	0.406
	Very often	-2.161***	0.832
	Cautious	-2.058***	0.569
	Seldom	-1.728***	0.505
	Never	(base)	
Diversification of farm	Always	-1.716**	0.562
	Very often	-0.787*	0.444
	Cautious	-0.610	0.720
	Seldom	-0.620	0.404
	Never	(base)	
Income group		-0.189	0.317
Age		0.004	0.023
Education		0.038	0.086
Mass media exposure		-0.190*	0.108
Log likelihood: -174.20, Prob>Chi^2: 0.000			

Note: ***, ** and * denote significance at 1 percent, 5 percent and 10 percent levels respectively

Being a member of Producer Company is associated with being 12.5 percent more likely to be in the increasing income status, 6 percent less likely to be in the stagnant income status and 6 percent less likely to be in the decreasing income status compared to the nonmembers. Membership in a farmer collective like producer company helps the farmers in getting good quality farm inputs at proper time and reasonable price, technical guidance and an assured market by means of procurement. Through this form of farmer collectives, farmers can reap benefits of economies of scale. Also, farmers have opportunities to get higher income by production and marketing of various coconut products through the company. This might have helped them to improve their income over years and to overcome risky situations.

Value addition is an important strategy for coconut farmers to ensure better income (CACP 2017). All the four categories of adoption of value addition gave significant results compared to those who never adopted value addition (base category). Those who always prepare value added products were found 41 percent more likely to report increasing income, 25 percent less likely to be in stagnant income and 16 percent less likely to be in decreasing income category compared to those who never adopted value addition. Similar pattern was found for other categories also, but with comparatively less probability as it proceeds from those who always adopt to those who seldom adopt. While coming to the diversification of farm; those who always adopt farm diversification were found 23

Table 7. Efficacy risk management strategies adopted: marginal effects from ordered logistic regression

Variable		Income increasing	No change in income	Income decreasing
Producer company		0.125**	-0.065**	-0.060**
Value addition of products	Always	0.412***	-0.253***	-0.159***
	Very often	0.404***	-0.247***	-0.157***
	Cautious	0.391***	-0.237***	-0.154***
	Seldom	0.344***	-0.201***	-0.143***
	Never	(base)	(base)	(base)
Diversification of farm	Always	0.234***	-0.137***	-0.096***
	Very often	0.121*	-0.062	-0.058**
	Cautious	0.095	-0.048	-0.047
	Seldom	0.097	-0.049	-0.048
	Never	(base)	(base)	(base)
Income group		0.027	-0.014	-0.013
Age		-0.001	0.0003	0.0003
Education		-0.005	0.003	0.003
Mass media exposure		0.028*	-0.014*	-0.013*

Note: ***, ** and * denote significance at 1 percent, 5 percent and 10 percent levels respectively percent more likely to be in increasing income category, 14 percent less likely to be in stagnant income category and 10 percent less likely to be in decreasing income category compared to those who never adopted farm diversification. Those who adopt it very often were found 12 percent more likely to be in increasing income group and 5.8 percent less likely to be in decreasing income status compared to the base category. Krishnakumar et al (2013) also had reported higher income realized by coconut farmers in Kerala through farm diversification interventions. A number of crops like tubers, spices, flower crops, etc. can be cultivated in coconut plantations, which will help to get a stable additional income. Also, intercultural activities for these intercrops may improve yield of coconut palms also. Unit increase in mass media exposure was found to be associated with 2.8 percent more likely to be in the increasing income status, 1 percent less likely to be in the stagnant income status and 1 percent less likely to be in the decreasing income status. Mass media exposure is an important way for farmers to get updated about better farming techniques, various schemes and a lot of valuable information, which can help them to improve their farm yield and profitability, and also to cope up with risky situations. Thus, these information obtained through mass media exposure might have helped the farmers to improve farm income over the years.

CONCLUSIONS

Risks are inherent in agriculture, and farmers of perennial crops like coconut face difficulty to make adjustments in order to cope with risky events. Lack of financial capital, high temperature and drought were the major issues leading to production risk. Price risk was arising mainly as a result of input price hike. Subsidy policies by the government, price volatility, price policies and fluctuations in production were the major causes of income risk. Government policies need to be streamlined so as to give maximum benefit to the target farmers. Interventions are needed to address capital issue of farmers and for availability of inputs at reasonable and steady price. Joining in farmer collectives (Producer Company), value addition and farm diversification were the major risk management strategies being adopted by the farmers. While looked at the efficacy of these strategies adopted, all these three strategies and also mass media exposure were found to be effective in managing income risk faced by farmers. With adequate government support, producer companies may can better help farmers further. Guidance and support for farm diversification and value addition will help farmers of perennial crops to cope with income risk and thus to improve their income and livelihood.

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Market Integration and Price Volatility in Tea Market of India

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Abstract: This paper examine the transmission and spatial integration analysis between tea producing (Guwahati, Kolkata and Chennai) and consuming (Mumbai, Delhi and Bhopal) markets using monthly price data from April 2005 to March 2020. Correlation analysis, Johansen co-integration test, Vector Error Correction Model and Granger causality were used for the analysis. Instability in the price series was measured by Cuddy Della-Valle index. The maximum instability in tea prices was in March in Guwahati market (18.48%)andthe seasonality index revealed that farmers got nearly average prices throughout the year.Johansen co-integration test revealed that all the selected markets were well integrated in the long run. Bhopal market was found to be the key market which influenced the price of all other markets by Granger Causality test. ARCH family model was found to be best fitted for estimating price volatility in the key market.

Keywords: ARCH, Co-integration, Granger causality, Instability, Volatility

India was the market leader at the international level with regard to production and consumption of tea till 2005. India was the world's second largest tea producer, with 1360.81 million kgs produced (Statista 2020). The tea market in India is huge with tens of thousands of tea gardens spread around the nation, including such popular varieties as Darjeeling and Assam. More than half of the tea produced in India remains in the country for consumption, effectively making this a country of a billion tea drinkers (World Atlas 2022). Tea occupies an important role in the Indian economy not only due to its capacity to earn foreign exchange, but also because it impacts the livelihoods of scores of people employed directly and indirectly by the industry. Tea prices were on the rise since July due to supply issues, which were considered as a requisite for the sustenance of the sector. Domestic tea prices from January to November period at auctions especially South India, was higher by Rs. 28.96 per kg at Rs. 130/kg, while North Indian price was up by Rs. 54.75 at Rs. 207.59 as compared to same period last year. However, in the last few auctions sales, there was a free fall in prices and quantity sold (Kumar S, 2020). In the first auction held in May, prices surged by over 52 per cent to Rs. 217.12 per kg in Guwahati. (Tea board of India 2020). In a similar auction in Siliguri, prices surged by around 39 per cent to Rs. 204.25 per kg. By mid-May, prices in Guwahati rose by 61 per cent to Rs. 217.12 per kg. Buyer demand has been extremely strong, which pulled up prices significantly (Rakshit 2020). Although there is geographical dispersion of markets, prices across different market centres exhibit long-run spatial linkages, suggesting that all the exchange locations are integrated and that prices provide relevant market signals (Ghosh 2010). The extent of integration gives signals for efficient resource allocation, which is considered essential for ensuring greater market efficiency, price stability and food security (Muhammad and Mirza 2014). Test of integration also plays a key role in determining the geographical level at which agricultural price policy should be targeted, at least in the short-run to ensure regular availability of food and price stability (Jha et al 2005). Therefore, the present paper attempts to understand the co-movement of prices among different domestic markets for tea crop in India. It also aims to estimate the volatility exhibited by the prices in the selected markets to provide suitable policy suggestions for pricing policy.

MATERIAL AND METHODS

The monthly time series data on prices and arrivals of tea for the period January 2009 to August 2020 were used for the present study obtained from Agmarknet portal of Ministry of Agriculture and Farmers Welfare, GoI. The study investigated market integration across six major wholesale markets, Guwahati (Assam), Kolkata (West Bengal), Chennai (Tamil Nadu), Mumbai (Maharashtra), Delhi and Bhopal (Madhya Pradesh). These markets were selected on the basis of location as well volume of produce handled. Guwahati, Kolkata and Chennai are located in tea producing area, whereas Mumbai, Delhi and Bhopal are located in the consuming area.

Analytical framework: Different analytical tools such as seasonality analysis, unit root test, Johansen co-integration test, Granger's causality analysis, vector error correction model and ARCH family model were used to examine the market behaviour. The analysis of data was performed by using E-views 9 and R software.

Instability analysis: The coefficient of variation (C.V.) is usually employed to estimate instability in time series data. However, a limitation of C.V. is that it over-estimates the level of instability in the time series data characterised by long term trends (Paul et al. 2013, Nimbrayan and Bhatia 2019). This limitation is overcome by the Cuddy-Della Valle index (CDVI) suggested by Cuddy and Della Valle (1978) which corrects the coefficient of variation.

$$CDVI = CV\sqrt{1-r^2}$$

where, r^2=coefficient of determination

Seasonality index: The range in seasonality was estimated by using the method suggested by Ali (2000):

Si = $(I_h - I_l)$

S_i = Seasonal indices

I_h = Highest value of seasonal index

I_l = lowest value of seasonal index

Test for stationarity: The first step in the time series analysis, before testing for co-integration and Granger causality, is to examine the stationarity of each individual time series selected for the analysis. Augmented Dickey-Fuller (ADF) unit root test (Dickey and Fuller 1979) was considered to examine the stationarity. The test was applied to check the order of integration by using the model:

$$\Delta P_t = \alpha_0 + \delta_1 t + \beta_1 P_{t-1} + \sum_{j=0}^{q} \beta_1 \Delta P_{t-1} + \varepsilon_t$$

Where,

P = the price in each market,

Δ= difference parameter (i.e., $\Delta P_1 = P_t - P_{t-1}$, $\Delta P_{t-1} = P_{t-1} - P_{t-2}$ and $\Delta P_{n-1} = P_{n-1} - P_{n-2}$)

α_0 = constant or drift

t = time trend variable

q = number of lag length and

ε_t = pure white error term,

The null hypothesis is that β_1 (the coefficient of P_{t-1}) is zero. The alternative hypothesis is: $\beta_1 < 0$. A non-rejection of the null hypothesis suggests that the time series under consideration is non-stationary (Gujarati 2004).

Johansen's co-integration method: Co-integration explains the extent of deviation from the long run equilibrium relationship by the non-stationary series. Once it was confirmed that all of the price-series were stationary at the level or at same order of differences, the maximum likelihood (ML) method of co-integration was applied to check long run wholesale price relation between the selected markets (Johansen 1988, Johansen and Juselius 1990). Maximum likelihood ratio test statistic is proposed to test number of co-integrating vectors. The null hypothesis of atmost 'r' co-integrating vectors against a general alternative hypothesis of more than 'r' co-integrating vectors is tested by trace statistics. The number of co-integrating vectors indicated by the tests is an important indicator of the extent of co-movement of prices. An increase in the number of co-integrating vectors implies an increase in the strength and stability of price linkages.

Vector error correction model: The co-integration analysis reflects the long-run movement of two or more series, although in the short-run they may drift apart. Once the series are found to be co-integrated, then the next step is to find out the short run relationship along with the speed of adjustment towards equilibrium using error correction model, represented by equations:

$$\Delta \ln X_t = \infty_0 + \sum \beta_{1i} \Delta \ln Y_{t-1} + \sum \beta_{2i} \Delta \ln X_{t-1} + \gamma ECT_{t-1}$$

$$\Delta \ln X_t = \beta_0 + \sum \infty_{1i} \Delta \ln X_{t-1} + \sum \alpha_{2i} \Delta \ln Y_{t-1} + \gamma ECT_{t-1}$$

where, ECT_{t-1} is the lagged error correction term; X_t and Y_t are the variables under consideration transformed through natural logarithm; and X_{t-i} and Y_{t-i} are the lagged values of variables X and Y. The parameter γ is the error correction coefficient that measures the response of the regressor in each period to departures from equilibrium. The negative and statistically significant values of γ depict the speed of adjustment in restoring equilibrium after disequilibria and if it is positive ad zero, the series diverges from equilibrium.

Granger causality test: After undertaking co-integration analysis of the long run linkages of the various variables, and having identified they are linked, an analysis of statistical causation was conducted. The Granger causality test conducted within the framework of a VAR model is used to test the existence and the direction of long run causal price relationship between the markets (Granger, 1969). F-test is used to check the significance of changes in one price series affect another price series. Also, this test identifies the key market, i.e., the market which influences the price of all other markets.

Measuring price volatility: ARCH family Model: Once the key market is identified, volatility of price series of that market is checked by testing the presence of heteroskedasticity through ARCH test. If heteroskedasticity has found in price series, then to deal with this, the popular and non-linear model is the autoregressive conditional heteroscedastic (ARCH) model, proposed by Engle (1982). The model was generalized by Bollerslev (1986) in the form of Generalized ARCH (GARCH) model for parsimonious representation of ARCH. In the GARCH model, the conditional variance is also a linear function of its own lags. As in ARCH, this model is also a weighted average of past squared residuals, but it has declining weights that never go completely to zero. Apart from these two models, there are other models such as TARCH, EGARCH and PARCH. The best fit model was selected out of these models based on AIC and SIC values. The forecasting performance of fitted models is assessed with respect to two traditional accuracy measures, viz., the root mean square error (RMSE) and the mean absolute percentage error (MAPE).

RESULTS AND DISCUSSION

Seasonality and instability analysis: The CDVI index showed that the prices are stable and had no fluctuation in prices throughout the year and seasonality index was almost around one in each month which means that the farmers receive nearly average prices through the year (Table 1).

Correlation analysis: The correlation coefficients between the markets were highly significant and ranged from -0.846 to 0.896. This also implies that price differential in the markets is not more than transportation cost and hence, the markets are said to be efficient (Table 2).

Augmented Dickey-Fullar test (ADF): As correlation analysis provides only rough estimates on price movements. To avoid spurious results there is a need to check whether the variables are stationary or not. (Guleria et al 2022). The ADF based unit root test procedure was applied to check whether the price series is stationary at their level, followed by their differences. So, the price series is stationary at first difference which means zero mean and zero variance. The t-statistic value for all the markets is significant implying that these series were stationary and free from consequences of unit root (Table 2). Therefore can proceed with co-integration

Johansen co-integration test: The integration among selected tea markets was analysed through the Johansen co-integration test (Table 3). Unrestricted co-integration rank test (Eigen value and trace statistic) indicated the presence of at least six co-integrating equations at 5 per cent level of significance. This indicated that tea prices in the selected market were having long run equilibrium and also implies

Table 1. Instability and seasonality in Tea price in selected markets

Month	Guwahati			Kolkata			Chennai			Mumbai			Delhi			Bhopal		
	CV	CDVI	SI	CV	CDVI	SI	CV	CDVI	SI	CV	CDVI	SI	CV	CDVI	SI	CV	CDVI	SI
January	24.78	15.42	1.02	12.62	4.72	1.01	15.06	9.52	1.01	20.79	10.88	1.00	17.69	4.82	1.00	28.44	11.34	0.98
February	24.26	15.21	1.01	13.06	4.62	1.00	15.13	9.22	1.01	19.96	10.37	1.01	17.94	5.63	1.00	26.98	10.64	0.99
March	24.74	18.48	1.02	12.97	4.62	1.00	14.20	8.11	1.01	20.50	10.71	1.00	18.28	6.18	1.00	27.23	14.41	1.02
April	28.24	12.96	0.96	13.54	4.76	0.99	9.83	5.82	0.99	20.66	10.99	1.00	18.40	6.02	1.01	27.23	14.41	1.02
May	28.72	11.99	0.97	13.52	4.69	0.99	9.79	5.71	0.99	20.48	10.86	1.00	18.00	5.97	1.01	27.23	14.41	1.01
June	26.82	12.23	1.00	13.46	5.36	1.01	9.98	5.84	0.99	20.13	10.66	0.99	17.90	5.19	1.00	27.03	14.65	1.02
July	25.44	11.31	1.00	13.86	5.52	1.01	10.18	5.99	0.99	19.72	10.57	0.99	18.34	4.91	1.00	25.84	14.29	1.04
August	23.65	10.46	1.01	13.86	5.52	1.00	10.26	6.05	0.99	19.47	10.29	1.00	18.63	3.85	0.99	24.17	11.41	1.03
September	21.41	12.91	1.02	14.14	5.14	1.00	11.20	6.59	1.00	16.60	8.13	1.01	17.63	3.70	1.00	24.99	11.03	1.02
October	21.41	12.91	1.02	12.46	5.15	1.01	12.27	7.30	1.00	16.59	9.30	1.01	16.35	3.81	1.01	25.13	11.09	1.01
November	21.05	14.58	1.02	12.53	4.90	1.01	12.63	7.72	1.00	17.18	10.21	1.01	16.33	4.24	1.00	25.95	11.50	1.00
December	21.20	15.47	1.02	12.53	4.90	1.00	14.18	8.96	1.00	15.52	9.34	1.02	15.91	4.32	1.00	25.95	11.50	0.99

CV-Coefficient of Variation (%), CDVI- Cuddy-Della Valle index and SI-Seasonality Index

Table 2. Correlation coefficients of monthly tea prices between selected markets

	Guwahati	Kolkata	Chennai	Mumbai	Delhi	Bhopal
Guwahati	1.000					
Kolkata	0.712*	1.000				
Chennai	-0.388*	0.736*	1.000			
Mumbai	0.592*	0.819*	-0.711*	1.000		
Delhi	0.773*	0.911*	0.742*	0.896*	1.000	
Bhopal	0.581*	0.859*	-0.846*	0.745*	0.801*	1.000

*indicates $p<0.05$

Table 3. ADF test to check the stationary of the data

Markets	t-Statistic	p value
Guwahati	-5.65	0.00
Kolkata	-13.09	0.00
Chennai	-7.96	0.00
Mumbai	-9.38	0.00
Delhi	-7.45	0.00
Bhopal	-3.39	0.01

*MacKinnon (1996) one sided p-values The whole data was Stationary

strength and stability of price linkages between selected tea markets.

Vector error correction model: Vector Error Correction Model (VECM) was employed to know the speed of adjustments among the markets for long run equilibrium among the selected markets. The number of lags in the VECM was taken to be two as the Akaike Information Criterion (AIC) was lowest at this order (2) in the system for all the selected markets i.e., Guwahati, Kolkata, Chennai, Mumbai, Delhi and Bhopal. The results of error correction terms were interpreted in order to study the nature of market (stable/unstable/random), endogeneity and the movement towards the long run equilibrium, i.e., efficiency of the market. Thereafter, the short-term causality in the prices of selected markets included in the system, i.e., which market impacts the price of other market was also explained.

$$\Delta \ ln\ Guwahati_t = -0.11\ ECT_{t-1} - 0.71 \Delta ln\ Guwahati_{t-1} + 0.74\ \Delta Kolkata_{t-1} - 0.87\ \Delta lnMumbai_{t-1} + -0.005\ \Delta ln\ Delhi_{t-2} + 0.04\ \Delta Chennai_{t-1}$$

$$\Delta \ ln\ Kolkata_t = -0.04\ ECT_{t-1} - 0.69 \Delta ln\ Kolkata_{t-1} - 0.02\ \Delta Mumbai_{t-2} + 0.87\ \Delta lnMumbai_{t-1} + -0.005\ \Delta ln\ Delhi_{t-2} + 0.04\ \Delta Chennai_{t-1} - 0.03 \Delta ln\ Guwahati_{t-1}$$

$$\Delta \ ln\ Bhopal_t = -0.66\ ECT_{t-1} - 0.13 \Delta ln\ Chennai_{t-1} - 0.03\ \Delta Guwahati_{t-2} + 0.18\ \Delta lnKolkata_{t-2} + -0.30\ \Delta ln\ Mumbai_{t-2}$$

$$\Delta \ ln\ Chennai_t = -0.05\ ECT_{t-1} - 0.23 \Delta ln\ Mumabi_{t-1} - 0.15\ \Delta Bhopal_{t-1} + 0.08\ \Delta Guwahati_{t-2} + -0.08\ \Delta ln\ Kolkata_{t-2}$$

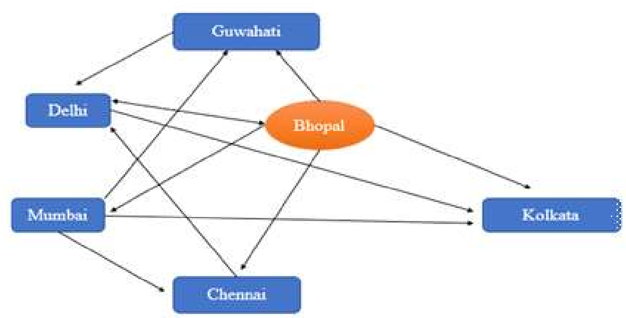
$$\Delta\ ln\ Delhi_t = -0.09\ ECT_{t-1} - 0.23 \Delta ln\ Delhi_{t-1} - 0.04\ \Delta Guwahati_{t-1} + 0.01\ \Delta Kolkata_{t-2} + -0.03\ \Delta ln\ Mumbai_{t-2} + 0.01\ \Delta Bhopal_{t-2}$$

$$\Delta \ ln\ Mumbai_t = -0.29\ ECT_{t-1} - 0.22 \Delta ln\ Bhopal_{t-2} - 0.28\ \Delta ln\ Chennai_{t-1} + 0.17\ \Delta ln\ Delhi_{t-1} + -0.02\ \Delta ln\ \Delta Guwahati_{t-1} + 0.15\ \Delta ln\ Kolkata_{t-1} - 0.39\ \Delta ln\ Mumabi_{t-1}$$

Guwahati, Kolkata and Chennai were found to be dependent on the other markets, the speed of adjustment is low in general i.e., 11, 4 and 5 per cent respectively. This is probably due to the reason that only one-way transaction exists between the markets and said to be producing markets which supplies the produce to the other markets. However, in the Bhopal, Mumbai and Delhi markets, the speed of adjustment is found to be higher i.e., 66 per cent and 29 per cent and 11 per cent respectively. As, Bhopal market is found to be consuming market the stored quantity might be released due to faster error correction mechanism takes place. Similar results were observed by Saxena and Chand (2017).The tea prices in Guwahati market were affected by the prices in Kolkata, Mumbai and Chennai market with lag of one month as well as that of Delhiprices with two lags. Kolkata market affected by the price of Bhopal and Guwahati at lag of one-month Mumbai and Chennai market affect Kolkata market with two lags. However, prices in Bhopal market were affected by Chennai with one month lag and two-month lag of Guwahati, Kolkata and Mumbai prices.

Granger Causality test: Among the selected tea markets, the tea price of Bhopal market showed bidirectional causality transmission with tea price of Delhi market (Fig. 1). The Bhopal market itself influenced the price of Mumbai, Chennai, Kolkata and Guwahati markets which shows the unidirectional relationship between them. Guwahati market uni-directionally influenced the price of Delhi market. Mumbai market uni-directionally influenced Chennai, Kolkata and Guwahati markets. Bhopal market was a key market which influenced the price of tea crop in all other selected markets.

Testing the ARCH effect in the key market: The Box-Jenkins approach has a basic assumption that the residuals



Single arrow shows unidirectional relationship and double arrow shows bidirectional relationship

Fig. 1. Unidirectional and bidirectional relationship between markets

Table 3. Johansen cointegration test (trace) of price variation in tea markets

Null hypothesis	Eigen value	Trace statistic	Critical value	Prob.**
None *	0.298147	171.6785	95.75366	0.00
At most 1 *	0.202394	124.2383	69.81889	0.00
At most 2 *	0.197901	93.93554	47.85613	0.00
At most 3 *	0.18836	64.38543	29.79707	0.00
At most 4 *	0.144382	36.4198	15.49471	0.00
At most 5 *	0.109399	15.52508	3.841466	0.0001

Trace statistics indicates six cointegrating eqn(s) at the 0.05 level
*denotes rejection of the hypothesis at the 0.05 level
**MacKinnon-Haug-Michelis (1999) p-values

remain constant over time. Thus, the ARCH – Lagrange multiplier (LM) test was carried out on the square of the residuals and to test whether residuals do in fact remain constant. The results of the test given in table 6 revealed the presence of ARCH effect in price series of Bhopal (key) market.

Fitting of GARCH model: The GARCH model was fitted on the Bhopal market priceseries and then forecasting ability was tested. From all ARCH family models, the GARCH model was identified to be the best fit on the basis of minimum value of AIC and SIC (Table 6).

The capture volatility present in the tea price series quite well as evident from the significant value of coefficient of squared of residual term at lag one in the variance equation. The average price of tea in each month will be around Rs. 102.09 per kg (antilog of 2.009) (Table 7). Forecasting ability

Table 5. ARCH - LM test for price series of Bhopal market

Lags	F-statistic	P value
1	-0.011	p<0.001
2	-0.015	p<0.001
3	0.036	p<0.001
4	-0.015	p<0.001
5	0.121	p<0.001
6	-0.016	p<0.001

Table 6. Selection of best fit model

ARCH family model	Akaike information criterion (AIC)	Schwarz information criterion (SIC)
ARCH	6.357	6.399
GARCH	5.581*	5.666*
TARCH	5.609	5.673
EGARCH	6.811	6.853

*indicates model selected for the estimating price volatility

Table 4. Pair-wise granger causality test of selected tea markets

Null Hypothesis:	F-Statistic	Prob.
CHENNAI does not Granger Cause BHOPAL	0.041	0.960
BHOPAL does not Granger Cause CHENNAI	11.239	0.000
DELHI does not Granger Cause BHOPAL	5.271	0.006
BHOPAL does not Granger Cause DELHI	4.705	0.010
GUWAHATI does not Granger Cause BHOPAL	0.453	0.637
BHOPAL does not Granger Cause GUWAHATI	10.568	0.000
KOLKATA does not Granger Cause BHOPAL	1.225	0.297
BHOPAL does not Granger Cause KOLKATA	7.686	0.001
MUMBAI does not Granger Cause BHOPAL	0.626	0.537
BHOPAL does not Granger Cause MUMBAI	2.802	0.064
DELHI does not Granger Cause CHENNAI	0.166	0.847
CHENNAI does not Granger Cause DELHI	2.465	0.089
GUWAHATI does not Granger Cause CHENNAI	0.489	0.614
CHENNAI does not Granger Cause GUWAHATI	0.712	0.493
KOLKATA does not Granger Cause CHENNAI	0.119	0.888
CHENNAI does not Granger Cause KOLKATA	0.220	0.803
MUMBAI does not Granger Cause CHENNAI	2.921	0.057
CHENNAI does not Granger Cause MUMBAI	0.392	0.676
GUWAHATI does not Granger Cause DELHI	6.967	0.001
DELHI does not Granger Cause GUWAHATI	1.230	0.296
KOLKATA does not Granger Cause DELHI	0.374	0.689
DELHI does not Granger Cause KOLKATA	2.268	0.108
MUMBAI does not Granger Cause DELHI	1.242	0.292
DELHI does not Granger Cause MUMBAI	1.196	0.306
KOLKATA does not Granger Cause GUWAHATI	2.157	0.120
GUWAHATI does not Granger Cause KOLKATA	0.793	0.455
MUMBAI does not Granger Cause GUWAHATI	3.285	0.041
GUJRAT does not Granger Cause MUMBAI	0.604	0.548
MUMBAI does not Granger Cause KOLKATA	5.058	0.008
KOLKATA does not Granger Cause MUMBAI	0.908	0.406

Table 7. Mean and variance equation for GARCH Model

Variable	Coefficient	Std. Error	Z-Statistic	p value
Mean equation				
Intercept	2.009*	0.13	15.456	0.000
Variance equation				
GARCH	0.592*	0.024	24.222	0.000
ϵ^2_{-1}	0.974*	0.237	4.108	0.000
R-squared	-0.016	Sum squared residuals		76.351
Adjusted R-squared	-0.0089	Log likelihood		-38.685
RMSE	7.411	MAPE		11.51

*p<0.05

of the model was judged on the basis of value of root mean square error (RMSE) and mean absolute per cent error (MAPE). In the present study, the value of RMSE and MAPE has been found to be 7.411 and 11.51. Low value of MAPE has been assured the high forecasting ability of the fitted model (Gabriel 2012).

CONCLUSION

The tea price remained stable in the selected markets. In January to March and September to December, low to medium instability was observed. During these months, farmers received a better price than usual. The correlation analysis revealed that market prices moved together and were highly integrated, implying that the price differential in the selected markets was not greater than the transportation cost. This signalled that the markets are efficient. The price series in the selected markets were stationary and co-integration test indicated that the tea prices in the selected markets had long run relationship. The speed of adjustment was highest in Bhopal market (66 per cent) followed by Mumbai (29 per cent) and Guwahati (11 per cent). Granger causality revealed that Bhopal market was the key market which influenced the price of the other selected markets. For checking price volatility, GARCH model was used which revealed that the average price of tea in each month will be around Rs. 102.09 per kg. However, priority should be given to Guwahati, Kolkata and Chennai markets where lower chance of correction of any disequilibrium. More farmers should be encouraged to participate in future trading and contract farming so as to reduce the variation in arrivals and prices.

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Modelling and Forecasting Wheat Production in Punjab State of India using Hierarchical Time Series Models

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Abstract: Due to the importance of wheat crop and Punjab being the leading wheat producer, this paper considers hierarchical time series data on wheat production in Punjab. The Punjab state wheat production data is organized in a hierarchy based on geographical regions. Top-down, bottom-up, middle-out, and optimal-combination approaches were used along with single series forecasting. An analysis of forecast performance shows that bottom-up approach outperforms other methods in terms of RMSE and MAE in out of sample forecast horizon. Finally, the state of Punjab has forecasted wheat production from 2019 to 2023 using a bottom-up approach.

Keywords: Bottom-up, Forecasting, Middle-out, Production, Wheat

Wheat (*Triticum astivum*) is a staple meal for nearly a third of the world's population, as well as a significant source of protein, niacin, and fibre in the diet. India is the world's second-largest wheat producer, blessed with a variety of agro-ecological conditions that give food and dietary security to the Indian people (Sharma and Sendhil 2015, Sendhil et al 2019), in particular in the recent past. Wheat has increased in this country to 99,70 million tons, 13.64 percent of global production, with a productivity level of about 3,371 kg/ha(www.fao.org.). Uttar Pradesh, Punjab, Haryana and Madhya Pradesh are major wheat producing states in India. (http://www.agricoop.nic.in). In Punjab, wheat production and productivity have increased consistently in the last five decades (Kaur et al 2015). Punjab has maintained the status of increased wheat productivity for many years (Sendhil et al 2019). Faridkot is top wheat growing district of Punjab with a production of 2635 thousand tons. Predicting the production behaviour of major crops is critical in tackling the global food safety crisis. Modeling and forecasting and application of phenomena, particularly in the agricultural sector, became significant in the latter half of the last century. Mishra et al (2015) considered the wheat productivity forecast time series model in India. With the introduction of hierarchical time series methodology, it has gained further boost, particularly in time series predictions. The hierarchy of time series models in various fields has been studied by Athanasopoulos et al (2009) and Moon et al (2012). Pal and Paul (2016) used hierarchical time-series to model and predict the production of sorghum in India and observed that the middle-out technology outperformed other approaches to hierarchical time series models and to traditional prediction methods. Mitra et al. (2017) use hierarchical time series models to predict the production of India's oilseeds and pulses and the bottom-up approach was superior to the other approaches of the hierarchical model of time series for modeling and forecasting the production of oilseeds in India, while the optimum combination for pulses production was the best. Gua and Xue (2014) applied Artificial Neural Network to compare spatial and temporal models of crop yield forecasting. Shrivastri et al (2022) compared the different time series model for forecasting of wheat production for India. In the present study the production of wheat has been predicted using hierarchical time series models in major regions and all districts of the state of Punjab.

MATERIAL AND METHODS

Hierarchical time-series: The level 0 (zero) refers to the aggregate series, level 1 the first stage, and the most disaggregated series is level K. A letter sequence identifies the individual series and the level of hierarchy. As in: A is for level 1 series A, AB is for level 2 series A, and so on (Fig. 1).

The observations were recorded at times t = 1,2,…n, and the objective is to forecast each series at each level at times t = n + 1, n +2,…,n + h. Suppose, the notation X is used to refer to a generic series within the hierarchy. Observation on series X are written as $y_{x,t}$. Thus, $y_{AB,t}$ is the value of series AB at time t. y_t denotes aggregate of all series at time t. Therefore,

$$y_t = \sum_i y_{ij,t}\ ,\ y_{i,t} = \sum_j y_{ij,t}\ ,\ y_{ij,t} = \sum_k y_{ijk,t}\ ,\ y_{ijk,t} = \sum_l y_{ijkl,t}\ ,$$

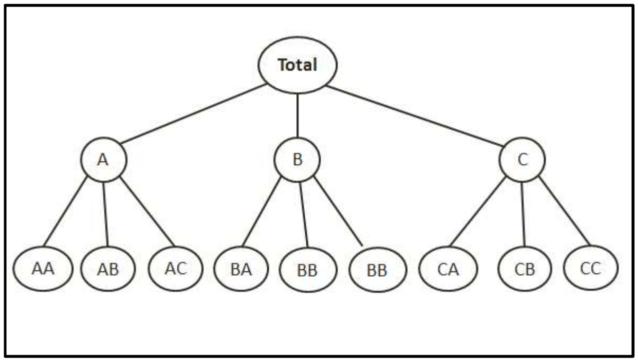

Fig. 1. A3-structure hierarchical tree diagram

The, observations at higher levels are obtained by adding up the series below.

Let m_i denotes the total number of series at level i (i=2,1,2,…,K) subject to the constraint, $m_i > m_{i-1}$, then the total number of series in the hierarchy is $m = m_0 + m_1 + \ldots + m_k$. In the above example m =13.

Let $y_{i,t}$ denotes the vector of all observations at level *i* and time t and $y_t = [y_t, y_{1,t}, \ldots, y_{kt}]$

$y_t = Sy_{k,t}$ (1)

where S is a "summing" matrix of order $m \times m_K$ used to aggregate the forecasts of the lowest level series. In the above example, $y_t = [y_t, y_{A,t}, y_{B,t}, Y_{C,t}, Y_{AA,t}, Y_{AB,t}, \ldots, y_{CC,t}]$ and the summing matrix is of order 13 × 9 and is given by

$$S=\begin{vmatrix} 1&1&1&1&1&1&1&1&1\\ 1&1&1&0&0&0&0&0&0\\ 0&0&0&1&1&1&0&0&0\\ 0&0&0&0&0&0&1&1&1\\ 1&0&0&0&0&0&0&0&0\\ 0&1&0&0&0&0&0&0&0\\ 0&0&1&0&0&0&0&0&0\\ 0&0&0&1&0&0&0&0&0\\ 0&0&0&0&1&0&0&0&0\\ 0&0&0&0&0&1&0&0&0\\ 0&0&0&0&0&0&1&0&0\\ 0&0&0&0&0&0&0&1&0\\ 0&0&0&0&0&0&0&0&1 \end{vmatrix}$$

The rank of S is m_k.

The $\hat{y}_{x,n}$ (h) be the h-step-ahead forecasts for the series y_x. A sample of t = 1,2,…, n is used to generate the forecasts. Therefore, $\hat{y}_{AA,n}(h)$ denotes the h-step-ahead base forecast of series y_{AA} using the sample $y_{AA,1}, y_{AA,2}, \ldots, y_{AA,n}$. For level i, all h-step-ahead base forecasts can be represented by $\hat{y}_{i,n}$ (h) and the h-step-ahead base forecasts for the whole hierarchy are given as $\hat{y}_{,n}$ (h), which contains all of the base forecasts stacked in the same sequence as y_t.

Using this notation, all existing hierarchical methods can be represented by the general form

$$\tilde{y}_n(h) = SP\hat{y}_n(h) \qquad (2)$$

where S is the summing matrix of order $m \times m_k$, as in Eq.(1), and P is a matrix of order $m_k \times m$.

The form of P differs depending on the hierarchical forecasting approach.

Bottom-up approach: For the majority of disaggregated series at the bottom of the hierarchy, bottom-up forecasting generates independent base forecasts that are then aggregated to generate revised forecasts for the remaining series Figure 1. After obtaining the h-step-ahead independent forecasts for each of the bottom level series namely $\hat{y}_{AA,n}$ (h), $\hat{y}_{AB,n}$ (h), …, $\hat{y}_{CC,n}$ (h) aggregate these forecasts upwards to obtain the h-step-ahead forecasts for the whole hierarchy as follows:

$$\tilde{y}_A(h) = \tilde{y}_{AA}(h) + \tilde{y}_{AB}(h) + \tilde{y}_{AC}(h)$$
$$\tilde{y}_B(h) = \tilde{y}_{BA}(h) + \tilde{y}_{BB}(h) + \tilde{y}_{BC}(h)$$
$$\tilde{y}_C(h) = \tilde{y}_{CA}(h) + \tilde{y}_{CB}(h) + \tilde{y}_{CC}(h)$$
$$\tilde{y}(h) = \tilde{y}_A(h) + \tilde{y}_B(h) + \tilde{y}_C(h)$$

For the bottom–level series the revised forecasts are same as the base forecasts (i.e. $\tilde{y}_{AA}$ (h) = $\hat{y}_{AA}$ (h)).

$$P = \left[0_{m_k \times (m-m_k)} / I_{m_k}\right] \qquad (3)$$

The general form of this approach is represented as

where $0_{i \times j}$ is the i × j null matrix. In this case, the role of is to aggregate the revised forecasts of all series in the hierarchy. For modeling, the bottom-up method uses the most disaggregated bottom level series data, so no information is lost.

Top-down approach: In the top-down approach, forecasts of the "Total" series y t are first generated, then disaggregated downward based on data proportions (Athanasopoulos et al 2009). This approach's general form is:

$$\mathbf{P} = \left[\mathbf{p} \,/\mathbf{0}_{\,m_k \times (m-1)}\right]$$

where $p = [p_1, p_2, \ldots, p_{mk}]$ are a set of proportions for the bottom level series. p gives the base forecasts for the "Total" series as revised forecasts for the hierarchy's bottom level. Her the top-level forecasts are disaggregated to obtain the lower-level series predictions. In this approach, the revised forecasts at the top level are equal to the highest base predictions, i.e., $\hat{y}_t(h) = \tilde{y}_t(h)$.

Middle-out approach: In the first step, intermediate hierarchical projections are generated and these forecasts disintegrate in order to achieve revised lower and aggregate forecasts at higher hierarchical levels for the purposes of computing revised forecasts. Basic forecasts are first produced for all the selected mid-level series. These basic predictions are added to get a down-to-earth approach to the revised series above the mid-level predictions. Then the mid-level forecasts are divided down to get a top-down approach to the revised forecasts for the lower mid-level series.

Optimal forecasts using regression: Hyndman et al (2011)'s approach allows all h-step-ahead base predictions to be expressed by the linear regression model

$$\hat{\mathbf{y}}_n(h) = \mathbf{S}\boldsymbol{\beta}_n(h) + \boldsymbol{\varepsilon}_h$$

where $\beta_n(h) = E(y_{k,n+h} / y_1, y_2, \ldots y_n]$ is the unknown mean of the bottom level K and ε_h has zero mean and covariance-variance matrix $Var(\varepsilon_h) = \sum_h$. Then the $\beta_n(h)$ is estimated by considering Eq.(5) as a regression equation, and thus obtain forecasts for all levels in the hierarchy. If was $\sum_h$ known, one can use generalized least squares estimation to obtain the minimum variance unbiased estimate of $\beta_n(h)$ as

$$\hat{\boldsymbol{\beta}}_n(h) = \left(\mathbf{S}'\boldsymbol{\Sigma}^{\dagger}_h\mathbf{S}\right)^{-1}\mathbf{S}'\boldsymbol{\Sigma}^{\dagger}_h\hat{\mathbf{y}}_n(h) \qquad (5)$$

where $\sum^{+}_{h}$ is the Moore-Penrose generalized inverse of $\sum_h$. A generalized inverse is used because $\sum_h$ is often (near) singular due to aggregation involved in y_n. This results the following revised forecasts

$$\tilde{\mathbf{y}}_n(h) = \mathbf{S}\hat{\boldsymbol{\beta}}_n(h) = \mathbf{SP}\hat{\mathbf{y}}_n(h) \text{ and } \mathbf{P} = \left(\mathbf{S}'\boldsymbol{\Sigma}^{\dagger}_h\mathbf{S}\right)^{-1}\mathbf{S}'\boldsymbol{\Sigma}^{\dagger}_h \qquad (6)$$

For hierarchical time-series data under this study, modeling and forecasting have been done using hts package (Hyndman *et al.* 2020) in R softwarehttps://cran.r-project.org/web/packages/hts/hts.pdf.

Forecasting accuracy was measured by RMSE and MAE respectively (Mishra et al 2021) of five forecasting methods for different forecasting horizon (h=1 to 6) using eq. (7) and (8).

$$MAE = 1/h\Sigma_{i=1}^{h}|y_{t+i} - \hat{y}_{t+i}| \qquad (7)$$

$$RMSE = [1/h_{i=1}^{h}\{(y_{t+i} - \hat{y}_{t+i})^2\}^{1/2} \qquad (8)$$

To meet the aim of present investigation, data was obtained from Open Government Data (OGD) Platform of India related to wheat production in the districts of Punjab state for the period from 1973 to 2018.The condition of Punjab is classified according to homogeneity, rainfall distribution, soil texture, crop pattern, etc into three agro-climate zones. These areas are sub-mountainous, central and south-west zones, also called wheat-maize areas, wheat-paddy and wheat-cotton (Table 1). Presently whole Punjab is divided into 22 districts. However, before 1992, there were 12 districts in Punjab state Table 1. Later, these 12 districts have been further divided into other districts. As the district wise wheat production time series data, before 1992, is consisted of 12 districts only, this many number of districts are considered in the present study. 1992 onwards, data series of newly formed districts have been merged into their respective old districts to obtain data for those 12 districts (Table 2). Punjab state is at the top level of this structure. Level 1 consists of the three wheat productivity zones. Level 2 consists of 12 nodes representing 12 districts of Punjab. For example, node AA represents the Ferozepur district. In

Table 2.

Total level		
1	Total	Punjab
Level 1: Zone		
2	A	South West (Zone I)
3	B	Central Zone (Zone II)
4	C	Sub Mountainous Zone (Zone III)
Level 2: District		
South West (Zone I)		
5	AA	Ferozepur+Fazilka (2011)
6	AB	Faridkot+Moga(1995)+Sri Muktsar Shahib(1995)
7	AC	Bathinda+Mansa (1992)
Central (Zone II)		
8	BA	Amritsar+Tarantaran (2006)
9	BB	Kapurthala
10	BC	Jalandhar
11	BD	Ludhiana
12	BE	Sangrur+Barnala (2006)
13	BF	Patiala+Fatehgarh Sahib
Sub Mountainous (Zone III)		
14	CA	Gurdaspur+Pathankot (2011)
15	CB	Hoshiarpur+Shaheed Bhagat Singh Nagar (1995)
16	CC	Rupnagar+SAS Nagar (2006)

*Figures in bracket represent the year of formation of different district of Punjab

Table 1. Wheat agro-climatic zones in Punjab

Name of zone	Productivity	Districts
South west (Zone I)	Mid productivity	Ferozepur, Faridkot, Bathinda
Central zone (Zone II)	High productivity	Amritsar, Kapurthala, Jalandhar, Ludhiana, Sangrur, Patiala , Fatehgarh Sahib
Sub mountainous zone (Zone III)	Low productivity	Gurdaspur, Hoshiarpur, Rupnagar

2011, Fazilka district came out as the result of division of original Ferozepur district into these two separate districts

RESULTS AND DISCUSSION

Level 1 shows production of wheat in 3 different zones separately of Punjab state. Time-series of total wheat production in Punjab is displayed in level 0. For each of the graphical representation, y-axis represents production in '000 tonne and x-axis indicates the time period (year). Tables 3 and 4 present forecasting accuracy (RMSE and MAE, Mishra et al 2021) respectively of five forecasting methods for different forecasting horizon (h=1 to 6). It the bottom-up approach outperformed the other methods in case of both RMSE and MAE. On an average (over the forecast horizon), we estimated a MAE=209.19 for bottom-up, followed by the middle-out with an MAE. According to the RMSE accuracy statistics, the bottom-up gives a value of 234.6 followed by the middle-out and the optimal method. Consequently, select the bottom-up method for forecasting the next five years' (2019-2023) wheat production in Punjab, 3 different productivity zones and in 12 districts separately (Table 5).

The, Zone-II would be leading among the zones of Punjab in wheat production with 8834.07 thousand tones in year 2023-24. By the same period, wheat production of whole Punjab state would reach 19037.1 thousand tones. Faridkot would be leading district with 2826.07 thousand tones production of wheat in the year 2023-24. An increasing future trend of wheat production in district-wise and zone-wise has

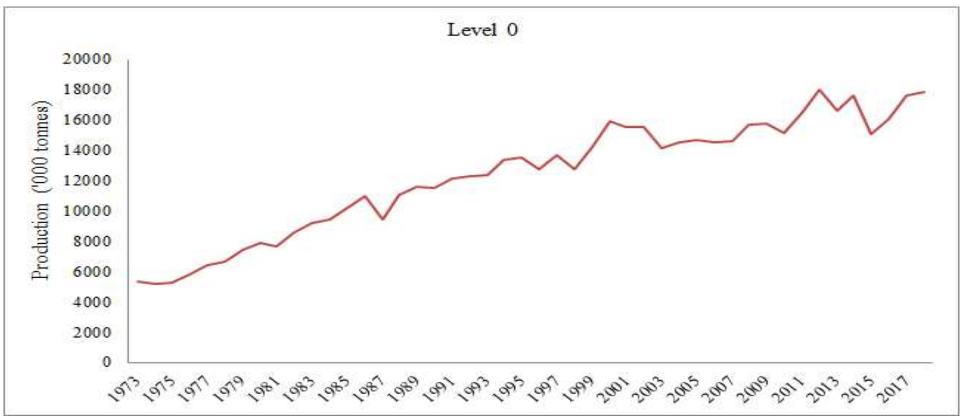

Fig. 2. Hierarchical time-series of wheat production at level 0

Table 3. Root mean square error (RMSE) for each forecast horizon

Methods	RMSE						
	Forecast horizon (h)						
	1	2	3	4	5	6	Average
Bottom-up	66.43	196.05	153.40	341.63	278.81	371.29	234.60
Top-down	76.78	212.83	161.03	340.22	286.94	473.30	258.52
Middle-out	68.96	226.99	176.49	333.55	289.22	363.55	243.13
Optimal	71.05	211.81	160.80	338.08	284.94	406.18	245.48
Independent	403.72	1059.24	617.77	1787.97	1494.33	1943.00	1217.67

Table 4. Mean absolute error (MAE) for each forecast horizon

Methods	MAE						
	Forecast horizon (h)						
	1	2	3	4	5	6	Average
Bottom-up	66.43	194.73	144.54	300.07	226.64	322.71	209.19
Top-down	76.78	208.37	142.86	299.13	237.89	434.35	233.23
Middle-out	68.96	220.94	159.32	291.64	241.60	317.91	216.73
Optimal	71.05	208.88	147.77	296.47	235.34	361.79	220.22
Independent	403.72	1058.59	615.82	1593.59	1214.42	1695.89	1097.00

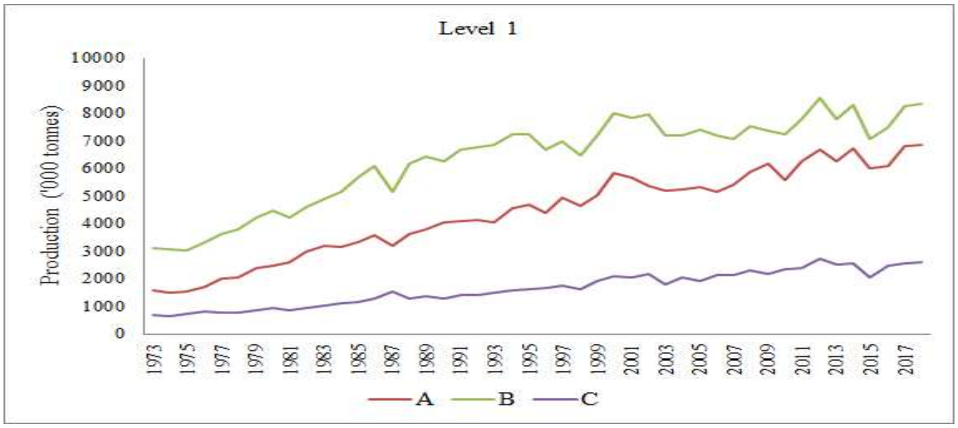

Fig. 3. Hierarchical time-series of wheat production at level 1

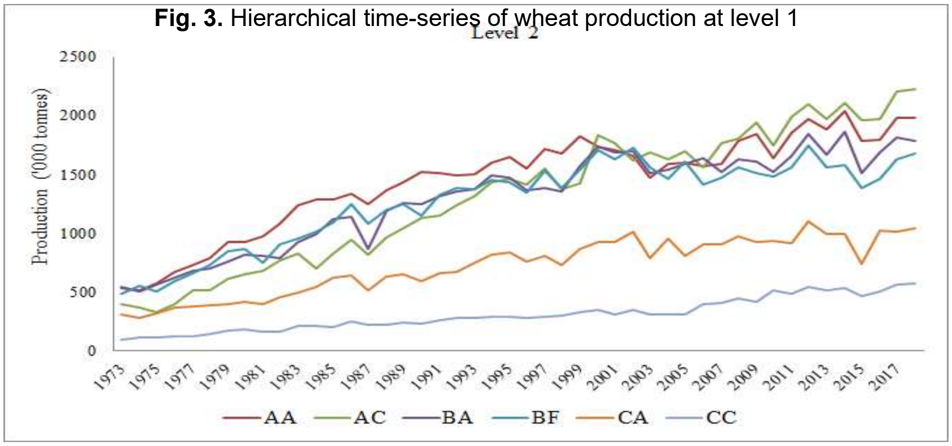

Fig. 4. Hierarchical time-series of wheat production at level 2 for selective districts of Punjab state

Table 5. Forecasting of wheat production at all levels during 2019-2023

Level		2019	2020	2021	2022	2023
Top level						
1	Total	17954.7	18225.4	18495.9	18766.5	19037.1
Level 1						
2	A	6900.43	7018.89	7137.35	7255.81	7374.27
3	B	8400.27	8508.72	8617.17	8725.62	8834.07
4	C	2654.01	2697.76	2741.39	2785.08	2828.74
Level 2						
5	AA	2014.25	2046.73	2079.2	2111.67	2144.14
6	AB	2647.63	2692.24	2736.85	2781.46	2826.07
7	AC	2238.54	2279.92	2321.3	2362.68	2404.06
8	BA	1823.54	1851.8	1880.04	1908.27	1936.51
9	BB	528.059	536.468	544.876	553.284	559.692
10	BC	861.23	862.12	863.52	865.01	867.3
11	BD	1288.63	1300.12	1311.12	1322.01	1334.12
12	BE	2225.55	2259.35	2293.17	2327.01	2360.83
13	BF	1673.27	1698.86	1724.44	1750.03	1775.61
14	CA	1044.74	1060.99	1077.24	1093.49	1109.74
15	CB	1024.89	1041.73	1058.58	1075.42	1092.27
16	CC	584.383	595.039	605.574	616.162	626.727

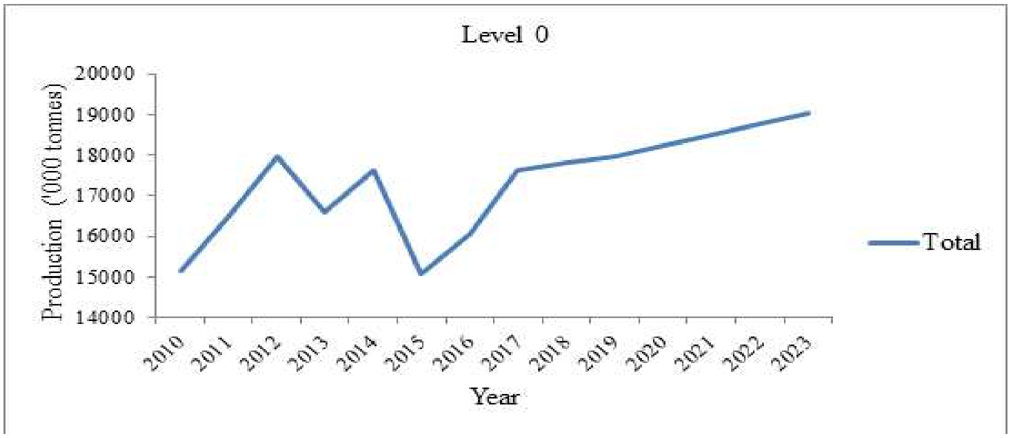

Fig. 5. Hierarchical forecasting of wheat production at level 0

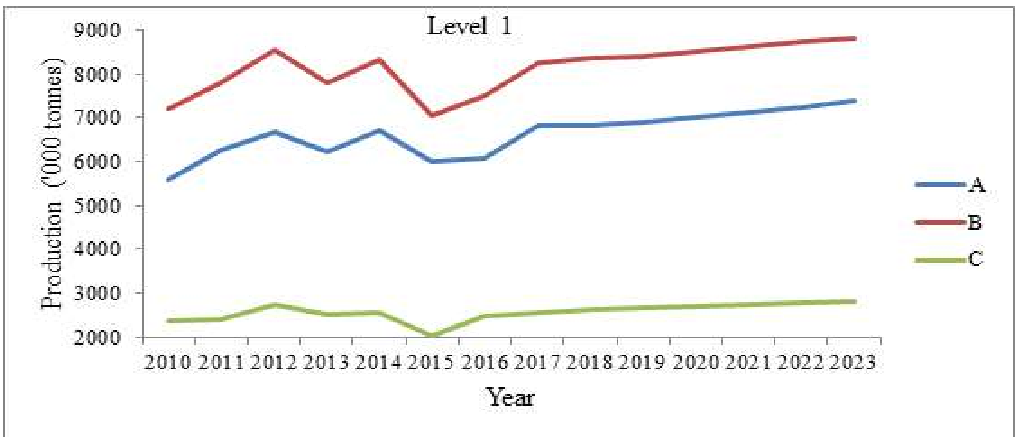

Fig. 6. Hierarchical forecasting of wheat production at level 1

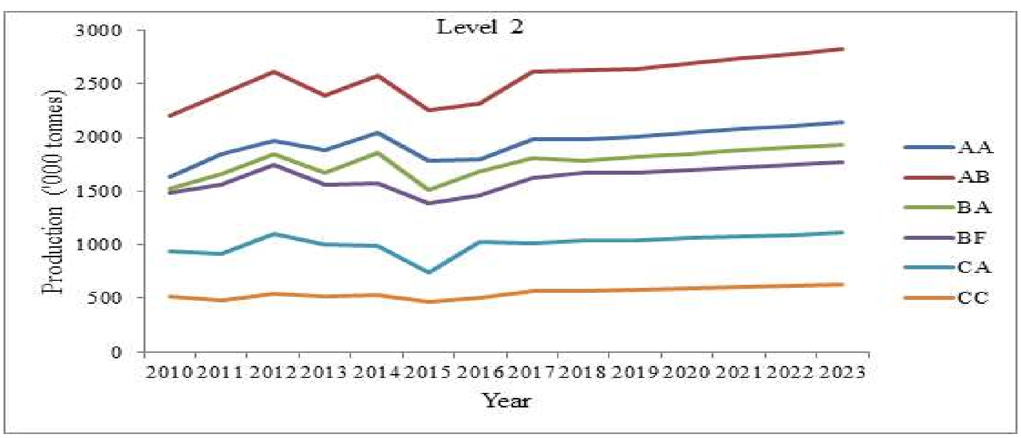

Fig. 7. Hierarchical forecasting of wheat production at level 2

been observed for Punjab (Figure 5, 6 and 7). The bottom-most level i.e. level 2 exhibits forecast value of wheat production for each of the districts, however, for clarity of graphical representation, only few of those are labeled. Level 1 shows forecasted production of wheat in different zones of Punjab. Predicted future values of total wheat production in Punjab are displayed in level 0.

CONCLUSION

Hierarchical time series modelling and forecasting of wheat production in Punjab state of India has been carried out. Among five different methods, bottom-up approach has outperformed the other methods in terms of forecast accuracy measure criteria viz., RMSE and MAE in out of sample forecast horizon. The next five years' forecast is also calculated for all three levels which show a growing trend in wheat production and this forecasting approach is ideal for short forecast time, since predictive accuracy is used to decrease as the forecast horizon increases. The projection can provide direct support in the formulation of national agriculture policies and provide good food security decision-making well in advance. The approaches utilized here can be used for forecasting the production of other crops for which hierarchical time series data are available.

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Fuzzy Logic for Sensory Evaluation of Rice Stored in Sub-Baric Storage Bin

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***Abstract*:** Sub-baric storage of food grains i.e., extreme levels of vacuum has gained importance these days because of its potential to preserve food by preventing the growth of insects, mold or bacteria by depriving oxygen. Cleaned and sorted rice grains were stored in the sub-baric storage bin (SBSB) at 600±50 mm of Hg as well as in bag storage structure (jute bag) at ambient condition as control. After 2 months of storage, the rice grains were taken out and evaluated for its sensory characteristics to compare the acceptance of both raw and cooked rice grains stored in SBSB and bag storage. The samples were R1-raw rice stored in SBSB; R2-raw rice stored in bag storage; C1-cooked rice from the grains taken out from SBSB; C2-cooked rice of grains from bag storage. Fuzzy logic sensory study was conducted for analysis and acceptability of these samples. The results concluded that rice sample stored in SBSB showed high acceptability in both raw and cooked form in comparison to rice stored in bag storage.

***Keywords*:** Fuzzy logic, Rice, Sensory evaluation, Sub-baric, Vacuum

Rice (*Oryza sativa*) is the most important human food crop in the world, directly feeding more people than any other crop. One of the main issues of storing rice is to protect it from pest and disease attacks; in particular, it is more prone to insect infestation and also aflatoxin contamination (Biancolillo et al 2019). Factors like humidity, heat, pests and aeration which are effective on storage conditions affect the quality and quantity of grain and shorten the storage period (Abedin et al 2012). In this aspect, proper storage practices are among the most important elements in food supply chain of grain which is a significant nutritional source. Sub-baric storage is defined as the storage condition which is maintained at extreme levels of vacuum between 600-650 mm Hg helps in depletion of oxygen in the storage systems through the application of negative pressure causes slower metabolic rate and finally cessation of basic metabolism and death of insects in a few days (Kumar et al 2017). Lack of oxygen had a significant impact on the mortality of insects and inhibits the development of fungi and yeast (Sidik 2000). The anaerobic environment of vacuum storage prevents the growth of microorganisms especially aerobic ones which are responsible for nutritional loss, off-odour and texture changes (Chetti et al 2014). Sub-baric storage technology will emphasize greater control, longevity of grains, cleanliness with respect to collected grains. Moreover, the effect of using toxic chemicals like methyl bromide and phosphine fumigants on grain include diminishing of germination ability, seeding viability and dietary risk are eliminated. The harmful end results of chemicals range from minor discomfort to cancer, endocrine, organic disorder which includes disruption of activity of organs in the body, e.g., kidney, liver or digestive tract can be terminated (Mbata et al 2005). At the end of storage, grains stored should be best in quality in terms of sensorial properties to gain acceptance from the consumers.

Sensory analysis with subjective method has great variability and therefore, requires a robust method of sensory evaluation. Moreover, subjective method of sensory analysis carries vagueness and ambiguity among judges and is quite uncertain. Fuzzy logic is an important tool by which indistinct and vague data can be analyzed and important conclusions regarding acceptance, rejection, ranking, strong and weak attributes of food can be drawn. In fuzzy modelling, linguistic variables (not satisfactory, good, medium, fair and excellent) are used for developing relationship between independent (taste, color and appearance, flavor, texture and overall acceptance) and dependent (acceptance, rejection, ranking, strong and weak attributes of food) variables (Das 2005, Routray and Mishra 2011). In fuzzy logic based sensory analysis the data is mathematically interpreted and analyzed as membership functions which are a representation of the non-numerical sensory observations of the panel members (Sugumar and Guha 2022). The developed fuzzy mathematical models perform remarkably well in the evaluation and ranking of food products (Fatma et al 2016). Such fuzzy sets provide the mathematical methods that can represent the uncertainty of human's expressions attributes of ready to eat (RTE) food that are evaluated by human senses are color and appearance texture, flavor, taste and overall acceptance (Lazim and Suriani 2009). The fuzzy model can be used to determine the importance of individual factors to the overall quality of a product. The objective of this study is to demonstrate the usefulness of developed fuzzy model in optimization and ranking of the sub-baric stored raw and cooked rice samples.

MATERIAL AND METHODS

Procurement and preparation of rice samples: Freshly harvested and dried rice was procured from open market in APMC yard, Yashwanthpura, Bangalore, Karnataka, India in March, 2019 to store in the sub-baric storage bin maintained at vacuum level of 600$\pm$50 mm Hg and at ambient conditions as control in bag storage structure (jute bags) for 2 months. Before experimentation the grains were properly cleaned and sorted so that the extraneous matter such as unhealthy grain, infected grain, chaff and sand were removed from the grains. After the storage period, the rice sample was taken out from the sub-baric storage bin as well as from bag storage for evaluation of sensory quality. The raw rice grains were washed twice with water in ratio of 1:2 (v/v) and until free of water. Approximately 250 g of raw rice grains (1:2 v/v) were cooked for conducting sensory evaluation.

Sensory evaluation: The samples were named and coded as raw grains stored in storage bin (R1), raw grains stored in bag storage (R2), cooked rice prepared from the grains stored bin (C1) and bag storage (C2). Twenty semi-trained panelists were selected included faculty and post graduate students from the Department of Processing and Food Engineering and Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore, in the age group between 22 and 40 years included 10 female and 10 males. The quality attributes selected for the organoleptic properties of raw rice were color, grain size and overall quality and whereas for cooked rice, color, grain size, taste, texture and overall quality. Both sub-baric and control stored rice samples were subjected to sensory evaluation in the raw and cooked form to a panel of twenty judges and not more than two samples were presented at a time and were also advised to wash off their mouth with water after sensory analysis of each sample (Jaya and Das 2003). The sensory scale was divided into 5 linguistic scale responses that range from not satisfactory, fair, medium, good and excellent, respectively. Similar scales were also established for the sensory attributes, which range from 1=not at all important, 2=somewhat important, 3=important, 4=highly important and 5=extremely important. After evaluating the sample, judges were asked to give marks for each quality attributes based on their own taste regarding rice.

Fuzzy comprehensive model for sensory scores: Fuzzy model for the present problem was having three sets: (i) Factor set U_f, (ii) Evaluation set V_f and (iii) Fuzzy transformation T_f. The factor set, U_f contains all of the quality attributes such as color, grain size, texture, taste and overall quality of the products. The evaluation set, V_f includes the scale actor for each of the quality attributes, such as Excellent, Good, Medium, Fair and Not satisfactory. For the fuzzy transformation (T_f) of the factor set (U_f) into evaluation set (V_f), numerical values assigned to the scale factors were Excellent (EX) = 1, Good (GD) = 0.9, Medium (MD) = 0.7, Fair (FR) = 0.4 and Not Satisfactory (NS) = 0.1.

Evaluation of Analysis

Fuzzy membership function (FMF), M_f: It was calculated by adding the individual scale factor given to each of the quality attribute of the product and dividing it by the number of judges who evaluated the product (Jaya and Das 2003).

$$M_f = \sum V_f \text{ / total of judges} \quad \dots (1)$$

Normalized fuzzy membership function (NFMF), N_f: NFMF was calculated by multiplying each of the fuzzy membership function with the assigned numerical value of the respective 'scale factor'.

$$N_f = M_f \times S_f \quad \dots (2)$$

Normalized fuzzy membership function matrix, O_f : Addition of the normalized fuzzy membership function of individual linguistic term of respective quality attributes for each of the product given for sensory evaluation formed the elements of the normalized fuzzy membership function matrix. All the element of the normalized matrix were calculated and written in the form of a matrix called normalized fuzzy membership function matrix having its row as quality attributes and the column as samples number.

$$O_f = \sum N_f \text{ for each quality attribute} \quad \dots (3)$$

Judgment membership function matrix, X_f: The column values of a sample were then added and the individual values of the same column were divided by the "Maximum" of the added value. These values formed the elements of the judgment membership function matrix. Thus, the matrix decided the rank of the chips.

$$X_f = O_f / \max \sum O_f \quad \dots (4)$$

Judgment subset, Y_f: The average of numerical weightage given by the judges for individual quality attributes: color, grain size, taste, texture and overall quality formed the judgement subset as judgement membership function explained in the above steps.

Quality-ranking subset, Z_f: The comparison was made between the individual elements of the judgment membership function matrix (X_f) and the respective elements of the judgment subset (Y_f). Thus, the minimum of them was taken to form the quality-ranking subset, Z_f.

Ranking of the sample: Highest rank (I) was assigned to the sample which had the maximum value in the quality ranking subset Z_f. Then the quality attribute, which gave the highest value, was considered as the reason for that sample to get the highest rank. The values of the JMF, were then compared with the average weightage given by the judges for each of the quality attributes. Based on this, the quality ranking sub set values were calculated.

Quality ranking subset (QR): Comparing the weightage average of quality attributes and the judgment membership function formed, the minimum of these two was assigned as the quality ranking subset value.

RESULTS AND DISCUSSION

Ranking of raw rice: The results of raw rice samples stored under SBSB and bag storage are presented. Various sensory attributes of normalised fuzzy membership function for color, grain size and overall quality of both SBSB and bag stored raw rice samples were 0.86 (R1) and 0.77 (R2); 0.88 (R1) and 0.86 (R2); 0.89 (R1) and 0.83 (R2), respectively (Table 1). The raw rice grains stored in SBSB (R1) had good sensory attributes compared with grains stored in bag storage. This was mainly due to anaerobic environment of sub-baric storage bin prevents the growth of microorganisms especially aerobic ones which are responsible for nutritional and sensory quality loss (off-color, off-odour and texture changes). The grains stored in bag storage at ambient conditions were more vulnerable to insects and micro-organisms as well as ambient conditions such as temperature and humidity. These two membership functions (M_f and N_f) led to calculation of Normalized Fuzzy Membership Function Matrix (O_f). The maximum and minimum of NFMF matrix (O_f) value were 2.63 and 2.46

Table 1. Scale factor, fuzzy membership function (FMF) and normalized membership function (NFMF) for quality attributes of raw rice samples stored in SBSB and bag storage

Sensory attribute	Sensory quality factor	Scale factor	Raw rice stored in SBSB (R1)			Raw rice stored in bag storage (R2)		
			No. of judges rated	FMF (M_f)	NFMF (N_f)	No. of judges rated	FMF (M_f)	NFMF (N_f)
Color	EX	1	4	0.2	0.2	0	0	0
	GD	0.9	10	0.5	0.45	10	0.5	0.45
	MD	0.7	6	0.3	0.21	8	0.4	0.28
	FR	0.4	0	0	0	2	0.1	0.04
	NS	0.1	0	0	0	0	0	0
Total			20		0.86	20		0.77
Grain size	EX	1	4	0.2	0.2	0	0	0
	GD	0.9	12	0.6	0.54	16	0.8	0.72
	MD	0.7	4	0.2	0.14	4	0.2	0.14
	FR	0.4	0	0	0	0	0	0
	NS	0.1	0	0	0	0	0	0
Total			20		0.88	20		0.86
Overall quality	EX	1	6	0.3	0.3	2	0.1	0.1
	GD	0.9	10	0.5	0.45	10	0.5	0.45
	MD	0.7	4	0.2	0.14	8	0.4	0.28
	FR	0.4	0	0	0	0	0	0
	NS	0.1	0	0	0	0	0	0
Total			20		0.89			0.83
O_f					O_{f1} = 2.63			O_{f2} = 2.46

EX- Excellent; GD-Good; MD- Medium; FR -Fair; NS- Not satisfactory; O_f - Normalized fuzzy membership function matrix O_{f1} and O_{f2} – Normalized fuzzy membership function matrix of raw rice stored in SBSB and bag storage obtained for raw rice stored in storage bin and bag storage, respectively. The matrix O_f was converted to Judgment Membership Function Matrix X_f. Raw rice sample (stored in storage bin) has the highest O_f value which was used for calculation of Judgement membership function (JMF) (Table 2). The values of judgement membership function were then compared with the average of weightage given by the panellist for each of the quality attributes (Table 3 and 4). The weightage average values for color, grain size and overall quality were 0.328, 0.328 and 0.343, respectively (Table 5). The order of preference of quality attributes for raw rice samples in general was overall quality > color = grain size. Comparing the weightage average of quality attributes and judgement membership function formed, it was observed that score of the sample R1 (raw rice samples stored in SBSB) was the highest (QR = 0.338) based on the score obtained for the quality attribute overall quality followed by R2 (raw rice stored in bag storage) with QR value 0.315. The quality response values i.e., color and grain size values of R1 were higher than R2. It may be concluded that, raw rice grains stored under SBSB has best quality and more

Table 2. Evaluation of judgment membership functions (JMF) of raw rice samples stored in SBSB and bag storage

Sensory attribute	Judgment Membership Functions (JMF), X_f	
	Raw rice stored in SBSB (R1)	Raw rice stored in bag storage (R2)
Color	0.327	0.293
Grain size	0.335	0.327
Overall quality	0.338	0.315

Table 3. Fuzzy membership function (FMF) and normalized membership function (NFMF) of different quality attributes

Quality attribute	Scale factor	No. of judges rated	FMF	NFMF
Color	EIMP	10	0.5	0.5
	HIMP	5	0.25	0.225
	IMP	5	0.25	0.175
	SIMP	0	0	0
	NIMP	0	0	0
Total		20		0.9
Grain size	EIMP	8	0.4	0.4
	HIMP	8	0.4	0.36
	IMP	4	0.2	0.14
	SIMP	0	0	0
	NIMP	0	0	0
Total		20		0.9
Overall quality	EIMP	12	0.6	0.6
	HIMP	6	0.3	0.27
	IMP	2	0.1	0.07
	SIMP	0	0	0
	NIMP	0	0	0
Total		20		0.94

Table 4. Evaluation of judgment membership functions (JMF) of raw rice samples stored in SBSB and bag storage

Quality attribute	Color	Grain size	Overall quality	Total
Sum of NFMF	0.9	0.9	0.94	2.74
JMF	0.328	0.328	0.343	

Table 5. Evaluation of quality ranking (QR) of raw rice samples stored in SBSB and bag storage

Quality attribute	Color	Grain size	Overall quality	Total	Ranking
Weightage average	0.328	0.328	0.343		
R1:QR	0.327	0.335	0.338	1	I
R2:QR	0.293	0.327	0.315	0.935	II

acceptable.

Ranking of cooked rice: Various sensory attributes of normalised fuzzy membership function for color, grain size, texture, taste and overall quality of cooked rice prepared from grains stored under both SBSB and bag storage were 0.93 (C1) and 0.81 (C2); 0.86 (C1) and 0.83 (C2); 0.73 (C1) and 0.66 (C2); 0.92 (C1) and 0.8 (C2); 0.92 (C1) and 0.79 (C2), respectively (Table 6). The cooked rice prepared from grains stored in SBSB (C1) had good sensory attributes compared with grains stored in bag storage. The maximum and minimum of NFMF matrix (O_f) were 4.36 and 3.89 obtained for C1 and C2, respectively. Cooked rice (C1-SBSB) has the highest O_f value which was used for calculation of Judgement membership function (JMF) (Table 7). Judgement Membership Function values were then compared with the average of weightage given by the panellist for each of the

Table 6. Scale factor, fuzzy membership function (FMF) and normalized membership function (NFMF) for quality attributes of cooked rice samples stored in SBSB and bag storage

Sensory attribute	Sensory quality factor	Scale factor	Cooked rice stored in SBSB (C1)			Cooked rice stored in bag storage (C2)		
			No. of judges rated	FMF (M_f)	NFMF (N_f)	No. of judges rated	FMF (M_f)	NFMF (N_f)
Color	EX	1	10	0.5	0.5	4	0.2	0.2
	GD	0.9	8	0.4	0.36	8	0.4	0.36
	MD	0.7	2	0.1	0.07	6	0.3	0.21
	FR	0.4	0	0	0	2	0.1	0.04
	NS	0.1	0	0	0	0	0	0
Total			20		0.93	20		0.81
Grain size	EX	1	8	0.4	0.4	2	0.1	0.1
	GD	0.9	10	0.5	0.45	10	0.5	0.45
	MD	0.7	0	0	0	8	0.4	0.28
	FR	0.4	0	0	0	0	0	0
	NS	0.1	2	0.1	0.01	0	0	0
Total			20		0.86	20		0.83
Texture	EX	1	0	0	0	0	0	0
	GD	0.9	6	0.3	0.27	8	0.4	0.36
	MD	0.7	12	0.6	0.42	4	0.2	0.14
	FR	0.4	2	0.1	0.04	8	0.4	0.16
	NS	0.1	0	0	0	0	0	0
Total			20		0.73			0.66
Taste	EX	1	4	0.2	0.2	0	0	0
	GD	0.9	16	0.8	0.72	10	0.5	0.45
	MD	0.7	0	0	0	10	0.5	0.35
	FR	0.4	0	0	0	0	0	0
	NS	0.1	0	0	0	0	0	0
Total			20		0.92			0.8
Overall quality	EX	1	4	0.2	0.2	2	0.1	0.1
	GD	0.9	16	0.8	0.72	6	0.3	0.27
	MD	0.7	0	0	0	12	0.6	0.42
	FR	0.4	0	0	0	0	0	0
	NS	0.1	0	0	0	0	0	0
Total			20		0.92			0.79
O_f					O_{f3} = 4.36			O_{f4} = 3.89

EX- Excellent; GD-Good; MD- Medium; FR -Fair; NS- Not satisfactory; O_f - Normalized fuzzy membership function matrix O_{f3} and O_{f4} – Normalized fuzzy membership function matrix of cooked rice stored in SBSB and bag storage quality attributes. The weightage average values for each of the quality attribute were calculated (Table 8 and 9). The weightage average values for color, grain size, texture, taste and overall quality were 0.1992, 0.1871, 0.2089, 0.189 and 0.215, respectively (Table 10). The order of preference of quality attributes for cooked rice samples in general is as follows: overall quality > texture > color > taste > grain size. Comparing the weightage average of quality attributes and judgement membership function formed, it was found that score of the sample C1 (cooked rice samples-SBSB) was the highest (QR = 0.2119) based on the score obtained for the

Table 7. Evaluation of judgment membership functions (JMF) of cooked rice samples stored in SBSB and bag storage

Sensory attributes	Judgment Membership Functions (JMF), X_f	
	Cooked rice stored in SBSB (C1)	Cooked rice stored in bag storage(C2)
Color	0.214286	0.18578
Grain size	0.198157	0.190367
Texture	0.168203	0.151376
Taste	0.211982	0.183486
Overall quality	0.211982	0.181193

Table 8. Fuzzy membership function (FMF) and normalized membership function (NFMF) of different quality attributes

Quality attribute	Scale factor	No. of judges rated	FMF	NFMF
Color	EIMP	6	0.3	0.3
	HIMP	6	0.3	0.27
	IMP	7	0.35	0.245
	SIMP	0	0	0
	NIMP	1	0.05	0.005
Total		20		0.82
Grain size	EIMP	4	0.2	0.2
	HIMP	4	0.2	0.18
	IMP	10	0.5	0.35
	SIMP	2	0.1	0.04
	NIMP	0	0	0
Total		20		0.77
Taste	EIMP	6	0.3	0.3
	HIMP	5	0.25	0.225
	IMP	5	0.25	0.175
	SIMP	4	0.2	0.08
	NIMP	0	0	0
Total		20		0.78
Texture	EIMP	7	0.35	0.35
	HIMP	7	0.35	0.315
	IMP	5	0.25	0.175
	SIMP	1	0.05	0.02
	NIMP	0	0	0
Total		20		0.86
Taste	EIMP	6	0.3	0.3
	HIMP	5	0.25	0.225
	IMP	5	0.25	0.175
	SIMP	4	0.2	0.08
	NIMP	0	0	0
Total		20		0.78
Overall quality	EIMP	7	0.35	0.35
	HIMP	8	0.4	0.36
	IMP	5	0.25	0.175
	SIMP	0	0	0
	NIMP	0	0	0
Total		20		0.885

Table 9. Evaluation of judgment membership functions (JMF) of cooked rice samples stored in SBSB and bag storage

Quality attribute	Color	Grain size	Texture	Taste	Overall quality	Total
Sum of NFMF	0.82	0.77	0.86	0.78	0.885	4.115
JMF	0.1992	0.1871	0.2089	0.189	0.2150	

Table 10. Evaluation of quality ranking (QR) of cooked rice samples stored in SBSB and bag storage

Quality attribute	Color	Grain size	Texture	Taste	Overall quality	Total	Ranking
Weightage average	0.1992	0.1871	0.2089	0.189	0.2150		
C1:QR	0.2142	0.1981	0.2119	0.1682	0.2119	1.004	I
C2:QR	0.1857	0.1903	0.1834	0.1513	0.1811	0.8918	II

quality attribute overall quality followed by C2 (cooked rice-bag storage) with QR value 0.1811. Also, the quality response values i.e., color, grain size, texture and taste values of C1 were higher than C2. It was concluded that, both raw and cooked rice from the sub-baric storage bin had best acceptability.

CONCLUSIONS

The present study demonstrates that the both raw and cooked rice grains of sub-baric stored sample were highly acceptable in terms of sensory quality parameters like color, grain size and overall quality for raw rice and color, grain size, texture, taste and overall quality for cooked rice. Between R1 and R2; C1 and C2 samples, both R1 and C1 which were taken out from sub-baric storage bin were scored highest value of judgement membership function (X_f) followed by R2 and C2. Finally, it was concluded that sub-baric stored raw rice could benefit high price at market and also cooked rice had superior quality.

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Potential of Biochar and Silicon on Productivity and Profitability of Organic Fenugreek (*Trigonella frenum-graecum* L.) in Southern Rajasthan

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Abstract: The aim of the present study accessed the effects of the integrated use of organic inputs such as silicon and biochar on the growth, yield and profitability of fenugreek. The field experiment was conducted in *rabi,* 2020-21 at MPUAT, Udaipur (Rajasthan). The treatments consisted of four levels of soil-applied biochar (control, 2, 3 and 4 t/ha) and four foliar-applied silicon sprays at 45 and 90DAS (water spray, 1.5, 2.0 and 2.5 %). Significantly maximum plant height (53.30 and 90.14 cm), dry matter accumulation (5.00 and 20.43 g) at 60 DAS and harvest, respectively, total chlorophyll content with SPAD meter at flower initiation (56.55), and seed yield (1944 kg/ha) were recorded with the application of 4 t /ha biochar at par with 3 and 2 t/ha. Moreover, the maximum plant height (49.43 and 90.23 cm), dry matter accumulation (4.65 and 20.18 g/plant) at 60 DAS and harvest, and seed yield (1919 kg/ha) were with 2.5% foliar application of silicon. Maximum seed yield (2155 kg/ha), net return (150490₹/ha), and BC ratio (2.66) were obtained with the treatment's combination of 2 t/ha soil applied biochar and 2.5% foliar applied silicon.

Keywords: Biochar, Fenugreek, Organic farming, Silicon

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop and member of the family Fabaceae, the order Leguminosae. Since ancient civilizations, fenugreek has been renowned for its several culinary and medicinal purposes, from preserving carcasses and mummies in Egypt to acting as a traditional ameliorate in the medical system of several Southeast Asian countries. This plant is primarily used as a spice in India and countries of the Mediterranean region. India leads the fenugreek production in the world with an area of 120 thousand hectares, contributing to the production of 188.48 thousand tonnes with average productivity of 1007 kg/ha as per the advance estimate of 2019-20 (Spice Board of India, 2020). Rajasthan leads in fenugreek production, having 150 thousand hectares area under cultivation and alone contributing up to 77 percent production, *i.e.*, 160 thousand tonnes with average productivity of 1066 kg/ha in 2016-17 (Sachan et al 2020). The recognition and demand for organic products in the world market are rising. Still, production is deficient under organic cultivation because of limited land area and poor crop productivity, which needs to be supplemented with incorporating advanced inputs to increase crop production per unit area under organic farming. Several organic inputs are being studied to increase yield and productivity without sabotaging the agricultural ecosystem to improve the organic cultivation of crops. In a similar line of research, two potential inputs, biochar and silicon, are considered in the organic cultivation of fenugreek.

Biochar is a pyrolyzed product of the biomass formed after heating at the temperature of 300-600^0 C in the absence of oxygen. It is a carbon-rich, dark-colored powdery product with several unique properties. Biochar is preferred as an amendment because of its low density, high porosity and aeration, significant adsorption and cation exchange capacity, providing favorable conditions for living microbiology in the soil, capacity to increase the soil carbon pool and nutrient availability and thus to improve the overall fertility of the soil. With the upliftment in soil's physical, chemical and biological properties, biochar can be categorized as potential input to increase the yield under organic farming conditions. Adequate nutrient management is necessary to enhance the productivity of the organic fenugreek crop. The foliar application of silicon can play a crucial role by promoting several desirable physio-chemical processes in plants to increase crop productivity and quality besides mitigating the stresses (Jinger et al 2021). The need for proper Si management to increase yield and sustain crop productivity appears necessary where Si diminution in the soil can occur due to intensive and continuous cultivation practices of high-yielding cultivars. As a result, these soils are generally low in plant-available Si (Korndorfer et al 2001). Keeping these facts, the present study was conducted to evaluate the effect of soil-applied biochar and foliar-applied silicon on fenugreek's growth, yield and economics.

MATERIAL AND METHODS

Experimental site and soil status: The field experiment was carried out during *rabi* season of 2020-21 at the organic farm unit, MPUAT, Udaipur, which lies in Rajasthan's South-Eastern zone (IV a). The soil of the experimental site was clay loam in nature, with a slightly alkaline reaction (pH 7.8) having a bulk density of 1.35 g/cc and electrical conductivity of 0.75dS m^{-1}. The nutrient status of the soil showed 0.70 %, 283.44, 22.2 and 279.3 kg/ha of organic carbon, available nitrogen, phosphorus and potassium content, respectively, depicting the overall medium nutrient status of the soil.

Preparation of biochar: In the present experimental field, legume biochar was used through slow pyrolysis in the biochar production system (Pratap Kiln) prepared in the CTAE, Udaipur. Biochar was made by partial oxidation of the biomass at high temperatures to convert it into a carbon-rich porous substance. Haulms of legume were fed to the pyrolysis reactor in the oxygen-limiting condition, where the temperature went up to 450^{0} C for 4 minutes. This process occurs in 3 stages, first where the moisture content of the biomass is reduced to <10% at the temperature of 180^{0} C, second here the biochar production starts with the breakdown of hemicellulose and cellulose at the temperature range of $180\text{-}360^{0}$ C and last stage where lignin breaks down at the temperature of 450^{0} C. In slow pyrolysis, the final yield of the converted biochar goes up to 35 %, which is more in comparison to fast pyrolysis**.** The biochar was then applied in each plot as per the detail of the treatments before the crop sowing and incorporation in the soil was done with the help of a rake.

Preparation of suspension of silicon: The source used for the foliar spray of active silicon was diatomaceous earth. Silicon present in this compound is in the form of silicates. It is a smooth white sedimentary talc with 80-90 percent silica, forming a milky white suspension when it dissolves in water. To make a stable suspension and to prevent it from settling down, Na CMC (Carboxyl Methyl Cellulose) was used at the rate of 0.05 percent as it is a natural polymer and helps stabilize the suspension. The desired amount of suspension was made by adding diatomaceous earth in water with 0.05 percent of Na CMC and sprayed in each plot as per the treatments with the help of a knapsack sprayer. Two foliar sprays were done at 45 and 90 DAS.

Experimental design and treatments: The experiment consists of two input factors, each at four levels, represented symbolically such as soil application of biochar, i.e., B_1, B_2, B_3 and B_4 (control, 2, 3 and 4 t/ha, respectively) and foliar spray of silicon, i.e., S_1, S_2, S_3 and S_4 (water spray, 1.5, 2.0 and 2.5 %, respectively), respectively, was laid out in a factorial randomized block design and replicated thrice. The recommended dose of 20 N and 40 P_2O_5 kg/ha was applied as basal at the time of sowing through organic sources like vermicompost, NADEP and neem cake. The crop variety Pratap Rajasthan Methi-45 (PRM-45) was sown using 25 kg seed /ha and harvested after 137 days of sowing. Biochar was applied and incorporated into the soil before the crop sowing. At the same time, silicon was foliar applied two times at 45 and 90 DAS in silicate suspension through diatomaceous earth (80% Si) as per the treatment recommendation. To make a stable suspension of silicate in water, sodium carboxymethyl cellulose (Na-CMC) was used to increase the solubility with an organic origin.

Observations: Dry matter accumulation was calculated by weighing the oven-dried samples (65°C). Total relative chlorophyll content was measured at the flower initiation stage with the help of a SPAD meter. Yield attributes and yield were recorded from the net plot area at and before the crop harvest.

RESULTS AND DISCUSSION

Effect of biochar levels: The maximum plant height (53.30 and 90.14 cm), dry matter accumulation (5.00 and 20.43 g) at 60 DAS and harvest, respectively and total chlorophyll content with SPAD meter at flower initiation (56.55) of the crop were observed with the biochar addition of 4 t/ha which were at par with 3 and 2 t /ha biochar (Table 1). Moreover, all the biochar levels failed to show significant variations in days to flower initiation and days to maturity in fenugreek. All the yield attributes viz., the number of pods/plant (30.42), seed weight/plant (6.11 g) and test weight (12.01g) were significantly maximum at 4 t/ha over control and at par with 3 and 2 t/ha of biochar addition in the soil (Table 2). In contrast, the number of seeds/pods failed to increase significantly. Higher seed yield (1994 kg/ha) and haulm yield (4816 kg/ha) of fenugreek were soil application of 4 t/haover control and at par with 3 and 2 t/ha. Similarly, biological yield (6810 kg/ha) was found to be maximum under 4 t/ha biochar at par with 3 t/ha. Harvest index (29.75%) was the maximum, with 2 t /ha biochar over control and the rest of the treatments. Economic analysis (Table 3) revealed that soil application of 2 t/ha biochar gave a maximum return (₹130409 /ha).

The increase in growth attributes like plant height and dry matter accumulation could be due to more nutrient availability by improving the CEC of the soil, total organic carbon and total nitrogen content (Schulz et al 2013) and also by providing favorable conditions to the soil microbiota mainly when biochar is applied in combination with the organic manures (Arif et al 2021). The biochar incorporation in the soil leads to vigorous vegetative growth resulting in higher photosynthate production and translocation from source to the sink, which is apparent in reproductive growth, *viz.,* the number of pods/plant, the number of seeds/pod and seed weight/plant, which were the vital yield attributes having a significant positive effect on seed yield. Similar results were obtained by Hiama et al (2019) and Ye et al (2020) regarding seed yield and biological yield.

Effect of silicon levels: The varying levels of foliar-applied silicon positively improved the fenugreek's growth (Table 1). Foliar application of 2.5% silicon recorded maximum plant height (49.43 and 90.23 cm) and dry matter accumulation (4.65 and 20.18 g/plant) over control and other treatments at 60 days after sowing and harvest, respectively. Although, silicon application had no significant impact on total chlorophyll content, days to flower initiation and days to maturity. The foliar-applied silicon increased the number of pods/plant (30.50) and seed weight/plant (6.12g) with silicon concentration at 2.5%. In contrast, the number of seeds/pod and test weight did not affect significantly. Maximum seed yield (1919 kg/ha), haulm yield (4806 kg/ha) and biological yield (6725 kg/ha) were reported with 2.5% foliage applied silicon over control and the rest treatments. The harvest

Table 1. Effect of biochar and foliar applied silicon on the growth attributes of organic fenugreek

Treatments	Plant height(cm)		Dry matter accumulation (g/plant)		Chlorophyll content at flower initiation (SPAD meter)	Days to flower initiation	Days to maturity
	60 DAS	At harvest	60 DAS	At harvest			
Biochar levels (t /ha)							
Control	40.97	82.94	4.20	15.84	51.51	44.17	136.58
2	43.77	88.21	4.34	19.81	54.41	44.75	136.75
3	51.89	88.24	4.67	20.00	54.79	44.83	136.92
4	53.30	90.14	5.00	20.43	56.55	45.17	137.08
CD (p=0.05)	1.86	2.09	0.16	0.68	2.29	NS	NS
Silicon levels (%)							
Water spray	46.10	84.67	4.43	18.25	54.00	44.75	136.75
1.5	45.61	86.57	4.48	18.58	54.21	44.58	136.67
2.0	48.79	88.07	4.65	19.07	54.52	44.83	136.42
2.5	49.43	90.23	4.65	20.18	54.53	44.75	137.50
CD (p=0.05)	1.86	2.09	0.16	68	NS	NS	NS

Table 2. Effect of biochar and foliar applied silicon on the yield attributes and yield of fenugreek

Treatments	No. of pods/ plant	No. of seeds/pod	Seed weight /plant	Test weight (g)	Seed yield (kg/ha)	Haulm yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)
Biochar levels (t /ha)								
Control	26.58	15.83	4.85	11.53	1328	3953	5281	25.25
2	29.50	16.08	5.65	11.89	1935	4575	6510	29.75
3	29.67	16.42	5.82	11.93	1967	4698	6665	29.51
4	30.42	17.00	6.11	12.01	1994	4816	6810	29.35
CD (p=0.05)	1.59	NS	0.47	0.19	63.85	242.78	241.74	1.34
Silicon levels (%)								
Water spray	28.25	16.00	5.31	11.76	1712	4333	6044	28.25
1.5	28.58	16.25	5.48	11.78	1757	4383	6140	28.54
2.0	28.83	16.33	5.59	11.86	1837	4520	6357	28.72
2.5	30.50	16.75	6.12	11.97	1919	4806	6725	28.35
CD (p=0.05)	1.59	NS	0.47	NS	63.85	242.78	241.74	NS

index of fenugreek did not significantly affect by the silicon application as a foliar spray. Net return (₹127367 /ha) was obtained highest with 2.5% foliar application of silicon (Table 3).

The effect of silicon could explain the increase in plant height and dry matter accumulation in enhancing the plant-water relationship, which modifies the cell division and elongation process. The silicon in the plant system acts as an immuno-booster and regulates anti-oxidant properties, which can positively increase plant growth. Diwan et al (2019) and Hussain et al (2021) reported similar results on increased plant height and dry matter accumulation. The increase in yield attributes and yield of fenugreek could be due to the role of silicon in stomatal activity by hindering sodium flux and enhancing intracellular CO_2 concentration, which further increases photosynthesis and water use efficiency (Ahmad et al 2013). Moreover, silicon plays a significant role in plant stress resistance and disease prevention, leading to better plant yield and quality. Silicon modifies the source-sink relationship and thus increases the mobilization of photosynthates and amino acids from the vegetative parts to the reproductive organs. The increase in net return could be due to the increased seed yield (1780 kg /ha) and haulm yield (4089 kg /ha) obtained under the foliar application of 2.5% silicon. Similar findings were also reported by Rao et al (2018).

Interaction effect of biochar and foliar applied silicon: Biochar and foliar-applied silicon positively interacted with parameters like seed yield (Table 4), net return and BC ratio (Table 5). The highest seed yield (2155 kg /ha) was obtained with the treatment combination of 2 t /ha biochar and 2.5% foliar applied silicon, which was at par with 3 t/ha biochar and 2.5% foliar applied silicon (2115 kg /ha) and 3 t/ha biochar and 2.0% foliar applied silicon (2113 kg /ha). While the maximum net return (₹150490 /ha) was found with the treatment combination (B_2S_4) of 2 t/ha biochar with 2.5%, foliar applied silicon over control and other treatment combinations.

The positive coupling action of biochar could explain the interaction effect on the seed yield on below-ground modifications to increase the nutrient availability and active silicon on the above-ground control on the stomatal activities and enhanced photosynthesis through higher light use efficiency (Maghsoudi et al 2016), synergetic effects on the plant growth and development (Sattar et al 2020), improved the crop productivity (Seleiman et al 2019). Due to the increased seed yield of fenugreek significantly by the combined application of biochar and active silicon, the

Table 3. Effect of biochar and foliar applied silicon on net return (₹/ha) and BC ratio

Treatments	Total cost (₹/ha)	Net return (₹/ha)	BC ratio
Biochar levels (t/ha)			
Control	36367	93834	2.58
2	56454	130409	2.31
3	66370	123448	1.86
4	76131	117242	1.54
CD (p=0.05)		614.90	0.01
Silicon levels (%)			
Water spray	55150	107560	1.95
1.5	55876	111194	1.99
2.0	56578	118813	2.10
2.5	56607	127367	2.25
CD (p=0.05)		614.90	0.01

Table 4. Interaction effect of biochar and foliar applied silicon on seed yield (kg /ha)

Biochar levels (t/ha)	Silicon levels (%)			
	Water spray	1.5	2.0	2.5
Control	1297	1339	1336	1341
2	1845	1858	1883	2155
3	1815	1826	2113	2115
4	1889	2006	2015	2066
CD (p=0.05)	127.69			

Table 5. Interaction effect of biochar and foliar applied silicon on net return (₹/ha) and BC ratio

Biochar levels (t /ha)	Silicon levels (%)							
	Net return (₹/ha)				BC ratio			
	Water spray	1.5	2.0	2.5	Water spray	1.5	2.0	2.5
Control	90690	94333	94340	95973	2.54	2.59	2.58	2.62
2	122027	123291	125830	150490	2.19	2.18	2.23	2.66
3	109694	110940	136548	136613	1.67	1.67	2.05	2.05
4	107831	116212	118536	126391	1.42	1.52	1.55	1.65
CD (p=0.05)	319.48				0.02			

interaction effect of both factors is also visible on the economic parameter resulting in a higher net return from the treatment combination of B_2S_4.

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Evaluation of Different Grain Substrates for Spawn Production and Yield Performance of Blue Oyster Mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] through Bio-Conversion of Agri/Industrial Wastes

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***Abstract*:** Blue oyster mushroom is a novel species that is widely gaining popularity nowadays owing to its simple and low production technology with high biological efficiency. Since spawn serves as the planting materials in mushroom production and its quality is the most important factor for the successful cultivation of any edible mushroom. Hence, the study was undertaken to determine with the objective to find out the best grain spawn for getting early and high yield crop of *Hypsizygus ulmarius* on different substrates. Seven locally available grains *viz*; barley, bajra, paddy, wheat, oat, maize and sorghum were used for spawn production. Bajra grains were found as an excellent substrate for spawn preparation as it supports faster and enhanced growth (11 days) followed by sorghum, wheat and maize grain substrate. Linear growth (50.52 mm) and growth rate (0.39 mm/h) of the fungus was found maximum on bajra grains as compared to other grains. Maximum yield (640.00 g/0.5 Kg substrate) and biological efficiency (128.00 %) was also recorded in bajra grains spawn on wheat straw substrate closely followed by wheat grains spawn on the same substrate. The performance of *H. ulmarius* on wood chips, curry leaves and saw dust was found minimum when spawned with either of the grain spawn.

***Keywords*:** Biological efficiency, Grain spawn, *Hypsizygus ulmarius*, Substrate, Yield

The global food and nutritional security of the growing population is a great challenge that looks for alternative crops as a source of food and nutrition. Mushrooms are among the favoured alternative crops. Mushrooms are fleshy, spore-bearing and multicellular fungi, enriched with quality proteins, vitamins, minerals and also possess therapeutic and medicinal properties (Aditya and Bhatia 2020, Aditya et al 2022c). Blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] is gaining popularity nowadays among small and marginal farmers because of its simplicity of growing on soyabean, sawdust, paddy straw, wheat straw and other agro-wastes (Singh et al 2018, Aditya et al 2022d). Being saprophytic it can easily be introduced in any part of the country. Its fast growth and high resistance against competitive micro-organisms are likely to make its cultivation more economical and less tedious. This mushroom has also very high biological efficiency compared to other oyster mushrooms which makes this fungus the mushroom of the future in the coming years (Aditya et al 2022a, Aditya et al 2022c).

Mushroom spawn is the mushroom mycelium growing on a given grain substrate which is used as a planting medium for growing mushrooms. Spawn production is a technologically advanced process that necessitates a high level of expertise, specific knowledge and attention on the part of those involved in it (Aditya et al 2022b). It is considered as the bedrock of the mushroom industry and the limiting factor to mushroom cultivation all over the world (Chinda and Chinda 2007). Spawn quality is the most important factor in the production of edible mushroom. In nature, mushroom use spores for generative multiplication and these are microscopic and very difficult to handle. As an alternative, spawn can be prepared using tissue cultures extracted from cap tissues. Grain spawn is in common use because of its ability to ramify the substrate faster and ease of planting. In India, not much research work has been done on the cultivation technology of blue oyster mushroom. It is in light of these facts, the aim of the current work was to evaluate the different grain substrates for spawn production and yield performance of blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] on different growing substrates under the sub-tropical zone of Himachal Pradesh, India.

MATERIAL AND METHODS

Study area: The study was conducted in the Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur under the aegis of Dr. Yashwant Singh Parmar

University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India during the year 2019-2021. The cultivation experiments were carried out under natural climatic conditions in the bamboo hut constructed for mushroom cultivation. Neri is located at an altitude of 650 m in the valley of Himachal Pradesh in the northern sub-tropical zone of India at 31.67°N and 36.48°E with an annual temperature of 16.8°C, precipitation 1342.72 mm and relative humidity is around 69 per cent.

Procurement, maintenance and preservation of culture: The pure culture of blue oyster mushroom (*Hypsizygus ulmarius)* was procured from the Directorate of Mushroom Research, ICAR complex, Chambhaghat, Solan (H.P). The culture, thus obtained was maintained on a potato dextrose agar (PDA) medium (subcultured periodically at an interval of 30-45 days). Full-grown culture was stored at 2-4°C in the refrigerator until used further for the entire work.

Spawn preparation: Seven different cereal grains i.e., wheat, paddy, sorghum, bajra, oat, maize and barley were evaluated to see their effect on spawn production of *H. ulmarius.* Healthy grains were cleaned, washed and rotten grains or those floating on the surface of the water were removed. As much as 10 Kg of each grain substrates were boiled in 15 liters of water and care was taken not to overcook the grains, so that they may not rupture. Excess water was drained off by keeping on a wire mesh for 1-2 h. The substrates were mixed with 2.0 per cent calcium sulfate and 0.5 per cent calcium carbonate on a wet-weight basis to obtain the desired pH and to avoid the stickiness of the grain substrates. Thereafter, grains were filled in graduated glass bottles (1000 ml capacity) plugged with non-absorbent cotton and sterilized at 22 psi for 2 h. After cooling, the bottles were shaken vigorously to restore the transparency of the glass and were inoculated with uniform-sized mycelial bits (5.00 mm) of *H. ulmarius* under aseptic conditions. These bottles were then incubated at 25±1° C in the BOD incubator, till the grains were fully impregnated with the mycelium of inoculated fungus culture (11-19 days).

Substrate preparation: Seven different agro and industrial wastes *viz*., wheat straw, maize straw, pine needles (*Pinus roxburghii*), wood chips, saw dust, lantana leaves (*Lantana camara*) and curry leaves (*Murraya koenigii*) were collected from the locality of College of Horticulture and Forestry Neri, Hamirpur, Himachal Pradesh and used as cultivation substrates. At first, all the growing substrates were chopped into small pieces (2-4 cm long) and thereafter soaked separately in water to get completely wet and then treated with a solution of formalin (0.5 %) and carbendazim (0.075 %). After about 18 hours, the substrates were taken out and the excess water in the substrate was drained off by placing the substrate on a clean wire mesh. After that, all of these chemically treated substrates were thoroughly spawned using a 5.0 percent spawn dose of each grain spawn. These spawned substrates were then filled in the mushroom growing bags of polythene having 2.0 Kg capacity (0.5 Kg dry substrate) and later shifted to the growing mushroom hut. Standard mushroom growing packages and practices were followed to raise the crop.

Observations recorded: Data were recorded in terms of time taken for spawn run (days), growth characteristics and linear growth (mm) at 24 h intervals until the mycelium ramified completely on the grains. The growth rate (mm/h) was further calculated as per the following formula:

$r_g = dgt_2 - dgt_1 / t_2 - t_1$

Where, dgt_2 = Diametric growth (mm) at time t_2; dgt_1 = Diametric growth (mm) at time t_1.

Yield of mushroom: This is the quantity/weight of mushroom produced per bag of substrate per harvest time.

Biological efficiency: The matured mushroom was harvested, which differed depending on the substrate type. The curl margin of the cap denoted mature mushrooms, which were harvested by twisting to uproot from the base. The yield was expressed in biological efficiency (%) and calculated (Chang et al 1981). The biological efficiency was calculated thus:

$$\text{Biological Efficiency (BE) \%} = \frac{\text{Fresh weight of harvested mushroom}}{\text{Dry weight of the substrate used}} \times 100$$

Statistical analysis: The experiments were conducted in a completely randomized block design with four replications and analyzed by using the statistical package of the program OPSTAT.

RESULTS AND DISCUSSION

Effect of different grain substrates for spawn production of *Hypsizygus ulmarius*: There was a significant difference in spawn development of *H. ulmarius* on different grain substrates, except paddy and oat grain substrates which took 17 and 17.5 days respectively for complete colonization of the grains by the test fungus (Table 1). Latter both treatments were statistically at par with each other. Among the different tested grain substrates, a significant minimum period for the spawn run of tested fungus was recorded in bajra grains (11 days) followed by sorghum (13 days) and wheat (15 days) grains. However, barley grain substrates took a significantly maximum time (19 days) for spawn development of *H. ulmarius.* The growth characteristics of *H. ulmarius* spawn varied considerably with respect to different grain substrates. A white mycelial growth was recorded on all types of grains under study but, it was more conspicuous in bajra, sorghum, wheat and maize grains spawn. The grains were tightly held with each other after a complete spawn run. The mycelia growth was faster, thick, strandy and dense white on bajra grains while, observed to be dense to thick in the case of sorghum, wheat and maize grains. However, comparatively slower, thin and fluffy growth was noticed and grains were also loosely held in oat, paddy and barley grains. A perusal of the data depicts that irrespective of the durations of incubation, significantly maximum (50.52 mm) linear growth was recorded in bajra grains followed by sorghum, wheat and maize grain substrates (Table 2). However, significantly minimum (17.97 mm) linear growth of the test fungus was in barley followed by oat and paddy grains. Keeping aside the spawn substrates, the linear growth rate of the fungus increased in all the grain substrates with the passage of time and it attained maximum growth in each grain substrates after 11 days of incubation. The linear growth was significantly higher in bajra grains (107.13 mm) followed by sorghum grains after 11 days of incubation while, minimum linear growth was observed in barley grains (1.27 mm) after 1 day of incubation. An intermediate level of linear growth was recorded in the rest of the grain substrates after different durations of incubation.

Additionally, growth rate of *H. ulmarius* on different grain substrates was also calculated on 24 h intervals up to 264 h (11 days). The maximum mean growth rate was observed in bajra grains (0.39 mm/h) followed by sorghum, wheat, maize and oat and paddy grain substrates, irrespective of the time of incubation (Table 3). The growth rate in the case of oat and paddy was statistically at par with each other and the minimum growth rate was in barley grain substrate (0.15 mm/h), irrespective of the different days of incubation. Irrespective of the spawn substrates, the growth rate of the test fungus increased significantly in all the test grain substrates with the passage of time being significantly minimum (0.09 mm/h) between 0-24 h and maximum (0.36 mm/h) between 240-264 h of incubation. The maximum growth rate (0.57 mm/h) was in bajra grains between 240-264 h of incubation significantly followed by same grain substrate (0.54 mm/h) between 216-240 h of incubation. The minimum growth rate was in barley grains (0.05 mm/h) between 0-24 h of incubation. An intermediate range of growth rate was recorded in the rest of the grain substrates after different durations of incubation.

Effect of different grain substrates on yield performance of *Hypsizygus ulmarius*: After screening of different grain

Table 1. Effect of different grain substrates on spawn production and growth characteristics of *Hypsizygus ulmarius*

Grain substrate	Time taken for spawn run (days)	Growth characteristics
Bajra	11.00	Thick, strandy and dense white mycelial growth on all grains.
Sorghum	13.00	Dense white mycelial growth on all grains but intact with each other.
Wheat	15.00	Thick, strandy white mycelial growth on all grains.
Maize	16.00	Dense, fluffy white mycelial growth on all grains.
Oat	17.00	Thin, fluffy white mycelial normal growth on all grains.
Paddy	17.50	White mycelial growth on all over the grains but not tightly held.
Barley	19.00	White mycelial normal growth on all grains.
CD (p=0.05)	0.51	

Table 2. Effect of different grain substrates on linear growth of *Hypsizygus ulmarius*

Grain substrate	Average linear growth (mm) after different incubation duration (days)											Overall mean
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	
Bajra	5.93	12.47	19.50	27.77	36.90	46.33	57.23	68.70	80.30	93.47	107.13	50.52
Sorghum	3.38	6.53	12.67	19.23	26.20	33.33	42.67	52.07	62.37	72.60	83.33	37.67
Wheat	3.23	6.18	9.70	15.60	22.30	29.67	37.23	44.83	52.90	61.57	69.83	32.09
Maize	2.37	4.70	8.40	12.73	18.20	24.70	31.27	37.97	45.03	52.40	59.57	27.03
Oat	2.30	5.00	7.80	11.53	16.57	22.20	28.00	34.23	41.03	48.40	55.63	24.79
Paddy	2.43	4.68	8.03	11.83	16.93	23.00	29.00	35.67	42.40	49.70	57.03	25.51
Barley	1.27	2.57	4.83	7.83	11.70	15.83	20.00	24.96	30.47	35.90	42.30	17.97
Overall mean	2.99	6.01	10.13	15.22	21.26	27.87	35.05	42.63	50.64	59.15	67.83	
CD (p=0.05)	Grain substrate 0.12		Duration 0.15		Interaction 0.39							

substrates under *in vitro* conditions, cultivation trials were conducted to assess the yield performance of *H. ulmarius* on different substrates viz., wheat straw, maize straw, pine needles (*Pinus roxburghii*), wood chips, saw dust, lantana leaves (*Lantana camara*) and curry leaves (*Murraya koenigii*) using commercial spawn prepared on different grain substrates. The yield was recorded and biological efficiency was further calculated and have been presented in Table 4. The results evinced that irrespective of the different substrates used, bajra grains spawn produced maximum average yield (468.28 g/0.5 Kg substrate) followed by wheat grains spawn which was statistically at par. Moreover, it was significantly followed by sorghum grains spawn and maize grains spawn (Table 4). Minimum yield was recorded in barley grains spawn (431.76 g/0.5 Kg substrate) which was statistically at par with oat grains spawn and paddy grains spawn. Irrespective of the different grains spawn used, wheat straw substrate produced maximum average yield (614.52 g/0.5 Kg substrate) significantly followed by maize straw substrate. However, minimum yield (337.95 g/0.5 Kg substrate) was recorded in wood chips substrate significantly followed by curry leaves and saw dust. Interaction of the table clearly depicts that maximum yield (640.00 g/0.5 Kg substrate) was recorded in wheat straw substrate spawned with bajra grains spawn which was statistically at par with the same substrate spawned with wheat grains spawn. Minimum yield spawned with barley grains spawn was recorded in wood chips substrate (321.00 g/0.5 Kg substrate) followed by the same substrate spawned with, paddy, oat and maize grains spawn respectively, which was also statistically at par

Table 3. Effect of different grain substrates on growth rate of *Hypsizygus ulmarius*

Grain substrate	Average growth rate (mm/h) between duration of incubation (h)											Overall mean
	0-24	24-48	48-72	72-96	96-120	120-144	144-168	168-192	192-216	216-240	240-264	
Bajra	0.16	0.27	0.29	0.34	0.38	0.39	0.46	0.46	0.48	0.54	0.57	0.39
Sorghum	0.07	0.13	0.25	0.27	0.29	0.29	0.38	0.39	0.42	0.42	0.44	0.30
Wheat	0.07	0.12	0.14	0.24	0.27	0.30	0.31	0.31	0.33	0.35	0.35	0.25
Maize	0.09	0.10	0.15	0.17	0.22	0.27	0.27	0.27	0.29	0.30	0.29	0.22
Oat	0.11	0.11	0.11	0.15	0.20	0.23	0.24	0.25	0.28	0.30	0.30	0.21
Paddy	0.10	0.10	0.13	0.15	0.21	0.25	0.25	0.27	0.28	0.30	0.30	0.21
Barley	0.05	0.05	0.09	0.12	0.16	0.17	0.17	0.18	0.21	0.22	0.26	0.15
Overall mean	0.09	0.13	0.17	0.21	0.25	0.27	0.29	0.31	0.33	0.35	0.36	
CD (p=0.05)	Grain Substrate 0.01		Duration 0.01		Interaction 0.02							

Table 4. Yield of *Hypsizygus ulmarius* as influenced by different grain spawn on different substrates

Grain spawn	Average yield (g/0.5 Kg substrate) in different substrates							
	Wheat straw	Maize straw	Pine needles	Saw dust	Wood chips	Lantana leaves	Curry leaves	Overall mean
Bajra	640.00 (128.00)	595.66 (119.13)	470.66 (94.13)	410.00 (82.00)	349.33 (69.87)	459.00 (91.80)	380.66 (76.13)	468.28
Sorghum	609.00 (121.80)	568.33 (113.67)	437.00 (87.40)	400.00 (80.00)	350.33 (70.07)	424.33 (84.87)	361.66 (72.33)	455.33
Wheat	626.66 (125.33)	587.33 (117.47)	465.00 (93.00)	420.66 (84.13)	347.33 (69.47)	457.66 (91.53)	368.33 (73.66)	466.23
Maize	616.66 (123.33)	587.00 (117.40)	420.33 (84.07)	407.00 (81.40)	340.00 (68.00)	412.33 (82.47)	353.00 (70.60)	448.04
Oat	613.33 (122.67)	531.33 (106.27)	419.33 (83.87)	396.00 (79.20)	334.33 (66.87)	421.66 (84.33)	350.00 (70.00)	438.00
Paddy	602.66 (120.53)	574.00 (114.80)	434.00 (86.80)	394.00 (78.80)	323.33 (64.67)	408.66 (81.73)	346.66 (69.33)	440.47
Barley	593.33 (118.67)	572.66 (114.53)	409.66 (81.93)	389.67 (77.93)	321.00 (64.20)	398.66 (79.73)	337.33 (67.47)	431.76
Overall mean	614.52	573.76	436.57	402.47	337.95	426.04	356.81	
CD (p=0.05)	Grain Spawn 9.86		Substrate 9.86		Interaction 26.09			

Figure given in parentheses represents biological efficiency (percentage) with each other. Biological efficiency was recorded to be maximum (128.00 %) in wheat straw substrate spawned with bajra grains spawn (Plate 1) followed by wheat straw substrate spawned with wheat grains spawn whereas minimum biological efficiency (64.20%) was achieved in wood chips spawned with barley grains spawn. Spawn, the vegetative seed material plays a vital role in mushroom cultivation and the substrate on which the spawn is prepared also affects the mushroom production to a significant extent. Grain spawn is of common use because of its ability to ramify the substrate faster and ease of planting (Bahl 1988). Several authors have tried a variety of grain substrates and agricultural wastes for preparation of spawn based on the easy availability, accessibility and lower cost for different edible mushrooms (Reddy et al 2020, Aditya et al 2022a). At present grains like wheat, maize, barley, oat, sorghum, paddy and bajra are commonly used for the commercial spawn production and cultivation of a variety of edible mushrooms (Aditya 2021).

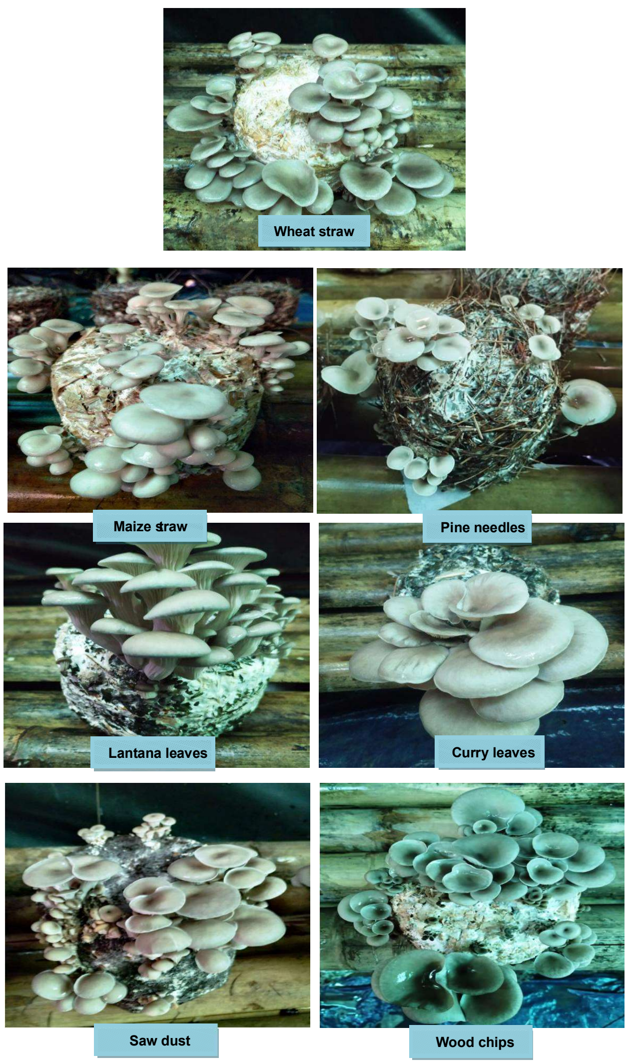

Plate 1. Mature sporocarps of *Hypsizygus ulmarius* on different substrates using bajra grains spawn

Spawn making is a rather complex task and not feasible for the common mushroom grower. It has been a primary concern in mushroom cultivation which is achieved by developing mushroom mycelia on a supporting medium under controlled environmental conditions. In all cases, the supporting material is sterilized grains which is preferred due to inherent biochemical properties and practical performance over others. The results of our present study are in accordance with the findings of Chauhan & Pant (1988) and Rathod et al (2002) who have also reported bajra and sorghum grains to be superior than other grains for spawn production of different *Pleurotus* spp. In the earlier reports, several other workers have also tested different grains for spawn development of *H. ulmarius* and found early spawn development in maize grains, sorghum grains and paddy grains (Sumi & Geetha 2017, Shendge 2018, Aditya 2021) as an ideal substrate for spawn production of blue oyster mushroom. Also Baghel (2017), further supports our results, as according to him bajra grains spawn took (11.2 days) followed by maize (13.4 days) and wheat grains spawn (14.5 days) for complete mycelia spread of *H. ulmarius* while, paddy grains took maximum time (17.60 days) for spawn run. These findings further support our results. In the present study, the highest yield and biological efficiency was recorded from the bajra grains spawn closely followed by the wheat grains spawn. The results of the present study are in accordance with the findings of Baghel (2017), Sumi & Geetha (2017) and Shendge (2018) who have also reported bajra, wheat and sorghum grains spawn exhibited higher yield and biological efficiency of *H. ulmarius.*

CONCLUSION

The mycelial growth of blue oyster mushroom (*H. ulmarius*) was faster on all the seven grain substrates used for spawn production. Bajra grains were the best substrate for spawn production as it supports much faster and enhanced growth of the fungus. The highest yield and biological efficiency of the mushroom was also recorded from the bajra grains spawn closely followed by wheat grains spawn on wheat straw substrate. The wood chips, saw dust and curry leaves substrates exhibited minimum yield on either of the grain spawn tested.

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Retrieving MODIS AOD and Evaluation of Ground-level $PM_{2.5}$ in Addition to the Identification of Potential Source Regions Over South India

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Abstract: In this paper retrieving Aerosol Optical Depth (AOD) from Moderate Resolution Imaging Spectroradiometer (MODIS),was used to estimate the ground-level $PM_{2.5}$ in the south Indian region, the AOD and other meteorological parameters are also considered, with various grid sizes helpful to find more accurate data prediction of $PM_{2.5}$ levels. The multiple regression methods are implemented in this article, the study has shown good agreement in the prediction of $PM_{2.5}$ at the Zoopark location, all other locations are in the moderate range. $PM_{2.5}$ particle's Source identification was done based on Potential Source Contribution Function (PSCF), Concentration-Weighted Trajectory (CWT), and Cluster analysis archive the receptor dominating source regions. The Central India and East Indian regions are more dominating source regions at receptor locations in the winter season. The predominant sources are local sources at the receptor location. The cluster analysis has shown the intensity of combined trajectories towards receptor location. Winter season cluster III was dominating among the all seasons at receptor location.

Keywords: $PM_{2.5}$, MODIS, Back-Trajectory, Cluster analysis

Atmosphere Aerosols are an important role in driving the pollution at ground level, dust loading coupled with black carbon emissions from local sources in northern India (Lau et al 2006) and Aerosol loading in East Asia (Kim et al 2007). The total loading of aerosol in climate is measured based on the AOD, AE, and SSA and may be based on surrounding regions these are the sea salt aerosol (sea), volcanic eruption, natural sources, and anthropogenic activities. The aerosol loading varies with source and climate conditions. The aerosol dust is transported from far away regions in the elevated layers (<3000m) towards the receptor regions (Sun et al 2001). However, the wind speed and wind direction are the dominating transportation of aerosols, it is proved by Xia et al (2011) that the south-westerly winds are dominating the central Tibetan Plateau. The AOD represents the columnar property of the atmosphere, few studies are retrieving AOD from remote sensing instruments to understand the columnar properties, aerosol vertical profile, and estimating the $PM_{2.5}$ (Wu et al 2016, Ferrero et al 2019). The field campaign study also helpful in AOD properties, from the literature study the ISRO-GBP field campaign measured AOD with MICROTOPs instruments, fine particles dominating with the fine mode around 0.23 mm and coarse mode around 1 mm(Singh et al 2006). Badarinath et al (2007) case study showing the dust storm over the south Indian region over Hyderabad, reveals that the diffuse-to-direct-beam ratio is the most appropriate parameter for dust monitoring, the variation in AOD also observed in dust and non-dust days it influences based on local meteorology. The cloud properties effect the presence of aerosols in the atmosphere, it influences on cloud droplet number concentration based on aerosol humidification (Gryspeerdt et al 2016). The particle charge state influence atmospheric humidity, which produces liquid bridging forces and electrostatic interactions among particles that may be negative or positive in charge, the agglomeration rate of particles would increase with a rise in the atmospheric humidity (He et al 2019). Such, aerosols influence agricultural production (Greenwald et al 2006). However, biomass burning significantly affects the aerosol optical properties locally as well in the downwind regions (Shaik et al 2019). The overall aerosol properties in Indian regions change based on their production mechanisms, removal and transport processes can contribute to these variations (Ramachandran et al 2012). East Indian regions have high aerosol concentrations during winter than during summer months in an urban environment (Pani and Verma 2014). The source identification based on a Trajectory study by Shaik et al (2019) revealed the transport pathways originated from central India and adjacent oceanic regions during the post-monsoon season. Ramachandran (2005) showed that the long-range transport of pollutants from the surrounding continental locations was found unhealthy indicating the influence of anthropogenic pollution. Banerjee et al (2015) concluded that based on a review of receptor models used in India, vehicular pollutions are dominating in Hyderabad region. The trajectory based approach identifies the source regions. In this article, assessment of the suitable MODIS instrument Terra/Aqua AOD and Meteorological parameters, to identify the ground-level $PM_{2.5}$ based on the multiple regression analysis over Hyderabad. The missing gaps filled with predicted $PM_{2.5}$and identified the potential sources of receptor location, based on backward trajectories, were CWT, PSCF, and cluster analysis are used in this study.

MATERIAL AND METHODS

Study area and source regions: In this study south Indian region, Hyderabad was chosen, Hyderabad city was developed and the urban city was full of traffic and industrial areas surrounded. The pollution may be due to local source regions dominating as well as transport of pollutants may influence the pollution in the city region. The average annual rainfall is 136.1 mm, average altitude of 542 meters, the highest being Banjara Hills at 672 meters. Hyderabad has the surrounding with different regions such as Industrial and rural areas, whereas the CPCB monitoring location (Fig. 1) operates under Telangana state pollution control Board (TSPCB). The region is full of developed area so the pollution is under control such that continuous monitoring stations are very useful for the assessment of urban impact. The study region is located in south Indian region which was Deccan tebetaian. The study region domination pollutants are the vehicular activities and nearby industrial sources, building contraction and metro construction emissions such anthropogenic sources are main contributions of Hyderabad pollution levels as well as the outside the city agriculture burning and wast burning around the city are the secondary level sources but the effect of all these sources transport by the wind from far away regions. The boundary states are Maharashtra in north, Karnataka in west, Andhra Pradesh in the south, Chahattisgarh states are east side located for Telangana state. Patancher industrial area located North West direction, small and medium industries are sourended by 40 km from the center of city Hyderabad, all other sides are agriculture land bounded. The biomass burning of Agriculture waste burning maybe influence.

MODIS AOD data collection: The satellite AOD product is provided by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra/Aqua instrument (Levy and Hsu et al 2017). The MODIS instrument is a multi-spectral radiometer, designed to retrieve aerosol microphysical and optical properties over land and ocean. The MODIS instrument produces global coverage in 1 or 2 days and captures most of the aerosol variability due to this high sampling frequency. In this study, the MODIS level 2 daily AOD data from Terra (MOD04_3K, MOD04_L2 Collection 6.1) and Aqua (MYD04_3K, MYD04_L2) are used, which is reported at 3K for 3Km and L2 for 10km. Were used the instated of missing AOD, 3x3 or 5x5 grid product. The MODIS AOD data product was downloaded from NASA LAADS (https://ladsweb.modaps.eosdis.nasa.gov/).

Meteorological data collection: The meteorological data was adapted from CPCB (https://app.cpcbccr.com/ccr/#/caaqm-dashboard-all/caaqm-landing) at Hyderabad. The meteorological parameters are temperature, humidity, pressure, wind speed, and wind direction and $PM_{2.5}$ are secondary data collectedfrom May 2018 to May 2019.

Backward trajectory: Global Data Assimilation System (GDAS) was used as meteorological data input to the model Hysplite trajectory (Stein et al 2015). The 7-day back trajectories at a height of the surface layer (100,500 and 1000 m) archive for source analysis. Stein et al (2015) highlight the recent applications of the HYSPLIT modeling system, including the simulation of atmospheric tracer release experiments, radionuclides, smoke originating from wildfires,

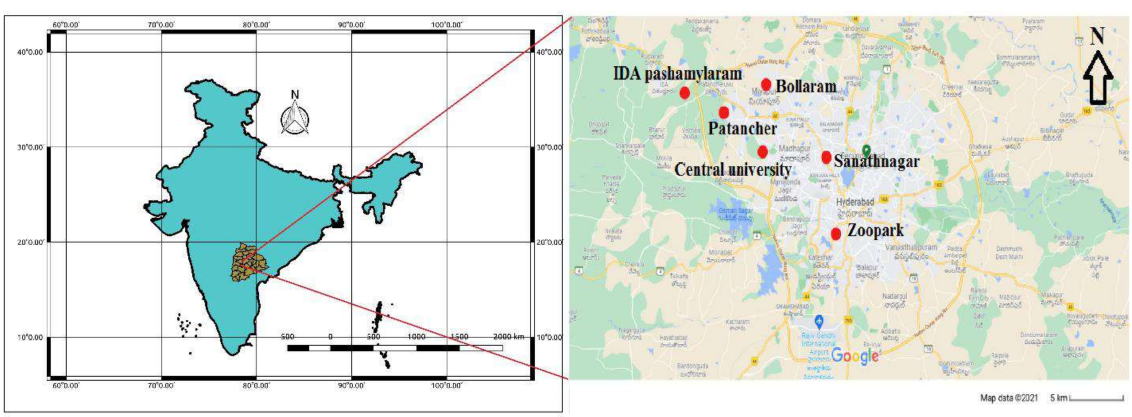

Fig. 1. Study location Hyderabad (six locations)

volcanic ash, mercury, and wind-blown dust. The back trajectories to very sophisticated computations of transport, mixing, chemical transformation, and deposition of pollutants and hazardous materials. Based on the above-generated 7-day-backward trajectories, the PSCF, CWT, and Cluster analysis were analyzed using the TrajStat tool (Wang et al 2009). The spatial resolution was 0.5×0.5° established to find the source paths. The PSCF grid values in the study domain are calculated by counting the segment endpoints. the outliers are removed based on IQR and Z-score methods. The filtered data was divided into 80% for model fitting and 20% for validation. The AOD and meteorology influence PM 2.5 concentration near ground level, were as the multiple regression analysis was used to predict the $PM_{2.5}$ at six locations in Hyderabad, the MODIS data extracted based on Python scripts, were as 5x5 and 3x3 grid data considered for fill the gaps in average grid AOD. The model validation based on statistical parameters (RMSE, NMB, d, and R) concluded the best fit model for MODIS collection.

The CWT, PSCF, and Cluster analysis based on predicted $PM_{2.5}$ concentrations identified the sources of $PM_{2.5}$ near the study region. The 7-day back-trajectories data from Hysplite model was archived. The whole study was divided based on the Indian meteorology department (IMD) four seasons (winter, pre-monsoon, Monsoon, and Post-monsoon). The layers are identified as long-range transport of dust at the receptor location. The threshold criterion for $PM_{2.5}$ was 60 ug/m^3 in PSCF. Han et al(2007)gave the calculation of endpoints in a particular grid cell, which allowed for calculating the PSCF. In the CWT method, each grid cell is assigned a weighted concentration by averaging the sample concentration. The trajectory endpoint time in the grid cells has been weighted by the $PM_{2.5}$ corresponding trajectory. Cluster analysis based on the Ecudian methodology was used in this study to cluster the seasonal dominant paths and contribution areas. The clustering technique shows the average trajectory paths.

RESULTS AND DISCUSSION

Variation of AOD and meteorological parameters: The AOD was maximum at Bollarm (0.54) and minimum in Zoopark (0.47) observed in this study. The meteorology of the six locations (Fig. 2) with standard deviations shows that the $PM_{2.5}$, AOD, BP, and SR parameters are more deviations at all locations but theless deviation within Ambient Temperature (AT), Relative humidity (RH). It may be due to the topography of land and climate conditions and as well as the long-range transport of the pollutants.

MODIS AOD for prediction of the $PM_{2.5}$: AOD-$PM_{2.5}$relation was a great correlation observed in some locations. The

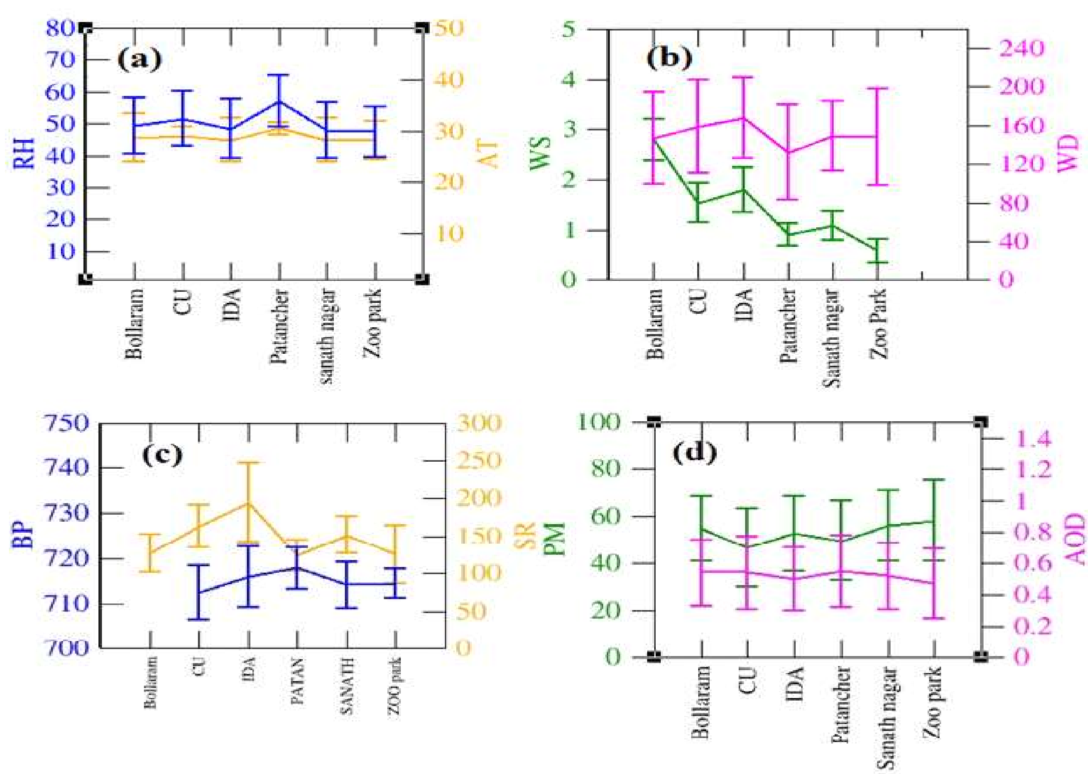

Fig. 2. Meteorological variation of all locations

MODIS all products (MOD_3K, MOD_L2, MYOD_3K, and MYOD_L2) had good correlations at Zoo park observed in this study. The Patancher and IDA locations are moderate correlation seen, other locations are below moderately correlation observed, it may be due to different meteorology around the regions and as well as the land properties are more uncertain in MODIS AOD retrievals (Sathe et al 2019).

The correlation coefficient R, RMSE, d, and NMB model validation parameters are shown (Table 1) the best-fit combination. The statistical parameters are used(Sathe et al

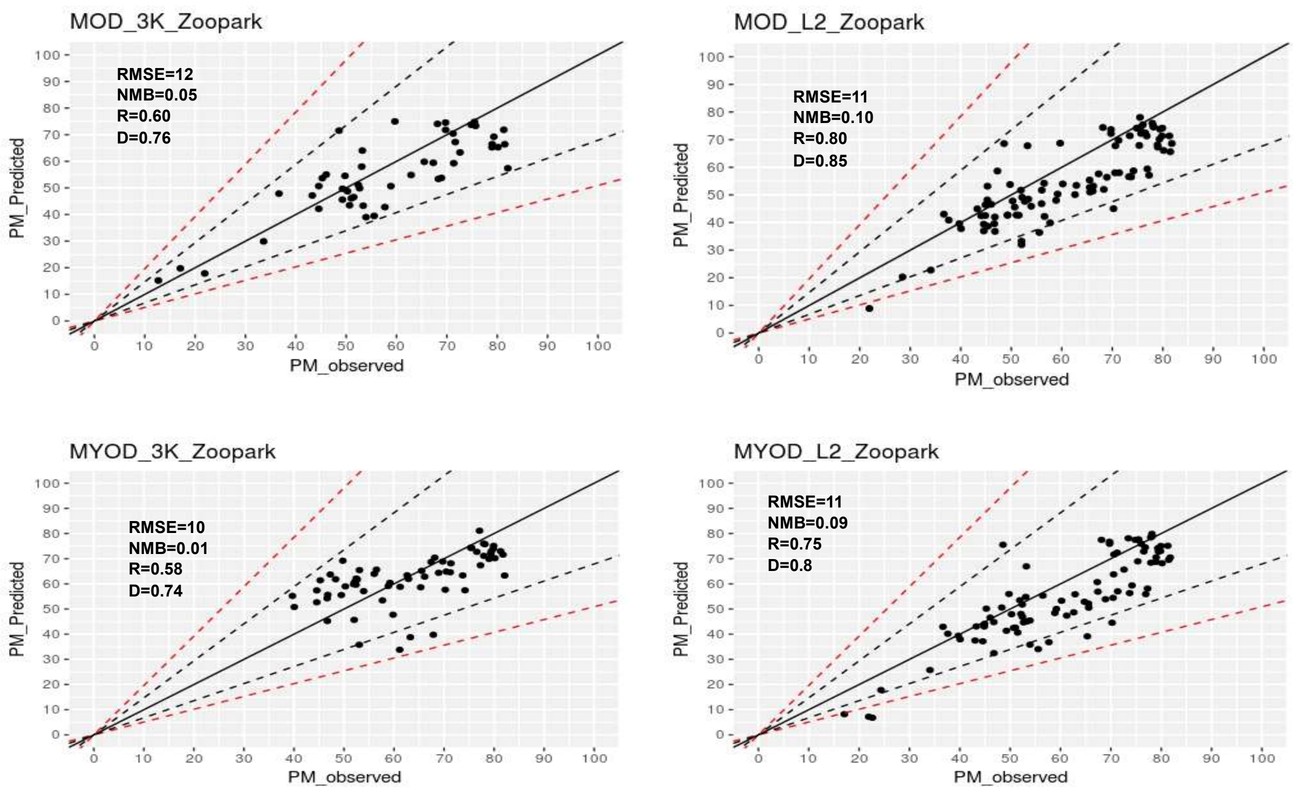

Fig. 3. Scatter plot for $PM_{2.5}$Predicted and Observed at Zoopark location for four MODIS AOD

Table 1. Model performance statistical parameters

	Parameter	Bollaram	Central univercity	IDA	Patancher	Sanath nagar	Zoo park
MOD_3K	RMSE	15	15	11	54	10	12
	D	0.53	0.59	0.81	0.21	0.78	0.76
	NMB	-0.15	-0.02	-0.02	-0.84	-0.04	0.05
	R	0.37	0.36	0.37	-0.43	0.69	0.60
MOD_L2	RMSE	12	14	12	55	11	11
	D	0.52	0.66	0.71	0.22	0.73	0.85
	NMB	-0.009	0.11	-0.03	-0.87	-0.03	0.10
	R	0.59	0.55	0.68	-0.39	0.62	0.80
MYOD_3K	RMSE	15	15	13	47	10	10
	D	0.52	0.73	0.62	0.25	0.81	0.74
	NMB	-0.12	0.14	-0.08	-0.67	-0.02	0.01
	R	0.53	0.46	0.42	-0.34	0.45	0.58
MYOD_L2	RMSE	14	16	11	44	11	11
	D	0.52	0.60	0.82	0.22	0.74	0.80
	NMB	-0.12	0.11	-0.04	-0.67	-0.01	0.09
	R	0.46	0.40	0.70	-0.50	0.65	0.75

2019) to identify the model performance. MOD_3K has all locations are negative NMB indicating except Zoopark the negative shows that models are in general estimating lower $PM_{2.5}$ concentration than observed the model was under predicting $PM_{2.5}$. For positive indicate that over predicting the $PM_{2.5}$ concentration than observed values. The correlation coefficient higher values shown at Zoopark were as low as the correlation shown in other regions. The RMSE has seen higher (54 ug/m^3) at Patancheru and other locations low in range (11-15 μg/m^3), Here we observed that the Patancheru RMSE value has peaked in all MODIS collections compared to other locations. The correlation was great at the Zoopark and lower relation at Patancheru. The RMSE variation range (11-14μg/m^3) except the Patancheru region. The MYOD_3K and MYOD_L2 have nearly similar values in RMSE, d, and NMB, where the good agreement in correlation coefficient in the MOD_L2 product was seen. the scatter plot for the Zoopark location (Fig. 3) the best fit model data from multiple regression analysis, it is seen from the model validation analysis. The scatter plot shows the 3% and 5% percentage error line from the standard line (0 % error), were as the most of the points within 3% error line, few points are above the 5% in MYOD_L2 product, the grater resolution data have more deviation from the standard line seen in this study.

Back Trajectory Analysis for Source Identification

Concentration-Weighted Trajectory (CWT): The CWT analysis (Fig. 4a, b, c, d) form all seasons identified in the winter season was dominating among the all seasons. The weighted trajectory paths are seen from East India regions and coastal regions are major sources. Were as other seasons being weightage of trajectory seen to be less. The pre-monsoon identifies the two paths which are from land and sea regions, which effect may be due to land and sea breeze effect nearby coastal regions, the one path from central India and the other is from the Bay of Bengal. The monsoon season will be a dominating trajectory from West India and the Arabian Sea. The post-monsoon will be affected by East Indian regions. The two seasons (winter and pre-monsoon) have two identification paths and the other two (monsoon and post-monsoon) have one transport path.

Potential Source Contribution Function (PSCF): The potential source contribution function shows the receptor source regions, shows the all seasons PSCF (Fig. 5 I, II, III, and IV) for Winter, pre-monsoon, monsoon, and post-monsoon seasons. The winter season sources are from coastal and East regions, were as the pre-monsoon contribution source areas seen to be high from sea regions other from the West region. There are very low source regions in monsoon and post-monsoon seasons.

Cluster analysis: Trajectory cluster analysis was applied to identify the clustering of the seasonal base analysis of the study period at the receptor location. The Winter, pre-monsoon, monsoon and post-monsoon seasons (Fig. 6 a, b, c and d) were presented. The legend shows the height of the trajectory with respective each cluster. The each cluster's contribution (Table 2) towards the receptor site in

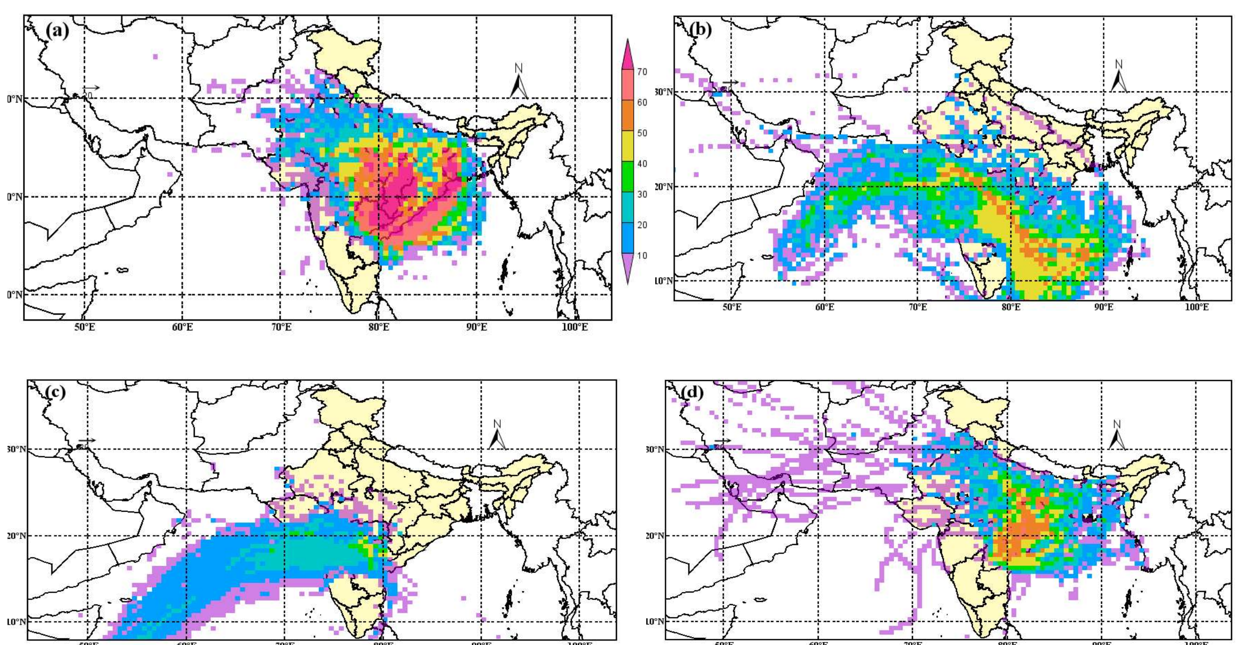

Fig. 4. CWT analysis with different seasons (same scale for all figures follows the Figure (a) legend).

percentage, with six clusters divided based on the direction. The clusters are dominating central India and North West Indian regions in the winter season. The pre-monsoon season has different direction clusters but the Bay of Bengal has the low-level trajectories seen in this season. The monsoon season has all clusters from the Arabian Sea region and West India. Most of the trajectories are from local regions, the Winter polluted cluster was Cluster I, the mean concentration as 72.05 μg/m^3 but the number of polluted trajectories is 179 which is the highest number of the trajectory seen in Winter season. The least polluted trajectory means 34.64μg/m^3 associated with cluster III in monsoon. The polluted clusters has the less number of trajectories but the intensity of pollutant transport is more, compared to other clusters.

Reduction in $PM_{2.5}$ under high RH due to the deposition of

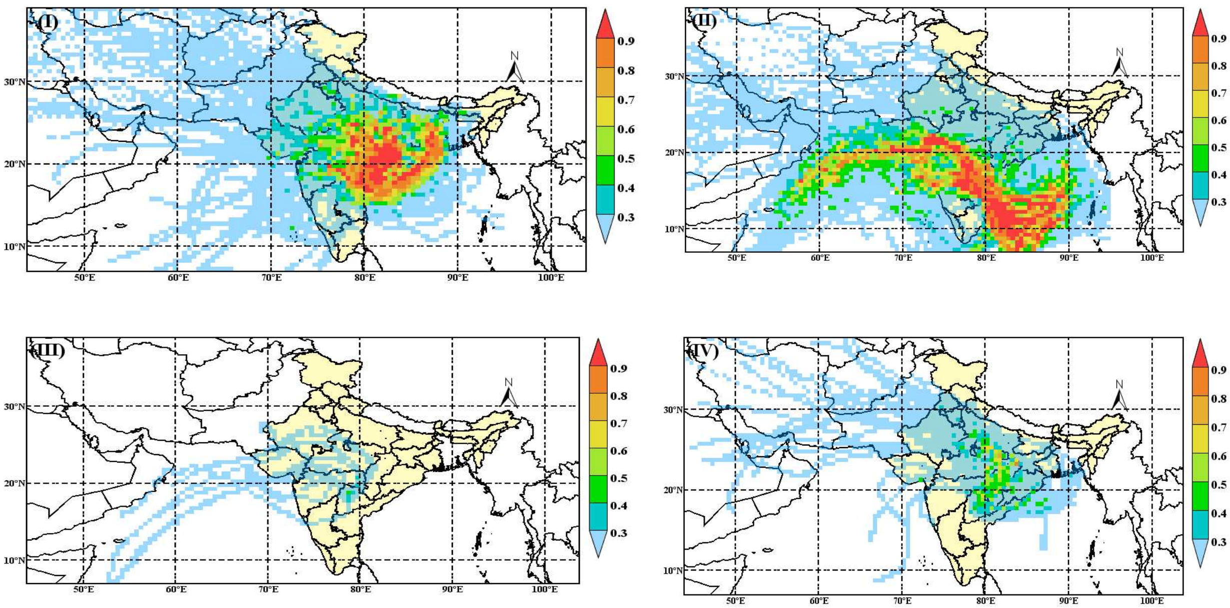

Fig. 5. PSCF analysis with different seasons at surface layer and elevated layer

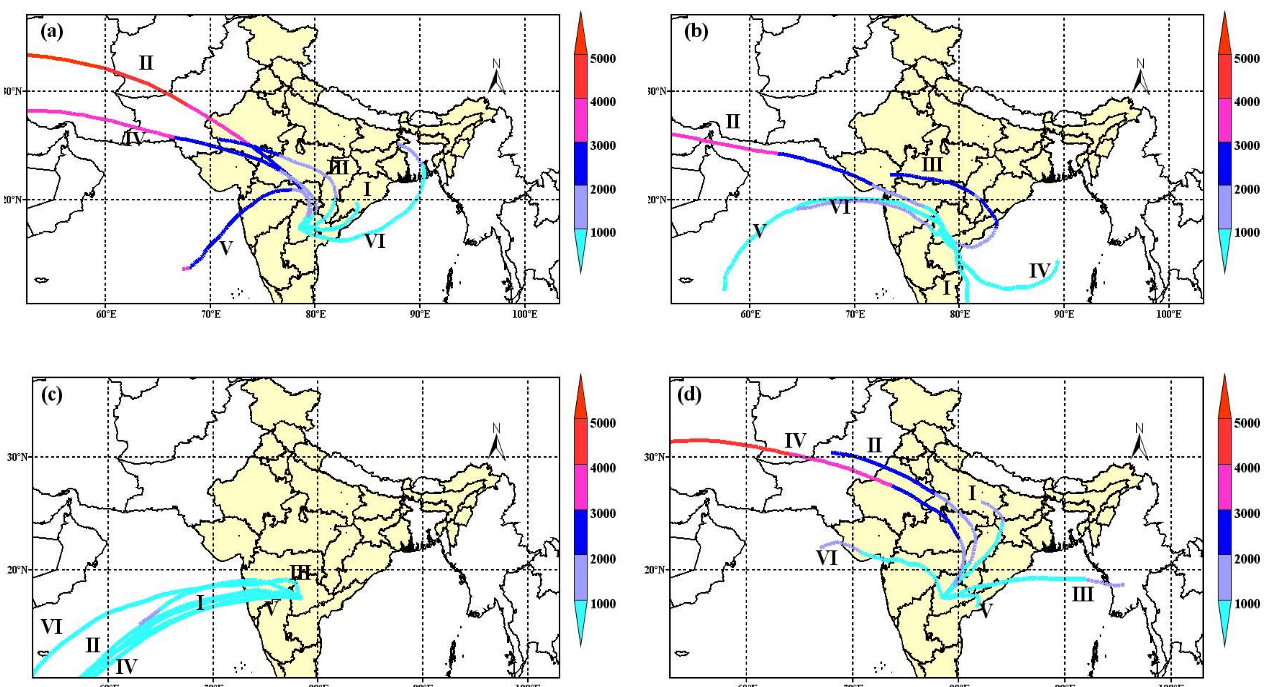

Fig. 6. Cluster analysis with different seasons at surface layer and elevated layer

Table 2. Polluted clusters and associated trajectory's numbers

Winter	Number_traj	Mean_value	Standard devation	Polluted_num	Polluted_mean_value	Polluted_stdev	Ratio (%)
1	202	72	8.4	179	74.2	4.3	21.6
2	45	65	11.6	29	72.1	5.5	8.33
3	79	68	10.3	61	72.5	5.8	38.1
4	163	64	12.4	98	73.3	5.5	13.52
5	42	63	14.3	26	73.4	5.9	7.22
Pre_monsoon							
1	118	43	7.6	0	0	0	18.29
2	49	48	10.8	6	69.4	7.8	7.91
3	51	50	10.4	9	66.4	4	7.91
4	199	48	11.3	38	66.9	4.4	31.3
5	115	42	11.5	11	67.8	7.7	19.22
6	98	47	9.7	11	66.7	5.9	15.3
Monsoon							
1	154	21	5.6	0	0	0	22.59
2	89	25	7.4	0	0	0	12.4
3	111	34	12.7	10	62.4	2.7	17.15
4	124	18	3.2	0	0	0	18.9
5	133	21	4.2	0	0	0	20.9
6	55	32	9.2	2	63.1	4.8	7.95
Post monsoon							
1	97	55	19.2	42	71.1	6.4	29.23
2	60	50	23.9	24	70.1	5.4	17.76
3	55	40	13.2	4	67.8	5.1	15.03
4	26	54	25.7	15	72.6	5.1	8.20
5	67	42	22.3	16	69.1	6.1	22.40
6	16	33.33	27.28	4	72.18	7.53	7.38

dust particles, the high average temperature in the patancheru area (30.51^{oC}) observed may be due to the industrial zone. The temperature inversion leads to higher values of pollutants in the winter season (Yadav et al 2019), PSCF has shown high source regions in the winter season compared to other seasons. The variation of meteorology gives the climate change in the northeast region (Deb and Sil 2019). Numerous studies also shown that meteorology is a great influence on the ground level particulate concentration and transboundary aerosols (Tiwari et al 2015, Yadav et al 2016). As well as several studies show that the correlation between AOD and ground-level $PM_{2.5}$ concentration and estimation of Particulate matters from AOD (Lv et al 2017, Shao et al 2017, Lu et al 2021, Tuna et al 2021).

Previous studies are shown based on back trajectory analysis of effective origin source regions and long-range transport of pollutants (Conte et al 2020, Hong et al 2019, Owega et al 2006). The satellite retrieval studies shown the better agreemt for hydrometeorological studies (Suchithra and Agarwal 2021). Road dust particles are effecting on Plant Species (Bemmansour et al 2021). Soni et al (2018) reported that the $PM_{2.5}$ and AOD nonlinear multi-regression model performance was better for the Jaipur region. The AOD influences the cloud properties (Balakrishnaiah et al 2012, Kumar 2013, Gopal et al 2016,).The observational study has shown the AOD-$PM_{2.5}$over china region (Xin et al 2016) concluded that there was a high consistency of AOD versus $PM_{2.5}$ and the correlation coefficient ranges from 0.64 to 0.70 across China. The European hot-spot areas are identified by Squizzato and Masiol (2015)based on the statistical approaches to understand the influence of external and local contribution of $PM_{2.5}$sources such as back-trajectories cluster analysis, PSCF, and CWT analysis, here we are also concluded that the most dominating sources based on trajectory calculation, and in our study. The back-trajectory-based source apportionment of airborne sulfur and nitrogen concentrations identified the source contribution at the receptor (Gebhart et al 2011). The dust outbreaks are identified in Spain (Cabello et al 2016). The tracking of Hazardous air pollutants from refinery fire based on trajectory by Shie and Chan (2013). The comparative study within two cities was analyzed for source identification (Kong et al 2013).

In this study found that the high contribution regions from East Indian regions and coastal regions in Winter season, the pre-monsoon origins from Bay of Bengal and central Indian regions, the most of air mass transport from these regions.

CONCLUSIONS

In this study, the assessment of ground-level $PM_{2.5}$ is based on meteorology and MODIS AOD. The multiple regression analysis was adopted in this study. The good perdition of $PM_{2.5}$at the Zoo park location was found, were as other locations are in the moderate range. On the other hand, source identification based on trajectory-based analysis as CWT, PSCF, and cluster analysis has shown in this study, the long-range transport of the $PM_{2.5}$and potential source regions from the East India and Coastal regions are the potential source regions at receptor locations, among all seasons winter season local pollutant are dominating. It is maybe due to anthropogenic and vehicular activities. The cluster analysis provided the main mechanism of similar types of trajectories transporting paths towards the receptor. Cluster III in the winter season was dominating at receptor location. The study was useful for the health policymakers to establish new guidelines. As well as the pollution assessment of the remote location.

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Bio-Monitoring of Air Quality Using Leaf Characteristics

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***Abstract*:** An evaluation of eight roadside ornamental trees was conducted for the purpose of evaluating their potential as bio-indicators of air quality. Tree leaves were analyzed to determine leaf area, stomatal density, and stomatal pore size. Leaf samples were collected from two sites: Ludhiana city (polluted site) and the university campus (control site). The mean stomatal density of selected tree species ranged from 200-412 mm^{-2}. In selected trees, mean stomatal density ranged between 246.88-351.56 mm^{-2} (control > polluted). Among selected tree species, the mean stomatal length ranged from 8.61-14.40 μm. The location-wise order of mean stomatal length in selected trees ranged between 9.88-11.66 µm (control > polluted). The mean stomatal width of selected tree species ranged from 4.03-1.39 µm. The mean stomatal width of selected trees ranged between 2.47-2.87 μm (control > polluted). Polluted sites showed reduced stomatal density, length, and width. In light of these findings, it can be concluded that tree stomatal characteristics can provide practical information about air quality.

***Keywords*:** Air pollution, Ornamental trees, Stomatal parameters, Roadside, Bio-monitor

The air pollution levels have increased many folds during the last five decades which has led to adverse effects on plants in terms of specie loss or change in community composition Air quality is generally measured using physicochemical methods but this doesn't take into account the effect of air pollution on living entities viz., plants (Balasooriya et al 2009). Therefore using biomonitoring which is defined as the response of living entities to air pollution method instead of the physiochemical approach can address this problem (Nali and Lorenzini 2007). Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases like carbon dioxide, and oxygen and also provide a vast area for impingement, absorption, and accumulation of air pollutants to reduce the pollution level in the atmosphere (Escobedo et al 2008). Plants are continuously involved in gaseous exchange through stomata, they add oxygen into the atmosphere and in turn, take up CO_2 and other harmful gases thereby helping in ameliorating the environment's gaseous composition. Thus plants can help in combating the problem of air pollution but in this process get affected because of their continuous exposure to a particular environment. In the polluted environment change in plants' morphological, physiological, and biochemical parameters can be observed ((Verma and Singh 2006, Stevovi et al 2010, Wuytack et al 2010). Thus, a plant can be used as an important bio-monitor for studying air pollution levels by studying their morphological and physiological parameters.

The extent of plant susceptibility towards air pollution is variable as air pollution affects plants mainly through the uptake of pollutants into the leaves through stomata. And plant stomata response to pollution is variable from plant to plant, plant age, prevailing environmental conditions, and most important concentration of air pollution (Verma and Singh 2006, Stevovi et al 2010). Several studies suggested that low concentrations of air pollutants may influence stomata indirectly through damage to subsidiary cells or the epidermal cells or changes in cell wall structures (Abeyratne and Ileperuma, 2006). Plant's response to air pollution can be studied more specifically through parameters viz., morphological (specific leaf area) and anatomical (stomatal density & pore surface) (Balasooriya et al 2009, Kardel et al 2010, Chaturvedi et al 2013, Arriaga et al 2014). In the present studies, passive biomonitoring i.e. by using existing avenue plantations was performed with a major focus on leaf characteristics.

MATERIAL AND METHODS

Study areas: The study area features a semi-arid climate with three distinct seasons; summer, monsoon, and winter. The average precipitation in the region is 809.3mm annually. Ludhiana is one of the worst polluted cities in India with particulate matter as high as six times normal with average maximum day temperature of 39.6°C and a minimum average temperature of 26.2°C. January is the coldest month followed by June as the hottest and November is the driest month in the region. National Highway 5 between

Chandigarh and Firozpur passing through Ludhiana city, Punjab (North latitude of 30°46'38" and the East longitude of 76°33'23") was taken as a study area (polluted site) while the Punjab Agricultural University campus was the control site to conduct present experiments.

Sampling: In the survey, the distribution of tree species along both sides of the Highway was recorded during 2019-2020. Out of the total, eight commonly growing trees (*Acacia auriculiformis, Alstonia scholaris, Cassia fistula, Cassia siamea, Chukrasia tabularis, Dalbergia sissoo, Heterophragma adenophyllum,* and *Putranjiva roxburghii*) were identified and chosen for the study with three replications of each treatment and each replication comprises of three trees at each polluted (NH-5) and control location (Punjab Agricultural University campus). To keep homogeny, trees of the same age, size, and spread were selected. In total seven tree species were selected for study with three replications of each treatment and each replication comprises three trees at each polluted and control site

Methodology

Leaf area (cm^2): The dimension of leaf length was measured from the leaf tip down the midrib of the leaf lamina to the leaf base point where the petiole attaches to lead. The leaf breadth was considered along the widest width crossways the lamina. Ten leaves were selected per tree for calculation.

Stomatal density (mm^{-2}): The rightly healthy leaves were collected from selected trees from experimental locations. Leaves were dried and fixed on aluminum stubs with the help of double-sided carbon tape followed by gold plating. Leaf samples were then observed under a scanning electron microscope (S-3400 N) by Hitachi. The stomatal density was estimated by counting the number of stomata per unit area and expressed in mm^{-2}.

Stomatal pore length (µm): Pore length was measured by stretching the cursor over the length of the stomatal pore.

Stomatal pore width (µm): Pore width was measured by stretching the cursor across the width of the stomatal pore.

Statistical analysis: The data were analyzed using factorial randomized block design. Mean comparison was performed by Tukey's test using SAS software computer version 9.2.

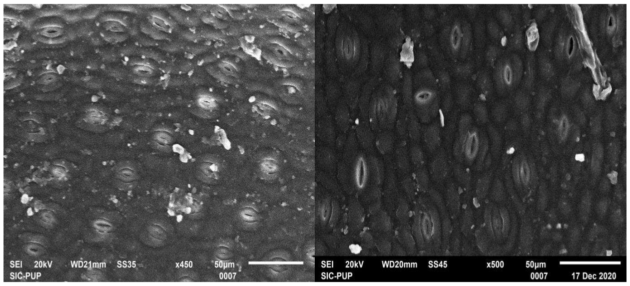
RESULTS AND DISCUSSION

The stomatal length, width, and density of selected trees were compared under controlled and polluted conditions (Table 1. Fig. 1-8). *Dalbergia sissoo* (15.23 µm) has the longest stomata at control location followed by with shortest in *Putranjiva roxburghii* (7.83 µm) at polluted location; stomatal width was recorded highest for *Chukrasia tabularis* (4.37 µm) at control location with least in *Cassia fistula* (1.30 µm) at polluted location . Stomatal density was highest in *Heterophragma adenophyllum* (475 mm^{-2}) at control location with lowest in *Dalbergia sissoo* (200 mm^{-2}). Overall mean higher stomatal length, stomatal width & stomatal density (11.66 µm, 2.87 µm & 351.56 mm^{-2}) was in leaves of trees growing at the control location as compared to the polluted location (9.88 µm, 2.47 µm and 246.88 mm^{-2}). In line with Wuytack et al (2010), observed that selected tree species were resistant to the polluted environment. The tree with the most resistance to polluted environment was *Cassia fistula* followed by *Heterophragma adenophyllum, Cassia siamea, Putranjiva roxburghii, Alstonia scholaris, Dalbergia sissoo, Acacia auriculiformis*and *Chukrasia tabularis*. Among different tree species, stomatal characteristics vary based on genetics or their adaptability to environmental conditions, but plants with higher stomatal frequencies / resistances are considered to be better adapted or tolerant of air pollution (Al

Table 1. Stomata pore length, width (µm) and stomatal density of selected roadside tree species at the control and pollution location

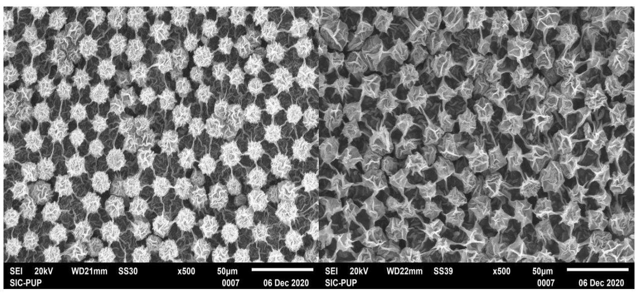
Tree species	Pore length (µm)		Pore width (µm)		Stomata density (mm^{-2})	
	Control	Polluted	Control	Polluted	Control	Polluted
Acacia auriculiformis	11.88	9.85	2.65	1.98	350.00	225.00
Alstonia scholaris	11.53	8.88	4.06	3.88	237.50	175.00
Chukrasia tabularis	11.25	7.85	4.37	3.68	400.00	300.00
Cassia fistula	11.61	10.63	1.48	1.30	325.00	225.00
Cassia siamea	13.17	12.51	4.12	3.78	400.00	275.00
Dalbergia sissoo	15.23	13.57	1.72	1.34	250.00	150.00
Heterophragma adenophyllum	9.29	7.97	2.20	2.04	475.00	350.00
Putranjiva roxburghii	9.38	7.83	1.98	1.77	375.00	275.00
Overall mean (Location)	11.66^a	9.88^b	2.82^a	2.47^b	351.56^a	246.88^b

Different letters in each column are significantly different at P≤0.05 by Turkey's Test



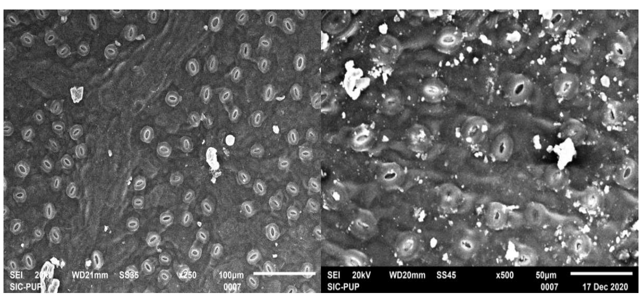
A) Control location B) Polluted location

Fig. 1. Stomata in leaves of *Acacia auriculiformis* at control and polluted locations



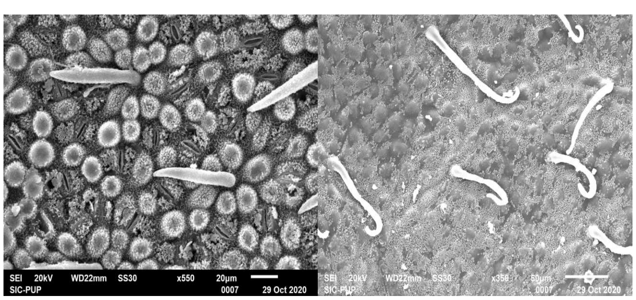
A) Control location B) Polluted location

Fig. 2. Stomata in leaves of *Alstonia scholaris* at control and polluted locations



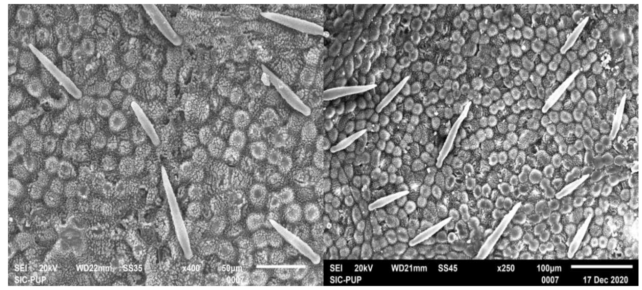
A) Control location B) Polluted location

Fig. 3. Stomata in leaves of *Chukrasia tabularis* at control and polluted locations



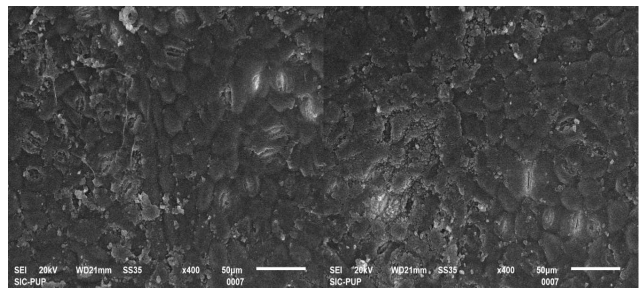
A) Control location B) Polluted location

Fig. 4. Stomata in leaves of *Cassia fistula* at control and polluted locations



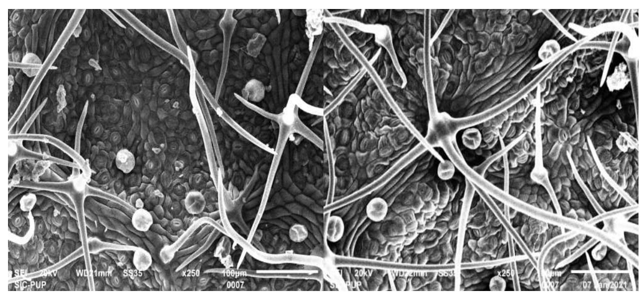
A) Control location B) Polluted location

Fig. 5. Stomata in leaves of *Cassia siamea* at control and polluted locations



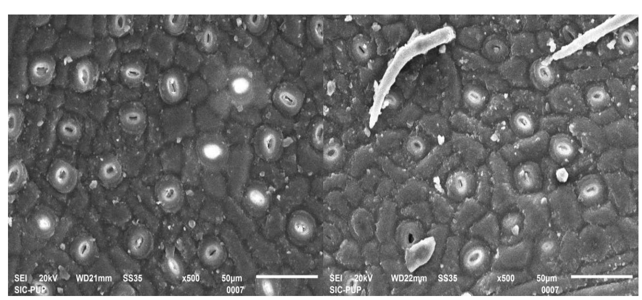
A) Control location B) Polluted location

Fig. 6. Stomata in leaves of *Dalbergia sissoo* at control and polluted locations



A) Control location B) Polluted location

Fig. 7. Stomata in leaves of *Heterophragma adenophyllum* at control and polluted locations



A) Control location B) Polluted location

Fig. 8. Stomata in leaves of *Putranjiva roxburghii* at control and polluted locations

Afas et al 2006, Raina et al 2008, Ha and Martinez 2018). The location of the trees had a significant impact on these parameters.In many previous studies, leaf stomatal characteristics were also reported to decrease as a result of pollution (Pourkhabbaz et al 2010, Wuytack et al 2010, Khushwaha et al 2018).The stomatal characteristics of plants are determined not only by their genetic makeup but also by the environmental conditions surrounding them.These trees can therefore be used as an indicator of air pollution based on their stomatal characteristics. The high CO_2 concentration in a polluted environment causes low reactivity of stomata. Therefore, reduction in stomatal density at the polluted site can be seen as an adaptation of plants for controlling pollutant absorption but cause a reduction in photosynthesis at the same time (Verma et al 2006).

CONCLUSION

The trees growing in the more polluted area (roadside) adapts themselves by a reduction in pore size and the number of stomata, which will enhance stomatal resistance and alternatively increases plant survival potential in the polluted environment. Therefore, can conclude that roadside trees' stomatal characteristics can be used as good bio-indicators for monitoring air quality and as resistance trees for developing greenbelts in polluted urban areas. *Cassia fistula* is the most tolerant tree for NH-5.

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Potentials of Biochar to Improve Productivity of Automobile Wastes Contaminated Ultisol under Mound Tillage Practice Using Cocoyam (*Xanthosoma sagittifolium*) as Test Crop in Abakaliki Southeast Nigeria

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Abstract: A field experiment was conducted in 2018 and 2019 cropping seasons to evaluate the effect of *biochar on the physicochemical properties of* automobile waste contaminated soil, yield , soil heavy metal (Cu, Zn, Fe, Pb) content and uptake by Cocoyam (*Xanthosoma sagittifolium*) in a mound tillage practice. The treatments were; Contaminated automobile waste soil (CO-control), contaminated soil amended with 5 tha-^{1} biochar (BO_5), contaminated soil amended with 10 t ha-^{1}(BO_{10}) and contaminated soil amended with 20 tha-^{1}biochar (BO_{20}). There was significant decrease in soil bulk density and increased soil hydraulic conductivity in biochar amended plots compared to the control. Exchangeable bases (Ca, Mg, K, Na), effective cation exchange capacity (ECEC), total N, available P, organic carbon and pH levels were significantly higher in biochar amended plots relative to the control. The significantly higher cocoyam yield was recorded in the biochar amended plots compared to control plots. Biochar amendment reduced the levels of Pb, Fe, pb and Cu to tolerable limits in soils. Similarly, uptake of Fe, Zn, cu and Pb by cocoyam in biochar amended soil was within acceptable limit for human consumption. It is recommended that agronomic practices that increases soil nutrient and decrease heavy metal uptake be used in automobile waste contaminated soils to obtain high crop yield and safe produce.

Keywords: Underutilized crops, Food security, Rural farmers, Acceptable limit, Mechanic village

Many indigenous plant species are in regular use by both human and non-humans. However, most of these plant species cultivated for food across the world have largely been neglected, unrecognized and underutilized. According to Agulana (2020) these indigenous and underutilized crops help in improvement and enhancement of health status of local population. In a study on food security; the challenge of feeding 9 billion people, Godfray et al (2010) reported that those indigenous and underutilized crops all over the world promote food security, enhance nutrition and help in generation of income for poor rural farmers. Cocoyam (one of the popular crops in Nigerian in late 1960's and early 1970's) is one of these neglected indigenous and underutilized plant species. At present it is at the verge of being forgotten. Ojenuga et al (1996) showed that heavy metals are naturally present in soils. In a study on transport of heavy metals in surface runoff from vegetable and citrus fields. He et al (2004) reported that anthropogenic activities result in high concentration of heavy metals in the environment. Poorly managed automobile workshops popularly called 'sites' abound in Abakaliki, the study area. These "sites" according to Ugoh and Moneke (2011) are source of constant release of used spent oil discharged from crank cases of cars, motorcycles and generators. Researches on heavy metal uptake by crops grown in automobile waste contaminated soils are few or non-existence in the study area. Similarly, studies on theeffects of biochar on Nigerian soils are very few and scanty. Fagbenro et al (2015) observed that available literature on biochar in Nigeria indicated that nearly all the biochar researches were potted/green house experiments. There is therefore, the need to carry out biochar studies in the field for an understanding of its effect on soil properties and crop yield. The aim of this research was to examine the effect of different rates of biochar on the properties of automobile waste contaminated soil and their impact on the yield and heavy metal uptake of cocoyam. The study will create awareness on the suitability of such soils for crop production especially cocoyam- an important, indigenous and underutilized crop that is almost being forgotten. Farming is the major activity of the people of the study area while the tillage system used by the farmers is mound tillage.

MATERIAL AND METHODS

Study area: The study was carried out on abandoned land that used by mechanics for workshop and disposal of automobile wastes for many years and adjacent to the Teaching and Research Farm of Faculty of Agriculture and Natural Resources Management, Ebonyi state university Abakaliki, Abakaliki is located by latitude 06^{0}4'N and longitude 08^{0}65'E in the derived Savannah zone of the Southeast agro-ecological area of Nigeria. The rainfall pattern is bimodal (April – July and September – November) with a short dry spell in August normally referred to as "August break". The total annual rainfall in the area ranges from 1500 -2000 mm, with a mean of 1,800 mm. At the onset of rainfall, it is torrential and violent, sometimes lasting for one to two hours (Okonkwo and Ogu 2002). The area is characterized by high temperatures with minimum mean daily temperature of 27^{0}C and maximum mean daily temperature of 31^{0}C throughout the year. Humidity is high (80%) with lowest (60%) levels occurring during the dry season between December to April, before the rain season begins. Geologically, the area is underlain by sedimentary rocks derived from successive marine deposits of the cretaceous and tertiary periods. According to the Federal Department of Agriculture and Land Resources (FDALR 1985), Abakaliki agricultural zone lies within 'Asu River group' and consists of olive brown sandy shales, fine-grained sandstones and mudstones. The soil is shallow with unconsolidated parent materials (shale residuum) within 1m of the soil surface. It belongs to the order, ultisol and is classified as Typic Haplustult (FDALR 1985). The vegetation of the place is primarily derived savannah with bush growth and scanty economic trees. The site has history of previously being used for mechanic workshop and disposal of automobile wastes for over twenty years before it was reclaimed. Existing grasses in some portions were cleared manually using cutlass. The debris left after clearing was removed before seedbed preparation. Farming is the main occupation of people of the study area.

Land preparation and application of treatments: The land used for the study measured 13.5 m by 15 m equivalent to 0.0203 ha. The experiment was arranged as Randomized Complete Block Design (RCBD) with four treatments and five replications. A total of 20 plots measuring 3 m x 3m each were used for the study. Plots were separated by buffer of 0.5 m and each replicate 1 m apart. The treatments were; Control (automobile waste contaminated soil-(CO) , 5 t ha-^{1}of biochar (BO_5) ,10tha-^{1}biochar (BO_{10}) and 20 t ha^{-l} biochar (BO_{20}) equivalent to 0,6,12, and 20 kg plot^{-1}. The treatments were spread uniformly and incorporated into their respective plots during cultivation. Mounds 16-20 cm high were prepared using hoe. Cocoyam sett weighing 20-25g was planted 30 cm apart and at a depth of 5cm to give a planting population of 834 corms/ha. Weeding was done manually at interval of 6 weeks with hoe. At maturity (18 months after planting) ten plants were harvested from each plot by shelling up the plant and uprooting it. This brings out the corms while the remains in the soil were dug out. The corms were dried in the sun for 2-3 days, weighed and the yield expressed in t ha-^{1}. The dried corn was ground in food processor with stainless cutter. Approximately 1g of the sample was digested with Conc HNO_3. The digest was analysed for heavy metal (Cu, Fe, Zn, Pb) content by subjecting it to flame AAS according to APHA (1985). The same procedure was repeated in the second cropping season without application of amendments to test the residual effect.

Soil sample collection: Initial soil samples were collected randomly from 8 observation points in the study site at 0-15 cm depth before the study. Auger samples were collected from 4 observation points at 0-15 cm .after harvest. The soil samples were thoroughly mixed to form a composite sample and used for routine analysis. Core samples were collected from four observation points at a depth of 6 cm in each plot at 45 and 90 days after planting (DAP) and used for the determination of soil physical properties. Biochar was collected from two different species of hard wood viz Oil palm (*Elaeis guineensis*) and Gmelina (*Gmelina arborea*) bought from a local distributor. The hardwood was pyrolysed at 350^{o}C, manually crushed to particles smaller than 2 mm and thoroughly mixed together. Later, characterization was carried out according to biochar material test categories and characteristic of the IBI Standards version 2.0 (2014). At the end of the end of each cropping season (after harvest) undisturbed core samples were collected and used for the determination of soil physical properties. Similarly, auger samples were collected from the plots air dried, sieved and used for determination of soil chemical properties.

Plant sample collection: At maturity eight cocoyam plants per plot were sampled and tagged. The corms were harvested, air dried and weighed to get the yield per plot. The yield data was expressed to hectare equivalent. The dried corm was analysed for heavy metal (Cu, Zn, Fe, Pb) content.

Laboratory methods: Bulk density (bd) was determined using the core method described by (Blake and Hartage, 1986).Hydraulic Conductivity was determined by the constant head technique (Klute and Dinken 1986). Exchangeable bases (Ca^{2+}, K^+, Mg^{2+}, Na^+) were determined by the method of Thomas (1982) and effective cation exchange capacity (ECEC) determined by summation. Soil reaction was measured according to the procedure of Henderson et al (1993). Total nitrogen was determined using the micro-Kjeldhal distillation method of (Bremner 1996). Available P was determined by the Bray-2 method as described by (Page 1982) while organic carbon was determined using the method described by Nelson and Somners (1982).

Determination of heavy metals: The corm heavy (Cu, Fe, Pb, Zn) metal content was determined using the analytical procedure by APHA et al (1985).

Statistical analysis: Data was analysed using Statistical Analysis System (SAS 1985).

RESULT AND DISCUSSION

Selected properties of the automobile waste contaminated soil and biochar: The soil texture is sandy clay loam . The soil pH was extremely acidic (USDA-SCS 1974). Higher values of Avail. P, total N and organic carbon (OC) were observed in the biochar compared to the soil (Table 1).

Effects of biochar on soil bulk density (bd) Mgm-^{3} and hydraulic conductivity (Cm hr-^{1}): Significantly lower bd values were observed inbiochar amended plots at 45 DAP in both cropping seasons compared to control (Table 2). Brady and Weil (2004) observed that biochar has lower bd (0.3 Mgm-^{3}) than mineral soils (1.3 M gm-^{3}) and thus can reduce soil bd. In a study on the effect of mechanic village activities on the soils of Abakaliki south eastern Nigeria, Njoku et al (2021) reported higher bd in spent oil contaminated soil relative to the control. Nellison et al (2015) reported decreased soil bd relative to the control when they studied the effect of woody biochar on the properties of a sandy loam soil and spring barley in a two year field experiment. Atkinson et al (2010) and Ndor et al (2015) also reported lower bd values in biochar amended plots compared to control as in present study.The significantly higher levels of hydraulic conductivity (HC) in biochar amended plots relative to control in both cropping seasons (Table 2). In the first cropping season HC ranged between 8.29-25.11 Cm hr^{-1}. In the second cropping season the order of increase in HC wasCO< BO_5< BO_{10}<BO_{20}. Nwite (2013) reported decreased HC in plots contaminated with automobile lubricant oil compared to non-lubricant oil contaminated soil. Njoku et al (2015) and Busscher et al (2011) showed increased HC in biochar amended plots relative to control plots.

Table 1. Selected properties of the automobile waste contaminated soil and biochar

Parameter	Soil (g kg^{-1})	Biochar
Coarse sand	364	Nd
Fine sand	200	Nd
Silt	366	Nd
Clay	70	Nd
Texture	Sand clay loam	-
pH	3.82	7.4
Ash content	Nd	23
Total N %	0.12	0.89
Avail. P mg kg^{-1}	5.30	28.0
OC (%)	3.60	23.9

Nd= Not determined

Effect of biocharon soil exchangeable bases and effective cation exchange capacity (ECEC) (Cmol kg^{-1}): The significantly (p=0.05) higher values of exchangeable bases and ECEC in amended plotsrelative to CO in both cropping seasons were observed (Table 3). In the first cropping seasonCa, K, Mg and Na ranged between, 0.11-0.27, 0.11-0.21, 0.10-0.23 and 0.09-0.24,respectively. In both cropping seasons the least exchangeable cation values were in the control. Mbah and Ezeaku (2009) characterized the physicochemical conditions of farmland affected by automobile waste and reported decrease in exchangeable bases and ECEC. Uchendu and Ogwo (2014) in a study on the effect of spent engine oil discharge on soil properties in automobile mechanic village reported decrease in exchangeable bases relative to non-spent engine oil discharged soil. Inal et al (2013) reported increase in exchangeable bases and cation exchange of soils amended with biochar and processed poultry droppings. Glaser et al (2002) opined that biochar inherently containing ash adds nutrients such as K, Ca and Mg to the soil solution thereby increasing the pH of the soil and providing readily available nutrients for optimum plant growth.

Effectbiocharon soil organic matter (OM%), pH ,available P (mg kg-^{1}) and total N (%): The significantly high

Table 2. Effect of biochar on soil bulk density (gcm^{-1}) at 45 and 90 days after planting (DAP) and Hydraulic conductivity (HC)

Treatment	2018		2019		HC	HC
	45 DAP	90 DAP	45 DAP	90 DAP	2018	2019
CO	1.47	1.51	1.49	1.52	8.29	5.09
BO_5	1.37	1.49	1.38	1.51	20.14	15.04
BO10	1.35	1.48	1.36	1.50	18.20	13.44
BO_{20}	I.33	1.37	1.35	1.49	25.11	18.70
FLSD (0.05)	0.02	NS	0.02	NS	2.23	1.02

pH values in biochar amended plots relative to the control were observed in the two cropping seasons respectively (Table 4). Similarly, significantly higher values of avail P, total N and OM was observed in amended soils relative to the control in both cropping seasons. The improvement in soil pH following application of the amendments corroborates the study of Chan et al (2008a).Using biochar as soil amendment, Vaccari et al (2011) reported elevated levels of pH in amended plots compared to control plots. Improvement in soil contents of available P, total N and organic matter observed in the study could be attributed to higher levels of these nutrients in the amendment in line with the observations of Peston and Schmdt (2006). Similarly Angst et al (2014) observed significantly higher values of SOC in soils amended with biochar compared to control.

Effect of biochar on the yield of cocoyam (Mg ha^{-1}): The significantly (p=0.05) higher Cocoyam corm yield in amended plots relative to control was observed (Table 5). The highest cocoyam corm yield (28.4) in the first cropping season was obtained in BO_{20} plots. This value (28.4) was 13%, 6% and 65% higher than yield values in BO_5, BO_{10} and CO, respectively. The order of yield increase in the second cropping season was $CO<BO_5<BO_{10}<BO_{20}$. The low yield obtained in CO plots is in line with the report of Vuoto et al (2005) that lubricant oil contaminated soil has serious fertility problems. The higher cocoyam corm yield in amended plots could also be due to high nutrient content of biochar and (Table 1) Blackwell et al (2008) reported that biochar can be used as amendment to improve soil quality and crop productivity. Yilangai et al (2014) reported significantly higher tomato yield in beds treated with charcoal than without charcoal. Similarly, Vinh et al (2014) observed increased vegetable yield following application of biochar. However, the result of this study differed from that of Jay et al (2015) who reported that short time application of biochar has no yield benefit.

Heavy metal uptake by cocoyam corm after the study: Heavy metal uptake by cocoyam corm differed significantly among the treatments in both cropping seasons (Table 6). In the first cropping season Cu varied between 1.04 and 2.52 with the highest value observed in CO. In the same season Zn, Fe, and Pb content of cocoyam corm varied between 8.08-28.5, 19.3-36.1 and 0.001-0.011, respectively. The highest corm content of Cu (1.90) was observed in CO in the second cropping season. Plants growing in a polluted environment can accumulate toxic metals at high concentration causing serious risks to human health if consumed Vahter et al (2007). Vousta et al (1996) reported higher levels of heavy metals in vegetables grown in contaminated soil relative to uncontaminated soil. Furthermore, Nwiko and Eguobi (2002) carried out a study

Table 5. Effect of biochar on the yield of cocoyam (Mg ha^{-1})

Treatment	2018	2019	Mean
CO	10.0	6..12	8.0
BO_5	26.6	13.7	20.1
BO_{10}	24.6	16.6	20.6
BO_{20}	28.2	18.4	23.3
FLSD (0.05)	1.96	1.67	

Table 3. Effect of biochar on soil exchangeable bases and effective cation exchange capacity –ECEC (Cmol kg-^{1})

Parameter	2018					2019				
	Ca	Mg	Na	K	ECEC	Mg	Na	K	Ca	ECEC
CO	0.11	0.10	0.09	0.11	0.41	0.06	0.05	0.08	0.07	0.26
BO_5	0.20	0.25	0.19	0.18	0.82	0.13	0.16	0.11	0.13	0.53
BO_{10}	0.23	0.28	0.16	0.21	0.78	0.18	0.13	0.16	0.15	0.50
BO_{20}	0.27	0.23	0.24	0.16	0.90	0.21	0.18	0.11	0.14	0.54
FLSD (0.05)	0.03	0.13	0.02	0.02	0.12	0.03	0.04	0.01	0.04	0.12

Table 4. Effect of biochar on soilorganic matter (OM), pH, available P and total N

Parameter	2018				2019			
	pH	Available P	TN	OM	pH	Available P	TN	OM
CO	3.76	1.02	0.10	0.09	3.65	0.09	0.06	0.07
$B0_5$	5.80	4.05	0.23	0.27	5.40	1.08	0.10	0.16
BO_{10}	5.50	5.O1	0.28	0.37	5.42	1.98	0.14	0.19
$B0_{20}$	6.01	5.06	0.29	0.40	5.50	1.65	0.19	0.23
FLSD (0.05)	0.12	0.03	0.01	0.03	0.02	0.40	0.10	0.30

Table 6. Heavy metal uptake by cocoyam corm after the study

Parameter	2018				2019			
	Cu	Zn	Pb	Fe	Cu	Zn	Pb	Fe
CO	2.52	28.5	0.011	36.1	1.90	16.8	0.009	22.0
BO_5	1.11	7.20	0.002	19.7	1.00	6.72	0.001	16.0
BO_{10}	1.04	8.05	0.002	20.0	1.01	7.56	0.002	18.1
BO_{20}	1.23	9.24	0.001	19.3	1.12	7.61	0.001	16.0
FLSD (0.05)	0.11	0.32	NS	1.23	0.22	0.14	NS	1.31

Table 7. Heavy metal content of the soil after the study (Mg kg^{-1})

Parameter	2018				2019			
	Cu	Fe	Zn	Pb	Cu	Fe	Zn	Pb
CO	270	76	400	380	252	362	60	302
BO_5	162	33	298	198	230	108	30	120
BO_{10}	191	30	203	180	103	186	27	98
BO_{20}	153	28	198	142	98	169	20	102
FLSD(0.05)	10,3	6.9	12,5	16.3	8.9	10.2	4.5	1.8

on lead contamination of soils and vegetations in an abandoned battery factory in Ibadan and revealed that edible plants grown in polluted soils are susceptible to heavy metal uptake. Crop content of heavy metals is dependent on crop uptake and its availability in soils. High levels of heavy metals in crops above critical limit cause human hazards. Ware (2007) showed that the recommended daily allowance for Cu is 900 ug (meq) a day for adolescence and adult. The observed Cu value in the study is within acceptable limit. According to US National Institute of Health (2018) the recommended average intake of Fe in foods and supplement is 19-20.5 mg/day. Result of this study showed that Fe toxicity is possible in cocoyam grown in automobile soil since it is above acceptable limit for human beings. The range of Zn concentration/consumable in food samples according to World Health Organization) (WHO-2011) is 5-15 mg/kg. Ajiwe et al (2018) reported that the average human intake of Zn is 7-16.3 mg/day. The observed Zn content of the corm in automobile soil constitute human health hazard since it is above acceptable limit. According to Park et al (2011) plants readily bio-accumulate large quantity of pb through their roots without much changes in their total yield. Nnabo (2015) reported that pb accumulation above permissible level causes body ailments and easily leads to weariness of the body tooth and bones. The total pb burden according to Bersenyi (2008) is 90-400 mg.

Soil heavy metal content after the study (Mgkg^{-1}): The present study indicated that significant higher heavy metals in the CO plots (Table 7). The higher levels of soil heavy metal content in CO could be attributed to the effect of spent lubricant oil discharge by the mechanics. Duru (2019) reported that monitoring of heavy metal levels in soil is of great concern because of their toxicity and ease of leaching into surface and ground water. Majolagbe et al (2014), Palm et al (2013) and Oti (2018) showed higher levels of heavy metals in automobile waste soil than non-automobile waste soil. Orjiakor and Atuanya (2015) observed that the daily activities of auto-mechanic battery have negative impacts on soil physico-chemical properties. Demie (2015) showed extremely high levels of heavy metals (above USEPA regulation standard) in soil contaminated in garage and auto-mechanic workshop of Shasheme city. Njoku et al (2021) and Oti (2016) reported higher levels of heavy metals compared to the control. The result of the study showed that pb toxicity in automobile waste soil is possible since it is above acceptable limit.

CONCLUSION

The application of biochar improved the physicochemical properties of automobile waste contaminated soil. It also increased cocoyam yield and decreased heavy metal (Cu, Zn, Pb, Fe) uptake by cocoyam to acceptable limits that does constitute health hazard. Results also showed that heavy metals (Pb, Zn, Cu and Fe) content in automobile waste contaminated soil was decreased to non-toxic limit when biochar was uses as amendment. The use of biochar as an agronomic practice to increase crop yield and ensure safe produce from automobile waste contaminated soil was recommended.

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Effect of Micro Plastics and Nano Plastics in Terrestrial Animals, Birds, Human and Implications on Integrated Functional Ecosystem

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Abstract: The impact of micro plastics (MPs) on the bivalve larvae at different developmental stages throughout their life history, especially for the metamorphic stage is not at all available. In organisms, consequences of plastic ingestion include exposure to environmental pollutants and toxin accumulation, causing endocrine disruption, inflammatory and physiological stress. The transfer of micro plastics has been shown to transfer across food webs, however, the micro plastic accumulations across terrestrial food webs have examined but it has limited studies only. The different type of micro plastic exposure, exposure time, as well as physiological and behavioral differences among organisms exposed to micro plastics can show some of these differences in effects. If the organism ingested by the micro plastics, its interact within the gastrointestinal tracts differently in terms of the anatomy and structure of the tract, its mechanical action, transit time and chemical enzymatic action. Further, the characteristics of micro plastics such as size, shape, solubility, and surface properties also play an important role in the toxicity of micro plastics. Micro plastic pollution in the soils harms the fitness of multiple soil organisms, animals and birds underscoring the ecological risk posed by micro plastics within terrestrial ecosystems.

Keywords: Micro plastics and Nano plastics, Terrestrial animals, Terrestrial birds, Human-beings and Ecosystem

Over the past 50 years, mass production and population growth have resulted in the explosion of micro plastics, both as marketable products and the humiliation of larger plastic materials, the various sources and unique properties of primary and secondary micro plastics will abode tasks on an underprepared waste infrastructure. In addition to reducing or replacing primary micro plastics and the large plastics from which secondary micro plastics are formed, the release of micro plastic fibres, tire fragments, and abrasives must also be addressed. One of the most abundant types of micro plastics in the environment is polyester fiber. A major fraction of the fibres inflowing to wastewater treatment plants completion in sewage sludge and used as soil fertilizer in many countries. As their effects in the terrestrial environment are still unwell understood, Still fibres have been recognized as one of the major type of plastics in soil. Their adverse effects on soil invertebrates and possible entry into terrestrial food webs are still unknown. Recent reports of micro plastics and the effects on soil invertebrates generally studied are polyethylene (PE), polystyrene (PS) and polyvinylchloride (PVC) non-fibrous particles (micro beads, films, pellets, fragments). With the massive ingesting of plastic products, global plastic manufacture currently has beaten 290 million tons annually and will touch 33 billion tons by 2050 (Rochman et al 2013). Due to the low reclamation rate (<5%) and high environmental persistence, plastic debris consists of the major marine litter (c.a., 80–85%) (Auta et al 2017). As a result, plastic pollution in the marine environment has been familiar as a primary environmental issue (Wright et al 2013a, 2013b), resulting in an economic loss of $US13 billion annually around the world (UNEP 2015). Acting as the universal component of plastic litter (Andrady 2011, Auta et al 2017), MPs can be of primary (industrially manufactured to be micro and/or nanosized) and/or secondary (breakdown from large plastic items) origin, their surfaces can be familiarized with different functional groups (e.g., -COOH, -NH_2) upon photo degradation and biodegradation (Andrady 2011). Secondary MPs are small plastic fragments which decomposed from larger plastics (Rillig et al 2017). Since their size range closely conforms to plankton (e.g. Algae) at the bottom of the marine food web, MPs thereby could be available to a wide range of marine biota (e.g. Copepods, crustaceans, fish and mammals) via feeding and trophic transfer (i.e., consumption of prey containing MPs) along the food chain (Wright et al 2013a, 2013b, Galloway et al 2017, Luan et al 2019).

Several investigational studies have been completed with high concentrations of micro plastics regarding those found in nature. However, Duis and Coors (2016) pointed out that micro plastics are highly persistent and their concentration is very likely going to rise considerably in the next decades. Some hotspots in which micro plastics are present at tremendously high concentrations already present. The future potential higher plastic levels, the results obtained in studies not using significant environmental concentrations should not be rejected. Elements affecting MP behaviour and passage in the atmosphere may also be comparable to those of fine particulate matter including vertical pollution concentration gradient (higher concentrations close to the land), wind speed (increasing of wind speed lead to a decrease in concentration), wind direction (downwind, upwind, and parallel directions), precipitation, temperature and humidity. Also, urban topography (e.g. tall buildings, trees and space between buildings) can disturb wind modulation and distribution of air pollutants in urban environments. Lighter polymers can be transported easily by the wind and additional contaminate the terrestrial and marine environments (Horton et al 2017, Karbalaei et al 2018).

Most plastics received in the oceans were manufactured, used, and often disposed on land. Hence, it is surrounded by terrestrial systems that micro plastics influences first interact with biota producing ecologically relevant impacts. The pervasive micro plastic contamination as a potential agent of global change in terrestrial systems highlights the physical and chemical nature of the respective observed effects, and the broad toxicity of nano plastics derived from plastic breakdown. The fate of micro plastics in aquatic continental systems, insights into the mechanisms of effects on terrestrial geochemistry, the biophysical environment, and ecotoxicology. General changes in continental environments are likely even in particle-rich habitats such as soils. The growing body of evidence indicating that micro plastics interact with terrestrial organisms that mediate essential ecosystem services and functions, such as soil-dwelling invertebrates, terrestrial fungi, and plant-pollinators (de Souza Machado et al 2018).

The crucial pollution which is widely distributes in the environment is the micro plastics (MP). Recently, the studies of MP have increased rapidly due to increasing awareness of the potential and growing risks of biological effects during storage and disposal. Still, due to the limitations in analytical methods and the methods of environmental risk assessment, the distribution and biological effects of MP are still debatable issues (Zhang et al 2019). This review focuses on the occurrence, sources, and transport of MPs in terrestrial environments and applicable regulations to mitigate the impacts of MPs. This study also highlights the importance of personality traits and cognitive ability in reducing the entry of MPs into the environment.

Microplastics: Micro plastics are nominally divided into two distinct sources, primary micro plastics, which are those produced below five (or one!) millimeters in size, and secondary micro plastics, those made due to the fragmentation of bigger macro plastics or “parent material”. Sources of primary micro plastics include the feedstock for the plastic manufacturing process (nibs, nurdles, and powder), scrubbing particles in cosmetic goods, household cleaning products, glitter, and shot blasting media. Secondary micro plastics may be divided into those formed from plastics during the useful life span of the parent material and those formed after its beating to the environment. Secondary micro plastics sources include abrasion of car tires, the wear of boat rigging and fishing gear during operation and the establishment of microfibers during domestic clothes washing. The latter has only recently come to light as a significant contributor to global micro plastic pollution. Man-made fibres are common in the production of clothing throughout the world and the action of washing results in the shattering of fibres within the garment.

Production of plastics has been growing for more than 60 years, as the durable, primarily petroleum-based material gradually substitutes materials like glass and metal. Nowadays, plastics are used widely in a growing range of applications such as packaging industry, building and construction, automotive industry, textiles, electrical and electronics, agriculture, household applications, health, and safety equipment (Dehghani et al 2017). Analysis of micro plastics from different environmental samples requires a sequence of procedures including sampling, separation, clean-up and identification. Although several studies on method development and/or comparison for sampling, separation, clean-up and identification have been carried out, it is still critical to improving methods to yield more precise and accurate results. Among these identification methods, the most widely used should be evaluated for their relevance to future studies. Recently, small-sized micro plastics have been found in the marine environment and the abundance of micro plastics increased exponentially with decreasing particle size. The smaller micro plastics are more difficult to identify. Ambiguous characteristics of non-plastics (resembling plastics) and plastics (resembling non-plastics) make it difficult to accurately identify micro plastics (Song et al 2015).

Micro plastics (MPs) pollution is an emerging environmental and health concern. Micro plastic influences on benthic organisms could shake higher trophic levels (e.g., trophic energy transfer or trophic interactions). Similar waves may also occur in pelagic habitats where micro plastics can reach densities higher than naturally occurring planktonic organisms (Lechner et al 2014). Micro plastics in freshwater may have carry-over various effects to terrestrial systems, as many freshwater organisms are prey to terrestrial insects, amphibians, reptiles, and birds. Some forest birds receive up to 98% of their resources from aquatic prey. Potential exists for micro plastic transfer across habitats via animal migrations, much the way anadromous fish transfer marine nutrients to freshwater systems. Other habitat-related effects of micro plastics include their role as a substrate for egg-laying organisms or as habitat for encrusting organisms, rafting communities and microbial communities (Carson 2013, Zettler et al 2013). Micro plastics serve as novel ecological habitats for microbes and may provide a substrate for opportunistic pathogens.

MPs are entering the human digestive system via several sources such as drinking water (Eerkes –Medrano et al 2018, Koelmans et al 2019), table salt (Yang et al 2015), seafood products (Li et al 2016a, Jabeen et al 2017, Barboza et al 2018), and even tea bags (Hernandez et al 2019). Consequently, there is growing concern about the intake of MPs and the related health risks to the human being (Carbery et al 2018). The alternative possible for the entry of MPs into human is the land- dwelling animal material, which is consumed broadly as well as used in making traditional medicine (Lu et al 2020) and also stated that micro plastic contaminants can be existing in chicken meat and feces (Yan et al 2020). The micro plastic content was observed in human lungs and stools (Schwabl et al 2019). The quantity of micro plastics in an organism's tissues or organs has so far been recognized as a challenging factorin the present situation (Lee et al 2019, Huang et al 2020).

Origin and distribution of micro plastics: In early 1940s, itself humans have been mass-producing plastics since and production has increased widely in subsequent years. Approximately 240–280 million tonnes of plastic have been produced annually since 2008, compared to an annual production rate of 1.5 million tonnes in 1950 (Cole et al 2011, Wright et al 2013b). About 50% of plastic produced as a main contributor is the packaging materials which is disposed after one time use. The plastics entering the natural environment has intermediate life spans and come from durable consumer products, such as electronics and vehicles is the another 20–25% (Hopewell et al 2009). Most plastics are extremely durable and can persist from decades to millennia in their polymer forms (Thompson et al 2004,Hopewell et al 2009). The durability of plastics causes to persist and contaminate environments worldwide. Marine habitations are mostly affected (Lithner et al 2011). Micro plastics constitute plastics that are <5 mm, as classified by the National Oceanic and Atmospheric Administration (NOAA), and they are present in a heterogeneous array of shapes and sizes (Betts 2008, Hidalgo-Ruz et al 2012, Wright et al 2013b). Some of them classify the micro plastics with an upper size limit of 1 mm (Browne et al 2009) and, upper size limits of 1 mm and 5 mm are currently acceptable to describe micro plastics in the studies. The most noticeable micro plastic forms contaminating the marine environment are spheres, pellets, irregular fragments, and fibres (Wright et al 2013b). They are universal throughout the global oceans, and micro plastics (<1 mm) in the water column and seabed have been detected to weigh 100 times and 400 times more than macro plastic debris (Van Cauwenberghe et al 2013). In water column, sediments, and the deep sea, with highest concentrations along populated coastlines and within mid-ocean gyres micro plastics are distributed (Cole et al 2011, Wright et al 2013b). The spatial distribution of micro plastics discovered that accumulation is higher at downwind sites and in areas with decreased water flow. A relationship has yet to be observed between micro plastic concentrations and grain size distribution and the extent of micro plastic contamination to the marine environment is still largely unknown (Browne et al 2008, 2011, 2021). Plastics are synthetic organic polymers, created by polymerization of monomers extracted from crude oil and gas (Cole et al 2011). Some of the most prominent plastic polymers found in the environment include polystyrene (most commonly used in packaging and industrial insulation), acrylic, polyethylene (used in facial scrubs), polypropylene (commonly used in fishing gear), polyamide (nylon), polyvinyl chloride (PVC), and polyester fragments (Browne et al 2008, 2011). Primary micro plastics are produced at a microscopic size and are integrated into a variability of facial exfoliating cleansers, air-blasting boat cleaning media, and are increasingly used in medicine as vectors for drugs (Cole et al 2011). Secondary micro plastics from when macro plastics undergo mechanical, photolytic, and/or chemical degradation, resulting in fragmented micro plastic pieces and fibres. There is evidence that a primary source of micro plastics is synthetic fibres from garments. The study quantifying micro plastic concentrations at 18 sites worldwide showed that a single synthetic clothing garment can release >1900 micro plastic fibres per wash. These microfibers enter the marine environment via wastewater discharge contain proportions of polyester and acrylic micro plastic fibres resembling proportions used in synthetic clothing (Browne et al 2011, Mathalon and Hill, 2014). In natural environment the plastic pollution is unavoidable. One of the most enduring threats faced by wildlife is the plastic pollution, because of their small size; MPs may presumably also enter the food web and thus potentially end up in human food. The risk that is not yet predictable, because the interaction of MPs with tissue and cells is poorly understood. Investigation of the interaction is further complicated by the fact that MPs are not single compounds but constitute mixtures of different plastic types, each often consisting of a blend of synthetic polymers, residual monomers, and chemical additives. In addition, their morphology may influence their effects.

Effects of micro plastics in terrestrial invertebrates: The micro plastic and nanoplastic pollution is a growing environmental concern that affects the wildlife because of the vast amount of plastic waste emitted into the environment and the increasing concern of potential harm. It has the potential to cause both physical and chemical harm to wildlife directly or via sorption, concentration, and transfer of other environmental contaminants to the wildlife that ingests plastic. Small particles of plastic pollution, termed micro plastics (>100 nm and or nanoplastics (<5mm) can form through fragmentation of larger pieces of plastic. These small particles are especially concerning because of their high specific surface area for sorption of contaminants as well as their potential to translocate in the bodies of organisms. These are challenging to separate and identify in environmental samples because their small size makes handling and observation difficult (Nguyen et al 2019).

The secondary micro plastics are more common in the natural environment; both the primary and secondary micro plastics have increased in frequency over time, bringing potentially injurious effects to wildlife (Andrady 2011, Tanaka and Takada 2016). The ingestion of micro plastics in wildlife has become familiar. The ingestion of micro plastics, organisms is exposed to toxic materials and it cause adverse effect on organisms from ingested plastics is of increasing concern. In marine ecosystems, wide research is currently being shown and in the terrestrial wildlife there is limited research on micro plastic abundance and diversity is shown. Furthermore, in top predatory animals there is lack of research in micro plastic presence. Through passing awareness to plastic pollution in both marine and terrestrial ecosystems, checking that how it is affecting to top predators in the food web and also further conservation efforts can be made to decrease micro plastic presence to other wildlife in similar habitats (Carlin 2020).

In terrestrial environments micro plastics are widespread contaminants but comparatively little is known about interactions between micro plastics and common terrestrial contaminants such as zinc (Zn). The experiments in adsorption fragmented HDPE bags c. one mm^2 in size showed similar sorption characteristics to soil. However, the combination with soil, concentrations of adsorbed Zn on a per mass basis were over an order of magnitude lower on micro plastics. Desorption of the Zn was minimal from both micro plastics and soil in synthetic soil solution (0.01 M $CaCl_2$), but in synthetic earthworm guts desorption was higher from micro plastics (40−60%) than soil (2−15%); suggesting micro plastics could increase Zn bioavailability. *Lumbricus terrestris* earthworms experimented for 28 days in mesocosms of 260 g moist soil containing 0.35 wt % of Zn-bearing micro plastic (236−4505 mg kg−1) ingested the micro plastics, but there was no evidence of Zn accumulation, mortality, or weight change. Digestion of the earthworms showed that they did not retain micro plastics in their gut. These findings indicate that micro plastics could act as vectors to increase metal exposure in earthworms, but that the associated risk is unlikely to be significant for essential metals such as Zn that are well regulated by metabolic processes (Hodson et al 2017).

On soil invertebrates little research has focused. The recent research reported that exposed the soil springtail, *Folsomia candida* to artificial soils contaminated with polyethylene MPs (<500 mm) for 28 d to explore the effects of MPs on avoidance, reproduction, and gut micro biota. Springtails exhibited avoidance behaviors at 0.5% and 1% MPs (w/w in dry soil), and the avoidance rate was 59% and 69%, respectively. Reproduction was inhibited when the concentration of MPs reached 0.1% and was reduced by 70.2% at the highest concentration of 1% MPs compared to control. The half-maximal effective concentration (EC50) value based on reproduction for *F. candida* was 0.29% MPs. At concentrations of 0.5% dry weight in the soil, MPs significantly altered the microbial community and decreased bacterial diversity in the springtail gut. Specifically, the relative abundance of Wolbachia significantly decreased while the relative abundance of Bradyrhizobiaceae, Ensifer and Stenotrophomonas significantly increased. The results also demonstrated that MPs exerted a significant toxic effect on springtails and can change their gut microbial community (Ju et al 2019). The studies found that micro-sized plastic particles moved into bio-pores within seconds and that this influx disrupted the movement of springtails (*Lobellas okamensis*). In the soil system the springtails moved to avoid becoming trapped, and this behaviour created bio-pores. The springtails within the influx of plastic particles into these cavities subsequently immobilized. This phenomenon was experimental at low concentration of plastic particles (8 mg/kg), and it likely occurs in actual soil environments. The findings of the study indicates that the behaviour of plastic particles in the soil not only disrupts the movement of springtails but also has wider implications for effective management of soils (Kim and An 2019). There is an evidence for the translocation to tissues and micro plastic spheres of 0.5 µm have been shown to translocate to the haemolymph and tissues of crab (Farrell and Nelson 2013). The food consumption and energy expenditure available for growth was reduced after crabs (*Carcinus maenas*) ingested food containing microfibers for 4 weeks. The ingestion of MPs may facilitate transfer of persistent organic pollutants to the organism as a secondary effect (Watts et al 2014). Medaka exposed to a mixture of polyethylene and PBTs (persistent bioaccumulative and toxic substances) bioaccumulated the chemicals and suffered liver toxicity including glycogen depletion, fatty vacuolation, and single cell necrosis (Rochman et al 2013). The recent experiments indicated that the MPs were detected in surface water, sediment, and tadpoles with abundances of fibres and fragments of polyester (PES) and polypropylene. Further the distribution of MPs in tadpoles resembled that of water rather than sediment and MPs supplier was also found to be from surrounding water. Such high abundances of MPs in resident tadpoles strongly support that MPs may transport through the food chain to higher aquatic or terrestrial tropic levels (Hu et al 2018).

As per Song et al (2019), the uptake of appreciable burdens of PET microfibers (MFs) and depuration through the digestive tract in snails, following the appearance of cracks and deterioration on micro plastic surfaces. The prolonged exposure to MFs inhibited feeding and excretion of snails and caused pathological damage in the gastrointestinal tract and also, MFs exposure can reduce T-AOC and GPx activity, but elevate MDA levels, which indicate that oxidative stress is involved in the toxic mechanisms. The findings recommend that in terrestrial ecosystems micro plastic pollution in soils harms the fitness of soil organisms, and highlight ecological risks of micro plastic fibres. The polystyrene of 5µm and 70nm at environmentally relevant concentrations are accumulated in zebrafish liver and gut, producing oxidative stress and inducing alterations of metabolic profiles and disturbing lipid and energy metabolism and similar effects were detected in mice, in one of the very few toxicity studies of micro plastics performed in rodents (Deng et al 2017).

The earthworms are the model organisms of the soil ecosystems, they have been predominantly used as the test species in investigating the effects of soil plastic pollution on organisms (Chae and An 2018). The exposure experiments in the micro plastics present in soils is observed that it can be ingested, transferred, and cause toxic effects (Rillig et al 2017) but in terrestrial invertebrates few studies have reported the accumulation of micro plastics. Rodriguez-Seijo et al (2017) reported that damage in the gut of the earthworm *Eisenia andrei,* causing damage in the epithelium and inflammation of the gut wall, which may be an indicator of accumulation of PE micro plastics (250-1000 mm) that due to the big size of the particles induced harm in the organism. Bioaccumulation of polybrominated diphenyl ethers (PBDEs) was observed in *Eisenia fetida* after 28 days of exposure to polyurethane foam (Gaylor et al 2013) Hepatic stress was detected in Japanese medaka exposed to polyethylene pellets (Rochman et al 2013) and histological alterations of the intestine were detected in European sea bass exposed to polyvinyl chloride pellets (Pedá et al 2016). The effect of plastic covers on soil microbes can propagate in different ways to higher tropic levels of the food web. E.g. by positively affecting overall arthropod diversity and doubling omnivorous insect but decreasing springtail, predatory nematode, ground beetle or earthworm abundances (Steinmetz et al 2016).

Nematodes can ingest MP particles which might adversely affect their reproduction. The toxic properties of MP on nematode reproduction in soils cannot be ruled out. The toxicity risk for conventional and biodegradable MP particles is likely to be the same, as MP toxicity is rather attributable to physical and indirect nutritional effects rather than to chemical effects. Even though its negative effects of MP on the body length of nematodes, since nematodes as key members of the soil food web, may be at risk under MP exposure (Schöpfer et al 2020). Experimental studies in the effects of MP on mussels, lugworms, copepods, and oysters have documented reduced feeding, survival, and fecundity, as well as promoted polychlorinated biphenyl bioaccumulation connected to MP uptake (Lenz et al 2016). PET microfibers can be ingested and depurated throughout the digestive system of terrestrial snails, after transit through the digestive tract, MFs presented cracks and deterioration on the surfaces of MFs. Moreover, prolonged exposure to MFs had negative impacts on feeding and excretion of snails and caused visible villi damages in the stomach and intestine. It's found that MFs exposure can reduce T-AOC and GPx activity, but elevated MDA levels in the liver. These results found that the micro plastic pollutants in the soil are transferred by land snails and that they have physiological effects.

A recent study has found that larvae of *Galleria mellionella* are capable of biodegrading low density PE film. Significant mass loss of plastic was witnessed over a 21-day period with own consumption of 0.88 and 1.95 g by 150 larvae fed only either PS or PE. The formation of C = O and C – O containing functional groups and long chain fatty acids as the metabolic intermediates of plastic in the residual polymers indicated depolymerisation and biodegradation. The changes in the gut microbiome revealed that Bacillus and Serratia were significantly associated with the PS and PE diets. Therefore, the supplementing the c-diet affected the physiological properties of the larvae and plastic biodegradation and shaped the core gut microbiome. The recent study indicated that MPs were detected in surface water, sediment, and tadpoles with abundances of fibres and fragments of polyester (PES) and polypropylene. Moreover, the distribution of MPs in tadpoles resembled that of water rather than sediment and MPs supplier was found to be from surrounding water. Such high abundance of MPs in resident tadpoles strongly support may transport through the food chain to higher aquatic or terrestrial trophic levels.

The effects of micro plastics of *Eriocheir sinensis* show the growth, accumulation and oxidative stress response in the liver. The accumulation of fluorescent micro plastic particles (diameter = 0.5 μm) in the gill, liver and gut tissues of *E. sinensis* were observed when crabs were exposed to a concentration of 40000 μg/L for 7 days. The toxicity test suggested that the rate of weight gain, specific growth rate, and hepatosomatic index of *E. sinensis* decreased with increasing micro plastic concentration (0 μg/L, 40 μg/L, 400 μg/L, 4000 μg/L and 40000 μg/L) for 21 days. The activities of AChE and GPT in crabs exposed to micro plastics were lower than those in control group. GOT activity increased significantly after exposure to a low concentration of micro plastics and then decreased continuously with increasing micro plastic concentrations. The activities of superoxide dismutase (SOD), aspartate transaminase (GOT), glutathione (GSH), and glutathione peroxidase (GPx) increased in specimens exposed to low concentrations of micro plastics (40 and 400 μg/L) compared to the control and decreased in organisms exposed to high concentrations (4000 and 40000 μg/L). The activities of acetylcholinesterase, catalase (CAT), and alanine aminotransferase were significantly lower in the organisms exposed to micro plastics compared to control animals. Upon the exposure of micro plastic concentrations increases, so the expression of genes encoding the antioxidants SOD, CAT, GPx and glutathione S-transferase in the liver decreased after first increasing. The study demonstrates that the accumulation in the tissues of *E. sinensis* and negatively affect the growth. In addition, the exposure to micro plastics causes damage and induces oxidative stress in the hepatopancreas of *E. sinensis* (Yu et al 2018)

The investigators reported that the ingestion of anthropogenic waste, primarily plastic bags and rope by gromedary camels in the United Arab Emirates (UAE) which has led to a regional mortality rate of 1%. The ingested waste was found to be a polybezoar, a collection of tightly packed indigestible materials which can include plastics, ropes, other litter and salt deposits trapped in the stomach or digestive tract forming a large stone-like mass. Further, polybeozars lead to gastrointestinal blockages, leak toxins and sepsis from increased gut bacteria, dehydration and malnutrition give camels a false sense of fullness, so they stop eating and slowly starve to death (Eriksen et al 2021).

Plastic have been observed in digestive tracts of cattle, sheep and goats (Jebessa et al 2018). Plastic materials cannot be digested and may take a long time to pass through the digestive tract or be retained indefinitely when caught in complex digestive tracts. Consequences of plastic ingestion include ruminal impaction, where indigestible plastic foreign bodies accumulate in the rumen which leads to indigestion, the formation of bezoars containing primarily synthetic materials, trumas, poor body condition, immune suppression, reduced health status, and mortality and death within two to three weeks due to organ failure. The ingested plastic rubbish release toxins into the circulatory system, which causes liver values (glutamate oxaloacetate transaminase-GOT (AST), gamma-glutamyl transferase (Y-GT), glutamate –pyruvate –transaminase-GPT (ALT) and kidney values (blood urea nitrogen –BON, creatine 0 to increase steadily, culminating in organ failure. The plastic mass or polybezoar can affect feeding behaviour, resulting in camels eating less until they stop eating completely, as the camel always feels full resulting in a false sense of satiation. The polybezoars which containing poly ethylene, poly propylene and ethylene vinyl acetate also reported. MPs refer to micro beads used in personal care products (PCPP) and plastic products (Jambeck et al 2015), such as shampoos, shower gels, lipsticks, facial masks, and various synthetic textiles. PE or PP are usually used as micro beads or glitter in cosmetics (He et al 2018). Synthetic fibres such as ester and nylon are often used in synthetic textiles (Gong and Xie, 2020). Micro beads used by humans can flow into the sewage and produce primary MPs as the fibres wear and fall off when washed in the laundry or home for synthetic textile clothing (He et al 2018). Therefore, the large-scale use of synthetic textiles and personal care products is also the way for MPs to enter the soil.

Generally the effects of polyester fibres on the soil invertebrates were slight. Energy reserves of the isopods were slightly affected by both fibre types, and enchytraeid reproduction decreased up to 30% with increasing fibre concentration, but only for long fibres in soil. The low ingestion of long fibres by the enchytraeids suggests that this negative impact arose from physical harm outside the organism, or indirect effects resulting from changes in environmental conditions. The short fibres were ingested by enchytraeids and isopods, with the rate of ingestion positively related to fibre concentration in the soil. The study shows that polyester fibres are not very harmful to soil invertebrates upon short-term exposure. The studies found clear evidence for fibre uptake in enchytraeids and isopods, indicating the entry of polyester fibres into terrestrial food webs and potential long-term risks for these organisms and their predators (Selonen et al 2020).

The most of the plastic litter originate from land based sources is considered to entering the aquatic system. That includes recreational activities on shores, inappropriate or illegal dumping of domestic and industrial litter, plastic manufacturing facilities, transportation as well as sewage treatment and surface runoff of street litter (Gesamp 2010). Plastics are also present in the wastes, which can be categorized as municipal, industrial, agricultural, construction and demolition waste. Plastic waste generated by 192 coastal countries worldwide in 2010 was estimated to 275 million tons out of which 2-5% were assumed to be mismanaged and ending up in the oceans (Jambeck et al 2015). Fate of plastic waste in the terrestrial environment is probably not different but not well studied or analysed (Karman et al 2016).

Effects of micro plastics on birds: Numerous studies have dealt with the ingestion of marine debris by seabirds, where micro plastics, essentially pellets and fragments, have been isolated from birds targeted for dietary studies, cadavers, regurgitated samples, and feces (Van Franeker and Law 2015, Herzke et al 2016). Kuhn and van Franeker (2012) found more plastic in the intestines of juveniles than in adults. Its indicate that possibly micro plastics contamination in birds occurs mostly between generations and that the regurgitation process may lead to a breakdown of micro plastics into even smaller particles. The majority of birds examined did not die as a direct result of micro plastic uptake and can be concluded that micro plastic ingestion does not affect seabirds as severely as macro plastic ingestion (Lusher 2015). Most studies of micro plastics in seabirds only analyse micro plastics in the digestive tract (Herzke et al 2016) and feces (Reynolds and Ryan 2018) and thus, at this stage, there is no evidence that micro plastics can cross the intestine barrier and/or enter the bloodstream and accumulate in different organs. No studies have demonstrated nanometre-sized micro plastics in seabird guts or feces and also in respect to terrestrial birds, so far only two papers reported the ingestion of micro plastics. Zhao et al (2016) found fibres and fragments of millimeters in length in the gastrointestinal tract of 17 terrestrial birds. They also observed a decrease in the proportion of natural fibres from the esophagus to the stomach and subsequently to the intestine, which suggests that they may be digestible, although further research in this field is still necessary.

Studies on marine bird species recognized that at least 44% ingest plastics and research recording plastic ingestion in shorebirds (Amélineau et al 2016, Lourenco et al 2017, Provencher et al 2018). The micro plastic accumulation in birds of prey is not in published research. Birds of prey offer interesting insights for potential conservation efforts dealing with plastic pollution. The raptors searching habitats also have the potential to serve as indicators as to where plastic pollution is of greatest concern. Comparing osprey, whose primary diet comes from fish, to red-shouldered hawks, whose primary food source is small mammals and amphibians, can show differential plastic abundances in either the marine, freshwater, or terrestrial ecosystem. Beyond that, this research can shed light on the ability of micro plastics to transfer along with food webs. Studies have shown that higher trophic level organisms have a greater chance of deleterious effects due to accumulation of toxins in micro plastics along with the food web (de Sa et al 2018). As top predators, birds of prey can expand our current understanding on potential bioaccumulation of toxins via micro plastic accumulation.

The diversity of predatory birds' diets suggests that micro plastics will therefore also be present in birds of prey. The majority of literature on micro plastic prevalence in the environment focuses on aquatic ecosystems (Andrady 2011,Li et al 2016b, M'Rabet et al 2018,). Therefore, hypothesized that *Pandion haliaetus* (osprey), which forages primarily on fish from both fresh and saltwater ecosystems, would have the greatest mean abundance of micro plastic per gram of gastrointestinal (GI) tissue when compared to other species. A common source of marine plastic pollution comes from the fragmentation of boat ropes, and therefore polypropylene, nylon, and PET fibres are expected to be most commonly found in *P. haliaetus* (Mathalon, and Hill, 2014). *Buteo lineatus* (red-shouldered hawk), *Strixvaria* (barred owl), *Mega scopsasio* (eastern-screech owl), *Bueto jamaciensis* (red-tailed hawk), and *Accipiter cooperii* (cooper's hawk) diet is composed predominantly of small rodents and terrestrial reptiles and therefore a greater mean abundance of “user plastics” (i.e. trash, recyclable materials) can be expected to be found in the gastrointestinal tract of such species (Carlin 2020).

The studies say that terrestrial birds of prey may experience the bioaccumulation of micro plastics than aquatic species foraging at comparable trophic levels. It was conducted in a highly urbanized environment where rodents have a higher likelihood of relying on sources of anthropogenic waste for sustenance, therefore, increasing their chance of exposure to micro plastics. As a result, birds of prey feeding within such terrestrial food webs may experience the higher levels of bioaccumulation of micro plastics in anthropogenic materials. Fish and their food sources may be exposed to lower concentrations of micro plastics than rodents because rodents experience a direct source of anthropogenic litter from foraging in trash-cans and landfills. Furthermore, terrestrial birds of prey are not only exposed to micro plastics through secondary sources but are often exposed directly from foraging in landfills (Karbalaei et al 2018).

Chickens seem to take up plastics mainly from the plastic residues on the soil surface and therefore, MP found in soils and those found in Chicken feces were not correlated. Earthworms seem to bio concentrate MPs stronger in casts if the MP content in soil is low. This fact was well described by Lwanga et al 2016. The small number of MPs found in soil (0–2 particles/g) led to a higher number of MP being detected in casts, chicken faeces, and chicken gizzards (ratios of 12.7, 105, and 5.1 respectively). This bio concentration could explain the higher concentration of larger MPs being found in casts than in soil. Further studies are required to better understand this behaviour. Under natural conditions, earthworms ingest the equivalent of their weight each day (Lavelle and Spain 2001). MPs measuring between 0.1 and 5 mm were found in the gizzard and feces. In Mexico, chicken consumption per capita is around 15 chickens per person per year (Gallardo Nieto 2004). This translates into annual possible ingestion of 840 plastic particles per person. Consumption of domestic chickens (gizzards) around the world in traditional dishes (Fischer 2010) may potentially expose humans to high concentrations of MPs, either directly by consuming gizzards such as in their study, or indirectly through bio augmented MPs from the chicken's digestive system into their tissues (Lwanga et al 2017).

Transferring of micro plastics: Transferring of micro plastics from the intestinal tract to the surrounding tissue or circulatory system after ingestion, it can remain in the digestive tract, be excreted, or absorbed from the digestive tract into the body tissue (Browne et al 2007, 2008). Lugworms (*Arenicola marina*) exposed to sediment containing pre-production PS particles (400–1300 µm, 7.4 % of sediment dw) ingested these micro plastics. However, no translocation of the relatively large PS particles from the gut to the tissue was recorded (Besseling et al 2013). Hämer et al 2014 fed marine isopods (*Idotea emarginata)* with food containing fluorescent PS microspheres (10 µm), PS fragments (1–100 µm), or acrylic fibres (0.02–2.5 mm). Micro plastics were detected in the stomach and intestine, but not in the midgut where nutrients are reabsorbed. Passage of the micro plastics to the midgut was most likely impeded by filter structures in the isopods' proventriculus. In *D. magna*, fluorescent-carboxylated PS nano- and microspheres (20 nm and 1 µm diameter) were mainly observed in the gastrointestinal tract, but also in structures assumed to be oil storage droplets. It was concluded that PS spheres can cross the gut epithelium (Rosenkranz et al 2009). Inshore crabs (*C. maenas*), which were fed with mussels (*Mytilus edulis*) pre-exposed to PS microspheres, translocation from the intestinal tract to hemolymph, hepatopancreas, ovary, and gills were demonstrated for microspheres with 0.5 µm diameter (Farrell and Nelson, 2013). By contrast, larger microspheres (8–10 µm diameters) were only detected in the intestine but not in the hemolymph of shore crabs (Watts et al 2015, Browne et al 2008) when kept mussels (*M. edulis*) for 3 h in a suspension of fluorescent PS microspheres (3.0 and 9.6 µm, 4.3 × 104 items/L). Microspheres were detected in the hemolymph and inside the hemocytes. The smaller microspheres occurred in significantly higher abundance in the hemolymph than the larger ones. Von Moos et al 2012 exposed mussels for 3–96 h to 2.5 g/L of HDPE fluff consisting of non-uniformly shaped particles with a size between 0 and 80 µm. The concentration of HDPE fluff corresponds to approx. 2.7×10^7 to 3.6×10^7 items/L (NR von Moos, personal communication). HDPE micro particles were detected on the gill surface and in blood lacunae of the gills, as well as in the intestine, digestive gland, and connective tissue. In a very recent study, mullets (*Mugilcephalus*) were held for 7 days in water containing 33.8 mg/L of PE or PS particles with a size of 0.1–1 mm (nearly 2500 particles/L Avio et al 2015). Micro plastics were not only found in the gastrointestinal tract (approx. 10 PE particles and 90 PS particles per fish) but also the liver of the fish (approx. 1–2 particles per fish for both PE and PS). Thus, based on the results of laboratory experiments, translocation from the intestinal tract to the circulatory system or surrounding tissue depends on the size of the micro plastics with an upper size limit for translocation that appears to be specific for the species or taxonomic group (Duis and Coors 2016).

Micro plastics and antimicrobials are widely spread environmental contaminants and more research on their toxicity is needed. In *Corbicula fluminea* were investigated that the uptake and effects of the antimicrobial florfenicol, micro plastics, and their mixtures. Micro plastics were found in the gut, lumen of the digestive gland, connective tissue, hemolymphatic sinuses, and gills surface of animals. Florfenicol caused a significant inhibition of cholinesterase (ChE) activity (~32%). Animals exposed to 0.2 mg/l of micro plastics showed ChE activity inhibition (31%), and no other significant alterations. Mixtures caused feeding inhibition (57–83%), significant ChE inhibition (44–57%) and of isocitrate dehydrogenase activity, and increased anti-oxidant enzymes activity and lipid peroxidation levels (Guilhermino et al 2018).

In a tropical home garden in Mexico, it is possible to find earthworm biomass of 5 to 31 gm^{-2}, (Huerta and Wal 2012), which means that 5 to 31 gm^{-2} of soil is taken up daily by earthworms. The earthworm casts then concentrate the MPs present in the soil as a consequence of direct ingestion of the soil and the MPs probably accumulate in earthworm tissues (Lwanga et al 2016). Small particle selection by earthworms seem to be always present (Shipitalo and Protz 1989, Barois et al 1993) and reflected the highest concentration of MP per gram of cast were found within the size of 10–50 µm. Chickens mainly ingest macro plastics found on the soil surface since plastic debris >5 mm was present in the chicken crops and gizzards. Nevertheless, MPs measuring between 0.1 and 5 mm were found in the gizzard and faeces. Therefore, we assume that MPs in chickens may originate from the transformation of MaPs (macro plastics) to MPs during the passage through the digestive canal, ending up in the gizzard as a mixture of MaPs and MPs and resulting in the excrement as MPs. Under laboratory conditions, plastics ingestion by chickens reduced food consumption and the volume of the gizzards since plastic particles are well retained in the gizzards. The second study found micro plastics in the chicken crop, gizzards and faeces (Lwanga et al 2017) the presence of micro plastics in the crop or gizzards is not surprising since the earthworms also contained micro plastics in their digestive tract. On the other hand, the presence of micro plastics in feces is an indicator that these particles are excreted. More research is required to confirm if the totality of particles is excreted or if a portion can accumulate in bird bodies (Ribeiro et al 2019). The presence of MPs and/or MaPs in chicken organs (i.e. gizzards) may have negative consequences for human health (Duis and Coors 2016). This carries a potential risk to human health when local people consume polluted gizzards that are not thoroughly cleaned. Even thoroughly cleaning the gizzards would not guarantee that all of the plastic debris and chemical residues would be removed because of hydrophobic and hydrophilic interactions. In studies of aquatic birds, some plastic-derived chemicals (i.e. polybrominated diphenyl ethers) were biomagnified in their tissues (0.3–186 ng/g-lipid) while plastic debris was found in their stomachs (0.04–0.59 g/bird), Tanaka et al (2013).

Physically, micro plastics can interfere with the digestive process of aquatic animals and cause intestinal blockage, reducing animal feeding and energy assimilation (Besseling et al 2013). Furthermore, the high intake of plastics might diminish the uptake of nutritious food, thus leading to reducing energy and fertility, as demonstrated by Lee et al 2013 and Cole et al 2015 in copepods. This situation might represent a problem for species of commercial interest, due to yield reduction and the consequent economic loss. These effects are generally related to the size of micro plastics. Particles with size above 150µm are probably not absorbed and might produce local inflammatory effects, in contrast, particles of smaller sizes might induce systemic exposure, and the smallest fractions as small as 1.5µm, might penetrate the organs (EFSA 2016). In this perspective, nano plastics represent the most concerning problem as pointed out by Bouwmeester et al 2015.

Impact on human-beings: The concern of micro plastics pollution is a key whether they represent a risk to ecosystems and human health. However, there is much uncertainty associated with this issue. Data on the exposure and effect levels of micro plastics are therefore required to evaluate the risk of micro plastics to environments and human health. The adverse effects on organisms that are exposed to micro plastics can be separated into two categories: physical effects and chemical effects. The former is related to the particle size, shape, and concentration of micro plastics and the latter is related to hazardous chemicals that are associated with micro plastics. Though data on micro plastic exposure levels in environments and organisms have rapidly increased in recent decades, limited information is available on the chemicals that are associated with micro plastics. The combination of various kind of polymers of different sizes and shapes that are joined to the action of a large amount of additives that originate from plastics results in a cocktail of contaminants that not only alter the nature of plastic but can leach into the air, water, food, and, potentially, human body tissue during their use or their disposal, thus exposing us to several chemicals together (Campanale et al 2020).

"World Health Organization" (WHO 2019) emphasized the ubiquitous micro plastics presence in the environment and aroused great concern regarding the exposition and effects of nano and micro plastics on human health (Sharma and Chatterjee 2017,Revel et al 2018, Rist et al 2018, Bradney et al 2019, Lehner et al 2019, Campanale et al 2020). One of the major nano and micro plastic entry points into the human system is represented by the ingestion of contaminated food (Silva-Cavalcant et al 2017, Wright and Kelly, 2017, Waring et al 2018, Toussaint et al 2019 Humans could also assume an estimated intake of 80 g per day of micro plastics via plants (fruits and vegetable) that accumulate MPs through uptake from polluted soil (Ebere et al 2019). The presence of micro plastics in marine species for human consumption (fish, bivalves and crustaceans) is now well-known (Smith et al 2018). As an example, in *Mytilus* *edulis* and *Mytilus galloprovincialis* of five European countries, the micro plastic number has been found to fluctuate from 3 to 5 fibers per 10 g of mussels (Nelms et al 2016). Therefore, following exposure via diet, uptake in humans is plausible, as evidenced by the capacity for synthetic particles smaller than 150 µm to cross the gastrointestinal epithelium in mammalian bodies, which causes systemic exposure. However, scientists speculate that only 0.3% of these particles are expected to be absorbed, while a lower fraction (0.1%) that contains particles that are bigger than 10 µm should be capable of reaching both organs and cellular membranes and passing through the blood–brain barrier and placenta (Barboza et al 2018). Exposure concentrations are predicted to be low, although data about micro and nanoplastics into the environment are still limited due to the analytical and technical complications to extract, characterize, and quantify them from environmental matrices (Campanale et al 2020).

The uptake of plastic particles by humans can occur through the consumption of terrestrial and aquatic food products, drinking water, and inhalation (Vethaak and Leslie, 2016). Despite seafood being a recognized source of contaminants to the human diet, the occurrence of micro plastics in seafood is neither quantified nor regulated (Ziccardi et al 2016). Seafood may be contaminated with micro plastics through the ingestion of natural prey, adherence to the organism's surface, or during the processing and packaging phase (Cole et al 2013, EFSA 2016). Organisms that are eaten the whole present a greater risk of exposure compared with those having had the digestive tract removed. With plastics already present in a diversity of seafood items, there is strong support for the transfer of micro plastic particles to humans. Potential health effects resulting from the bioaccumulation and biomagnification of micro plastics and chemical contaminants in the human body. The translocation of PS and PVC particles <150 µm from the gut cavity to the lymph and circulatory system. Very fine particles are capable of crossing cell membranes, the blood-brain barrier and the placenta, with documented effects including oxidative stress, cell damage, inflammation and impairment of energy allocation similar to that reported for marine organisms (Vethaak and Leslie 2016).

There is mounting evidence of the occurrence of plastic particles in marine organism that are part of the human food chain and this might also represent a potential threat to human health via biomagnification. A possible exposure pathway of humans to micro plastic is represented by the diet, especially since there are studies available that demonstrate the presence of micro plastic in commercially important fishes, shrimps and mussels (Devriese et al 2015, Romeo et al 2015, Van Cauwenberghe and Janssen 2014). Microscopic fibers ranging from 200-1500 µm have been found in mussels (average 3.5 fibres/10 g mussel) from Belgian stores which was in the same range as wild caught mussels in the same study (De Witte et al 2014). Furthermore, synthetic fibers were reported in 63% of commercially important brown shrimp caught in the Southern North Sea and Channel area (Devriese et al 2015). There are also studies that reported non-marine sources of micro plastic in the food chain. For example nineteen honey samples were analyzed for colored fibres and fragments and colored material was found in all of the samples (Liebezeit and Liebezeit 2013). Exposure to hydrophobic contaminants can be a direct result of the ingestion of contaminated micro plastic particles, while secondary exposure can occur by ingesting fish, birds or other organisms that have accumulated contaminants within their tissue from previously egested micro plastics (Ziccardi et al 2016). Once inside the human digestive tract, intestinal uptake of the ingested particles may occur. Translocation of various types of micro-particulates across the mammalian gut has been demonstrated in multiple studies involving rodents (particle size 0.03- 40 mm), rabbits (particle size 0.1-10 mm), dogs (particle size 3-100 mm) and humans (particle size 0.16-150 mm). Using 2 mm latex microspheres in rodents, it was shown that intestinal translocation of micro plastics is low (0.04-0.3%) (Carr et al 2012). However, contrasting reports exist on the upper size limit of particles capable of being translocated and the magnitude of this type of transport. Through the M-cells micro plastics can enter the lymphatic system. This transport is governed by particle size: in rats, larger particles (5-10 mm) remained in Peyer's patches, while smaller particles

As potential sources of the contamination natural and synthetic fibers in clothing that become airborne, materials that were used during the production process and bottles that might have been already contaminated or became contaminated during the cleaning process were pointed out. A study on 15 different table salts in China demonstrated the presence of micro plastics in the samples (Yang et al 2015). The amount of micro plastics ranged from 550 – 681 particles/ kg in sea salts, 43 – 364 particles/kg in lake salts and 7 – 204 particles/kg in rock/well salts. Sea salts were found to be significantly higher contaminated with micro plastics than other salts which underline the contamination of marine products. In sea salts particles measuring less than 200 µm were detected to be the predominant type of micro plastic, accounting for 55% of the particles with PET as the most abundant polymer type followed by PE and cellophane.

Furthermore, a few studies have demonstrated the presence of micro plastics in freshwater systems which might be a reason to raise concern about the presence of micro plastic in drinking water since human population is highly dependent on freshwater systems for drinking water supply and food resources (Eerkes-Medrano et al 2015).

The interaction of plastic particles themselves with tissues and cells in humans is still poor. However, the physical effects of particles observed to date in human cells and tissues and animal models give insight into the possible risks of particle exposure in humans. The studies show that plastic particles can cause lung and gut injury, and especially very fine particles can cross cell membranes, the blood-brain barrier, and the human placenta. Observed effects include oxidative stress, cell damage, inflammation, and impairment of energy allocation functions. very fine plastic particles carrying chemical substances can cross cell membranes and may enhance the chemicals' bioavailability, analogous to nano-sized polymeric drug delivery vehicles that facilitate uptake, distribution, and delivery of pharmaceutical agents in human systems (Vethaak and Leslie 2016). Although the effects of consuming MPs on human health are largely unknown, potential pathways for harm have been suggested (Wright et al 2017, Prata 2018).Once MPs are in the gut, they can release constituent monomers as well as additives and absorb toxins, which can cause physiological harm ranging from oxidative stress to carcinogenic behaviour (Wang et al 2018). The MPs can further penetrate the human body via cellular uptake in the lungs or gut as well as by paracellular transport in the gut (Wright and Kelly 2017). The degree of uptake will vary according to the shape, size, solubility, and surface chemistry of MPs. Particles on the scale of a few microns or less may be directly taken up by cells in the lungs or gut, while particles up to 10 µm may be taken up by specialized cells in the Peyer's patch of the ileum (Powell et al 2010). Particles as large as 130 µm can enter tissue through paracellular transport in the form of perception, although the rate of particle transfer to blood over 24 h may be as low as 0.002% Given the data limitations surrounding the size classes of micro plastic particles present in consumed items, it is still unclear to what extent our estimate of human consumption of MPs poses a risk to human health (Cox et al 2019). Once particulate plastics and associated trace elements enter marine organisms, they can then make their way up the food chain where humans eventually ingest them. Human exposure can occur not only through the consumption of seafood but also through consuming water, beer, or salt contaminated with particulate plastics. Once in the gut, particulate plastic may have the potential to affect the digestive and immune systems of humans. However, the effects surrounding the exposure of humans to trace element-sorbed particulate plastics are largely unknown (Bradney et al 2019).

The intake of micro plastics by humans is by now quite evident. The entry point may be through ingestion (through contaminated food or via trophic transfer), through inhalation, or through skin contact. Following the intake of micro plastics into the human body, their fate and effects are still controversial and not well known. Only micro plastics smaller than 20 µm should be able to penetrate organs, and those with a size of about 10 µm should be able to access all organs, cross cell membranes, cross the blood–brain barrier, and enter the placenta, assuming that a distribution of particles in secondary tissues, such as the liver, muscles, and the brain is possible. Not enough information is available to fully understand the implications of micro plastics for human health, however, effects may potentially be due to their physical properties (size, shape, and length), chemical properties (presence of additives and polymer type), concentration, or microbial biofilm growth. How toxic chemicals adsorb/desorb onto/from micro plastics is not well known, but plausible mechanisms include hydrophobic interactions, pH variations, the ageing of particles, and polymer composition. Furthermore, not enough studies have fully explained the primary sources of pollutants that are present on micro plastics and whether their origin is extrinsic from the surrounding ambient space, intrinsic from the plastic itself, or, more probably, from a combination of both and from a continuous and dynamic process of absorption and desorption that is related to the spread of the particles into the environment and to their consequent exposure to weathering (Campanale et al 2020).

Further effects related to the plastic polymer itself are not described however knowledge can probably be extracted from the field of medical transplants using polymer materials of different types. Another concern in regard of exposure of micro plastics to humans is the plastic-associated chemicals (PACs) such as bisphenol A and phthalates. These compounds are well-known as endocrine disruptors and interfere with the hormone system. In one population-based human study levels of BPA and several phthalate metabolites were associated with lipid infiltration of the vascular wall and therefore suggesting that these chemicals play a role in atherosclerosis (Lind and Lind 2011). Furthermore BPA was reported to be positively associated with cardio vascular disease and prevalent myocardial infarction in a cross sectional analysis of 1455 adults (Lind and Lind, 2012, Kärrman et al 2016).

CONCLUSION

The research is required to explain the terrestrial fate and effects of micro plastics due to the widespread presence, environmental persistence and various interactions with continental biota, micro plastic pollution might represent an emerging global change threat to terrestrial ecosystems. The studies indicated that micro-sized plastic particles may affect the soil environment, and this could be linked to the behaviour of plastics in the soil system and how these particles are influenced by biological responses. Soil-dwelling organisms play a key role in modifying the soil system by constructing bio-pores, and these structural changes are potentially related to the behaviour of plastic particles. Most plastic polymers have hydrophobic (unwatchable) characteristics which can cause changes in soil structure such as bulk density and particle aggregation. As these soil properties are directly linked to the behaviour of soil organisms, the relationship between biological behaviour and plastic contamination in soil systems should be determined. The effects of MP exposure on earthworms, *Lumbricus terrestris*, and showed that MPs significantly increased mortality and reduced the growth rate of the earthworms. Additionally, the bioaccumulation of the smallest MPs (smaller than 50 mm) by earthworms may influence the fate and risk of MPs in the terrestrial system. However, more information is needed to assess the risk of MPs in soils. Plastic debris is a prolific, long-lived pollutant that is highly resistant to environmental degradation, readily adheres to hydrophobic persistent organic pollutants and is linked to morbidity and mortality in numerous soil organisms. The prevalence of MPs within the natural environment is a symptom of continuous and rapid growth in synthetic plastic production and mismanagement of plastic waste. Humans have evolved with oral exposure to dietary micro particles and nanoparticles as a normal occurrence but the ever-growing exploitation of nanotechnology is likely to increase exposure further, both qualitatively and quantitatively. Moreover, unlike the situation with respirable particles, relatively little is known about gastrointestinal intake and handling of nanoparticles. Further, extensive experimental and in depth research investigations regarding the impact of long term micro plastics ingestion along with food/feed sources, trophic transfer and their metabolic implications on animal production such as poultry, goat, sheep and cattle and livestock management attributes in functional ecosystem are needed.

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Fodder Quality Assessment Through Remote Sensing: A Review

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Abstract: This paper reviews the approach and techniques of remote sensing based fodder quality estimation. Timely knowledge of the fodder quality is important to meet the demands in the animal feeding. Remote sensing (RS) is a promising technology for assessing farm level fodder quality as compared to conventional methods. Various studies have been conducted on RS based fodder quality assessment at the farm level in the last two decades. In most of the studies handheld spectroscopy has been used for the small farm area, and aircraft or an unmanned aircraft vehicle (UAV)-based Hyperspectral imagery (HSI) or spectroscopy for the large area. In most of the studies, spectroscopy has been commonly used for fodder quality assessment to extract crude protein (CP), neutral detergent fibre (NDF), dry matter (DM) and acid detergent fibre (ADF) parameters for limited areas. While aircraft or UAV-based spectroscopy is being used for the large areas. These studies also suggest that multi-spectral imagery (MSI) data can be captured using UAVs and the Sentinel-2A/B satellite, while HSI data can be acquired by handheld or UAV-based hyperspectral cameras.

Keywords: Acid detergent fibre, Crude protein, Dry Matter (DM), Fodder quality, Hyperspectral, Neutral Detergent Fibre (NDF), Unmanned Aerial Vehicle (UAV)

Livestock has long been a symbol of wealth and power in all civilizations and India is blessed with a wide variety of livestock, such as "56.7% of world's buffaloes, 12.5% cattle, 20.4% small ruminants, 2.4% camel, 1.4% equine, 1.5% pigs and 3.1% poultry (Report of Animal Husbandry & Dairying 12th Five Year Plan, 2012-17)". The significance of livestock in India is well recognized for agriculture sector (ICAR-IGFRI, Vision 2050, 2013). As per the 19th livestock census report, India has the world's largest livestock population (512.06 million). Despite having the world's largest livestock population and highest milk production (176.35 million tonnes in 2018-19), the productivity of Indian livestock is lower than the global average and even lower than that of European countries (Rajendran and Mohanty 2004, Pratap and Jha 2005).

This largest population of livestock in the world plays a variety of roles in ensuring food security by supply of milk and meat and also eliminating unemployment in India. Accurate pasture management and feed planning are important aspects in increasing the profitability of livestock production. Updated information on concurrent field conditions such as harvest time, fertilizer rates and fodder procurement will help farmers to manage their feed for livestock (Schellberg et al 2008). Because, the agriculture sector is the backbone of India and plays a vital role in providing livelihood to more than half of India's population despite its declining contribution to India's GDP (Sasmal 2016). Livestock is the most important of the various enterprises under the vast umbrella of the agriculture sector.

In 2050, people will need about 400 million tonnes of milk and 14 million tonnes of meat. While in 2011, about 122 million tonnes of milk and 5 million tonnes of meat production were made only (IGFRI, vision 2050). Supplying nutritious fodder is an essential part of the dairy industries, while reducing the cost of quality livestock products requires a feed-based cost-effective feeding approach as feed alone accounts for 60-70% of the milk production cost. Therefore, any effort to increase fodder availability and reduce fodder costs will helpful in increasing profit margins for livestock owners as well. Presently, the country is facing a shortage of 35.6% green forage, 10.95% dried crop residue and 44% concentrated feed material (IGFRI vision 2050). In India, only 4.2 to 4.4% of crop land is devoted for fodder cultivation and there is a rare chance to expand the fodder growing area due to intensive use of agricultural land for food and other crops. Due to the scarcity of cropland for food production and the adverse effects of climate change, increased attention has been paid to forage production in controlled environmental settings in the recent years (Ahamed et al 2023).

Low production of quality and quantity of green fodder is one of the major reasons to prevent high production of dairy animals in India (Gupta et al 2019). This problem can be prevented by adopting proper agricultural practices in producing high quality fodder such as maize, oats and berseem. Maize is one of the most popular fodder crop in India as well as in the world. It has a higher growth rate and yield and is more adaptable, digestible, and palatable (Chaudhary et al 2014). "On the dry weight basis, average nutritional content in fodder maize is 20.5-24.7% dry matter (DM), 5.5-8.7% crude protein (CP), 23.1-30.2% crude fibre (CF), 64. 1-72.8% neutral detergent fibre (NDF), 38.3-46.8% acid detergent fibre (ADF) and 6.0-8.0% ash (Chaudhary et al 2011)".

Although, the optimum quality and quantity of maize forage production depends on several factors. such as planting date, adequate availability of moisture and important micronutrients (Fales and Fritz 2007). Some highlights of the findings from the different researchers in this field are depicted as under with their citations for assessment of quality of fodders.

Remote sensing technology based fodder quality assessment: In the past two decades, remote sensing satellite-based forage quality assessment approaches have emerged as a viable method for large-scale mapping (Jennewein et al 2021). Because remote sensing has potential to provide pasture quality information, which is of great interest for researchers. However, there is a need for further refinement in tools like spectral, spatial and temporal resolution of remote sensing satellites for better prediction accuracy of forage quality (crude protein, fibre etc.). Optical remote sensing sensors are using reflected light from the visible (0.4 - 0.7 μm) to shortwave infrared (1.4-2.5 μm) region to detect variations in foliar chemistry (Jennewein et al *2021*). Youngentob et al (2012) have carried out research for the extraction of digestible protein (DP) and digestible dry matter (DDM) using hyperspectral remote sensing from leaf to canopy-level. Which is reflected light in very narrow (3-10 nm) spectral bands (Jennewein et al *2021*). Some recent works suggested that DDM can also be estimated using multispectral imagery obtained from unmanned aerial vehicles (Insua et al 2019, Michez et al 2020). Although, this technology is best suited to provide accurate solutions at low cost and high temporal coverage (Pullanagari et al 2012).

Several methods exist to evaluate the quantity, quality and acreage of forage (Kumar et al 2022, Karwariya et al 2022). It is important to have precise and up-to-date information about the quality of fodder to meet the demand of livestock (Singh et al 2020). Conventionally, farmers use visual criteria, such as plant phenological stage and leaf color, to evaluate forage quality. Moreover, agronomists and nutritionists evaluate the quality of fodder through laboratory-based chemical analysis and NIR (near-infrared reflectance) spectroscopy. Methods that take place in a lab are used to figure out the chemical composition of fodder and how well it can be digested.

Acid detergent fibre (ADF) represents cellulose, lignin, and silica, which is essential parameters that is negatively correlated with fodder crop's digestibility. The amount of protein in fodder is another important factor that needs to be taken into account when making rations with good quality fodder.

Protein comprises amino acids, which are key elements of all cells and tissues. Protein is an important part of ruminants' diets because it gives them nitrogen, which is needed for their metabolism and the making of milk and meat. Many studies on grassland are based on field spectroscopy. Handheld spectrometers are sensitive in the wavelength range of approximately 350 nm to 2500 nm, with high spectral and radiometric resolution. This instrument is being used by agronomists to predict fodder yield and quality parameters such as CP, ADF, NDF, CF within a limited spatial area (Safari et al 2016, Zhou et al 2019).

Although, Remote sensing satellite is a cost-effective approach to assess forage yield and quality. In which, optical

Satellite based fodder quality estimation

and microwave remote sensing sensors can be used for monitoring and mapping forage yield and quality. Nowadays, spectroscopy is being used more commonly for quality evaluation using CP, NDF and ADF parameters, but it has some limitations for point level observations over large areas. Aircraft or drone-based applications of spectroscopy can reduce these limitations. In addition to point level observation, it is also proving 2D image with spectral reflectance value of pixels' in numerous bands. Airborne imaging spectroscopy is being used worldwide for forage quality assessment. But manned aerial-based observations require extensive pre-planning. On the other hand, UAVs allow observation from low altitude for large areas with relatively low cost. Which is globally adopted for vegetation mapping and biomass estimation using multispectral sensors.

Use of Hyperspectral and Unmanned Aerial Vehicle (UAV)

Hyperspectral imaging is a technique used to capture and analyze the spectral signature of an object or scene. It involves collecting and analyzing the electromagnetic spectrum over a wide range of wavelengths, typically in the visible and near-infrared range. Nowadays, UAVs and a hyperspectral imaging approach are being utilized to capture canopy reflections of fodder in the range of 450 nm to 800 nm for robust fodder quality and yield estimation by Geipel et al 2021 and observed that the maximum accuracies of estimation were achieved by general models based on the pooled data and by means of PPLSR model for estimation of fresh (FM) and dry matter (DM) yields, as well as crude protein (CP), dry matter digestibility (DMD), neutral detergent fibre (NDF), and indigestible neutral detergent fibre (iNDF) content. Wijesingha et al 2020 were used five predictive modelling regression algorithms (Partial least squares, Gaussian process, Random forest, Support vector machine, and Cubist) to develop quality estimation models. UAV with a hyperspectral sensor has been used to capture the spectral imageries from the grasslands, and crude protein (CP) and acid detergent fibre (ADF) concentration of the fodder was evaluated.

Drone-based Hyperspectral Data Capture and Processing

Nasi et al 2018 used Integrated spectral and 3D features using airborne miniaturized multispectral, hyperspectral and colour (RGB) cameras to estimate crop biomass and nitrogen content. In which excellent results were achieved by integrating hyperspectral and 3D features. Askari et al 2019 were utilised Hyperspectral imageries (HSI) and multi-spectral imagery (MSI) for the development of forage quality evaluation. Wherein, The MSI datasets were acquired using UAV sensors and the remote sensing satellites such as Landsat, LISS-III and Sentinel-2 etc. But the HSI datasets were acquired using a handheld hyperspectral sensor.

Some recent work has suggested that the use of push-sweep hyperspectral instruments mounted on manned aircraft has highest potential to estimate the fodder quality (Pullanagari et al 2018). This method needs a fully equipped aircraft, which is expensive and not easily available everywhere. Which is a major limitation of the use of UAVs for the assessment of fodder quality.

Application of Different Prediction Models for Assessment of Fodder Quality

Normalized difference vegetation index (NDVI), soil-adjusted vegetation index (SAVI), Partial least squares regression (PLSR), random forest regression (RFR), normalized-difference sand index (NDSI), Spectral ratios (SRs), Leaf area index (LAI), and artificial neural network (ANN) are well known predictive model for fodder quality assessment using high resolution satellite data. Apart from these predictive models, random forest, Gaussian processing regression (GPR), Support vector machine (SVM), Classification and regression tree (CART), and naïve Bayes (NB), and cubist regression (CBR), that have not been analysed with remote sensing satellite data for the estimation of fodder quality. Recently, a study has conduct by Wijesingha et al 2020 to estimate CP and ADF of fodder crop using UAV-borne imaging spectroscopy data and found that the resulting models can precisely estimate CP and ADF. The accuracy of that model are almost similar to obtained accuracy with the use of field spectroscopy. Punalekar et al 2018 were used integrated optical remote sensing (RS) satellite datasets (hyperspectral and Sentinel-2) with a radiative transfer model (PROSAIL) to estimate leaf Area Index and biomass for dairy industry. The hyperspectral and remote sensing spectral datasets has been utilized to achieve LAI through PROSAIL approach, which is compared with field based observations of LAI. Lugassi et al 2019 examined the relationship between CP, NDF, and reflectance in the visible–near-infrared–shortwave infrared (VIS–NIR–SWIR) spectral range using lab, field based measurement, and RS data and developed a statistical models using various calibration and validation data. Furthermore, they have utilized NDVI, SAVI and WDRVI indices as substitutes to estimate CP and NDF.

In addition, Machine learning techniques can be used to estimate the quantity and quality of fodder cropland using UAVs imaging spectrometry and spectral imaging. In this process, RS data can be obtained at least 4-times during the primary growth period and 3-times in re-growth season. Reference measurements can be included fresh and dry biomass and several quality parameters, such as DM, NDF, indigestible NDF, and nitrogen uptake. Various Machine learning algorithms were trained using reference measurements using training data. Recently Oliveira et al 2020 conducted a study for estimation of biomass, nitrogen content and digestibility using hyperspectral and 3D datasets and found that the results are better with a combined dataset of hyperspectral and 3D data than with multispectral and 3D data. Raab et al 2020 conducted a study to evaluate the potentials of combining Sentinel-1 and 2 datasets to estimate the quantity and quality of fodder crops. In which, study showed that sentinel-2 satellite datasets-based obtained parameters were adequate for predicting Organic ADF and CP concentration from field observations. A slight improvement in accuracy was found by adding the Sentinel-1 radar dataset to estimate ADF and CP. However, the combined Sentinel-1 and Sentinel-2 datasets did not improve well for dry matter estimation. Therefore, the Optical Remote Sensing Sensor (Sentinel-2) dataset may be sufficient to accurately estimate fodder quality.

CONCLUSION

The Crude Protein, Dry Matter, Neutral Detergent Fibre and Acid Detergent Fibre can be estimated for rapid and robust forage yield and quality estimation from grasslands and at different cutting regimes with combining of UAV-borne hyperspectral imaging spectroscopy (HSI), Radar Sentinel-1 and Multispectral imagery (MSI) of Sentinel-2 satellite. The MSI dataset will be obtained using UAV and Sentinel-2A/B satellite, while the HSI dataset can be acquired by handheld hyperspectral camera. Prediction models can be developed using PLSR statistical method and machine learning algorithms. In addition to this, vegetation indices NDVI, SAVI and WDRVI can be used for better results in fodder quality estimation.

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Ecological and Behavioural Aspects of Five Striped Palm Squirrel, *Funambulus pennantii* Wroughton in Natural Habitat in Agricultural Landscape

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***Abstract*:** Present study on ecology and behaviour of five striped palm squirrel, *Funambulus pennantii* was conducted in agricultural landscape in district Ludhiana and Sangrur (Punjab, India) from October, 2020 to September, 2021. Seasonal activity, time spent on different kinds of activities, vegetation preferred, interaction with other animals, nest analysis, and home range of five striped palm squirrel were studied. There was bimodal activity in summer season and unimodal activity in winter season. Squirrels spent most of their time in feeding, exploring and resting. They mostly fed on leaves, bark and fruits of trees. Squirrels were found to interact with different species of birds. Mostly human presence had no effect on their activity. The nest height ranged from 3.6 -10.5 m. Home range of male and female squirrels varied from 12.0-15.2 m and 10.1-16.0 m, respectively. The squirrels changed their day time activity with season, thermal conditions and day length. The mid-day resting period was observed to avoid excessive heat in all the seasons except winters. The present study on ecology and behaviour of squirrels may be helpful in suggesting different management methods against them.

Keywords: Activity pattern, Ecology, Feeding, Home range, *Funambulus pennantii*, Nesting

Squirrels (Class Mammalia; Order Rodentia; Family Sciuridae) are considered to be the excellent model organism for examining ecological and behavioural difference in the wildlife. Squirrels are present in all biogeographic zones except Australia, Madagascar, Antarctica, and other oceanic islands and deserts (Yousefi et al 2013). Worldwide, there are about 285 species of squirrels within 58 genera (Wilson and Reeder 2011). Among three pest species of squirrels in India, the northern palm squirrel, *Funambulus pennantii* is the most abundant (Parshad 1999). Their key diagnostic features include a bushy tail and five conspicuous stripes running along their bodies (Yadav et al 2019). Palm squirrels are arboreal and usually found near human habitations. They have sharp, curved, non-retractable claws that help them to climb and feed. Palm squirrels are highly mobile, agile, and quite vocal. Like other species in the order Rodentia, palm squirrels have one pair of chisel shaped incisor teeth in each jaw that grow constantly (Yadav et al 2019). *F. pennantii* have opportunistic diets, consuming a range of foods that vary depending on season. These are mainly herbivores and are known to feed on seeds, leaves and soft fruit, but may consume certain animal matter such as bird eggs/chicks and insects, especially locusts. Food resources along with reproductive efforts and environmental parameters are the key biotic and abiotic factors that have considerable effect on home range of squirrels (Edelman and Koprowski 2006). Home range in male is affected by spatial distribution and presence of females. The pattern of substantial seasonal changes in home-range was same for both male and female squirrels (Lurz et al 2000, Koprowski et al 2008). The selection of a site for nest construction is often based on the choice of specific features of the environment (Goodenough et al 2009, Skorka et al 2011). The selected site is the best compromise between minimum predation and maximum access to food and thereby increasing the animals' fitness (Barea and Watson 2013). Nest is constructed by the female using pieces of cloth, jute fibers, small twigs of plants, dry grasses, plastic rope etc. preferably on tree branches, holes in tree trunks, or inside the crevices in the walls of the old buildings (Sharma 2004). Litters are protected in the nest by the females. The composition of tree species and structural attributes play an important role in the use of the habitat by squirrels (Kumara and Singh 2006). Crops preferred by palm squirrels include pineapple, mango, pomegranate, apple, guava, blackberries, grapes, sugarcane, groundnut, maize, etc. (Chakravarthy 2004). To reduce the damage caused by squirrels to these crops, it is important to know about the key characteristics of the landscape that support their population along with their seasonal activity pattern and food preference. Such information on basic ecological and behavioural aspects of *F. pennantii* is limited. Present study was therefore conducted to record the seasonal activity pattern, food preference, home range and nesting of *F. pennantii* in their natural habitats in agricultural landscape.

MATERIAL AND METHODS

Seasonal activity: The seasonal activity pattern of *F. pennantii* was studied by keenly observing their activities in a natural habitat in a plant nursery located at Punjab Agricultural University (PAU), Ludhiana, Punjab (India). Animals were observed since their exit from nesting sites in the morning till their return back in the evening. Observations were recorded from 5:00 am to 7:00 pm at weekly intervals from October, 2020 to September, 2021. Mean time of sunrise and sunset and day length in each month were used to correlate the activity periods.

Time allocation to different activities: Activities of squirrels like exploring (running and climbing), feeding (foraging, actively ingesting food or processing food items), resting (remaining motionless on the substrate for more than 5 min), moving (walking and hoping), calling, grooming and chasing were recorded in the selected plant nursery at PAU campus on alternate days during the months of March-April, 2021. The observations were made for one hour each during morning (06:00 am-09:00 am) and evening (03:00 pm-06:00 pm) and time spent on each kind of activity along with their frequency were determined by following the activities of one animal at a time (total 4-5 animals). Mean time and frequency allocated to different activities were determined from fortnightly data and the comparison in different activities was made between morning and evening hours. Squirrels were surveyed using the focal animal observation method (Altmann 1974) through naked eye from a distance of about 5-10 m.

Preference for vegetation: To record the preference of squirrels for different types of vegetation in PAU campus, six quadrants each of size 13×13 feet covering the whole plant nursery were selected. In each quadrant, different type of vegetation like trees, herbs and nursery plants were present. Data on proportion of time spent by squirrels on each type of plant were recorded by observing each quadrant for 30 min on alternate days during March-April, 2021.

Home range: Home range of squirrels in the plant nursery was recorded by measuring the distance moved from the tree used for nesting or resting purpose to the place of activity. Average distance travelled by male and female squirrels (10 each) in the morning and evening hours was measured.

Nesting: Nests of five striped palm squirrel were located initially by perceiving their vocalizations in the evening hours in the selected plant nursery and agricultural landscape at PAU campus and villages of district Ludhiana and Sangrur (Fig. 1), Punjab (India). The timing of nest entries and exit were observed at regular basis. Nest analysis was done only for those nests which were found actively used by the squirrels. Nests were then photographed and monitored without disturbing the squirrels. Various parameters such as height of the tree, height of the nest and material used for nesting were recorded.

Association with other animals: Observations were also made on the association of squirrels with other animals such as different bird species present in the plant nursery.

Statistical analysis: The data was analysed by using SPSS version 27 software. Values were considered significant at 5% level of significance.

RESULTS AND DISCUSSION

Seasonal pattern of time and activity allocation: During autumn (October, 2020), the average time of sunrise and sunset was 6:29 am and 5:54 pm, respectively with day length of 11:22 hours. Squirrels were found active from 7:00 am-12:00 pm in the forenoon and 2:00 pm-6:00 pm in the afternoon (Tables 1 and 2). In winter (November, 2020-

Table 1. Seasonal pattern of activity of squirrels in the forenoon hours at selected plant nursery in PAU campus

Months	Sun rise (h:min)	5:00-6:00AM	6:00-7:00AM	7:00-8:00AM	8:00-9:00AM	9:00-10:00AM	10:00-11:00AM	11:00-AM 12:00PM
October 20	6:29	×	×	√	√	√	√	√
November 20	6:53	×	×	√	√	√	√	√
December 20	7:16	×	×	√	√	√	√	√
January 21	7:23	×	×	√	√	√	√	√
February 21	7:06	×	×	√	√	√	√	√
March 21	6:34	×	×	√	√	√	√	×
April 21	5:57	×	×	√	√	√	√	×
May 21	5:31	√	√	√	√	√	×	×
June 21	5:08	√	√	√	√	√	×	×
July 21	5:34	√	√	√	√	√	×	×
August 21	5:32	√	√	√	√	√	×	×
September 21	6:10	√	√	√	√	√	×	×

February, 2021), average time of sunrise varied from 6:53 am to 7:23 am and of sunset from 5:26 pm- 6:13 pm with day length of 10:11-11:06 hours. Activity started at about 7:00 am and which increased from 9:00 am to 1:00 pm hours. A drop in the activity was noticed from 1:00 pm onwards until 5:00 pm after which no squirrel was seen. In summer season (March-June, 2021), sunrise in March-April, 2021 was observed between 5:57 am-6:34 am and sunset between 6:34 pm-6:54 pm with day length of 12:11-12:26 hours. Activity began at 7:00 am and increased rapidly thereafter. The morning peak in the activity during 7:00-8:00 am was higher than that observed during 11:00 am-2:00 pm. A second peak in activity occurred between 4:00-5:00 pm, after which activity declined. During monsoon season (July-September, 2021), sunrise was observed between 5:32 am-6:10 am and sunset between 6:31 pm-7:19 pm with day length of 12:21-13:55 hours. Squirrels were active from 5.00 am-10:00 am in the morning and from 3:00 pm-6:00 pm in the evening. Squirrels showed a bimodal activity in all the seasons except for the winters. In winters, the peaks were increasingly displaced toward mid-day, eventually resulting in the unimodal patterns of activity. The short, uninterrupted active phase in winter may probably be an adaptation for conserving energy by minimizing the period of heat loss. Long et al (2003) also observed that squirrels reduce their activity during the mid of the day in summer season to avoid intense heat and start their day activity late in winter season. Thermal environment can have a strong influence on activity of tree squirrels. In summers, the active phase was more than twice as long as in winter, and was usually broken by a period of rest. In summer, because of the longer day lengths, the squirrels have the opportunity to separate their feeding activity into two distinct periods (Skibiel et al 2002).

Time and frequency allocation of different activities: Time spent and frequency during exploring, feeding, moving and chasing were significantly more during the evening hours than in the morning hours. On the contrary, time spent and frequency of calling and grooming by squirrels were significantly more during morning as compared to that in evening hours (Fig. 1 and 2). The monthly encounter rate of squirrels throughout the study period showed that exploring and feeding were the primary activities of squirrels. Calls of squirrels were mostly harsh when they were frightened. Females used short-duration intermittent sounds to entice the male while playing and before mating. Squirrels have been seen chasing and driving away other squirrels feeding in their territory. The activities got delayed during summer and monsoon as compared to autumn and winter due to very hot temperature in summer and heavy rainfalls in monsoon. The length of day sets the period available for feeding and therefore affects the activity pattern. The decreased day length in winter may lead to less time available for feeding and thus forcing the squirrels to concentrate their activity into a single period. Data also suggests that squirrels forage most in low to moderate temperatures and avoid higher temperatures. This is consistent with the finding that squirrel foraging activity is decreased in the afternoon when temperatures are highest (Palei et al 2015) and also suggested that activity pattern of squirrels may vary depending on geographical location, climate, food resources, and interaction with predator species.

Time allocation for feeding on different kinds of vegetation: Squirrels were observed to feed primarily on 16 species of plants, mostly trees (Table 3). Parts of 14 species

Table 2. Seasonal pattern of activity of squirrels in the afternoon at selected plant nursery in PAU campus

Months	12:00-1:00PM	1:00-2:00PM	2:00-3:00PM	3:00-4:00PM	4:00-5:00PM	5:00- 6:00PM	6:00-7:00PM	Sun set (h:min)	Day length (h:min)
October 20	×		√	√	√	√	×	05:54	11:22
November 20	√	√	√	√	√	×	×	05:26	10:35
December 20	√	√	√	√	√	×	×	05:27	10:11
January 21	√	√	√	√	√	×	×	05:57	10:24
February 21	√	√	√	√	√	×	×	06:13	11:06
March 21	×	√	√	√	√	×	×	06:34	12:11
April 21	×	√	√	√	√	×	×	06:54	12:26
May 21	×	×	×	√	√	√	√	07:14	13:39
June 21	×	×	×	√	√	√	√	07:34	14:07
July 21	×	×	×	√	√	√	×	07:29	13:55
August 21	×	×	×	√	√	√	×	07:07	13:14
September 21	×	×	×	√	√	√	×	06:31	12:21

of trees were used for feeding, while the remaining two species *Bauhinia variegate* (Kachnar) and *Livistona chinensis* (China Palm) contributed marginally towards the overall diet of squirrels. Leaves and bark were generally available almost round the year and therefore they formed the bulk of the squirrel's diet while flowers, fruits and seeds were the supplementary food items. Eventually squirrels adapted to the diet according to the abundance of different food items in relation to seasonal changes. Flowers and leaves of tun, grasses, harshingar, rubber palm and champaca were eaten in considerable quantities over bark and seed. In quadrant I, four types of trees, one herb (common rose) and grasses were present. The proportion of time spent on feeding two trees i.e., toon and Ashoka and the grasses were comparatively high. Squirrels in quadrant II mostly feed on banyan, palm nursery and grasses. The squirrel activity on rubber palm and harshingar were approximately 40 and 50%, respectively. The relatively high number of squirrels recorded on tun plant was probably related to the highest number of trees in nursery. Squirrels were observed to feed on bark and leaves of all plants present in III quadrant. Tun was the most preferred tree used for resting. Only four types of trees (jamun, mango, banyan and neem) were present in quadrant IV. Squirrels were rarely encountered on neem as it was used only for resting purpose. Activity was observed on all plants in quadrant V except China palm. In quadrant VI, only palm nursery plants were observed. Squirrels were found feeding on leaves of these plants (Fig. 3). Feeding time of squirrels included handling, masticating, or swallowing of food items.

Table 3. Different kinds of vegetation present in six quadrants at plant nursery in PAU campus

Common name	Scientific name	Family
Ashoka	*Saraca asoca*	Fabaceae
Banyan	*Ficus benghalensis*	Moraceae
Champaca	*Mangonlia champaca*	Mangnoliaceae
China palm	*Livistona chinensis*	Arecaceae
Common rose	*Rosa* sp.	Rosaceae
Harshingar	*Nyctanthes arbortristis*	Oleaceae
Jamun	*Syzygium cumini*	Myrtaceae
Kachnar	*Bauhinia variegate*	Fabaceae
Mango	*Mangifera indica*	Anacardiaceae
Mulbery	*Morus alba*	Moraceae
Neem	*Azadirachta indica*	Meliaceae
Palm (Nursery plants)	*Livistonia chinensis*	Arecaceae
Rubber palm	*Hevea brasiliensis*	Euphorbiaceae
Sohanjana	*Moringa oleifera*	Moringaceae
Tun	*Toona ciliate*	Meliaceae

Majority of the time to feeding activity was allocated in the evening hours. The most preferred vegetation for feeding and exploring were trees of tun, Ashoka, banyan, jamun, champaca and grasses, while the trees of neem and kachnar were used for resting. Squirrels generally fed on leaves, bark

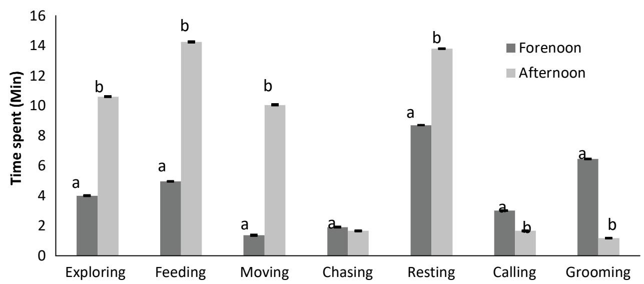

Fig. 1. Comparison of time spent on different activities by squirrels during forenoon and afternoon hours

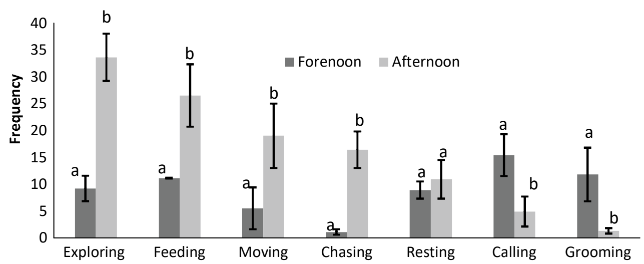

Fig. 2. Comparison of frequency of different activities of squirrels during forenoon and afternoon hours

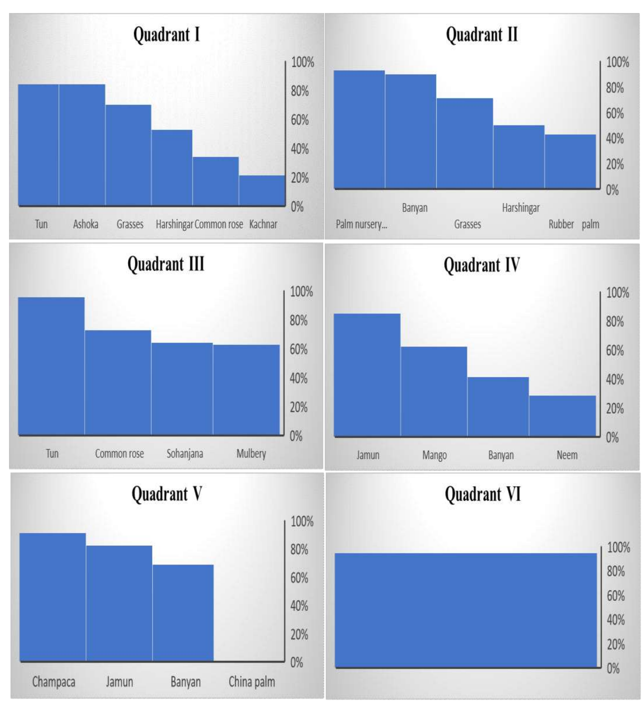

Fig. 3. Proportion of time spent by squirrels on different types of vegetations

and fruits of the trees. The rough bark of jamun trees helps to escape from terrestrial predators such as dogs. Its dense and intermingled canopy also helps the squirrels to extend the area of home range and escape from predators like cats which can climb the trees (Thorington et al 2012). The plant nursery at PAU campus provides a high-quality habitat or ecosystem structure with abundance of food material. Ecologically these are the most important factors that contribute towards higher animal diversity in the nursery.

Home range: The home range for male and female squirrels was worked out in plant nursery at PAU campus in the morning and evening hours (Table 4). On average, males covered maximum 12.0 m distance in the morning and 15.2 m distance in the evening hours whereas, the females covered maximum 16.00 m distance in the morning and 10.10 m distance in the evening hours. The significant difference was found in home ranges of male and female squirrels as well as in the home range of one sex between morning and evening hours. These home ranges of male and female squirrels were overlapping with each other. In the present study, females traveled a longer distance in the morning and shorter in the evening as compared to males. Similar home-range size for both male and female squirrels was observed by Koprowski et al (2008). Jacques et al (2017) observed the mean size of home range of adult *F. pennantii* ranging from 0.07 to 0.22 m.

Table 4. Home range of male and female squirrels

Sex (n = 10 each)	Distance travelled from nests and resting places (m)	
	Morning	Evening
Male	12.00± 1.10[a]	15.20±1.00[b]
Female	16.00±3.60[c]	10.10±2.40[d]

Values are expressed as Mean±SD
Values with different superscripts (a-d) in a row as well as in a column differ significantly at P<0.05

Nesting: A total of 24 nests of *F. pennantii* were sighted at different places i.e., at PAU campus (13), village Phullanwal (four), district Ludhiana and Balian (seven), district Sangrur. Squirrels preferred significantly more the trees with wider canopy to build their nests (Table 5). The material used for constructing nests was cloth, cotton, rope, grasses, twigs and synthetic fibrous material. Maximum number of nests (6) was found on Indian jujube trees. Squirrels preferred trees with maximum number of branches for nest building. Nests were found supported by 'V' shaped interlocking branches of the trees and located at a height ranging from 3.6-10.5 m. There was considerable variation in height at which the nests were made. The height of nesting trees was between 4-8 m, while maximum nesting was observed even at height of >9 m. This may be due to easy movement to and from the nest in all directions, and escape from predators. Further, tree branches provided substantial support for the nest to withstand harsh weather conditions. Similar to present study, Mohan and Singh (2018) also observed that the squirrels make nests using leaves, twigs, threads, mosses and other soft materials. Many of the Holarctic squirrel species such as *Sciurus arizonensis* (Cudworth and Koprowski 2011) and *Sciurus aberti* (Edelman and Koprowski 2006) select trees with more interlocking branches and more access routes. Nests in upper canopy are known to provide proper protection from terrestrial predators and provide moderate weather conditions (Cudworth and Koprowski 2011). In present study also nest heights did not show any correlation with tree heights and nests at a lower region of smaller trees. These small squirrels may be able avoid heat stress by direct sunlight, rain and wind and get protection from avian predators. These factors might possibly contribute to the selection of a lower nesting height. Our study additionally revealed that *F. pennantii* selected nesting substrates irrespective of its proximities to roads and buildings.

Table 5. Nest analysis of squirrels at different locations

District	Village/Location	Common name of tree	Scientific name of tree	Number of nests	Tree height (m)	Nest height (m)
Ludhiana	PAU campus	Neem	*Azadirachta indica*	2	9.14±0.00	7.60±0.00
	PAU campus	Ber	*Ziziphus mauritiana*	4	8.65±0.38	7.30±0.67
	PAU campus	Tahli	*Dalbergia sissoo*	2	9.10±0.00	7.60±0.85
	PAU campus	Ashoka	*Saraca asoca*	1	11.50	9.10
	PAU campus	Kikar	*Acacia karroo*	1	8.80	5.70
	PAU campus	Mango	*Mangifera indica*	1	12.10	10.30
	Phullanwal	Pomegranate	*Punica granatum*	1	4.50	3.60
	Phullanwal	Cheeku	*Manilkara zapota*	3	10.70	5.67±2.51
Sangrur	Balian	Kikar	*Acacia karroo*	2	8.20±0.84	4.40±0.64
	Balian	Ber	*Ziziphus mauritiana*	2	7.15±0.63	3.81±1.07
	Balian	Dharek	*Melia azedarach*	1	9.10	7.00
	Balian	Pilkan	*Ficus virens*	1	9.10	10.00

Values are expressed as Mean±SD

Table 6. Interaction of squirrels with different bird species in plant nursery at PAU campus

Bird species	Number of birds	Number of squirrels around birds	Kind of interaction
Common Myna (*Acridotheres tristis*)	3.90±0.56	2.80±0.30	Feeding and chasing
House Crow (*Corvus splendens*)	3.60±0.96	2.50±0.10	Sitting together
Rose-ringed Parakeet (*Psittaciformes manillensis*)	1.20±1.00	3.00±0.50	Sitting together
Jungle Babbler (*Turdoides striata*)	5.20±3.30	4.20±1.20	Feeding together
Red vented Bulbul (*Pycnonotus jocosus*)	1.00±0.80	4.60±1.10	Sitting together and chasing

Values are expressed as Mean±SD

Interaction of squirrels with other animals: Squirrels were spotted in plant nursery with five distinct bird species, namely Common Myna, House Crow, Rose ringed Parakeet, Jungle Babbler and Red vented Bulbul (Table 6). Birds and squirrels shared the same environment when it comes to feeding habits. The presence of humans did not seem to impact squirrel activity much, which can be due to habituation.

CONCLUSIONS

The present study provides an insight of the activity patterns, foraging preferences, home range and nesting characteristics of squirrels in an agricultural landscape. Further research on various ecological and behavioural aspects will help to understand their ecological adaptations to agricultural areas and their role in ecosystem health. Knowledge about ecology and behaviour of squirrels may be helpful in suggesting different management methods against them.

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