

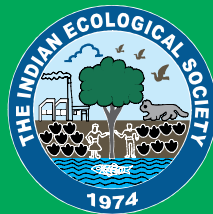
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Seasonal Variation and Diversity of *Triplos* (Dinoflagellata) in Relation to Environmental Drivers in the Hooghly River Estuary, Bay of Bengal


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ABSTRACT: Planktonic dinoflagellate *Triplos* spp. were sampled and documented from three selected sites within the Hooghly Estuary, Bay of Bengal. The study focused on their diversity, abundance patterns, and the environmental factors regulating them. 14 species of *Triplos* were identified, with *Triplos furca* and *Triplos fusus* being the dominant. Seasonal patterns indicated that post-monsoon (POM) and pre-monsoon (PRM) exhibited the highest diversity, characterized by low dominance ($D = 0.18$ to 0.23), high Simpson diversity index ($1-D = 0.78$ to 0.82), and elevated Shannon diversity index values ($H' = 1.75$ to 1.89). In contrast, monsoon (MON) displayed the highest evenness ($E = 0.90$ to 0.94) but reduced diversity. *Triplos fusus* showed maximum abundance (highest cell density 1.92×10^3 Cells/L) during the post-monsoon (POM) season, when nutrient levels and salinity were elevated and the water temperature was relatively lower. Multivariate Non-metric Multidimensional Scaling (NMDS) ordination with environmental vectors displayed a clear separation of seasonal groups, primarily influenced by temperature, salinity, and nutrient gradients. Similarity Percentage (SIMPER) analysis identified *Triplos fusus*, *Triplos furca*, and *Triplos lineatus* are the major contributors to seasonal dissimilarity in the assemblages. Individual rarefaction analyses applied to the abundance further revealed higher species accumulation during late POM and early PRM months. Overall monsoon-influenced hydrological changes are the dominant force regulating *Triplos* diversity and community composition in the Hooghly Estuary.

Keywords: Abundance, Diversity, Salinity, Nutrients, NMDS, post-monsoon.

1. INTRODUCTION

Dinoflagellates are a diverse group of single-celled myzozoan protists and a major component of the marine phytoplankton community. They play essential roles in primary production and nutrient cycling, acting as a link between microbial food webs and higher trophic levels (Taylor et al., 2008). Genus *Triplos* (formerly known as *Ceratium*) under family Ceratiaceae, a group of cosmopolitan thecate dinoflagellate is of particular ecological interest due

to its distribution, high morphological variability, and marked seasonal abundance in estuarine and coastal waters (Gómez, 2012). Owing to their distinct cell structure and morphological features, these dinoflagellates are easily distinguished from others. Different *Triplos* spp. possess variable-sized short or long apical and antapical horns. *Triplos* spp. are highly sensitive to environmental change, and their occurrence and diversity are commonly linked to changes in hydrographic conditions, nutrient inputs, and anthropogenic pressures (Shin et al., 2016; Hallegraeff, 2020).

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Estuarine areas serve as high productive transition zones that connect freshwater with marine ecosystems. The Hooghly River estuary is one of the important estuarine systems on the northeastern shore of the Bay of Bengal. This westernmost part of the Gangetic river delta has a lot of mixing of fresh water and plenty of human activity, like fishing boats, trawlers, and the disposal of urban wastewater (Chatterjee et al., 2013; Henderson et al., 2021). Salinity gradients, tidal flows, and nutrient fluxes create a unique biological niche that harbours a wide range of phytoplankton communities in this area. Dinoflagellates are ecologically significant group of phytoplankton, though they receive less attention compared to diatoms. Specifically, thorough studies on *Triplos* distribution, seasonal abundance, and diversity in response to environmental factors are lacking (Naik et al., 2011; Rath et al., 2021). The present study tries to address this gap by integrating seasonal field observations with multivariate statistical analyses to evaluate species-environment relationships in the Hooghly estuary.

Understanding the ecology of *Triplos* in the Hooghly estuary can provide the knowledge of estuarine ecosystem productivity and functioning. Their seasonal abundance affects primary production and energy transfer, which affects higher trophic levels such as zooplankton and fish population. The current investigation examines seasonal variation, diversity, and abundance of *Triplos* species in the Hooghly estuary in response to environmental factors that could enhance the understanding of dinoflagellate ecology.

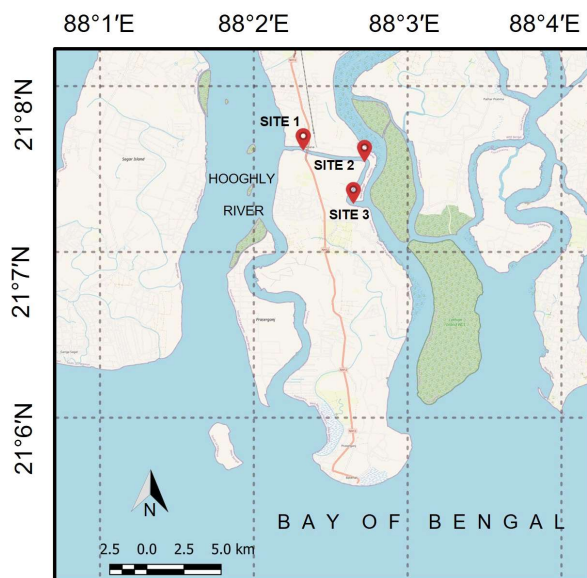


Figure 1. Map of the study area

2. MATERIAL AND METHODS

2.1. Study Area

The investigation was conducted from October 2021 to September 2023 in the lower estuarine stretch of the Hooghly River near Namkhana, West Bengal. Three seasonal observations were considered: the pre-monsoon season (PRM: February–May), the monsoon (MON: June to September) and the post-monsoon season (POM: October–January). Sampling was carried out at three locations: Station 1 (21.76056°N, 88.23628°E) adjacent to Namkhana Bridge, Station 2 (21.754776°N, 88.267445°E) near Madanganj, and Station 3 (21.727693°N, 88.266245°E) close to Dwariknagar Ferry Ghat. The study sites receive the impact of freshwater inflow coming from the Hooghly River and saltwater entering from the Bay of Bengal (Fig. 1).

2.2. Sampling Methods

We followed standard procedures to collect water samples. Surface water temperature, pH, salinity, total dissolved solids (TDS), and electrical conductivity (EC) were recorded using a portable multi-parameter probe of HANNA. Dissolved oxygen (DO) and nutrient concentrations were estimated by laboratory analysis using standard methods. Nitrate, phosphate, and silicate content of water were determined spectrophotometrically after filtration of samples, following the methods of Grasshoff et al. (2009). Dinoflagellate samples were collected through sieving 50 liters of water through bolting silk and converted to plankton concentrate, then preserved with 10% Lugol's iodine (Williams et al., 2016). Dinoflagellate cells were counted using a Sedgewick Rafter chamber under a Dewinter Educator Plus and Leica DMi8 microscope. Species identification and nomenclature were done using available literature (Hasle et al., 1996; Gómez, 2021).

2.3. Statistical Analysis

The Shapiro–Wilk test was applied to test for normality in the data of *Triplos* species. If data did not follow a normal distribution, the non-parametric Kruskal–Wallis test was employed. Post-hoc pairwise comparisons with Bonferroni-adjusted p -values were done when the Kruskal–Wallis test showed significance. Dominance (D), Simpson's index (1–D), Shannon–Wiener diversity index (H'), Brillouin index (B), Pielou's Equitability index (J), and Margalef richness, Menhinick index were employed to quantify diversity and species richness. Species rarefaction curve was constructed using pooled seasonal data to visualize species

richness across different months. To visualize the seasonal patterns of species assemblage, species counts were converted to relative abundance (%). Then Similarity Percentage (SIMPER) analysis (based on Bray–Curtis dissimilarity) was conducted to see which *Triplos* species contributed the most to the differences among seasons, summarizing their average dissimilarity, individual contributions, and cumulative influence. For a broader view of community structure, multivariate statistics Non-metric Multidimensional Scaling (NMDS) with environmental variables as vectors were performed using log (x+1) transformed abundance data to show the alignment with the species and seasonal patterns. Different statistical analyses were carried out in MINITAB, PAST (version 5.0), and Microsoft Excel (2010).

3. RESULTS AND DISCUSSION

3.1. Abundance Structure and Variation of *Triplos*

Test statistics for the Shapiro-Wilk test (W) involving monthly mean abundance of all *Triplos* spp. ranged between 0.642 and 0.771 (N = 14, $p < 0.001$) in different seasons, revealing their non-parametric nature. The Kruskal–Wallis test (tie-corrected) revealed significant seasonal variation in the abundance of *Triplos* ($H_c = 34.01, p < 0.0001$), indicating marked variability in community structure within the estuarine system. Dunn's post hoc analysis with Bonferroni correction indicated significant differences ($p < 0.05$)

between MON samples and both PRM and POM, demonstrating significant seasonal variation in dinoflagellate abundance across the sites. The study documented 14 species of *Triplos* belonging to several infrageneric sections, like *eugrammus*, *fusiformia*, and *macroceros* (Table 1). *Triplos fusus* was the most observed species with maximum cell density (1932 cells/L) in POM, followed by *Triplos furca* (963 cells/L) and *Triplos trichoceros* (733 cells/L) all exhibiting peak abundance during POM. These three taxa were the most abundant among all and contributed the majority of the *Triplos* assemblage (Baek et al., 2008). Observed seasonal patterns of relative abundance of all *Triplos* and their SIMPER outputs given in Table 1. *T. fusus* exhibited the 36.77% of the average Bray-Curtis dissimilarity, with notably greater abundances during POM and PRM. *T. furca* and *T. trichoceros* were the next two important contributors, each accounting for more than 16% of the dissimilarity, suggesting their rapid shifts in relative abundance in response to fluctuations in salinity and nutrient concentration across seasons. A limited number of species predominated the inter-seasonal variations. *T. mulleri* and *T. lineatus*, added moderate contributions with their fluctuating relative abundances, while all other species have contributed less than 5%. *T. fusus*, *T. furca*, and *T. trichoceros* (cumulatively contributing 69.5%) suggest that these taxa serve as dominant class in the estuary. The impact

Table 1. Relative abundance of all documented *Triplos* along with their SIMPER contribution

Species	Relative abundance (%)			SIMPER contribution		
	POM	PRM	MON	Average dissimilarity	Contribution (%)	Cumulative (%)
<i>Triplos fusus</i>	34.18	34.84	21.19	19.8	36.77	36.77
<i>Triplos furca</i>	20.77	16.52	28.07	8.812	16.37	53.14
<i>Triplos trichoceros</i>	15.4	20.18	20.94	8.81	16.36	69.51
<i>Triplos mulleri</i>	9.53	8.62	7.98	5.205	9.668	79.17
<i>Triplos lineatus</i>	10.13	8.25	20.22	4.22	7.84	87.01
<i>Triplos brevis</i>	3.19	3.09	1.6	1.936	3.596	90.61
<i>Triplos longipes</i>	3.78	2.23	0	1.915	3.557	94.17
<i>Triplos macroceros</i>	0.54	4.11	0	1.782	3.309	97.48
<i>Triplos falcatus</i>	0.57	0.42	0	0.3066	0.5695	98.05
<i>Triplos declinatus</i>	0.44	0.62	0	0.2952	0.5484	98.59
<i>Triplos inflatus</i>	0.56	0.27	0	0.248	0.4607	99.05
<i>Triplos azoricum</i>	0.29	0.41	0	0.1935	0.3595	99.41
<i>Triplos gibberum</i>	0.38	0.18	0	0.1721	0.3197	99.73
<i>Triplos minutus</i>	0.25	0.24	0	0.1436	0.2667	100

of rare species like *T. inflatus*, *T. azoricum*, *T. gibberum*, and *T. minutus* (<0.5%) underscores their limited contribution to overall community turnover (Haque et al., 2021). The combined patterns of abundance, relative contribution, and SIMPER results indicate significant population shifts, especially during POM, highlighting the suitable growth conditions for dominant *Triplos* spp. These seasonal changes are ecologically important and can have strong effect on the estuarine productivity, grazing activity, and the overall trophic structure.

3.2. Variation in Environmental Parameters

Surface water physicochemical properties showed strong seasonal variation, typical of tropical estuarine systems driven by the monsoon (Fig. 2). Water temperature ranging from a minimum of 18.7°C (POM) to a maximum of 33.6°C (MON). Dissolved oxygen (DO) ranging from 4.1 mg/L during August (MON) to a maximum of 7.46 mg/L recorded in January (POM) while the average value 5.0–6.5 mg/L, with higher concentrations consistently observed during the colder PRM and early POM months. pH exhibited moderate spatial-seasonal variation, generally oscillating between 7.0 and 7.8. TDS, EC, and salinity values followed a more or less symmetrical increase and decrease pattern. The nutrient concentrations exhibited marked seasonal variability, with the highest values recorded during the monsoon (MON) months. Nitrate from 26 to 47 µM, and

silicate levels between 53 and 114 µM. Phosphate exhibited a similar trend, reaching its highest during MON (3.6–3.8 µM) and declining gradually in POM and becoming the lowest during PRM (1.3 µM). Temperature is the key component affecting numerous aquatic organisms, especially the marine phytoplankton group (Hays et al., 2005). A large number of *Triplos* species were observed from December to March, when temperatures ranged between 20 and 26°C. This seasonal enhancement is attributable to reduced temperature and enhanced mixing, while the marked decline during the MON aligns with elevated temperature, increased organic loading, and intensified microbial decomposition, lowered salinity, and enhanced freshwater inflow, which together favour oxygen enrichment and elevated phytoplankton productivity (Kibler et al., 2012). The similar pattern of pH and DO suggests a common regulatory control by temperature. This kind of negative association with pH and DO is characteristic of productive estuarine waters where photosynthesis decreases carbonate chemistry. Fluctuations in EC, TDS, and salinity levels are affected by the temperature, quantity of freshwater inputs, tidal power, and rapid rate of evaporation. In PRM, low freshwater influx, rising temperature, and a higher rate of evaporation caused a lowering of salinity level that was further dropped in MON due to the large flow of freshwater and gradually increased in late POM (Manna et al., 2010). The elevated concentrations of nitrate and phosphate during

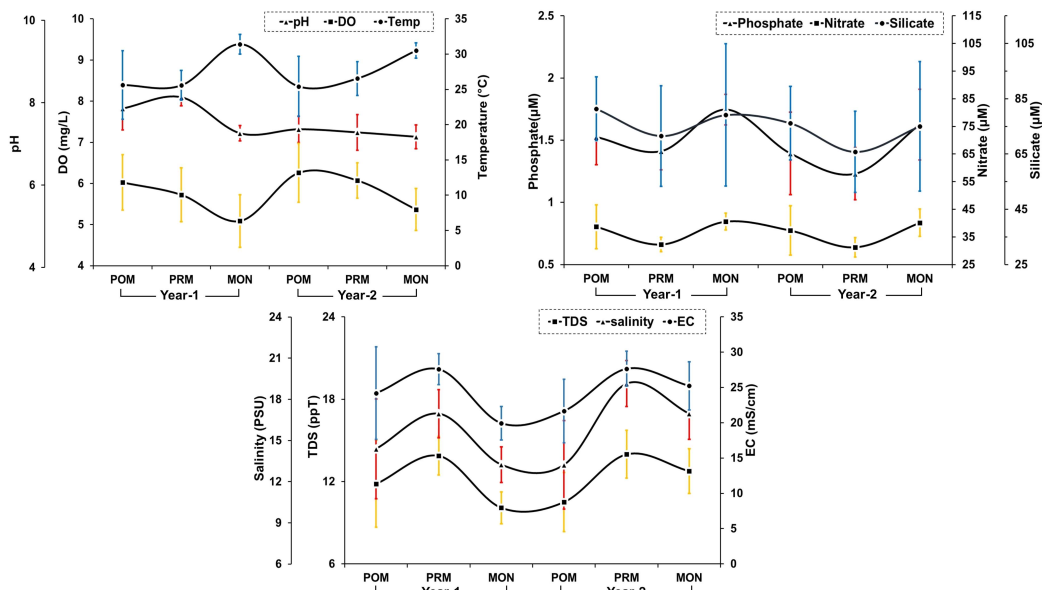


Figure 2. Fluctuation of physicochemical parameters of water during different season

MON are ecologically significant for dinoflagellates, many of which possess efficient nitrate uptake systems (Abbasi and Ki, 2022). The nutrient-rich, stratified MON environment therefore provides favourable conditions for opportunistic growth, whereas the more oligotrophic PRM restricts productivity and may influence the seasonal succession of *Triplos* species (Baek et al., 2009).

3.3. Dominance Patterns and Species Richness

Based on the abundance pattern of each *Triplos*, an alluvial diagram was constructed (Fig. 3), which revealed different species classes in the assemblage. *T. fusus* and *T. furca* were dominant with their wider bandwidth in POM and PRM. Contributions made by *T. lineatus*, *T. trichoceros*, and *T. mulleri* during POM and PRM, primarily in the frequent classes and occasionally dominant classes. This indicates their moderate abundance but significant ecological significance. On the other hand, species such as *T. minutus*, *T. azoricum*, *T. gibberum*, and *T. inflatus* are always few and compose the rare category. During PRM and POM, their bands become smaller and decline dramatically with a total absence in MON. The figure illustrates that POM and PRM are distinguished by the expansion of species while MON restricts the community to a state of low abundance, with just a few taxa that are able to withstand harsh

conditions (Naik et al., 2020). The seasonal succession demonstrates the significant impact of hydrological fluctuations on *Triplos* dynamics in the estuary, with POM conditions beneficial to proliferation and growth before the onset of monsoon dilution.

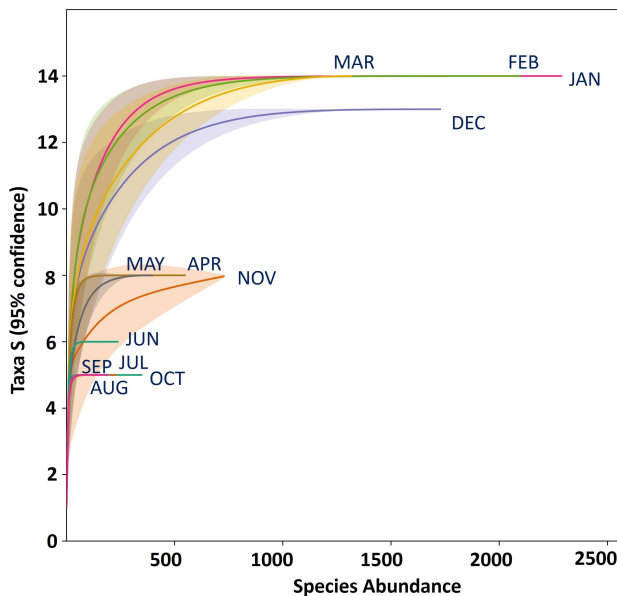


Figure 4. Individual rarefaction curve illustrating temporal variation in species accumulation pattern

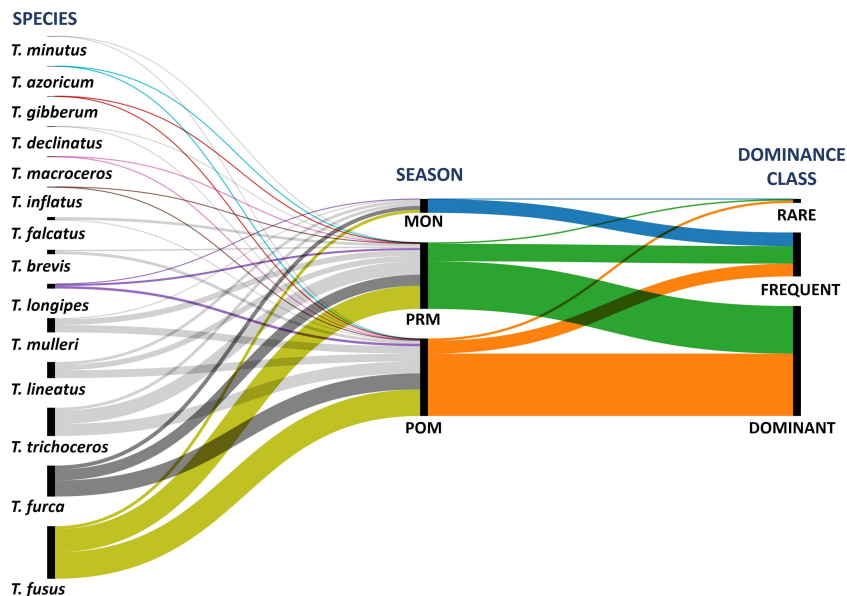


Figure 3. Alluvial diagram of all *Triplos* spp. redistribution by absolute abundance, band width proportionate to species contribution to the seasonal assemblage (Identified dominance classes as Dominant > 1000, Frequent 100-1000, and Rare < 100)

Individual rarefaction curves (Fig. 4) constructed from monthly mean abundance show distinct variation in species accumulation across the sampling period. The curves for January, February, and March demonstrate higher asymptotes, suggesting an increased estimated richness during the late POM and early PRM periods. In contrast, the curves for June–September level off at much lower values, reflecting very few species during peak MON. Intermediate patterns in October–November suggest transitional richness as favourable environmental conditions shift during POM phases. The monthly differences in rarefaction patterns reflect the strong seasonal influence on *Triplos* community structure. Higher richness in POM and early PRM likely corresponds to stable hydrographic conditions, enhanced salinity, nutrient availability, and reduced turbidity, which collectively favour greater species coexistence. The monsoon-related drops in richness (June to September) are due to the inflow of freshwater, the lowering of salinity, and the high turbidity load, all of which are known to reduce dinoflagellate diversity. The gradual recovery observed during the post-monsoon months (October, November) indicates reduced turbidity and re-stabilization of nutrient loads as hydrological conditions become more stable (Patil & Anil, 2011; Bharathi & Sarma, 2019).

3.4. Seasonal Diversity Indices

Changes in diversity and richness indices also showed

significant seasonal shifts (Fig. 5). Simpson's diversity ($1-D$) stayed more or less same all year, with values for POM between 0.74 and 0.80, for PRM between 0.72 and 0.82, and for MON between 0.76 and 0.80. Shannon's index (H') showed clear separation, with values of 1.58–1.89 in POM and PRM and 1.51–1.69 in MON. The Brillouin index (B) showed a clear pattern, with the highest values in the POM and PRM and the lowest values in the MON (1.39–1.55). Interestingly, Pielou's evenness (E) and Equitability index (J) was always higher in MON, ranging respectively from 0.47 to 0.97 and 0.71 to 0.97. The highest values of Margalef richness were in PRM (1.02–1.82), and the lower was in MON (0.71–0.92). Menhinick's index showed similar patterns, with higher values in POM and PRM (0.26–0.42) and lower values in MON (0.30–0.39). The highest value for dominance (D) was in PRM (up to 0.2831), and the lowest value was in MON (about 0.198). The higher Shannon, Simpson, and Brillouin indices observed during POM and PRM suggest more diverse and intricate assemblages characterized by low dominance, suggesting suitable hydrographic conditions that facilitate the coexistence of various taxa. The decrease in diversity and species richness observed during MON can be connected with significant physical stress associated with rainfall, freshwater influx, lowering salinity, turbidity, and hydrodynamic disturbance (Sahu *et al.*, 2014). Though MON displayed very few taxa, but showed the highest evenness, suggesting a uniform

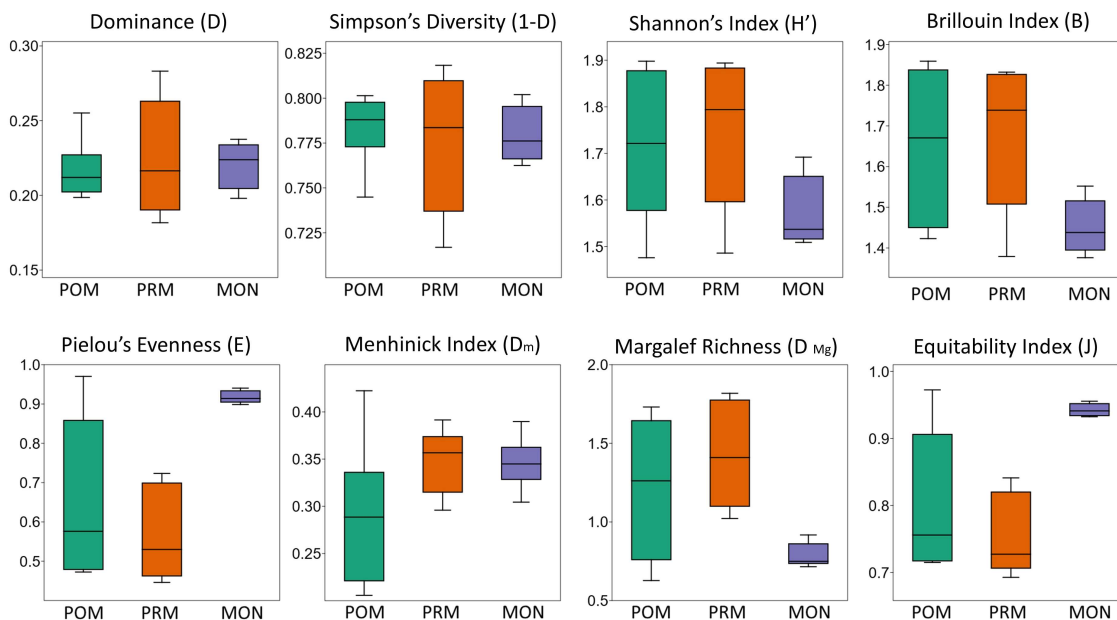


Figure 5. Seasonal variation in diversity and richness indices

distribution along with low abundance assemblage. These marked increase in diversity from late POM (December-January) to its peak in early PRM (February-March) reflects the transition to more favourable water conditions that support the growth and proliferation of *Triplos* populations. The seasonal rise in abundance and diversity is consistent with previous studies that relates phytoplankton growth with positive changes in salinity, stability, and nutrient availability (Sathish et al., 2022; Pradhan et al., 2023; Huang

et al., 2024). The investigation reveals that the increase in *Triplos* abundance and diversity during the late post-monsoonal months reflects the specific adaptive response of these dinoflagellate cells to varying hydrological conditions, demonstrating their unique ecology in the relatively unexplored Hooghly estuarine system.

3.5. Species Ordination and Correlation

The NMDS ordination showed that the *Triplos*

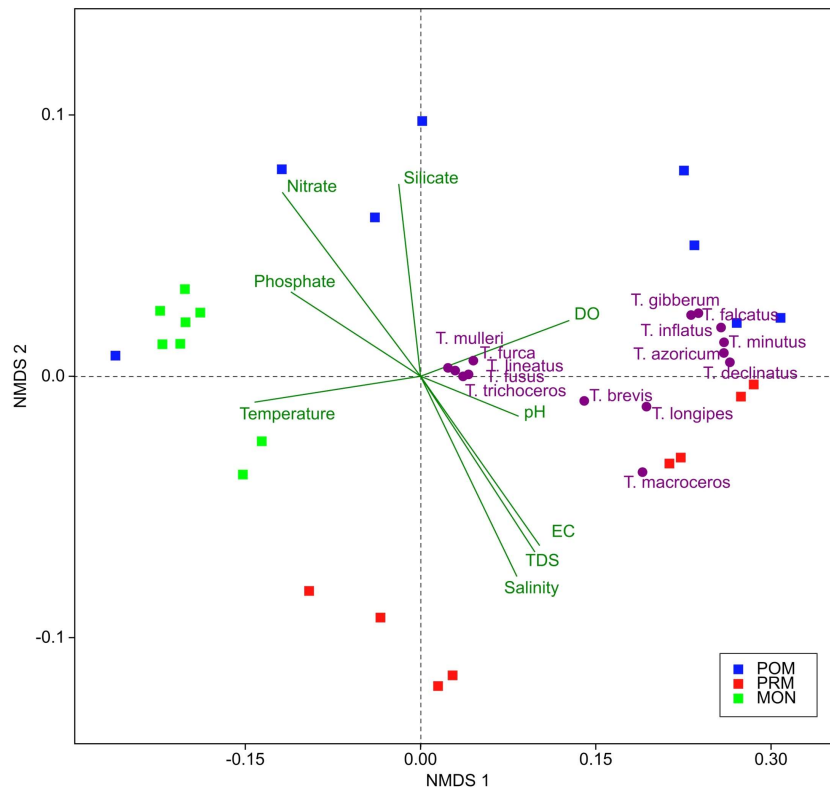


Figure 6. NMDS plot with environmental vectors showing abundance of all *Triplos* spp. in different seasons

Table 2. Environmental vectors fitted to the NMDS ordination showing axis correlations, r², significance

Rank	Environmental variable	NMDS1 (r)	NMDS2 (r)	r ²	p-value
1	Temperature	-0.93919	-0.06548	0.88637	0.001
2	Nitrate	-0.78213	0.46531	0.82824	0.001
3	Dissolved oxygen (DO)	0.8415	0.1411	0.72803	0.001
4	Electrical Conductivity (EC)	0.67391	-0.42809	0.63742	0.001
5	Total Dissolved solids (TDS)	0.64681	-0.44438	0.61584	0.001
6	Phosphate	-0.73361	0.21327	0.58367	0.001
7	Salinity	0.54506	-0.50626	0.55339	0.001
8	pH	0.55485	-0.10104	0.31807	0.001
9	Silicate	-0.12352	0.48635	0.25179	0.001

assemblages were clearly separated into three distinct seasonal clusters along the two NMDS axes (Fig. 7). The distribution of samples reveals significant changes in community structure over time due to variations in nutrient levels and water conditions. Environmental vectors applied to the ordination space elucidated the gradients that support the observed patterns. Axis 1 exhibited strong positive correlations with DO (0.84), EC (0.67), TDS (0.65), salinity (0.55), and pH (0.55), and strong negative correlations with temperature (-0.94), nitrate (-0.78), and phosphate (-0.73). This axis shows a gradient of salinity and dissolved oxygen, highlighting the differences between POM saline, oxygenated water, and the nutrient-rich, lower-salinity conditions observed during the MON. Axis 2 exhibited a positive correlation with silicate (0.49) and nitrate (0.47), while showing a negative correlation with salinity (-0.51), EC (-0.43), and TDS (-0.44) suggesting the presence of a secondary nutrient-ion concentration gradient (Kim et al., 2023). The rank of the different environmental vectors in the NMDS ordination along with axis correlations and significance was given in Table 2. The POM samples clustered toward the positive side of Axis 1 and the negative side of Axis 2, corresponding closely with vectors for salinity, DO, EC, and TDS. This indicates that POM assemblages are primarily influenced by high salinity, increased ionic concentration, and enhanced oxygen availability. Species such as *T. fusus*, *T. furca*, *T. brevis*, *T. mulleri*, and *T. trichoceros* were located near these vectors, indicating a strong preference for POM hydrographic conditions (Bharathi et al., 2022).

POM samples showed a transition from the MON conditions to PRM, and characterized by increasing salinity and intermediate nutrient concentrations. MON samples were grouped together on the negative side of Axis 1 and the positive side of Axis 2, right next to the nitrate and silicate vectors. This indicates that MON assemblages are shaped primarily by nutrient enrichment, reduced salinity, and cooler temperatures characteristic of heavy freshwater inflow. The NMDS data collectively illustrate that the composition of the *Triplos* community is significantly influenced by seasonal fluctuations in hydrographic parameters, including salinity, temperature, dissolved oxygen, and nutrient concentrations (Rothenberger et al., 2014). The recognized seasonal patterns validate the changes in abundance data and the SIMPER analysis, emphasizing the unique ecology of each *Triplos* throughout the annual cycle (Sidik et al., 2008). By integrating

multivariate analyses, the study identifies temperature, salinity, and nutrient gradients as key drivers of species restructuring. These findings are especially relevant under current conditions of increasing pollution and climate-driven changes in freshwater flow and temperature, providing an updated baseline for future ecological assessments.

As phytoplankton form the primary trophic base of estuarine ecosystems and play a fundamental role in supporting zooplankton populations and higher trophic levels. Seasonal variations in *Triplos* abundance may influence food availability for grazers and contribute to dissolved oxygen dynamics through photosynthetic activity. These shifts in abundance can also affect zooplankton and juvenile fish through changes in natural food supply, which in turn supports open estuarine capture fisheries. While such trophic assessments are more straightforward in pond aquaculture, phytoplankton-based insights from this study can still help in understanding natural feeding conditions and may provide useful guidance for fisheries management in dynamic estuarine systems. Although zooplankton and fish productivity were not assessed in the present study, the documented phytoplankton patterns provide important insight into the potential trophic functioning of the open dynamic estuarine ecosystem regulated by tidal exchange, freshwater discharge, and hydrodynamic variability, making direct extrapolation more complex.

Present study focuses on the seasonal dynamics of *Triplos*, direct evaluation of higher trophic linkages and fisheries implications requires further dedicated studies incorporating multi-trophic datasets and long-term monitoring. Future research integrating long-term datasets with AI-based predictive modelling and remote sensing approaches could forecast bloom dynamics and improve understanding of estuarine phytoplankton responses under changing climatic and environmental conditions.

4. CONCLUSION

Triplos spp. exhibit clear seasonal pattern in abundance and community structure as due to changing hydrographic conditions. The post-monsoon period supports higher species richness, diversity, and abundance, indicating favourable physicochemical conditions for the growth of these dinoflagellates. Bloom-forming taxa such as *T. furca* and *T. fusus* contributed substantially to seasonal dissimilarity, highlighting their ecological significance in nutrient-sensitive estuarine environments facing

anthropogenic pressure. Since phytoplankton form the base of the aquatic food web, understanding the ecology of *Tripes* enhance the understanding of estuarine productivity, trophic functioning and the potential support to fish populations. The study also highlights the need for long-term ecological monitoring that can enhance our knowledge of estuarine phytoplankton dynamics in the tropical monsoon-influenced Hooghly estuary under increasing stress due to climatic change.

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CRediT authorship contribution statement

GB: Investigation; Data curation; Formal analysis; Validation; Visualization; Writing draft, SKS: Conceptualization; Methodology; Review & editing; Supervision

Conflict of interest

The authors declare that they have no known competing financial or personal relationships that could have influenced the work reported in this paper.

Data availability statement

Data will be made available by the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted tools were employed in the writing of the text or in the creation or alteration of any figures, photos, or artwork.

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Spatial and Seasonal Dynamics of Ichthyofaunal Diversity in the Upper Subarnarekha River, Jharkhand, India

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ABSTRACT: Five stations across a 40 km stretch of the upper Subarnarekha River, Ranchi district, Jharkhand, were sampled monthly from January to December 2021. Sampling covered three seasons: pre-monsoon (January-May), monsoon (June-September), and post-monsoon (October-December). Across 60 sampling events, 62 fish species were recorded, belonging to 38 genera, 19 families, and 9 orders. Cyprinidae dominated, accounting for 28 species — 45% of total richness. The highest diversity was at Tatisilwai (Site 4; $H' = 3.21$, $1-D = 0.89$, $J' = 0.84$), where mixed rocky-muddy substrate and well-developed macrophyte beds support a notably even assemblage. Namkum (Site 3; $H' = 2.36$, $1-D = 0.79$) recorded the poorest diversity; domestic sewage from Ranchi city enters this reach visibly and consistently. Post-monsoon was the richest season across all five stations (repeated-measures ANOVA, $F(2,8) = 9.23$, $p = 0.008$), driven by improved discharge, better oxygen conditions, and the appearance of monsoon-season recruits. Sensitive species — *Tor tor*, *Bagarius bagarius*, and *Chitala chitala* — were confined to the two least disturbed upstream stations. NMDS ordination (Bray-Curtis; stress = 0.11) separated the impacted stations (3 and 5) sharply from the intact ones (1 and 4), with sand mining and effluent discharge the primary drivers. The dataset gives a repeatable baseline for managing fish communities in this stretch of the river.

Keywords: Ichthyofaunal diversity, Subarnarekha River, Species richness, Seasonal variation, Habitat heterogeneity, Invasive species, Freshwater conservation.

1. INTRODUCTION

Freshwater fishes are among the most threatened vertebrate groups on the planet, yet they receive far less conservation attention than mammals or birds. More than 15,000 species have been described (Froese & Pauly, 2019; Dudgeon et al., 2006), but habitat loss, pollution, and invasive species are shrinking that number faster than the literature can track. In Asia, the situation is acute. The Mekong, Ganges, and Yangtze basins together hold a disproportionate share of global freshwater fish diversity (Nelson et al., 2016; Pusey & Arthington, 2003), and India alone records over 2,500 freshwater species (Pinder et al.,

2019)- a figure that would surprise most people outside the field.

The Subarnarekha starts near Ratu in Ranchi district, at roughly 610 m on the Chotanagpur Plateau, and runs about 395 km eastward through Jharkhand, West Bengal, and Odisha before reaching the Bay of Bengal (Singh & Giri, 2018; Banerjee et al., 2022). Its catchment covers around 19,500 km². The Kharkai, Roro, and Kanchi rivers are its main tributaries in the Jharkhand section. In that upper stretch, the river crosses rocky plateau terrain with shallow riffles, deep pools, and patches of intact riparian forest — conditions that favour a diverse fish fauna. Local tribal communities have fished here for generations, mostly using

traditional gear, and the river still holds good populations of commercially valuable species (Saba & Sadhu, 2017).

What the river holds is worth listing plainly. Major carps - *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* - are present throughout. Catfishes, including *Clarias batrachus* and *Heteropneustes fossilis*, dominate slower sections. The clear, fast reaches near the source still carry rheophilic species: *Mastacembelus armatus*, *Ompok bimaculatus*, and, where conditions allow, *Tor tor* - a species that has become genuinely scarce across much of its range.

That scarcity has causes. Industrial activity near Jamshedpur, unregulated sand extraction, the Chandil Dam, and domestic sewage from Ranchi have all damaged the river at different points. *Oreochromis mossambicus* has established itself in disturbed reaches and is displacing native species. Populations of *Tor tor*, *Bagarius bagarius*, and *Gudusia chapra* have declined noticeably (Bera, 2022). The pressures are not abstract - they are visible at the water's edge.

Most published work on the Subarnarekha's fish fauna covers the West Bengal and Odisha sections (Bera, 2022). The upper Jharkhand reach, which is ecologically quite different from the lowland stretches, has received comparatively little attention. Devi & Pandit (2018) documented 41 species from this general area, but their survey was shorter in duration and covered fewer stations. The present study sampled five stations monthly for a full year, with the aim of documenting spatial and seasonal variation in assemblage structure and relating that variation to measurable habitat and anthropogenic gradients. The results are compared against earlier records from this and other Indian river systems.

2. MATERIALS AND METHODS

2.1. Study Area

The study covered approximately 40 km of river within

Ranchi district, from the source at Ratu downstream to the Silli block (Figure 1). The upper part of this reach is rocky and largely intact; the lower part passes through or near semi-urban areas affected by agriculture, sand mining, and domestic waste disposal. The region has a monsoon climate, with 1,200-1,400 mm of rain falling mostly between June and September. Outside that window, flows drop considerably, and the river character shifts from fast and turbid to slow and clear. Five stations were positioned to capture this gradient (Table 1).

2.2. Fish Sampling

Each station was sampled once per month from January to December 2021, giving 12 visits per station and 60 sampling events in total. At each visit, fish were collected for two hours using cast nets (2 cm and 4 cm mesh), gill nets (3-6 cm mesh), drag nets, and hook-and-line. Gear selection was consistent across visits. In addition to experimental sampling, specimens purchased from local fishermen at each station were examined, identified, and recorded separately; these were cross-checked against field collections to avoid double-counting. Fish were identified to species level using Jayaram (2010) and FishBase (Froese & Pauly, 2019). Specimens that could not be identified reliably in the field were preserved in 10% formalin for laboratory examination. Where possible, fish were returned to the river after identification.

2.3. Water Quality and Habitat

At each sampling event, the following parameters were recorded in situ: water temperature (°C), pH, dissolved oxygen (mg/L), electrical conductivity (µS/cm), and turbidity (NTU), using calibrated field meters (pre-calibrated against standards each morning). Flow velocity was measured at three cross-section transects per station

Table 1. Characteristics of the five sampling stations

Site	Location / Coordinates	Substrate	Land use	Main disturbances	Pressure
S1 – Ratu 23.3645°N, 85.2257°E	Rocky upland, river origin	Boulders & gravel	Forest / scrubland	Negligible	Low
S2 – Itki 23.3492°N, 85.3084°E	Shallow reach near village	Sand & silt	Agriculture & settlement	Agricultural runoff, subsistence fishing	Low–Moderate
S3 – Namkum 23.3278°N, 85.3889°E	Slow, urbanised section	Silt & mud	Ranchi city periphery	Domestic effluent, fishing pressure	High
S4 – Tatisilwai 23.3210°N, 85.4663°E	Meandering reach, macrophytes	Mixed rock & mud	Semi-rural, riparian forest	Limited sand mining	Moderate
S5 – Silli 23.2974°N, 85.5510°E	Variable flow, semi-rural	Sand & gravel	Agriculture & settlement	Sand extraction, bathing, fishing	Moderate–High

using a current meter. Habitat was described by substrate type (boulder, gravel, sand, silt, mud), estimated riparian canopy cover (%), and presence of macrophytes. These habitat descriptors were noted at each visit and used to contextualise assemblage patterns.

2.4. Diversity Indices

All indices were calculated from individual fish counts (number basis, not weight). Five metrics were computed for each station-season combination: Shannon-Wiener $H' = -\sum p_i \ln p_i$; Simpson's 1-D where $D = \sum p_i^2$; Margalef's richness $D_{mg} = (S-1) / \ln N$; Pielou's evenness $J' = H' / \ln S$; and Bray-Curtis dissimilarity for pairwise between-station comparisons. Here S is species count, N is total individuals, and p_i is the proportional abundance of species I.

2.5. Statistical Analysis

Differences in species richness among stations were tested with one-way ANOVA, followed by Tukey's HSD to identify which pairs differed. Seasonal effects were tested with repeated-measures ANOVA, with station as the within-subject factor and season as the repeated measure. Pearson's correlation was used to examine the relationship between an urban proximity index (ranked by distance from Ranchi city centre) and Shannon H' . Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was

used to ordinate station-season samples. All analyses were done in R version 4.2.0 (R Core Team, 2022), with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Species Composition

Sixty-two species were recorded across all stations and seasons (Table 2). They belong to 38 genera, 19 families, and 9 orders. Cyprinidae was the largest family by species count - 28 species, or 45% of the total (calculated as $28/62 \times 100$). This is consistent with the dominance of cyprinids in other Peninsular Indian rivers (Pinder et al., 2019) and with earlier records from the downstream West Bengal section of this river (Bera, 2022). Bagridae contributed 7 species; Channidae and Siluridae 4 and 3 respectively.

The distribution of sensitive species tells the more important story. *Tor tor* (IUCN: Vulnerable) and *Bagarius bagarius* (IUCN: Vulnerable) were found only at Sites 1 and 2. *Chitala chitala* (IUCN: Near Threatened) appeared at Sites 1 and 4. *Schistura savona* and *Amblyceps mangois* were restricted entirely to Site 1. None of these turned up at Sites 3 or 5. At those impacted stations, *Gambusia affinis* and *Oreochromis mossambicus* were common year-round. That replacement - rheophilic natives giving way to introduced generalists - is the clearest signal of degradation in the data.

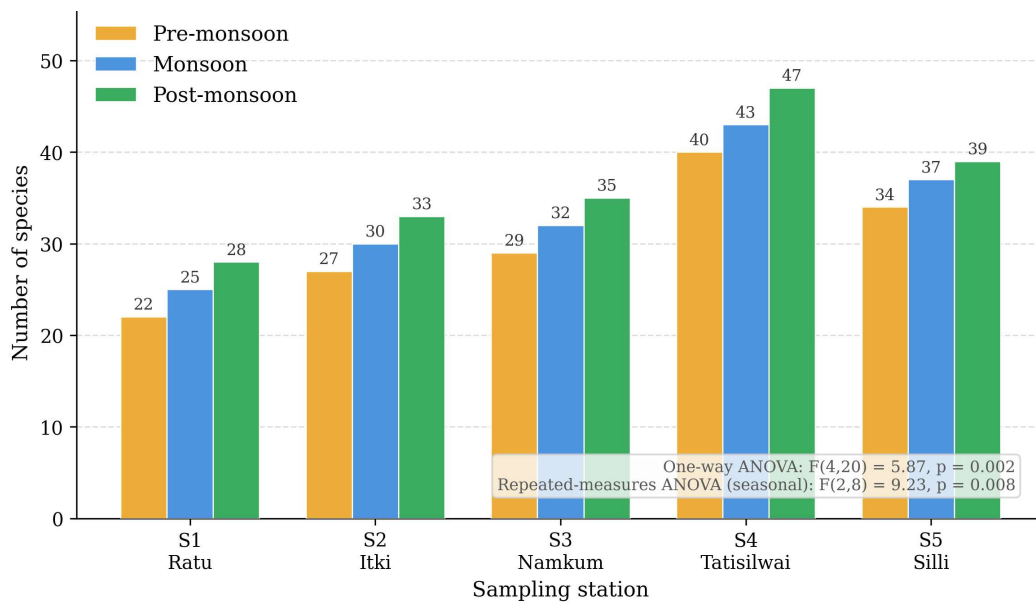


Figure 1. Seasonal species richness at each station (pre-monsoon, monsoon, post-monsoon). Data labels give species count. One-way ANOVA: $F(4,20) = 5.87, p = 0.002$. Repeated-measures ANOVA for seasonal effect: $F(2,8) = 9.23, p = 0.008$

Table 2. Fish species recorded from the upper Subarnarekha River (January–December 2021)

Species	Family	Common name	IUCN	Abundance	Sites	Tolerance	Order
<i>Labeo rohita</i>	Cyprinidae	Rohu	LC	+++	1–5	Moderate	Cypriniformes
<i>Catla catla</i>	Cyprinidae	Catla	LC	+++	1–5	Moderate	Cypriniformes
<i>Cirrhinus mrigala</i>	Cyprinidae	Mrigal	LC	+++	1–5	Moderate	Cypriniformes
<i>Labeo bata</i>	Cyprinidae	Bata	LC	++	1–5	Moderate	Cypriniformes
<i>Puntius sophore</i>	Cyprinidae	Pool barb	LC	++	1–5	Moderate	Cypriniformes
<i>Puntius ticto</i>	Cyprinidae	Two-spot barb	LC	++	2–5	Moderate	Cypriniformes
<i>Tor tor</i>	Cyprinidae	Mahseer	VU	+	1, 2	Low	Cypriniformes
<i>Barilius bendelisis</i>	Cyprinidae	Hamilton barila	LC	+	1, 2	Low	Cypriniformes
<i>Salmostoma bacaila</i>	Cyprinidae	Large razorbelly	LC	++	1–3	Low–Moderate	Cypriniformes
<i>Rasbora daniconius</i>	Cyprinidae	Slender rasbora	LC	++	2–5	Moderate	Cypriniformes
<i>Chagunius chagunio</i>	Cyprinidae	Chaguni	LC	+	1, 2	Low	Cypriniformes
<i>Garra mullya</i>	Cyprinidae	Torrent fish	LC	+	1	Low	Cypriniformes
<i>Devario devario</i>	Cyprinidae	Sind danio	LC	+	1–3	Low–Moderate	Cypriniformes
<i>Esomus danrica</i>	Cyprinidae	Flying barb	LC	++	3–5	High	Cypriniformes
<i>Amblypharyngodon mola</i>	Cyprinidae	Mola carplet	LC	+++	2–5	High	Cypriniformes
<i>Puntius conchoniis</i>	Cyprinidae	Rosy barb	LC	++	3–5	High	Cypriniformes
<i>Puntius chola</i>	Cyprinidae	Swamp barb	LC	++	3–5	High	Cypriniformes
<i>Pethia phutunio</i>	Cyprinidae	Spottedsail barb	LC	+	3–5	Moderate	Cypriniformes
<i>Laubuca laubuca</i>	Cyprinidae	Indian glass barb	LC	+	2–4	Moderate	Cypriniformes
<i>Osteobrama cotio</i>	Cyprinidae	Pearly razorbelly	LC	+	2–4	Moderate	Cypriniformes
<i>Aspidoparia morar</i>	Cyprinidae	Morar	LC	+	2–4	Moderate	Cypriniformes
<i>Salmophasia sardinella</i>	Cyprinidae	Small razorbelly	LC	++	2–5	Moderate	Cypriniformes
<i>Danio dangila</i>	Cyprinidae	Moustached danio	LC	+	1, 2	Low	Cypriniformes
<i>Crossocheilus latius</i>	Cyprinidae	Scale-lip	LC	+	1, 2	Low	Cypriniformes
<i>Rohtee ogilbii</i>	Cyprinidae	Ogilby's rohtee	LC	+	1–3	Low–Moderate	Cypriniformes
<i>Schistura savona</i>	Nemacheilidae	Stone loach	LC	+	1	Low	Cypriniformes
<i>Lepidocephalichthys guntea</i>	Cobitidae	Guntea loach	LC	+	1–3	Low–Moderate	Cypriniformes
<i>Bagarius bagarius</i>	Sisoridae	Goonch catfish	VU	+	1	Low	Siluriformes
<i>Pseudolaguvia shawi</i>	Sisoridae	Rock catlet	NE	+	1	Low	Siluriformes
<i>Gagata cenia</i>	Sisoridae	Indian gagata	LC	+	1–3	Low	Siluriformes
<i>Mystus tengara</i>	Bagridae	Tengara catfish	LC	++	1–4	Moderate	Siluriformes
<i>Mystus vittatus</i>	Bagridae	Striped catfish	LC	++	2–5	Moderate	Siluriformes
<i>Rita rita</i>	Bagridae	Rita catfish	LC	+	1–3	Low	Siluriformes
<i>Sperata seenghala</i>	Bagridae	Giant river catfish	LC	+	1, 2	Low	Siluriformes
<i>Amblyceps mangois</i>	Amblycipitidae	Torrent catfish	NE	+	1	Low	Siluriformes
<i>Clarias batrachus</i>	Clariidae	Walking catfish	LC	++	2–5	High	Siluriformes
<i>Heteropneustes fossilis</i>	Heteropneustidae	Stinging catfish	LC	++	3–5	High	Siluriformes
<i>Ailia coila</i>	Schilbeidae	Gangetic ailia	LC	+	2–4	Low–Moderate	Siluriformes
<i>Wallago attu</i>	Siluridae	Wallago	LC	+	1–3	Low	Siluriformes

Cont...

Table 2. Fish species recorded from the upper Subarnarekha River (January–December 2021)

Species	Family	Common name	IUCN	Abundance	Sites	Tolerance	Order
<i>Ompok bimaculatus</i>	Siluridae	Glass catfish	LC	+	1, 2	Low	Siluriformes
<i>Chitala chitala</i>	Notopteridae	Clown knifefish	NT	+	1, 4	Low	Osteoglossiformes
<i>Notopterus notopterus</i>	Notopteridae	Grey featherback	LC	+	2–4	Moderate	Osteoglossiformes
<i>Gudusia chapra</i>	Clupeidae	Indian river shad	LC	++	2–4	Moderate	Clupeiformes
<i>Hilsa ilisha</i>	Clupeidae	Hilsa shad	LC	+	4, 5	Moderate	Clupeiformes
<i>Channa striata</i>	Channidae	Snakehead murrel	LC	++	2–5	High	Anabantiformes
<i>Channa punctata</i>	Channidae	Spotted snakehead	LC	++	3–5	High	Anabantiformes
<i>Channa marulius</i>	Channidae	Great snakehead	LC	+	1, 2	Low	Anabantiformes
<i>Mastacembelus armatus</i>	Mastacembelidae	Tyre-track eel	LC	+	1, 2	Low	Synbranchiformes
<i>Mastacembelus pancalus</i>	Mastacembelidae	Striped spiny eel	LC	+	2–4	Low–Moderate	Synbranchiformes
<i>Macrognathus aculeatus</i>	Mastacembelidae	Lesser spiny eel	LC	+	4, 5	Moderate	Synbranchiformes
<i>Monopterusuchia</i>	Synbranchidae	Cuchia eel	LC	+	3–5	High	Synbranchiformes
<i>Glossogobius giuris</i>	Gobiidae	Tank goby	LC	++	3–5	High	Gobiiformes
<i>Rhinogobius flumineus</i>	Gobiidae	River goby	LC	+	1–3	Low	Gobiiformes
<i>Mugil cephalus</i>	Mugilidae	Flathead mullet	LC	+	4, 5	Moderate	Mugiliformes
<i>Nandus nandus</i>	Nandidae	Gangetic leaf fish	LC	+	2–4	Moderate	Perciformes
<i>Ambassis nama</i>	Ambassidae	Burmese glassy fish	LC	+	3–5	High	Perciformes
<i>Parambassis ranga</i>	Ambassidae	Indian glassy fish	LC	+	3–5	High	Perciformes
<i>Xenentodon cancila</i>	Belonidae	Freshwater garfish	LC	+	2–4	Moderate	Beloniformes
<i>Colisa fasciata</i>	Osphronemidae	Banded gourami	LC	+	3–5	High	Anabantiformes
<i>Gambusia affinis</i>	Poeciliidae	Mosquitofish	LC	+++	3, 5	Very High	Cyprinodontiformes
<i>Oreochromis mossambicus</i>	Cichlidae	Mozambique tilapia	LC	+++	3, 5	Very High	Cichliformes
<i>Colisa lalia</i>	Osphronemidae	Dwarf gourami	LC	+	3–5	High	Anabantiformes

Abundance: + Rare (1–2 individuals per sampling event); ++ Common (3–10); +++ Abundant (>10). IUCN: LC Least Concern; NT Near Threatened; VU Vulnerable; NE Not Evaluated

Table 3. Species richness and diversity indices by station and season

Parameter / Season	S1	S2	S3	S4	S5	H' (annual)	1-D (annual)	J' (annual)
Richness – Pre-monsoon	22	27	29	40	34	2.14	0.79	0.68
Richness – Monsoon	25	30	32	43	37	2.68	0.83	0.74
Richness – Post-monsoon	28	33	35	47	39	2.89	0.87	0.77
H' (Shannon–Wiener, annual)	2.04	2.51	2.36	3.21	2.78			
1-D (Simpson's, annual)	0.72	0.81	0.79	0.89	0.84			
Margalef's Dmg	3.82	4.73	4.12	6.18	5.44			
Pielou's J'	0.60	0.72	0.71	0.84	0.79			

3.2. Spatial Variation in Ichthyofaunal Diversity

Species richness ranged from 22 at Site 1 during pre-monsoon to 47 at Site 4 during post-monsoon (Table 3; Figure 1). One-way ANOVA confirmed the between-station differences are real ($F(4,20) = 5.87, p = 0.002$); Tukey's HSD identified Site 4 as significantly richer than Sites 1 and 3. Annual diversity indices are given in Table 3 and Figure 2.

Site 4 - Tatisilwai - is the most diverse station by every measure ($H' = 3.21, 1-D = 0.89, Dmg = 6.18, J' = 0.84$; Figure 2). It is a broad, meandering reach with pools of varying depth, mixed rocky and muddy substrate, and well-established macrophyte beds along the margins. That structural variety allows multiple guilds to coexist: rheophilic species use the rocky runs, benthic feeders work the muddy pools, and macrophyte beds shelter juveniles and small cyprinids. Habitat complexity and fish diversity are tightly linked in Indian riverine systems (Barton & Anderson, 2016; Jackson et al., 2001), and this station fits that pattern exactly.

Site 1 - Ratu - needs some explanation. It is ecologically intact, yet it records lower total richness (maximum 28 species) than the disturbed mid-reach stations. This is not a contradiction. Site 1 holds only sensitive rheophilic species, most present at low densities. The tolerant generalists and invasive species that inflate cumulative counts at Sites 3 and 5 are simply absent here. Low species richness at a pristine headwater station is not a failure - it reflects natural zonation. The species that are there - *Tor tor*, *Schistura savona*, *Amblyceps mangois* - cannot be found anywhere else in the survey reach.

Site 3 recorded the lowest diversity among the mid-reach stations ($H' = 2.36, 1-D = 0.79, J' = 0.71$). The reduced evenness index is telling: it is not that species are absent, but that a handful of tolerant species dominate numerically while the rest are barely represented. That pattern is typical of enriched, degraded urban river sections, where organic loading suppresses dissolved oxygen and eliminates the more sensitive taxa (Allan & Castillo, 2007; Poff et al., 2010). The negative correlation between urban proximity and H' across stations ($r = -0.76, p = 0.01$) confirms the direction of this gradient.

3.3. Seasonal Variation in Ichthyofaunal Diversity

Post-monsoon was the richest season at every station (Table 3; Figure 4). Repeated-measures ANOVA confirmed this seasonal effect is consistent rather than site-specific ($F(2,8) = 9.23, p = 0.008$; $F(2,8)$ representing 2 seasonal contrasts and 8 error degrees of freedom). Pre-monsoon was consistently the poorest season, with monsoon intermediate.

Three things happen in October-December that push richness up. River levels drop to moderate flows after the monsoon flood pulse, increasing habitat diversity and making sampling more efficient across gear types. Dissolved oxygen rises as water temperature falls and turbulence increases, which allows oxygen-sensitive species to reoccupy areas they vacated during the warm, turbid monsoon period. And the monsoon breeding season is followed by juvenile recruitment: fish hatched in July-September appear in the catch as small but catchable individuals by October. Shrestha et al. (2013) documented

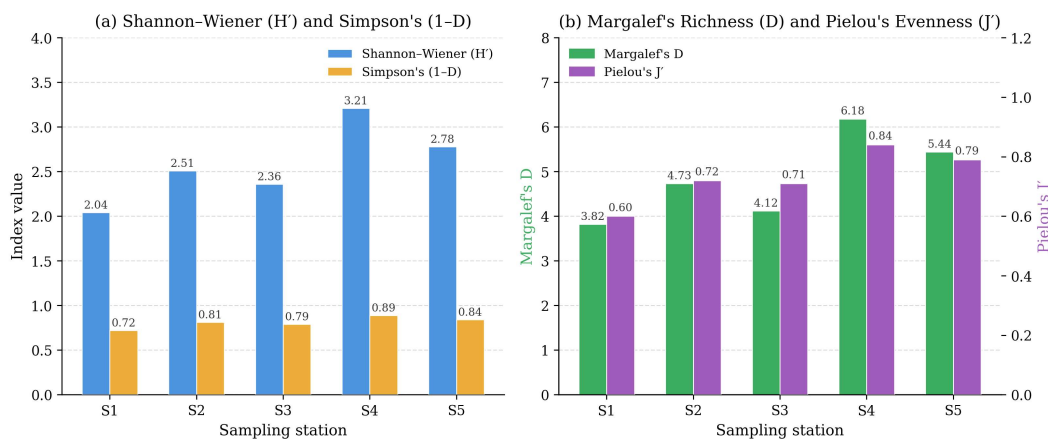


Figure 2. Annual diversity indices by station. Left panel: Shannon–Wiener H' and Simpson's $1-D$. Right panel: Margalef's D (left axis) and Pielou's J' (right axis)

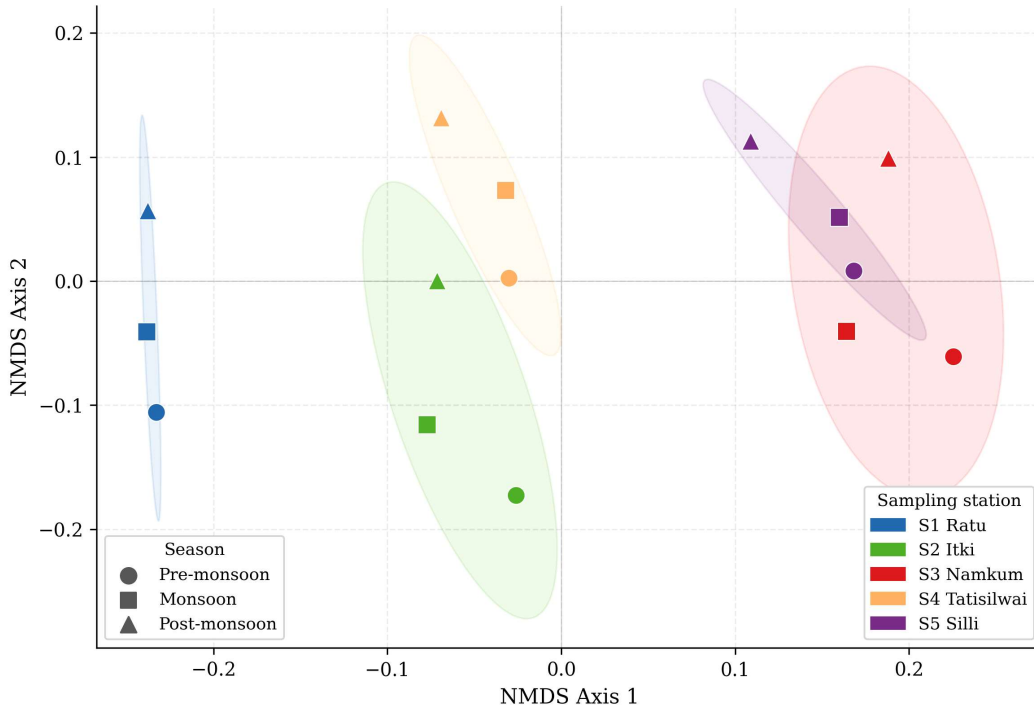


Figure 3. NMDS ordination based on Bray–Curtis dissimilarity (stress = 0.11). Each point is one station–season combination. Shaded ellipses mark per-station groupings. Symbols: circle = pre-monsoon; square = monsoon; triangle = post-monsoon

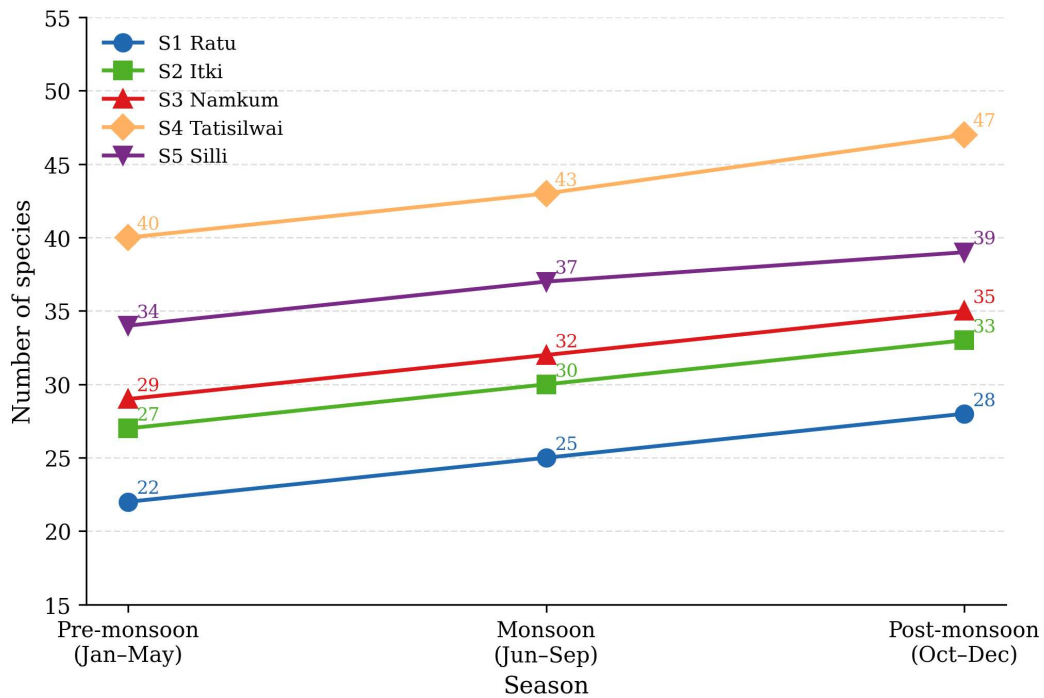


Figure 4. Seasonal trends in species richness across all five stations, showing the consistent post-monsoon peak

the same post-monsoon pattern in Nepali rivers; Dahanukar et al. (2004) found comparable trends in Deccan Plateau systems.

The monsoon season itself presents a sampling paradox. Productivity in the river is highest at this time - runoff delivers nutrients, invertebrate biomass is elevated, and food availability is good. But high discharge, elevated turbidity, and fast flows reduce catch efficiency with passive gear and push many species into marginal habitats not covered by fixed sampling stations. So the catch is lower, but that does not mean fish abundance is lower. Pre-monsoon low-flow conditions simply concentrate fish into remaining deep pools, which actually makes them easier to sample - yet total richness is lowest then, because many species that move into the river from floodplain areas during the monsoon have not yet arrived.

3.4. Assemblage Patterns: NMDS and Community Structure

NMDS ordination (Bray-Curtis, stress = 0.11) produced a clear pattern (Figure 3). Sites 1 and 4 group together despite being 14 km apart. The similarity is not accidental: both stations have complex habitat structure, low-to-moderate disturbance, and sustain sensitive and moderately sensitive species. Sites 3 and 5, by contrast, cluster on the opposite side of the ordination, driven by the dominance of *Gambusia affinis* and *Oreochromis mossambicus* at both locations. Site 2 falls between these extremes, consistent with its transitional character.

Pairwise Bray-Curtis dissimilarity between Sites 1 and 3 was 0.62. Between Sites 3 and 5 it was 0.28 - these two stations are more similar to each other than to any of the upstream stations, even though they are separated by over 20 km of river. Shared disturbance produces convergent communities. The stressors driving that convergence are not limited to urbanisation and agricultural runoff; sand mining at Sites 2 and 5 destabilises substrate and reduces benthic invertebrate availability, and the cumulative effect of these pressures appears to be additive. Moyle & Leidy (2011) and Pinder et al. (2019) make the same point for Indian freshwater systems broadly: it is rarely a single stressor but the combination that matters.

3.5. Comparison with Earlier Work on the Subarnarekha

Bera (2022) recorded 48 species from the Paschim Medinipur section of the Subarnarekha - a lower-elevation

stretch with more lentic influence. This survey records 62 species from the upper Jharkhand reach. The difference is largely explained by the Chotanagpur Plateau topography: faster, more heterogeneous flows in this stretch support rheophilic guilds absent from the lowland section. Devi & Pandit (2018) reported 41 species from the upper basin; the gap between that figure and the current 62 reflects a full annual sampling cycle across five stations rather than a shorter-duration survey. Notably, both *Tor tor* and *Bagarius bagarius* appear more regularly in this upper reach than in downstream records, which is consistent with their preference for rocky, fast-flowing water and their sensitivity to organic enrichment.

4. CONCLUSION

Sixty-two fish species were recorded across five stations in the upper Subarnarekha River over a full annual sampling cycle. Site 4 (Tatisilwai) was the most diverse station by all four indices ($H' = 3.21$, $1-D = 0.89$, $Dmg = 6.18$, $J' = 0.84$); its mixed substrate and macrophyte cover provide the habitat variety that supports a broad species assemblage. Post-monsoon was the richest season at all stations. Site 3 (Namkum), receiving direct domestic effluent from Ranchi, was the poorest in diversity among the mid-reach stations despite sitting downstream of more species-rich locations. Sensitive species including *Tor tor*, *Bagarius bagarius*, and *Chitala chitala* were absent from all impacted stations; their persistence at Sites 1 and 2 depends entirely on those reaches remaining relatively undisturbed.

Three things follow from these results. The headwater reach at Ratu (Site 1) and the Tatisilwai reach (Site 4) are the stations that matter most for conservation. Both support species found nowhere else in this survey; both are under moderate but growing pressure. Designated riparian buffers for these two reaches would be a practical first step. The effluent problem at Namkum (Site 3) is not complicated to diagnose - domestic sewage enters the river visibly - but it has not been addressed. Until it is, Site 3 will continue to act as a barrier between the relatively intact upstream and downstream sections, preventing natural recolonisation. Finally, the invasive species situation at Sites 3 and 5 is manageable now but not indefinitely.

Oreochromis mossambicus spreads quickly once established; active removal and monitoring at these two stations is more cost-effective than attempting to control a river-wide population later. The data presented here provide the baseline needed to track whether any of these interventions actually work.

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CRedit authorship contribution statement

Preeti Kumari: Conceptualisation, Methodology, Field data collection, Formal analysis, Writing - original draft, Writing - review and editing.

Conflict of interest

The author declares no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

No AI tools were used in data collection, analysis, or interpretation.

Data availability statement

Data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary Material Link

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Detecting the Dynamics: Geospatial Monitoring of the Newly Assigned Ramsar Site, “The Wadhvana Wetland” in Central Gujarat, India

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ABSTRACT: Wetland ecosystems are one of the most important areas that provide various ecosystem services as well as habitat for both aquatic and terrestrial life forms. In spite of multiple functions, like biodiversity conservation, water quality improvement, erosion control and climate regulation through CO₂ sequestration, wetland ecosystems are under threat. This study attempts to analyse the wetland dynamics of the Wadhvana Ramsar site using geospatial techniques. The land use land cover (LULC) and Normalized Difference Vegetation Index (NDVI) were investigated through LANDSAT 5 ETM+ of 1990, 2000, 2010, and LANDSAT 8OLI/TIRS of 2020. The LULC for the study area extent is prepared using the Sentinel-2A 2023 dataset. The LULC is classified using a supervised classification method with a maximum likelihood algorithm. The red and infrared bands of Landsat imagery from three different time periods were used to calculate NDVI. Results show that the wetland ecosystem in the study area decreased by 3.07 km² (~45%) with the average rate of ~0.615 km²/year. In contrast, agricultural land decreased by 5.56 km², at an average rate of ~0.428 km²/year between 1990 and 2020. About 0.83km² of wetland is converted to cultivated land, whereas 1.78 and 3.78km² of Water bodies and Barren lands are converted into cultivated land, indicating the biotic pressure on the wetland. To minimise the rapid loss of wetlands and water bodies in the study area, proper land use planning and environmental education should be promoted.

Keywords: Wetland, Change detection, Turbidity, Moisture, Vegetation, Ramsar site.

1. INTRODUCTION

Since the beginning of the twentieth century half of the world's wetlands have been lost (Davidson, 2014) and more than 60% of the remaining wetland ecosystems are being used unsustainably (MEA (Millennium Ecosystem Assessment) 2005). The fate of wetlands is determined by human beings who exploit water resources beyond their needs, which may lead to future crises (Murray et al., 2014). Thus, the preservation of wetlands is an important issue to

address these days. Information and understanding of environmental changes in wetland are necessary to allow the protection and remediation of ecosystems (Ramachandra & Solanki, 2007). Wetlands are known to provide high-value ecosystem services because of their position in the landscape (Zedler et al., 2006) and allow sediments and other materials to accumulate and settle, providing cleaner water for fish, wildlife and people. However, wetlands worldwide have been intensively modified by various land-use and land-cover (LULC) change activities for more than a century,

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owing to the ever-increasing population and economy (Hong et al., 2021). LULC have in turn, resulted in changes in the economic and ecological values of various ecosystem services (Leh et al., 2013; Hasan et al., 2020). Changes in land use and their spatial consequences for the wetland ecosystem are a major concern of geographers and environmental conservationists (Cui et al., 2017). Wetlands are diverse and dynamic ecosystems that play a critical role in maintaining ecological balance and supporting a wide range of plant and animal species. They can be found in various forms, such as marshes, swamps, bogs, and mangroves. These ecosystems are often transitional areas between terrestrial and aquatic environments. Wadhvana Wetland, located near the town of Dabhoi in central Gujarat, was designated a Ramsar site in 2021 for its significant ecological, cultural, and socioeconomic value. Wadhvana Wetland, as a Ramsar site, is meant to be conserved and managed for the long-term benefit of both nature and people.

Earlier studies and reports indicate that this wetland faces multiple threats, including degradation, pollution, and anthropogenic activity during the dry season (Purandara, 2008). Several studies are carried out at Wadhvana wetland including aquatic bird diversity, water quality and floral diversity (Dabgar, 2012; Soniya et al., 2024; Suthar et al., 2019; Vankar et al., 2019), however, there is a paucity in

research data available through which one can understand the dynamics of this wetland with respect to land use and land cover change. For understanding the role of Land Use, Vegetation and other wetland parameters through digital image analysis of remotely sensed data, which serves as a significant marker in the conservation of Wetland or Ramsar sites. Highly functioning wetlands typically have high NDVI values, while disturbed or low-functioning wetlands have lower NDVI values. The aim of the present study is to analyze the wetland dynamics by means of the current and temporal Land Cover, surface vegetation and water cover. The findings of the present study highlight the status of the current situation of the wetland and represent the decadal changes over the years. Understanding these changes is crucial for formulating effective conservation strategies and sustainable management practices to preserve and restore these vital ecosystems.

2. MATERIALS AND METHODS

2.1. Study Area

Wadhvana Wetland, also known as Wadhvana Lake or Reservoir ($22^{\circ} 09' 42.2''N$, $73^{\circ} 28' 32.9''E$), is located in the state of Gujarat, India (Figure 1). It is situated in the Dabhoi tehsil, in the district of Vadodara, approximately 75 kilometers (47 miles) from the city of Vadodara. It is 45km

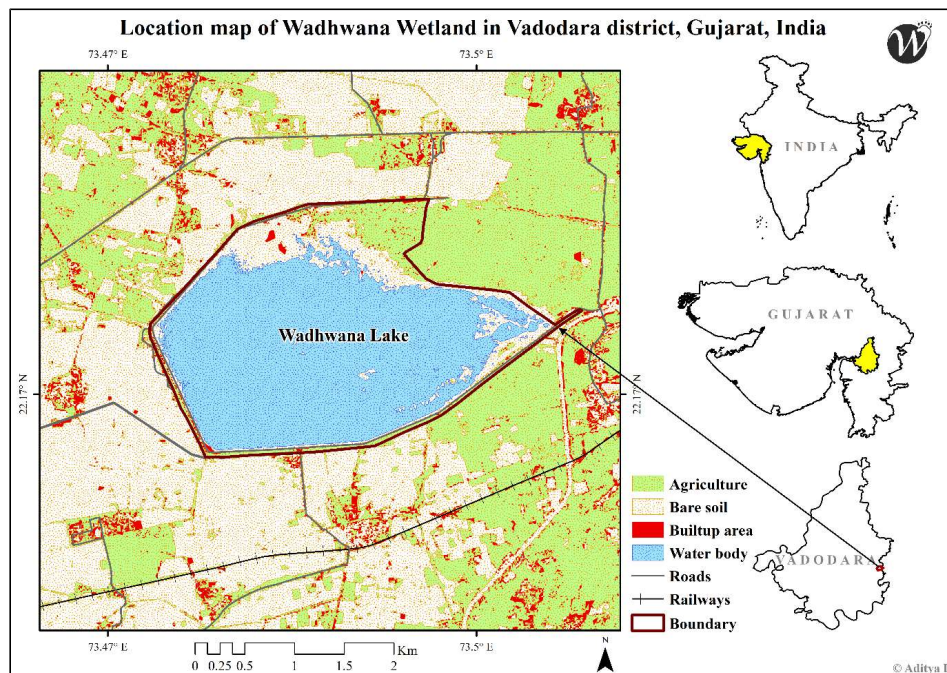


Figure 1. Location map of Wadhvana Wetland, a Ramsar site located in central Gujarat

away from Vadodara city on Vadodara-Dabhoi-Chhota Udepur state highway. Dabhoi town is approximately 15 km from Wadhvana wetland. It was originally created in 1910 by the former King of Baroda State, Maharaja Sayajirao Gaekwad III. Historical records indicate that Shrimant Maharaja Sir Sayajirao Gaekwad III carried out the excavation works between 1909 and 1910 (Suthar et al., 2019), primarily for irrigation purposes. In recent years, the wetland has gained significant ecological recognition and was designated as a Ramsar Site in 2021. The references to more recent development relate not to its initial creation, but to conservation and management initiatives undertaken by the Government of Gujarat. Wadhvana Wetland was officially declared a wildlife sanctuary in 2005, marking a key step in its protection. The wetland covers an area of approximately 5.8 km² with a perimeter of about 11 km (Tatu, 2012).

Wadhvana reservoir is located in Dabhoi tehsil of Vadodara district in central Gujarat. The major inlets of the wetland are the Mahi and Narmada canals, which are feeder canals of the lake. The maximum depth of the wetland measured during the study is 20ft, and the tank area is 1430 acres. It irrigates about 88.15km² land of 25 villages in its vicinity (Mudaliar & Pandya, 2023). The wetland is predominantly used for fishing, irrigation, and the supply of drinking water to nearby villages. The climate in the area is characteristic of hot-dry with the precipitation during the south-west monsoon, i.e. June to September. The average temperature in the area ranges from 12°C to 33°C; however, it can reach 40°C during peak summer and drop to 10°C in winter. The precipitation pattern in the region is primarily governed by large-scale monsoonal circulation rather than the wetland itself. However, the presence of the wetland influences local microclimatic conditions, such as enhancing humidity, moderating temperature, and facilitating localized evapotranspiration processes. These factors may contribute to minor local variations in moisture availability and atmospheric conditions, but do not

significantly control the overall precipitation regime (Murray et al., 2014).

Suthar et al. (2019) have recorded six species of hydrophytes viz, *Hydrilla verticillata*, *Potamogeton nodosus*, *Ipomoea aquatica*, *Nelumbo nucifera*, *Nymphaeoides indica* and *Typha domingensis*. Dabgar (2012) studied floral diversity and recorded 73 genera and 82 species of flora belonging to 43 families. Wadhvana wetland provides wintering ground to many migratory bird species such as Dalmatian Pelican (*Pelecanus crispus*), Greater spotted Eagle (*Clanga clanga*), Pallas's fish-eagle (*Haliaeetus leucoryphus*), Grey-headed fish-eagle (*Ichthyophaga ichthyaetus*), Common Pochard (*Aythya ferina*) and Ferruginous Duck (*Aythya nyroca*).

2.1.1. Soil type

The wetland soil is a mixture of black cotton soil and clay with abundant organic matter. It also contains sporadic deposits of calcareous Kankar. This suggests the presence of areas with accumulated calcium carbonate (Prasad et al., 2014). The wetland includes alluvial tracts, implying influence from nearby water sources, enriching the soil with nutrients. Clay aids water retention, benefiting aquatic life and vegetation.

2.2. Data Acquisition

The satellite datasets of LANDSAT 5TM/+ETM, LANDSAT 8 OLI/TIRS and Sentinel 2A along with Cartosat 1 DEM were used to carry out the interpretation and analysis of the study. LANDSAT, SENTINEL, and Cartosat datasets were acquired from USGS Earth explorer, ESA (European Space Agency) and Bhuvan ISRO's data portal, respectively (Banerjee et al., 2020; Ghayour et al., 2021). Other vector land-use layers, such as roads and railways, were directly digitised from the OpenStreetMap (OSM) server in QGIS. The LANDSAT datasets were acquired over a 10-year time interval (1990, 2000, 2010, 2020) and were further used to prepare the Land Use Land Cover (LULC) map. The LANDSAT data has the spatial resolution of 30 m and the

Table 1. Data sources and their descriptions

Data type	Sensor	Acquisition year & Period	Path and row	Resolution	Source
LANDSAT 5	ETM+	1990 (Oct-Nov) Post-Monsoon	148 & 45	30 m	USGS
LANDSAT 5	ETM +	2000 (Oct-Nov) Post-Monsoon	148 & 45	30 m	USGS
LANDSAT 5	ETM+	2010 (Oct-Nov) Post-Monsoon	148 & 45	30 m	USGS
LANDSAT 8	OLI	2020 (Oct-Nov) Post-Monsoon	148 & 45	30 m	USGS
SENTINEL 2A	MSI	2023 (Not for analysis, only for general study area LULC)	N/A	10 – 60 m	ESA

SENTINEL data has that of 10-60 m. All satellite images used in this study were acquired during the post-monsoon season (October-November), when vegetation cover is at its peak due to adequate soil moisture availability following southwest monsoon rainfall. This period was specifically selected to enhance spectral separability among land-cover classes, particularly vegetation, agricultural land, and barren surfaces. During this season, edaphic factors such as soil moisture retention and soil characteristics play a significant role in controlling vegetation growth patterns, thereby improving the accuracy of LULC classification. The use of consistent seasonal imagery across different years ensures comparability and minimizes misclassification arising from seasonal variability. The Data sources are briefly listed in Table 1. The preprocessing and analysis were carried out using ArcGIS® 10.8.

2.3. Data Set and Classification

For the analysis of LULC change, satellite images (Landsat 5 and 8) of 30 years is procured from the Earth Explorer USGS website (<https://earthexplorer.usgs.gov/>). The composite band images are prepared for the downloaded satellite images, and the masking tool in the ArcGIS® 10.8 is used to extract the study area. Supervised classification is used to identify the temporal LULC changes. The features of the LULC classification on the

images are verified using Google Earth Pro. The change detection method is used to calculate per cent changes in LULC of analyse land transformation across various categories. The Normalised Difference Vegetation Index (NDVI) is generated using Sentinel-2 MS data along with False Colour Composite (FCC).

2.4. Pre-processing

The LANDSAT 5 & 8 and SENTINEL 2A datasets are pre-processed to improve the accuracy of the outcomes. Geo-referencing is done of the study area using the extraction tool to clip the satellite imagery with respect to the Area of Interest (AOI). The next step involves Radiometric Calibration, conversion of Digital Numbers (DN) to Top of Atmosphere (TOA) and Surface Reflectance, for which the Calibration technique is implied to convert DN to Radiance values. Surface reflectance or TOA is only processed when the influence of the atmosphere is removed from the imagery, thus, radiometric and atmospheric corrections are processed (Chander et al., 2009; Richards, 1999). The pixel values indicate the Surface reflectance values instead of DN, after atmospheric correction.

2.5. LULC Classification and Change

The changes that are observed over the years represent a serious threat to the ecosystem's sustainability, as the

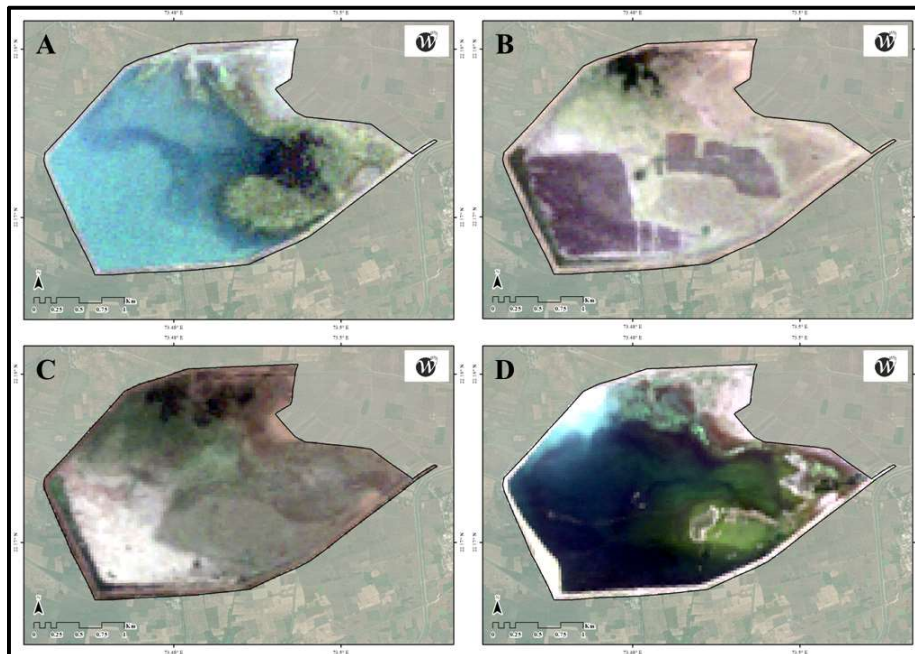


Figure 2. The figure shows the False Color Composite (FCC) required to generate the maps (A=1990; B=2000; C=2010; D=2020)

vegetation developed naturally gives way to man-made vegetation, for example (Gouda et al., 2021). In order to carry out the classification of the year 1990, 2000, 2010, and 2020, all the bands of imageries of the LANDSAT 5& 8 satellites were stacked using the Band Composite tool and thus, a False Color Composite (FCC) (Figure 2) was created using the combinations of red, blue and green bands of the visible spectrum. Specifically, for LANDSAT 8 OLI imagery, the stacked imagery was Pan-Sharpener, thereby converting the 30 m resolution to 15 m, as it includes a Panchromatic Band (Band 8) with a spatial resolution of 15 m. So, the image Pan-Sharpener was carried out in LANDSAT 8 in order to generate accuracy in the classification (Rahaman et al., 2017).

Classification is the process of assigning each pixel to an individual class based on a set of criteria. Maximum Likelihood Classification (MLC) is one of the most widely used methods of classification algorithms, in which a pixel is assigned to the class with the highest probability. This is a basic pixel method which relies by assuming the data of each class from each band has a normal distribution (Mondal et al., 2013). The training classes were assigned to the imagery, which assigns a value to each pixel based on similar spectral signatures, and 4 to 5 classes are classified in the imagery. After creation of LULC, the rasters of all the 4 imageries were converted to polygons using the polygonization tool and the Stratified Random Sampling technique was used, and around 50 points were generated for proving the accuracy of the classification statistically using the Kappa coefficient method of Error matrices (Fal et al. 2019; Miranda et al. 2016). Furthermore, this method of accuracy assessment was applied to all the classified layers of the years 1990, 2000, 2010 and 2020, along with the subsequent imageries (1990 - 2000), (2000 - 2010), (2010 - 2020), and (1990 - 2020) was subtracted from each other using the Intersect tool. Then, the attribute table of the resulting layer was processed to create a new field, changed classes using

the expression (1990 + “ - “ + 2000) and so on, to interpret in such a way that a particular class has been changed into another class during this 10-year interval (Anderson et al. 1976; Banerjee et al., 2020). The area of each class, along with the change in each area of all four years, was also calculated in km².

2.6. Normalized Difference Vegetation Index (NDVI)

Spectral indices are dimensionless variables derived from the reflectance of two or more bands. NDVI was developed for quantifying vegetation conditions over broad areas and was shown to have a strong correlation with green biomass (Karnieli et al. 2010; Rouse et al. 1974)The NDVI is essential to identify different land cover types in the study area, and it ranges from -1.0 to +1.0 (Kriegler et al. 1969). The NDVI is calculated on a per-pixel basis as the normalized difference between the red band (0.64-0.67 μm) and near infrared band (0.85-0.88 μm) using the formula as follows (Eq.1):

$$NDVI = \rho_{NIR} - \rho_{RED} / \rho_{NIR} + \rho_{RED}.....(1)$$

According to the LANDSAT 5 and 8 satellite imagery, the NIR, which is the Near Infrared Band, refers to Bands 4 and 5, and the visible band, which is the RED band, refers to Bands 3 and 4. So, the formula used in the raster calculator is as follows (Eqs. 2 and 3):

$$NDVI = \text{float}(\text{Band 4} - \text{Band 3}) / \text{float}(\text{Band 4} + \text{Band 3}), \text{ for LANDSAT 5 TM/+ETM....(2)}$$

$$NDVI = \text{float}(\text{Band 5} - \text{Band 4}) / \text{float}(\text{Band 5} + \text{Band 4}), \text{ for LANDSAT 8 OLI.....(3)}$$

3. RESULTS AND DISCUSSIONS

3.1. Analysis of Land Use and Land Cover (LULC)

The study area was classified into five classes, viz., water body, shallow water, bare soil, sparse vegetation, and dense vegetation, over of 10-year temporal range (1990, 2000, 2010, and 2020). In the year 1990, it was observed that the shallow water covered the maximum area of 3 km², while

Table 2. LULC change in the study area in the last three decades

LULC type	1990		2000		2010		2020	
	Area (km ²)	Area (%)	Area (km ²)	Area (%)	Area (km ²)	Area (%)	Area (km ²)	Area (%)
Bare soil	0.463	7	4.015	63	-	-	1.349	21
Deep water	0.635	10	1.298	20	1.486	23	-	-
Dense vegetation	-	-	-	-	1.697	27	0.754	12
Shallow water	3.045	48	0.461	7	-	-	3.972	63
Sparse vegetation	2.201	35	0.572	9	3.159	50	0.233	4

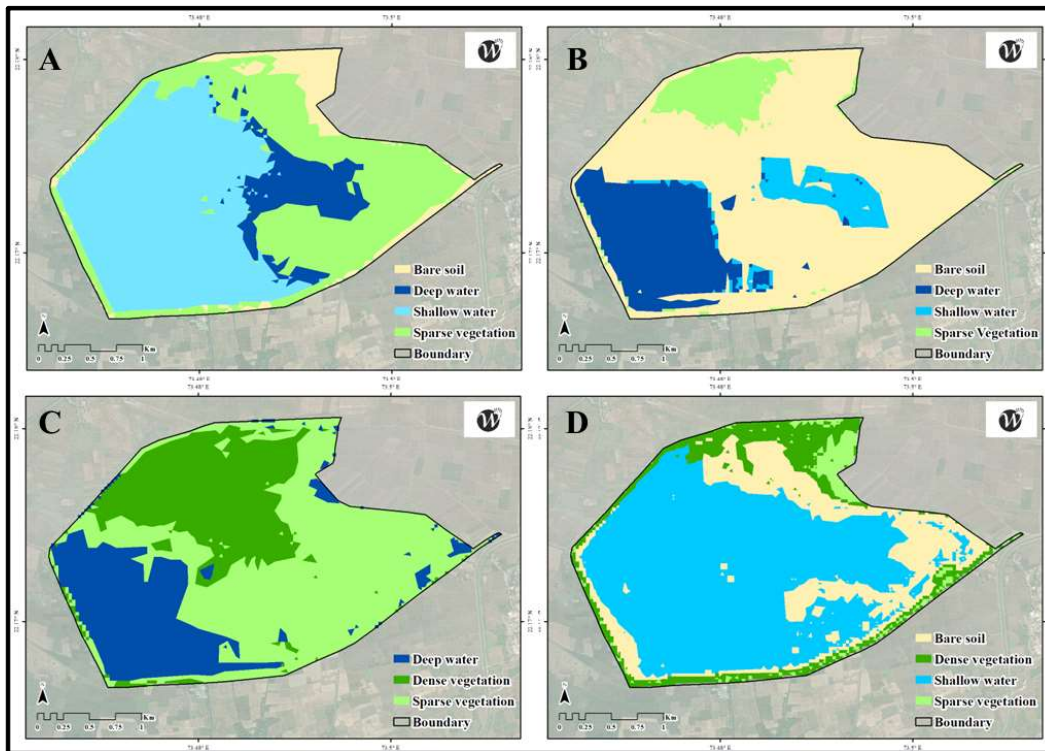


Figure 3. The figures show the land use and land cover from the year 1990-2020 in Wadhwana Wetland (A=1990; B=2000; C=2010; D=2020)

Table 3. Description of the rate of change in the area of LULC types in the past three decades

LULC type	1990-2000 area (km ² /year)	2000-2010 area (km ² /year)	2010-2020 area (km ² /year)
Bare soil – Bare soil	0.393	-	-
Bare soil – Deep water	0.004	0.255	-
Bare soil – Sparse vegetation	0.050	2.665	-
Bare soil – Dense vegetation	-	1.068	-
Deep water – Bare soil	0.323	-	0.191
Deep water – Deep water	0.004	1.185	-
Deep water – Shallow water	0.293	-	1.186
Deep water – Sparse vegetation	0.015	0.108	0.048
Deep water – Dense vegetation	-	0.005	0.051
Dense Vegetation – Dense vegetation	-	-	0.285
Dense Vegetation – Shallow water	-	-	1.054
Dense Vegetation – Bare soil	-	-	0.339
Shallow water – Bare soil	1.424	-	-
Shallow water – Deep water	1.266	0.025	-
Shallow water – Shallow water	0.126	-	-
Shallow water – Sparse vegetation	0.230	0.307	-
Shallow water – Dense vegetation	-	0.130	-
Sparse Vegetation – Bare Soil	1.854	-	0.817
Sparse Vegetation – Deep water	0.023	0.015	-
Sparse Vegetation – Shallow water	0.042	-	1.732
Sparse Vegetation – Sparse vegetation	0.271	0.063	0.169
Sparse Vegetation – Dense vegetation	-	0.490	0.401

the bare soil covered the minimum area of 0.5 km². In the year 2000, a sudden increase in the area of bare soil was observed (4 km²) and a corresponding decrease in the area of shallow water (0.5 km²). This can be understood from the rate of change in area, as shown in Table 3.

In the year 2010, a new class of dense vegetation was observed, whereas on the other hand, the class of bare soil had disappeared, and the maximum area was covered by the sparse vegetation or grassland of 3 km². Alongside, the water body and dense vegetation cover the least area of approximately. 1.5 km². Finally, in the year 2020, the class water body disappeared, and bare soil and shallow water were observed accordingly. Here, the maximum area is

covered by shallow water (4 km²), and the least area is covered by vegetation (0.5 km²).

From the overlay analysis, change detection maps of LULC of the corresponding ten-year intervals and the reference year to the final year (1990 - 2020) were prepared (Figure 4). These maps reveal the change that occurred in the LULC pattern in the respective ten years of interval along with the change from 1990 to 2020, say, the class such as water body, shallow water, bare soil, etc., has changed to bare soil, sparse vegetation, etc., or remained as it is, respectively (Figure 3). Alongside, the calculation of area change reveals the increase or decrease in the area of each class in the temporal range (Table 2).

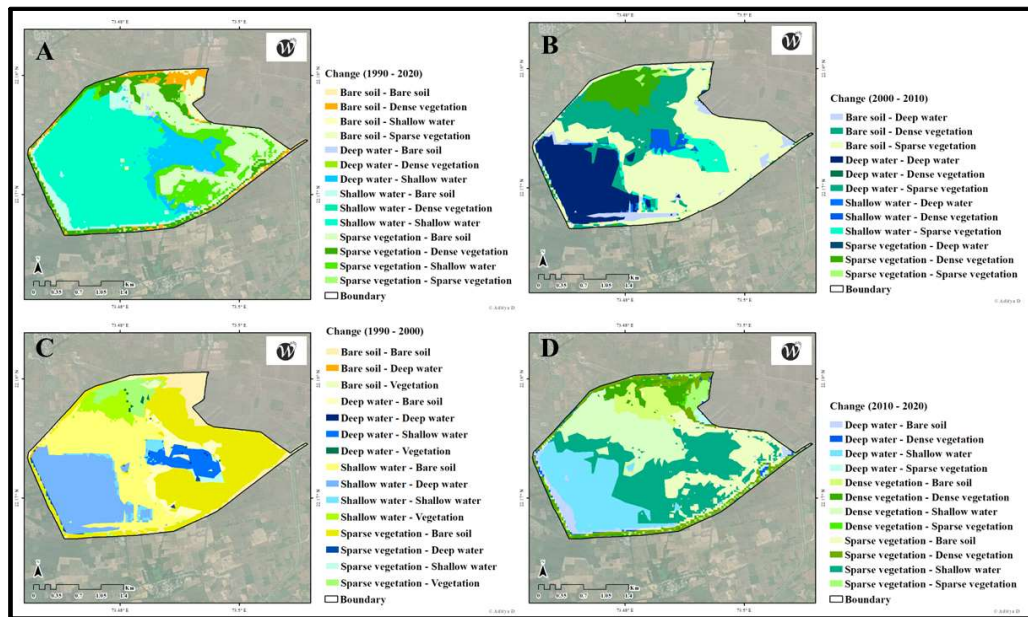


Figure 4. The figures show the change in land use and land cover from the year 1990-2020 in Wadhvana Wetland (A=1990; B=2000; C=2010; D=2020)

Table 4. Error (Confusion) matrix for the accuracy assessment of LULC for 1990, 2000, 2010, and 2020

LULC type	1990		2000		2010		2020	
	Producer accuracy (%)	User accuracy (%)	Producer accuracy (%)	User accuracy (%)	Producer accuracy (%)	User accuracy (%)	Producer accuracy (%)	User accuracy (%)
Bare soil	100	92	91	84	-	-	90	76
Deep water	76	100	100	84	94	94	-	-
Dense vegetation	-	-	-	-	100	88	85	92
Shallow water	100	76	81	100	-	-	92	100
Sparse vegetation	92	92	92	92	89	100	92	92

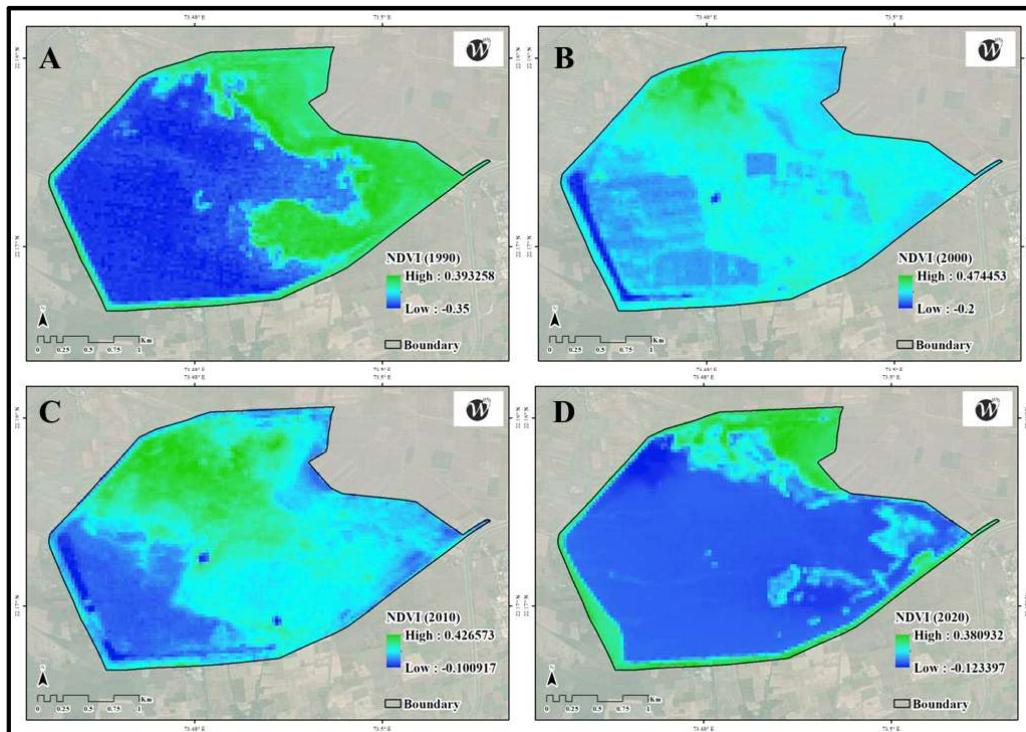


Figure 5. The figures show the change in vegetation through NDVI from the year 1990-2020 in Wadhwana wetland (A=1990; B=2000; C=2010; D=2020)

The confusion matrix or error matrix resulting from the random sampling technique reveals the overall accuracy and Kappa statistics, which are a vital part of post-classification accuracy assessment (Table 4). The Kappa statistic, often known as the value, contrasts observed accuracy with expected accuracy (random chance). The value range of Kappa, which quantifies the variety of the two classifiers, is between -1 and 1 (Carletta, 1996). The overall accuracy of the LULC for the years 1990, 2000, 2010, and 2020 are 90.38%, 90.38%, 94.12% and 90.38%, respectively. Moreover, it can be inferred that the overall accuracy of classifying all four images is nearly 90%. Similarly, the Kappa coefficient of all four images is 0.87, 0.87, 0.91, and 0.88, respectively, ranging from 0.8 to 0.9; the level of agreement between the classifiers is interpreted as Strong or basically, it infers that the classifiers are highly accurate (McHugh, 2012; Miranda et al., 2016).

3.2. Normalized Difference Vegetation Index (NDVI)

The NDVI were calculated for all four years, 1990, 2000, 2010, and 2020, from the LANDSAT spectral regions of NIR and Visible Red Bands. The NDVI values of the year 1990 range from -0.35 to 0.4. Correspondingly, the NDVI values

of the year 2000, 2010, and 2020 range from -0.2 to 0.47, -0.1 to 0.42, and -0.12 to 0.38, respectively. Here, the negative values indicate the water bodies, and the positive values (average ranging from 0.18 to 0.47) indicate the presence of vegetations (Ashok et al., 2021). From all four images, the positive NDVI values range from 0.47. According to Pettorelli (2013), shrubs, grasslands, and senescing crops are represented by NDVI values ranging from 0.2 to 0.5.

4. CONCLUSION

In conclusion, this comprehensive study examined the dynamic shifts in the Wadhwana Ramsar wetland ecosystem over four decades, using a combination of geospatial techniques and remote sensing data. The analysis has clearly established the interrelationship between LULC transitions and vegetation dynamics, where a significant decline in wetland extent (~45%) and the conversion of water bodies and barren land into cultivated areas reflect increasing anthropogenic pressure. The observed variations in NDVI values further indicate shifts in vegetation health and density, collectively demonstrating how these factors have altered the hydrological and ecological balance of the wetland over time. The findings underscore the challenges faced by

wetlands globally, as they grapple with anthropogenic pressures, climate fluctuations, and urban expansion.

The results unveiled a compelling narrative of transformation - a story of shrinking wetland areas and evolving vegetation patterns. The transition from water bodies to barren lands, from lush vegetation to cultivated fields, paints a vivid picture of the multifaceted impact of human activities. Amid these changes, the study identified strategic land-use planning and environmental education as pivotal tools to safeguard the fragile wetland ecosystem.

Furthermore, the application of the Normalised Difference Vegetation Index (NDVI) provided a quantitative lens to observe the ebb and flow of green cover. The positive NDVI values, indicative of flourishing vegetation, accentuated the ecological richness of the wetland when human intervention was minimised. These findings amplify the importance of preserving wetlands not only for their inherent biodiversity but also for their role in climate regulation, water purification, and the intricate web of ecosystem services they offer. As Wadhvana Wetland takes on its new designation as a Ramsar site, this study's insights serve as a beacon for responsible conservation and management practices.

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CRedit authorship contribution statement

Aditya Dharaiya: Conceptualization, Data collection, Data curation, Methodology, Software, Writing - original draft. **Shalu Mesaria:** Conceptualization, Data collection, Data curation, Formal analysis. **Pratikkumar Desai:** Conceptualization, Data collection, Data curation, Formal analysis, Project Administration, Resources, Validation. **Ravirajsinh Rathod:** Investigation, Resources, Supervision, Validation, Visualization. **Sasi Kumar K:**

Investigation, Resources, Supervision, Validation, Visualization. **Nishith Dharaiya:** Conceptualization, Investigation, Resources, Supervision, Validation, Visualization.

Conflict of interest

The authors declare that they have no known competing financial or personal relationships that could have influenced the work reported in this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no AI tools have been used in the writing process, except for grammar and spelling checks.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Population Fluctuation of Various Insect Pests of Summer Sorghum in North Gujarat, India

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ABSTRACT: The present study was conducted during the summer of 2024 at the Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Gujarat, to investigate the population dynamics of major insect pests and natural enemies of sorghum, and their relationships with abiotic factors. Observations on pest incidence were recorded weekly from crop emergence to harvest using six quadrats, with five plants examined in each quadrat. The results revealed that shoot fly (*Atherigona soccata*) damage reached a maximum of 40% dead hearts during the 15th standard meteorological week (SMW), while stem borer (*Chilo partellus*) infestation peaked at 40.00% during the 19th SMW. The shoot fly complex, including *Ectecephala* spp. and *Oscinella* spp., showed peak activity during the 17th SMW. Among foliage feeders, ash weevil population reached a maximum of 0.73 weevils' plant⁻¹ during the 18th SMW, while leafhopper population peaked at 0.56 hoppers plant⁻¹ during the 21st SMW. The natural enemy, like spiders, attained a maximum population of 0.23 spiders' plant⁻¹ during the 18th SMW. Correlation analysis indicated a significant negative relationship between wind speed and shoot fly incidence, whereas bright sunshine hours showed a significant positive correlation with stem borer and leafhopper populations. Maximum relative humidity was positively associated with spider abundance.

Keywords: Sorghum, Insect pests, Seasonal incidence, Abiotic factors, Correlation, Shoot fly complex, Stem borer.

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] (2n=20) belongs to the grass family (Poaceae) and is an important cereal crop in India. It is popularly known as jowar, great millet, Indian millet, milo, durra and shallu. Sorghum is a climate-smart C₄ crop that can produce grain and fodder in harsh environments under low-input conditions, delivering high net returns. In India, the major sorghum-producing states are Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, Telangana, Tamil Nadu, Gujarat, Uttar

Pradesh, Rajasthan and Haryana. In Gujarat, sorghum is cultivated across all three seasons: *kharif* (summer monsoon season), *rabi* (winter season), and *zaid* (short season between *rabi* and *kharif*), covering an area of about 42,660 ha, with a production of 66,130 tonnes and an average productivity of 1,550 kg ha⁻¹ (Anonymous, 2025). Sorghum fodder is highly nutritious, containing more than 50% digestible nutrients, along with approximately 8% protein, 2.5% fat, and 45% nitrogen-free extract. However, the sorghum crop is constrained by various insect species under both field and stored conditions. Damage from these

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causes results in serious losses (Daware et al., 2011). In India, shoot fly and stem borer significantly reduce yield, at times as high as 100% in grain and crop stand (Padmaja et al., 2010). In the northern dry zone of Gujarat, the insect pests, namely, shoot fly: *Atherigona soccata* Rondani (Diptera: Muscidae), Sorghum stem borer: *Chilo partellus* Swinhoe (Crambidae: Lepidoptera), Ear headworm: *Helicoverpa armigera* (Noctuidae: Lepidoptera) and aphids: *Melanaphis sacchari* (Zehntner) are the important regular pests. The losses due to shoot fly alone were to the tune of 22.11 to 83.94% (Jotwani & Sukhani, 1971; Mote et al., 1981 and 1982). Sorghum stem borer, *C. partellus* (Crambidae; Lepidoptera) is one of the most important pests in Asian and African countries (Arabjafari & Jalali, 2007). Among sucking pests, the aphid *M. sacchari* (Zehntner) (commonly known as the sugarcane aphid) is becoming a constraint in rabi sorghum-growing areas. The aphid affects not only grain and fodder yields but also fodder quality. Sorghum aphid caused 23-70% grain loss and 20% fodder yield loss, and led to deterioration of fodder quality (Balikai & Lingappa, 2002). The earlier authors also found that biotic and abiotic factor plays crucial role in the population build-up of insect pests and predators.

2. MATERIAL AND METHODS

2.1. Experimental Site and Season

Agronomy Instructional Farm, SDAU,

Sardarkrushinagar, during the summer season of 2024. The experimental site is loamy-sandy and supports the plant stand with sprinkler irrigation. Overall, the study highlights that abiotic factors significantly influence pest and natural enemy dynamics in sorghum. Understanding these relationships can help predict pest outbreaks and develop effective, timely pest management strategies for sustainable sorghum production.

2.2. Experiment Details

Sorghum variety, Swarna, was raised as per the SDAU package of practices, and Fixed Plot Observational Design was used for this study. The crop was sown in the 1st week of March and kept free of insecticide sprays. The crop was sown in a plot measuring 9 m × 7.5 m with a spacing of 45 cm row to row and 15 cm plant to plant.

2.3. Observation Procedure

Observations were recorded from the initial occurrence of insect pests up to the crop harvesting stage at different weekly intervals during morning hours between 6:00 am and 8:00 am from different quadrates. A total of six quadrats were selected, with five plants per quadrat examined for pest incidence. The population of shoot fly and stem borer were recorded by their damage symptoms (dead heart) per cent, shoot fly complex recorded as (maggots/quadrat), ash weevil, leaf hoppers recorded as (number plant⁻¹) and

Table 1. Seasonal incidence of sorghum insect pests and shoot fly species in relation to weather parameters during *summer 2024*

Month	WAS	SMW	Shoot fly	Stem borer	<i>Etecephala</i> spp	<i>Oscinella</i> spp	Ash weevil	Leaf hopper	Spider
			Dead heart (%)	Maggots / plant	Number plant ⁻¹	Number plant ⁻¹			
March 2024	1	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	13	10.00	0.00	0.00	0.00	0.00	0.00	0.00
April 2024	3	14	16.67	0.00	0.00	0.63	0.16	0.00	0.03
	4	15	40.00	0.00	0.23	0.96	0.23	0.00	0.03
	5	16	36.67	6.66	0.86	1.73	0.60	0.10	0.10
	6	17	30.00	16.67	2.93	4.63	0.67	0.16	0.03
May 2024	7	18	23.33	26.67	0.63	3.50	0.73	0.23	0.23
	8	19	13.33	40.00	0.00	2.20	0.56	0.36	0.10
	9	20	6.67	33.33	0.00	0.73	0.50	0.50	0.03
	10	21	0.00	26.67	0.00	0.00	0.36	0.56	0.00
	11	22	0.00	20.00	0.00	0.00	0.26	0.36	0.00
June 2024	12	23	0.00	3.33	0.00	0.00	0.13	0.16	0.00
	13	24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean			13.59	13.33	0.35	1.10	0.32	0.18	0.04

WAS: Weeks after sowing; SMW: Standard meteorological weeks

spiders were recorded as (number plant⁻¹).

2.4. Meteorological Observations

Meteorological data from March to June 2024, viz., temperature (maximum and minimum), relative humidity, bright sunshine hours and wind speed, were obtained from the Department of Agricultural Meteorology, SDAU, Sardarkrushinagar, and are provided in Table 4.

2.5. Statistical Analysis

All the data collected during the study period were managed using Microsoft Excel version 2019 and analysed by the Statistical Program for Social Sciences (SPSS

version 32. The independent variables were the weather parameters like temperature (maximum and minimum), relative humidity, bright sunshine hours and wind speed at the study site. The dependent variables were pests' prevalence and infestation level. The association between the occurrence of insect pests on the crop at the study site was analysed using simple linear regression (Pearson, 1895).

3. RESULTS AND DISCUSSION

The incidence of various insect pests and natural enemies of sorghum is mentioned in Table 1, Figure 1, and their

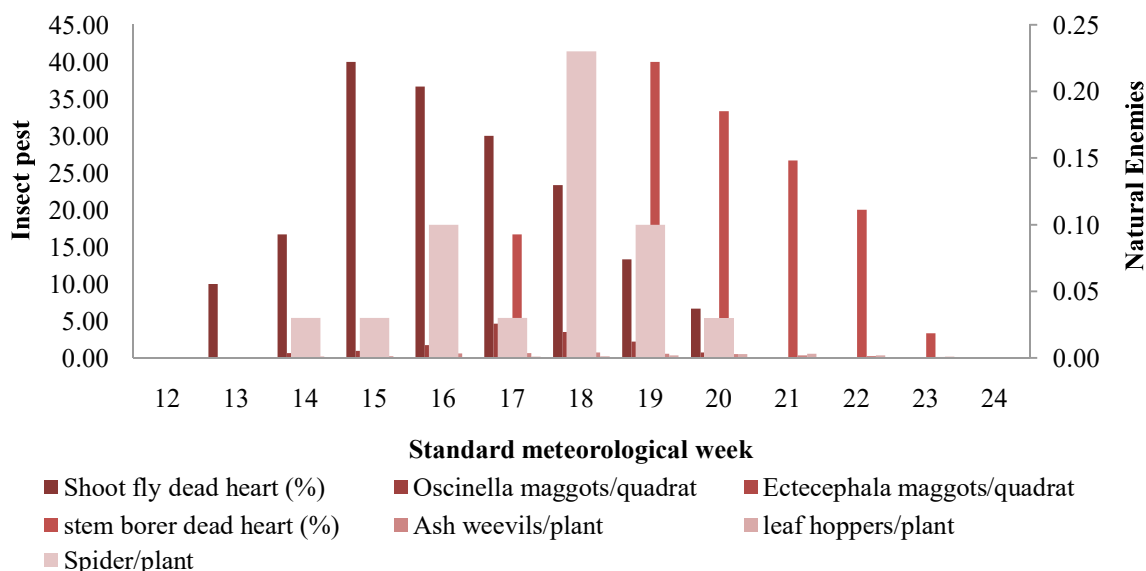


Figure 1. Graphical illustration of insect pests infesting summer sorghum 2024

Table 2. Correlation of weather parameters with insect pests infesting summer sorghum 2024

Insect pest/Natural enemy			Abiotic factors					
			Bright sunshine hours	Wind speed (Km h ⁻¹)	Temperature °C		Relative humidity (%)	
					Maximum	Minimum	Maximum	Minimum
Borers	Dead heart (%)	Shoot fly	-0.113 ^{NS}	-0.625*	-0.361 ^{NS}	-0.375 ^{NS}	0.103 ^{NS}	-0.097 ^{NS}
		Stem borer	0.611*	0.150 ^{NS}	0.400 ^{NS}	0.275 ^{NS}	-0.034 ^{NS}	-0.423 ^{NS}
Shoot fly species	Maggots / quadrat	<i>Etecephala</i>	0.067 ^{NS}	-0.382 ^{NS}	-0.343 ^{NS}	-0.180 ^{NS}	0.081 ^{NS}	-0.178 ^{NS}
		<i>Oscinella</i> spp	0.133 ^{NS}	-0.429 ^{NS}	-0.411 ^{NS}	-0.279 ^{NS}	0.234 ^{NS}	-0.215 ^{NS}
Foliage feeders	Number plant ⁻¹	Ash weevil	0.411 ^{NS}	-0.166 ^{NS}	-0.009 ^{NS}	0.073 ^{NS}	0.265 ^{NS}	-0.270 ^{NS}
		Leaf hopper	0.717**	0.355 ^{NS}	0.677*	0.573*	-0.052 ^{NS}	-0.300 ^{NS}
Natural Enemy	Number plant ⁻¹	Spiders	0.105 ^{NS}	-0.191 ^{NS}	-0.333 ^{NS}	-0.183 ^{NS}	0.571*	-0.045 ^{NS}

* Significance at 5 % level of significance (‘r’ = 0.553); ** Significance at 1% level of significance (‘r’ = 0.684)

correlation with weather parameters is described in Table 2.

3.1. Shoot Fly and Its Complex Species

The shoot fly dead heart percentage gradually increased, peaking at 40.00 % dead hearts during the 15th SMW. A declining trend was observed from the 18th SMW, with the dead heart % reducing to 23.33%. This highlights that the early vegetative phase of sorghum is particularly vulnerable. A significant negative correlation ($r = -0.625$) was observed between shoot fly dead heart incidence and wind speed. A positive but non-significant association was recorded between dead hearts and maximum relative humidity, while the remaining weather parameters had no significant influence on shoot fly dead heart formation. Monitoring of the shoot fly complex population revealed that *Etecephala* spp maggots were first observed during the 15th SMW and attained their peak during the 17th SMW (4.63 maggots/quadrant) and completely declined during the 18th SMW to 0.63 maggots/quadrant. Correlation with weather parameters revealed that among all the weather parameters, bright sunshine hours ($r = 0.067$) and maximum relative humidity ($r = 0.081$) showed a positive and non-significant relation with *Etecephala* spp maggots. *Oscinella* spp were first observed during 14th SMW and attained their peak during 17th SMW with (4.63 maggots/quadrant), and a declining trend was observed during 19th SMW to 2.20 maggots/quadrant. Weather parameters correlation with *Oscinella* spp resulted in bright sunshine hours ($r = 0.133$) and maximum relative humidity ($r = 0.234$) showing a positive and non-significant relationship. The regression equation for shoot fly (*Atherigona soccata*), indicated that maximum temperature (X_1), minimum temperature (X_2), relative humidity (X_3) and wind speed (X_6) had negative effects on shoot fly incidence, whereas bright sunshine hours (X_4) and rainfall (X_5) showed a positive influence. The model explained about 60 % ($R^2 = 0.60$) of the variation in

shoot fly incidence, indicating a moderate influence of weather parameters. In the case of shoot fly complex species such as *Oscinella* spp. and *Etecephala* spp., the regression models explained 61% ($R^2 = 0.61$) and 76% ($R^2 = 0.76$) of the variability in their populations, respectively. Among these, *Etecephala* spp. showed the highest R^2 value, suggesting that weather parameters had a relatively stronger influence on its population dynamics compared to other pests (Table 3).

3.2. Stem Borer

The stem borer dead heart percentage gradually increased, peaking at 40.00 % dead hearts during the 19th SMW. The dead heart percentage started to decline from the 21st SMW to 26.67 % dead hearts. The late vegetative phase and early reproductive stage of sorghum are particularly vulnerable to the stem borer. Correlation analysis indicated that bright sunshine hours showed a significant positive relationship ($r = 0.611^*$) with stem borer dead heart % formation. Wind speed, maximum temperature and minimum temperature also exhibited positive but non-significant correlations ($r = 0.150$, $r = 0.400$ and $r = 0.275$, respectively) with stem borer dead heart incidence. For the stem borer (*Chilo partellus*), the regression model indicated that most of the weather variables had a positive influence on pest incidence except wind speed. However, the coefficient of determination was relatively low ($R^2 = 0.47$), suggesting that other factors, such as crop stage or natural enemies, may also influence stem borer populations (Table 3).

3.4. Ash Weevil

Ash weevil population first observed during 14th SMW and lasted up to a week before harvest, with a population peak (0.73 weevils plant⁻¹) during 18th SMW and a decline of population to 0.36 weevils plant⁻¹ during 21st SMW. A positive but non-significant relationship is established

Table 3. Multiple regression equation of insect pests of sorghum with various environmental factors infesting summer sorghum

Insect	Multiple regression equation	Coefficient of determination R^2
Shoot fly	$Y = 31.49 - 3.21X_1 - 11.30X_2 - 1.26X_3 + 2.42X_4 + 0.87X_5 - 0.67X_6$	0.60
<i>Oscinella</i> spp	$Y = 24.14 - 0.15X_1 - 0.95X_2 - 0.70X_3 + 0.66X_4 + 0.01X_5 - 0.16X_6$	0.61
<i>Etecephala</i> spp	$Y = 24.35 - 0.13X_1 - 0.59X_2 - 0.64X_3 + 0.60X_4 + 0.06X_5 - 0.11X_6$	0.76
Stem borer	$Y = -69.70 + 4.59X_1 + 0.53X_2 + 1.13X_3 + 0.14X_4 + 0.17X_5 - 0.55X_6$	0.47
Ash weevil	$Y = 1.30 + 0.02X_1 - 0.13X_2 - 0.06X_3 + 0.08X_4 + 0.01X_5 - 0.02X_6$	0.47
Leaf hopper	$Y = -1.66 + 0.09X_1 + 0.01X_2 + 0.02X_3 + 0.09X_4 - 0.00X_5 - 0.00X_6$	0.79
Spider	$Y = -0.80 - 0.00X_1 - 0.01X_2 + 0.01X_3 - 0.00X_4 - 0.01X_5 - 0.00X_6$	0.61

Table 4. Details of weather parameters during *summer 2024*

Month	WAS	SMW	Bright sun shine hours	Wind speed	Maximum temperature	Minimum temperature	Maximum relative humidity	Minimum relative humidity
March 2024	1	12	9.8	1.6	35.0	14.5	69	35
	2	13	8.7	0.8	39.0	18.5	65	40
April 2024	3	14	9.3	1.9	36.5	20.0	73	48
	4	15	9.2	0.5	40.0	19.5	70	40
	5	16	8.5	2.4	39.0	22.5	76	43
	6	17	10.0	0.8	37.0	20.0	70	37
May 2024	7	18	10.5	1.9	36.5	21.0	89	42
	8	19	9.2	2.3	40.0	19.5	65	38
	9	20	11.0	2.3	43.5	25.0	66	34
	10	21	11.4	2.8	45.5	27.5	70	39
	11	22	11.5	5.1	42.0	27.0	78	39
June 2024	12	23	8.3	3.8	39.5	26.0	78	59
	13	24	6.9	4.0	42.0	26.0	74	44

WAS: Weeks after sowing; SMW: Standard meteorological weeks

between bright sunshine hours ($r = 0.411$), minimum temperature ($r = 0.073$) and maximum relative humidity ($r = 0.265$) with the ash weevil population. Similarly, the ash weevil population showed a weak association with weather parameters, as reflected by a lower coefficient of determination ($R^2 = 0.47$). This indicates that abiotic factors had a limited influence on its population build-up during the study period (Table 3).

3.5. Leaf Hoppers

Leafhopper incidence was first recorded during the mid-vegetative stage (16th SMW) and persisted until harvest. The population peaked during the 21st SMW (0.56 hoppers plant⁻¹) and declined in the 22nd SMW (0.36 hoppers plant⁻¹). Correlation analysis revealed a highly significant positive association with bright sunshine hours ($r = 0.717^{**}$), as well as significant positive correlations with maximum temperature ($r = 0.677^*$) and minimum temperature ($r = 0.573^*$). Wind speed showed a positive but non-significant relationship ($r = 0.355$) with leafhopper population. Leaf hopper population exhibited the highest coefficient of determination ($R^2 = 0.79$), suggesting that weather parameters strongly influenced leaf hopper population dynamics. Most of the weather variables showed a positive relationship with leaf hopper incidence, indicating favourable conditions for their multiplication (Table 3).

3.6. Spider

The natural enemy (spiders) was spotted during 14th

SMW, where the peak population of spiders (0.23 spider plant⁻¹) was attained during 18th SMW, and a decline of population was observed during 19th SMW, with (0.10 spider plant⁻¹). Maximum relative humidity showed a significant positive correlation ($r = 0.571^*$) with spider population, and a positive but non-significant correlation ($r = 0.105$) with bright sunshine hours. In the case of natural enemies, spiders showed a moderate relationship with weather parameters ($R^2 = 0.61$). Although the regression coefficients were small, the model indicated that weather conditions had some influence on spider population dynamics in the sorghum ecosystem.

Overall, the regression analysis indicated that weather parameters had varying levels of influence on different insect pests and natural enemies in sorghum, with the strongest effect observed on leaf hopper populations. These findings highlight the importance of considering abiotic factors while predicting pest incidence and developing effective pest management strategies.

These findings are in line with Khan et al. (2024), as the shoot fly infestation peaked after the 4th week of sowing, and some of the weather parameters were not significant. The shoot fly infestation in terms of mean % of plants with dead heart formation had a significant negative correlation ($r = -0.928$) with minimum temperature, the mean number of eggs per five plants had a significant negative correlation with minimum temperature ($r = -0.834$) and average temperature ($r = -0.909$). Further these results are in accordance with Meena and Bhamare (2024) in which the incidence of shoot fly complex like *A. soccata*, *Oscinella sp.*

and *Ectecephala* sp. in case of time of incidence, number and their correlation revealed a significant negative relationship (-0.639*) between wind speed and the population of *A. soccata* maggots and it was observed that no significant negative correlation between rainfall ($r = -0.192$), maximum temperature ($r = -0.473$), and minimum temperature ($r = -0.371$) and the *A. soccata* maggot population. Nevertheless, there was no significant negative association between rainfall ($r = -0.170$), maximum temperature ($r = -0.391$), and lowest temperature ($r = -0.200$) and the maggot population of *Oscinella* spp. on sorghum. Similar results were reported by Rakesh et al. (2022) in terms of time of pest occurrence, like shoot fly, leaf hoppers and their correlation with the abiotic factors and found that morning and evening relative humidity and rainfall showed a significant positive correlation ($r = 0.642$, 0.643 and 0.701 , respectively) with the *A. biguttula biguttula* population. Further, minimum temperature showed a positive correlation ($r = 0.280$) while the maximum temperature and evaporation showed, a negative correlation ($r = -0.340$ and -0.451 , respectively) with the *A. biguttula biguttula* population but statistically found to be non-significant. Integrating pest monitoring with abiotic factor analysis and crop development stages can improve pest management strategies for the sorghum crop.

4. CONCLUSION

From the results, it is concluded that the shoot fly incidence was observed more in the vegetative stages of the crop period compared to later stages. The weather parameters, such as wind speed and maximum temperature, exerted varying degrees of influence on shoot fly dynamics across different growth stages of the crop's growing period. The significant negative correlations with wind speed underscored its role in shaping population trends. Bright sunshine hours had played a vital role in the population dynamics of stem borer for their development and persistence at later stages of crop growth. None of the shoot fly complex (*Ectecephala* spp and *Oscinella* spp) maggots were significantly influenced by weather parameters that prevailed during the early stages of crop growth. Ash weevil prevailed from the early stages of crop growth to the later stages and was not significantly influenced by any of the weather parameters. Bright sunshine hours showed a highly significant positive correlation with leaf hopper population, whose abundance was higher during the later stages of the cropping period. The maximum and minimum temperatures also affected pest survival and dispersal. Natural enemies

like spiders also prevailed during the cropping period, from the initial to the later stages. Weather parameters like maximum humidity paved the way for spider survival.

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CRediT authorship contribution statement

Koosi Sai Thilak: Conceptualization, Data collection, Methodology, Software, Writing original draft, Formal analysis, Tabulation, Data curation and Graphical Analysis. **Bindu Panickar:** Supervision, Fund provision and visualization. **Chandaragi M K:** Supervision, Reviewing and editing. **Jyotika Purohit:** Reviewing and editing. **G Balraj:** Reviewing and editing.

Conflict of interest

The authors have no conflicts of interest to declare.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors used Grammarly only for checking grammar and sentence structure during the preparation of this manuscript. No content was generated by AI tools. After using Grammarly, the authors carefully reviewed and edited the entire manuscript themselves to ensure clarity and correctness. The authors take full responsibility for the content of the manuscript.

Data availability statement

The data supporting the findings of this study are available within the article (Tables 1-4 and Figure 1). Any additional datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

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Ecological Health Assessment of Vandiyur Lake, Madurai, India: A Multi-parametric Approach Using Physico-chemical and Planktonic Indicators

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ABSTRACT: Vandiyur Lake, a critical urban wetland in Madurai, faces ecological degradation mainly from encroachment and untreated sewage discharge. This study evaluates the lake's ecological health from December 2023 to March 2024 using physico-chemical profiling and planktonic analyses. Over the study period, both the physico-chemical profiles and planktonic community structures showed significant spatial and seasonal variability. In sampling station II, the highest organic loading was observed, with total alkalinity (321 mg/L), free CO₂ (22.25 mg/L), total nitrogen (1.16 mg/L), total phosphorus (1.01 mg/L), and BOD (6.66 mg/L) coinciding with suppressed dissolved oxygen (6.82 mg/L). A total of 25 phytoplankton and 45 zooplankton species were recorded. Phytoplanktons were dominated by Cyanophyceae and Bacillariophyceae, including *Euglena* sp., *Microcystis* sp., *Closterium* sp., *Asterionella* sp., and *Anabaena* sp., while Rotifera was the most diverse zooplankton group, including *Brachionus* spp., *Daphnia* sp., and *Diatomus* sp. These taxa are widely recognized as reliable bio-indicators of eutrophication and organic pollution. Diversity indices indicated that Station I had higher species diversity and evenness, reflecting a relatively stable ecosystem. In contrast, Station II showed high species richness but lower diversity and evenness, suggesting dominance of pollution-tolerant species due to anthropogenic stress. Overall, these findings suggest an ecological imbalance caused by nutrient enrichment and sewage discharge, emphasizing the urgent need for management strategies to restore the lake's ecological health.

Keywords: Bio-indicators, Eutrophication, Shannon-Wiener Index, Vandiyur Lake, Water quality, Zooplankton.

1. INTRODUCTION

Water is the most vital component of the global ecosystem, serving as a fundamental resource for domestic, agricultural, and industrial needs. Historically, lentic water bodies like lakes and ponds have been utilized for diverse purposes ranging from irrigation and fish farming to ritualistic and aesthetic value (Narayan et al., 2007; Bishnoi & Malik, 2008). Bishnoi and Malik (2008) report that rapid industrialization, burgeoning human populations, and

intensive use of agrochemicals have been contributing to the significant contamination of these freshwater ecosystems.

The quality of water is defined by its physical, chemical, and biological characteristics (Rajagopal et al., 2010a, 2010b). When these parameters deviate from established safety limits, they pose a direct threat to human health and aquatic biodiversity. Consequently, evaluating the Water Quality Index (WQI) has become a standard method for determining the suitability of water sources for consumption

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and ecological maintenance (Tyagi et al., 2013). Understanding the biological phenomena of a lake requires a deep dive into its hydro-chemical profile, as the chemistry of water dictates the metabolism of the entire ecosystem (Rajagopal et al., 2010b; Basavaraja et al., 2011).

Recent environmental assessments in India have highlighted nutrient enrichment (eutrophication) and acidification as primary drivers of freshwater degradation. These issues are largely attributed to the discharge of untreated domestic sewage and industrial effluents (Shekhar et al., 2008; Laskar & Gupta, 2009). Biological monitoring or biomonitoring, offers a more holistic view of lake health than chemical testing alone. Aquatic organisms, particularly plankton, are highly sensitive to environmental disturbances. Phytoplankton, as primary producers, form the foundation of the aquatic food web, and their diversity responds rapidly to changes in nutrient levels, particularly silica, nitrates, and phosphates (Chellappa et al., 2008). Certain species, such as those identified by Palmer (1969), serve as definitive bioindicators of organic pollution. Similarly, zooplankton communities play a critical role in energy flow and cycling of matter. Their distribution is governed by a complex interplay between climatic conditions and the physico-chemical variables of the aquatic ecosystem (Neves et al., 2003). Despite extensive research on freshwater bodies across India, ecological data regarding the wetlands, specifically the lakes of southern Tamil Nadu, remain relatively sparse (Smitha et al., 2007; Rajagopal et al., 2010b).

Vandiyur Lake, the second-largest lake in Madurai, serves as a crucial lifeline for groundwater recharge. It is primarily used for agricultural irrigation in Anuppanadi, while also supporting the local water supply for residential areas including Gomathipuram, Thendral Nagar, Tahsildar Nagar, and Melamadai. However, over the past two decades, the lake has undergone significant ecological degradation driven by urban encroachment and untreated sewage discharge. While previous studies have documented specific taxonomic groups such as birds, fish, and aquatic flora (Jaffer Ali et al., 2019; Selvamurugan, 2023; Seenivasan, 2024), a comprehensive understanding of the interplay between hydro-chemical properties and planktonic community structures remains elusive (McConnell & Meenakshi, 2024). Consequently, this study was designed to analyze physico-chemical parameters and plankton diversity to provide a baseline assessment of the trophic status and ecological integrity of Vandiyur Lake.

2. MATERIALS AND METHODS

2.1 Description of the Study Area and Sampling Site

The study was conducted at the Vandiyur Lake (VL), Madurai, Tamil Nadu (9.93°N, 78.12°E). As the second-largest lake in Madurai, it spans approximately 577 acres (reduced from its original 640 acres due to encroachment). The lake has a catchment area of 96.89 km², a storage capacity of 107.03 mcf, and a bund length of 2077 m. It primarily receives runoff from the Sathaiyar Dam and surplus channels from the Parasurampatti, Sambakulam, Thallakulam and S. Kodikulam tanks.

Vandiyur Lake serves as a multi-use resource for the local community, primarily supporting agricultural irrigation, cattle grazing, artisanal fishing, and domestic activities. Despite a 2004 Madras High Court directive aimed at curbing industrial and hospital effluent discharge, the lake remains under significant ecological pressure. Current threats include the ingress of untreated domestic sewage, runoff from nearby flower and fish markets, and non-point source pollution from open defecation within the riparian zones.

To assess water quality and plankton diversity, the lake was categorized into four sampling zones based on geographic orientation (Figure 1):

- Zone I (South site):** Situated near the Gomathipuram residential area, this zone is influenced by urban

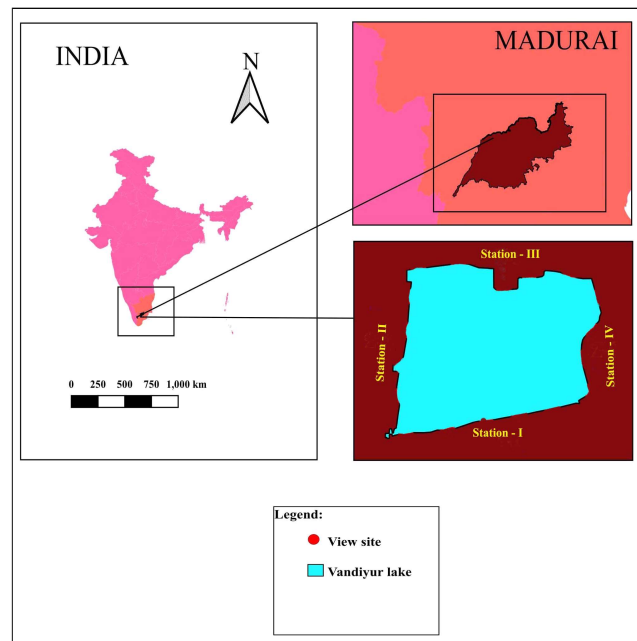


Figure 1. Map showing the location of Vandiyur Lake, Madurai, Tamil Nadu, Indian and specific study stations (I-IV)

housing, commercial showrooms, and the local fish market located along the southern banks.

2. **Zone II (West site):** Located adjacent to Sundaram Park, this site is primarily impacted by proximity to the Apollo and Sugapriya hospitals on the western banks.
3. **Zone III (North site):** Positioned near high-traffic landmarks including the Mattuthavani bus stand, flower and fish markets, and Meenakshi Mission Hospital on the northern banks.
4. **Zone IV (East site):** Adjacent to the Pandi Kovil and Madurai Ring Road, this zone is characterized by its proximity to schools, residential apartments, and Guru Hospital on the eastern shores.

2.2. Water Sample Collection

Water samples were collected monthly over a four-month period from December 2023 to March 2024. To ensure consistency, all sampling was performed during the morning hours (08:00 AM to 10:00 AM). Sub-surface water was collected using sterile, wide-mouth, screw-capped glass bottles from a depth of 5–10 cm. The samples were then transported immediately to the departmental laboratory for physicochemical analysis, following the methods described by Rajagopal et al. (2010a).

2.3. Water Quality Analyses

Air and surface water temperatures were recorded on-site using a mercuric centigrade thermometer. Initial pH was screened using pH indicator strips at the site and later confirmed in the laboratory using a digital pH meter. Rainfall data for the 2023–2024 study periods were obtained from the Meteorological Department of the Madurai District Administration Office. Other parameters, including water colour, odour, total solids (TS), salinity, alkalinity, dissolved oxygen (DO), biological oxygen demand (BOD), free CO₂, etc., were analysed following standard protocols prescribed by the American Public Health Association (APHA, 2017).

2.4. Plankton Collection and Identification

For planktonic analysis, 50 litres of water (integrated from surface, middle, and bottom layers) were filtered through a standard plankton net (25 µm mesh size) on-site at each sampling station during the morning hours (08:00 AM to 10:00 AM). The concentrated samples were transferred to polyethylene bottles and preserved immediately with 5% formalin for subsequent qualitative and quantitative analyses:

1. **Qualitative analysis:** Planktonic organisms were identified to the genus or species level under a compound microscope using standard taxonomic keys and manuals (Adoni et al., 1985, Agarker et al., 1994).
2. **Quantitative analysis:** Plankton density was estimated using a Sedgwick-Rafter counting cell, following the methodology described by Rajagopal et al. (2010a).

2.5. Statistical Analysis

The planktonic community structure was assessed through diversity indices calculated using PAST (Paleontological Statistics) software (Hammer et al., 2001). The following indices were determined: (1) Shannon-Wiener Index (H') to evaluate species diversity, (2) Menhinick's Index (D) to measure species richness and (3) Pielou's Evenness Index (J') to assess the distribution of individuals among species. Planktonic species abundance data were compiled and managed using SPSS software (Version 11.0). To identify significant differences in mean values across the sampling stations, the data were subjected to Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Assessment of Physico-chemical Parameters

Physico-chemical water quality parameters are very important in assessing the constituents of water and also determining the level of pollutants or contaminants. The physico-chemical parameters and plankton diversity variation have been found in Vandiyur Lake; these variations were influenced by both biotic and abiotic components, either directly or indirectly. In the present study, the air temperature (AT) and water temperature (WT) showed a progressive increase from December to March, consistent with the transition from winter to the pre-monsoon season. AT ranged from 29°C to 33°C, while WT remained slightly lower, peaking at 29°C in March. This synchrony is typical of shallow lentic systems, as noted by Welch (1952), who reported that smaller water bodies such as ponds respond rapidly to changes in atmospheric temperature due to their low thermal mass. The peak temperature in March likely accelerated the metabolic rates of the dominant planktonic communities, particularly Cyanophyceae and Rotifera (Naik et al., 2020). According to Ganesh et al. (2015), rising temperatures and nutrient concentrations create the perfect conditions for opportunistic species, shifting the ecosystem from a balanced state to a eutrophic one.

There was a significant peak of rainfall in December (105.1 mm). High precipitation likely reduced nutrient concentrations temporarily and increasing water clarity as a result of this “dilution effect”. As rainfall stopped and evaporation increased toward March, dissolved solids accumulation increased as the water level dropped. A similar observation was made by Jhingran (1991), who noted that the post-monsoon drawdown in Indian lakes often leads to nutrients accumulation, resulting in algal blooms (Jargal et al., 2021). Correspondingly, water color shifted from cloudy in December to slightly brown by February/March across all stations. This change is attributed to the accumulation of chromophoric dissolved organic matter (CDOM) and suspended solids during water level recession. Such discoloration is commonly associated with high organic loading and decomposition processes, indicating an advanced trophic state (Hossain et al., 2013; Shampa et al., 2024).

The pH of Vandiyur Lake showed a clear spatio-temporal shift, ranging from slightly acidic/neutral in December (minimum 6.12 at Station IV) to strongly alkaline in March (maximum 8.90 at Station II). While the mean pH (7.10–7.96) remained suitable for aquatic life; however, elevated March values indicate environmental stress. This rise is attributed to intensified phytoplankton photosynthesis during the pre-monsoon period, which increases alkalinity (Wetzel, 2001, Naik et al., 2020). Similar alkaline conditions (pH 8.0–9.0) are typical of eutrophic tropical waters and can enhance phosphorus availability, thereby promoting algal blooms (Verma et al., 2012; Wetzel, 2001). In contrast, the lower pH observed in December (6.12) is likely due to heavy rainfall (105.1 mm), which introduces organic acids and dilutes alkaline salts (Hulyal & Kaliwal, 2011).

The alkaline nature of Vandiyur Lake (ranges pH 7.10 to 7.96) indicates good buffering capacity, supported by the high total alkalinity across stations. Elevated alkalinity at Stations II and III (>300 mg/L) suggests localized nutrient inputs from domestic sewage and agricultural runoff (Kulshrestha et al., 1989). From December to March, a clear concentration effect was observed, with rising temperatures enhancing photosynthetic CO₂ uptake and evaporation, leading increased alkalinity and hardness (Hulyal and Kaliwal, 2011). Higher values at mid-lake stations (II/III) point to possible pollution hotspots or stagnant zones. Total hardness showed a similar trend, peaking in March (80 mg/L at Station II) (Table 1), likely due to evaporative concentration of calcium and magnesium ions (Jargal et al., 2021), consistent with patterns reported in Indian reservoirs

(Hulyal & Kaliwal, 2011).

Dissolved oxygen (DO) levels across the four sampling stations ranged from a minimum of 5.5 mg/L at Station II in January to a maximum of 8.8 mg/L at Station I in December. Despite increasing water temperatures, DO levels showed a slight rise toward the month of March, likely due to enhanced phytoplankton photosynthesis activity, which is typical of nutrient-enriched waters (Wetzel, 2001). In contrast, the biological oxygen demand (BOD) ranged from 3.0 mg/L to 8.0 mg/L, with higher mean values at Stations II (6.66 mg/L) and III (5.37 mg/L), indicating greater organic pollution. These values fall within the moderately polluted range (Kulshrestha et al., 1989) and correspond with observations of slightly putrid odour and brownish water, suggesting localised inputs of biodegradable organic matter from domestic sewage or agricultural runoff.

Total dissolved solids (TDS) ranged from 1.0 to 3.8 mg/L and salinity from 0.21 and 3.0 mg/L, indicating a predominantly freshwater environment. Station II recorded comparatively higher salinity (mean: 0.55 mg/L), reflecting greater input of dissolved salts. A slight rise in TDS toward March suggests a concentration effect driven by increased evaporation (Promilton et al., 2025). Free CO₂ values increased markedly from December to March, with higher mean values at Stations II (22.25 mg/L) and III (18.75 mg/L), likely due to enhanced microbial decomposition of organic matter (Shi et al., 2025). This is supported by the elevated BOD at these stations. Despite rising CO₂ levels, the pH remained alkaline, indicating strong buffering capacity, possibly maintained by concurrent photosynthetic activity (Shi et al., 2025).

Total nitrogen (TN) and total phosphorus (TP) were consistently highest at Station II, with peak values of 1.55 mg/L and 1.13 mg/L in December, likely due to nutrient-rich runoff during heavy rainfall. Both parameters remained elevated at Station II throughout the study period, indicating substantial human activity in this area of Vandiyur Lake (Hulyal & Kaliwal, 2011). Such nutrient enrichment, coupled with rising temperatures, promotes high primary productivity (Wetzel, 2001). Notably, the average phosphorus concentration (~1.0 mg/L) far exceeds the eutrophication threshold of 0.1 mg/L (Li et al., 2024), confirming that Vandiyur Lake is in a highly eutrophic state and requires urgent management intervention.

3.2. Assessment of Planktonic Community Structure

The planktonic assessment of Vandiyur Lake recorded

Table 1. Physico-chemical parameters of the various sampling stations of Vandiyur Lake from December 2023 to March 2024

Parameters	Sampling station-I				Sampling station-II				Sampling station-III				Sampling station-IV								
	D	J	F	M	MV	D	J	F	M	MV	D	J	F	M	MV	D	J	F	M	MV	
Colour	C	C	SB	SB	--	SB	SB	SB	SB	--	SB	SB	SB	SB	--	C	C	SB	SB	SB	--
Odour	N	N	N	N	--	N	N	SR	SR	--	N	N	SR	SR	--	No	N	N	N	N	--
AT (°C)	29	30	31	33	30.75	29	30	31	33	30.75	29	30	31	33	30.75	29	30	31	33	30.75	30.75
WT (°C)	27	27	28	29	27.75	27	27.2	28	29	27.75	27	27	27	28	27.75	27	27	27	29	27.75	27.75
Rainfall (mm)	105.1	8.9	13.2	24.2	133.32	105.1	8.9	13.2	24.2	133.32	105.1	8.9	13.2	24.2	133.32	105.1	8.9	13.2	24.2	133.32	133.32
pH	6.5	7.2	7.5	7.8	7.25	6.85	7.5	8.5	.9	7.96	6.55	7.25	8.1	8.5	7.6	6.12	7	7.5	7.8	7.10	7.10
TDS (mg/L)	1.0	3.2	3.2	3.8	2.80	2.03	2.71	3.33	3.45	2.88	2.05	2.4	3.02	3.05	2.63	1.8	2.0	2.5	2.58	2.22	2.22
Total alkalinity (mg/L)	180	188	220	212	200	289	304	355	336	321	280	300	316	327	305.75	211	220	233	276	235	235
Salinity (mg/L)	0.22	0.25	0.3	0.32	0.27	3.0	0.5	0.6	0.8	0.55	0.25	0.31	0.61	0.72	0.47	0.21	0.23	0.25	0.3	0.24	0.24
DO (mg/L)	8.8	6.4	7.5	8	7.67	8	5.5	6.3	7.5	6.82	8	5.8	6.5	7.5	6.95	8.1	6.5	7.5	7.9	7.5	7.5
BOD (mg/L)	3	3.2	3.5	4.1	3.45	5	6.45	7.2	8	6.66	4	4.5	6.2	6.8	5.37	3.1	3.5	4	4.75	3.83	3.83
Total Hardness (mg/L)	9	60.5	63	75	64.37	65	75	76	80	74	63	73	73	78	71.75	60	62	68	73	65.75	65.75
Free CO ₂ (mg/L)	8	10	12	15	11.25	14	22	25	28	22.25	10	18	22	25	18.75	10	12	15	18	13.75	13.75
Total nitrogen (mg/L)	0.91	0.75	0.85	0.98	0.62	1.55	0.94	1.03	1.12	1.16	1.23	0.75	0.92	1.01	0.97	0.94	0.65	0.71	0.78	0.77	0.77
Total phosphorous (mg/L)	0.97	0.68	0.74	0.92	0.82	1.13	0.93	0.98	1.02	1.01	1.02	0.85	0.91	0.98	0.94	0.95	0.75	0.78	0.91	0.85	0.85

Note: Abbreviation: SB: Slightly brown; SRS: Slightly putrid smell; C: Cloudy; N: None; AT: Air Temperature; WT: Water Temperature; TSC: Total dissolved Solid Content; DO: Dissolved Oxygen; BOD: Biological Oxygen Demand; D: December; J: January; F: February; M: March; MV: Mean value

Table 2. Phytoplankton species diversity at various sampling stations in Vandiyur Lake from December 2023 to March 2024

Phytoplankton	Sampling station-I						Sampling station-II						Sampling station-III						Sampling station-IV								
	D	J	F	M	DE	D	J	F	M	DE	D	J	F	M	DE	D	J	F	M	DE	D	J	F	M	DE		
CLASS: CHLOROPHYCEAE																											
<i>Closterium</i> sp.,	5	2	4	4	5	4	5	8	5	15	8.25	5	5	5	6	8	8	6	6	4	4	3	3	6	6	3	4
<i>Spirogyra</i> sp.,	-	-	5	4	2.25	2	2	2	4	4	3	-	-	-	2	2	2	1	1	-	-	5	3	3	4	4	3
<i>Stigeoclonium</i> sp.,	-	-	1	2	0.75	-	-	-	-	-	-	1	1	1	4	2	2	2	2	5	5	5	4	4	5	5	4.75
<i>Zygnema</i> sp.,	-	-	3	5	2	-	-	-	-	-	-	4	3	4	4	3	3.5	5	4	2	3	3	3.5	3	3	3	3.5
CLASS: CYANOPHYCEAE																											
<i>Gonium</i> sp.,	-	-	1	-	0.25	-	-	-	1	2	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.5	
<i>Oscillatoria</i> sp., 1	4	4	4	4	4	1	-	-	4	4	2.25	5	2	2	2	4	3.25	5	4	7	5	7	5	5	5	5.25	
<i>Oscillatoria</i> sp., 2	-	-	5	5	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	2	10	3	-	-	-	-	3	
<i>Oscillatoria</i> sp., 3	-	-	1	5	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.5	-	-	-	-	0.5	
<i>Oscillatoria</i> sp., 4	-	-	3	10	3.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.75	-	-	-	-	0.75	
<i>Microcystis</i> sp.,	3	2	3	2	2.5	10	12	8	25	13.75	6	4	14	18	10.5	3	-	2	2	1.75	2	2	2	2	2	1.75	
<i>Anabaena</i> sp.,	-	2	1	5	2	8	8	10	16	10.5	5	3	6	10	6	-	3	2	4	2.25	-	-	-	-	-	2.25	
<i>Merismopedia glauca</i>	1	-	-	2	0.75	2	-	-	4	1.5	-	-	-	2	0.5	-	-	-	-	-	-	-	-	-	-	-	
<i>Nostoc caeruleum</i>	1	-	-	1	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.5	-	-	-	-	-	0.5	
CLASS: BACILLAROPHYCEAE																											
<i>Skeletonema</i> sp.,	-	-	1	25	6.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Amphora</i> sp.,	-	-	3	5	2	-	-	-	-	-	-	-	2	1	-	-	0.75	-	-	-	-	-	-	-	-	-	
<i>Synedra</i> sp.,	3	2	3	3	2.75	-	-	-	-	-	-	2	2	2	4	2.5	5	3	1	2.5	-	-	-	-	-	-	
<i>Nitzschia</i> sp., 1	-	-	1	10	2.75	3	3	4	10	5	-	-	-	-	-	-	-	-	1	0.5	-	-	-	-	-	0.5	
<i>Nitzschia</i> sp., 2	3	2	2	3	2.5	5	4	4	5	4.5	4	2	1	1	2	2	-	-	1	-	-	-	-	-	1		
<i>Navicula</i> sp.,	1	2	3	3	2.25	4	3	4	8	4.75	1	-	8	6	3.75	3	-	3	2.25	-	-	-	-	-	2.25		
<i>Cyclotella</i> sp.,	4	4	-	4	3	-	-	-	-	3	0.75	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	
<i>Asterionella</i> sp.,	-	-	3	3	1.5	6	6	6	8	12	8	3	5	3	8	4.75	-	2	2	1	-	-	-	-	-	1	
CLASS: PENNALES																											
<i>Thalassiothrix</i> sp.,	-	-	-	-	-	-	-	-	1	1	0.5	-	-	-	-	4	1	-	-	-	-	-	-	-	-	-	
CLASS: TREBOUXIOPHYCEAE																											
<i>Chlorella</i> sp.,	-	-	3	3	1.5	5	3	10	12	7.5	3	3	3	8	10	6	-	1	2	1.25	-	-	-	-	-	-	
CLASS: EUGLENOPHYCEAE																											
<i>Euglena</i> sp.,	2	1	4	4	2.75	5	3	7	12	6.75	5	-	-	-	8	3.25	-	3	5	2	-	-	-	-	-	2	
<i>Phacus</i> sp.,	-	-	2	2	1	3	-	5	8	4	3	3	5	5	4	-	1	3	1	-	-	-	-	-	-		
TNI-MW	27	21	56	115	59	13	51	75	140	43	35	66	95	34	32	43	65	20	21	21	21	17	17	20	20		
TNS-MW	10	9	21	23	13	11	15	15	17	13	12	15	17	9	10	17	21	21	21	21	21	17	17	20	20		
TNS-SW	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24		
TNI-SW	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219	219		

Note: Abbreviation: D: December; J: January; F: February; M: March; DE: Density; TNI-MW: Total Number of Individual in Month-wise; TNS-MS: Total Number of Species in Month-wise; TNI-SW: Total Number of Individuals in Site-wise; TNS-SW: Total Number of Species in Site-wise

Table 3. Zooplankton species diversity at various sampling stations in Vandiyur Lake from December 2023 to March 2024

Zooplankton	Sampling station-I						Sampling station-II						Sampling station-III						Sampling station-IV					
	D	J	F	M	DE	D	D	J	F	M	DE	D	D	J	F	M	DE	D	D	J	F	M	DE	
CLASS: ROTIFERA																								
<i>Brachionus rubens</i>	2	3	3	5	3.25	4	6	6	5	5.25	5	3	6	6	5	4	2	2	2	5	3.5			
<i>Brachionus calyciflorus</i>	4	3	4	3	3.5	7	3	5	10	6.25	7	3	4	5	4.75	5	3	2	2	3	3.25			
<i>Brachionus kostei</i>	-	-	-	-	-	-	-	2	2	1	2	-	1	3	2	3	2	2	1	2	2			
<i>Brachionus urceolaris</i>	2	2	2	2	2	5	7	10	10	8	5	4	8	8	6.25	-	-	2	2	1	1			
<i>Brachionus quadridentatus</i>	4	2	3	3	3	8	6	8	12	8.5	4	2	6	8	5	2	4	4	2	3	3			
<i>Brachionus durgae</i>	2	-	-	2	1	-	-	-	-	-	2	2	2	3	2.25	-	-	-	-	-	-			
<i>Brachionus bidentatus</i>	4	2	2	4	3	8	3	5	7	5.75	4	5	5	5	4.75	-	-	2	2	1	1			
<i>Brachionus plicatilis</i>	3	2	4	3	3	5	6	6	6	5.75	-	-	-	-	-	3	5	5	6	4.75	4.75			
<i>Brachionus forficula</i>	5	5	5	5	5	2	2	4	3	2.75	-	-	-	-	-	2	4	4	3	3.25	3.25			
<i>Brachionus durgae isigakiensis</i>	4	3	2	3	3	-	-	-	-	-	1	3	2	1	1.75	-	-	-	-	-	-			
<i>Brachionus</i> sp., 1	13	9	9	7	9.5	7	8	6	6	6.75	8	7	7	6	7	8	4	7	8	6.75	6.75			
<i>Brachionus</i> sp., 2	-	-	-	1	0.25	-	-	2	2	1	1	2	-	2	1.25	-	-	1	2	0.75	0.75			
<i>Brachionus</i> sp., 3	-	-	1	2	0.75	-	-	-	-	-	-	-	1	1	0.5	-	-	1	2	0.75	0.75			
<i>Brachionus</i> sp., 4	-	-	-	-	-	1	1	1	1	1	-	-	2	2	1	-	-	3	3	1.5	1.5			
<i>Brachionus</i> sp., 5	-	-	1	2	0.75	3	2	2	5	3	1	-	1	1	0.75	-	-	2	5	1.75	1.75			
<i>Brachionus</i> sp., 6	-	-	1	2	0.75	1	-	1	1	0.75	-	-	-	1	0.25	-	-	10	5	4	4			
<i>Brachionus</i> sp., 7	-	-	1	5	1.5	2	-	2	2	1.5	-	-	2	1	0.75	-	-	2	2	1	1			
<i>Brachionus</i> sp., 8	-	-	-	-	-	-	-	-	-	-	1	-	2	2	1.25	-	-	1	1	0.5	0.5			
<i>Filimia longiseta</i>	2	2	1	2	1.75	3	-	2	3	2	3	-	3	-	1.5	-	2	1	2	1.25	1.25			
<i>Monostyla</i> sp.,	1	-	-	2	0.75	5	3	8	8	6	2	-	3	5	2.5	-	-	1	2	0.75	0.75			
<i>Lepadella</i> sp.,	-	-	2	2	1	4	1	3	5	3.25	3	-	3	3	2.25	1	1	1	1	1	1			
CLASS: CLADOCERANS																								
<i>Alonella</i> sp.,	-	-	2	2	1	3	2	2	2	2.25	4	6	2	4	4	3	2	2	4	2.75	2.75			
<i>Diaphanosoma sarsi</i>	1	-	-	1	0.5	7	5	5	6	5.75	5	7	5	4	5.25	4	2	2	2	2.5	2.5			
<i>Daphnia magna</i>	2	-	2	2	1.5	5	4	6	10	6.25	6	3	2	2	3.25	1	-	1	2	1	1			
<i>Daphnia</i> sp.,	3	2	2	3	2.5	7	9	9	7	8	5	3	3	3	3.5	-	-	1	2	0.75	0.75			
<i>Moina brachiata</i>	-	2	5	-	1.75	-	-	-	1	0.25	-	2	5	-	1.75	4	2	3	2	2.75	2.75			
<i>Moina</i> sp., 1	7	10	6	6	7.25	1	3	3	2	2.25	6	9	5	5	6.25	9	6	3	4	5.5	5.5			

Cont..

Table 3. Zooplankton species diversity at various sampling stations in Vandiyur Lake from December 2023 to March 2024

Zooplankton	Sampling station-I					Sampling station-II					Sampling station-III					Sampling station-IV				
	D	J	F	M	DE	D	J	F	M	DE	D	J	F	M	DE	D	J	F	M	DE
<i>Moina</i> sp., 2	-	-	2	5	1.75	2	-	2	2	1.5	-	-	2	2	1	-	-	-	-	-
<i>Simocephalus</i> sp.,	2	-	-	1	0.75	-	-	-	-	-	-	-	-	-	-	3	2	-	-	1.5
CLASS: COPEPODS																				
<i>Cyclops</i> sp.,1	6	4	4	1	3.75	5	3	6	6	5	7	2	4	10	5.75	7	4	2	2	3.75
<i>Cyclops</i> sp.,2	-	-	2	10	3	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.5
<i>Heliodiaptomus</i> sp.,	4	-	-	2	1.5	-	-	-	-	-	-	-	1	-	0.25	4	1	-	-	1.25
<i>Rhinediaptomus indicus</i>	3	-	-	-	0.75	-	-	-	-	-	-	-	-	1	0.25	-	-	-	2	0.5
<i>Sinodiaptomus</i> sp.,	3	3	3	1	2.5	-	-	-	-	-	-	-	-	-	-	3	-	4	-	1.75
<i>Diaptomus</i> sp., 1	6	2	2	3	3.25	8	5	6	12	7.75	4	3	8	8	5.75	6	4	2	5	4.25
<i>Diaptomus</i> sp., 2	-	-	-	2	0.5	-	-	2	2	1	1	-	3	3	1.75	-	-	-	-	-
<i>Mesocyclops leuckarti</i>	5	4	5	2	4	8	5	3	10	6.5	4	1	6	8	4.75	-	2	2	2	1
<i>Nauplius</i> sp.,	4	2	5	2	3.25	-	-	1	1	0.5	2	-	2	-	1	2	2	5	1	2.5
<i>Acartia</i> sp.,	-	-	-	1	0.25	5	2	5	8	5	3	-	3	5	2.75	-	-	-	-	-
CLASS: OSTRACODA																				
<i>Cypris</i> sp.,1	5	2	2	3	3	3	3	2	5	3.25	-	-	-	-	-	3	2	1	1	1.75
<i>Cypris</i> sp.,2	-	-	4	10	3.5	-	-	1	2	0.75	-	-	-	-	-	-	-	-	1	0.25
<i>Cypris</i> sp.,3	-	-	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.5
<i>Strandesia elongate</i>	1	-	-	-	0.25	-	-	-	-	-	1	1	-	1	0.75	-	3	3	3	2.25
<i>Cypris protuberata</i>	3	1	4	-	2	-	-	-	-	-	4	2	2	2	2.5	-	-	-	-	-
<i>Heterocypris dentatocarinatus</i>	2	-	-	1	0.75	2	-	1	2	1.25	-	-	-	-	-	-	-	-	-	-
TNI-MW	103	65	93	115		121	89	126	165		101	70	111	121		77	57	85	94	
TNS-MW	28	20	31	38		27	22	31	33		28	20	32	32		20	20	32	36	
TNS-SW			42					33					36					38		
TNI-SW			376					503					403					313		

Note: Abbreviation: D: December; J: January; F: February; M: March; DE: Density; TNI-MW: Total Number of Individual in Month-wise; TNS-MS: Total Number of Species in Month-wise; TNI-SW: Total Number of Individuals in Site-wise; TNS-SW: Total Number of Species in Site-wise

25 phytoplankton species across six major classes with a total density of 961 individuals. Cyanophyceae (9 species) was the most diverse group, followed by Bacillariophyceae (8 species) and Chlorophyceae (4 species), reflecting typical dominance patterns in nutrient-rich or eutrophic waters (Baba & Pandit, 2014). These groups exhibit broad ecological tolerance across salinity gradients (Stanca et al., 2013; Pratiwi et al., 2018), with Cyanophyceae favoring warm, stable conditions and Bacillariophyceae thriving in cooler, well-mixed, nutrient-rich environments (Guedes et al., 2018). Station I showed the highest species diversity (n=24 species) but lowest species density (219 individuals), whereas Station II had the highest density (329 individuals) despite lower diversity (16 species). Seasonally, phytoplankton density was lowest in January (range: 21–51 individuals) and peaked in March, reaching 140 individuals at Station II. Dominant genera included *Microcystis*, *Anabaena*, *Closterium*, *Nitzschia*, *Navicula*, *Asterionella*, and *Skeletonema*, whose proliferation particularly at Station II indicates organic pollution and advanced eutrophic conditions, as supported by earlier and recent studies (Shekhar et al., 2008; Nikolopoulou et al., 2025; Maire et al., 2025). This finding confirms that Vandiyur Lake's is in an advanced eutrophic state, necessitating urgent conservation and management measures to protect its ecological integrity.

The zooplankton community of Vandiyur Lake exhibited high taxonomic diversity, comprising 45 species across four major groups: Rotifera, Copepoda, Cladocera, and Ostracoda, with a total abundance of 1,595 individuals. Rotifera (21 species) was the most diverse group, followed

by Copepoda (10 species), Cladocera (8 species), and Ostracoda (6 species). Their dominance is consistent with their high ecological adaptability and rapid reproductive capacity, enabling them to thrive in nutrient-rich and eutrophic environments (Phan et al., 2021; Montemezzani et al., 2015). Station I recorded the highest species richness (42 species) but the lowest species abundance (n=376 individuals), whereas Station II showed the highest species abundance (503 individuals) despite lower species richness (33 species), indicating ecological imbalance. Seasonally, zooplankton density was lowest in January and peaked in March, with a maximum of 165 individuals at Station II. The community was dominated by *Brachionus* spp. (e.g., *B. calyciflorus*, *B. rubens*, *B. plicatilis*), along with *Daphnia magna*, *Diaphanosoma sarsi*, *Cyclops* sp., *Diaptomus* sp., and *Cypris* sp. The proliferation of *Brachionus* spp., particularly at Station II, indicates high organic loading and advanced eutrophication, as these taxa are well-known saprobic indicators tolerant of low dissolved oxygen and elevated nutrient levels (Sampaio et al., 2002). The occurrence of *Brachionus plicatilis* further suggests elevated total dissolved solids and alkaline conditions (Sládeček, 1983), consistent with observations from polluted tropical waters (Sampath et al., 2000).

Despite predation pressure, the persistence of Cladocerans such as *Daphnia magna* and *Diaphanosoma sarsi* is likely supported by abundant phytoplankton, including *Microcystis* and *Anabaena* (Jeppesen et al., 2011). The presence of *Cyclops* and *Diaptomus* species reflects a resilient community adapted to fluctuating environmental conditions, with cyclopoids known to tolerate polluted

Table 4. Comparative analysis of diversity indices for Phytoplankton and Zooplankton across four sampling stations in Vandiyur Lake

Category	Sampling station	No. of species	No. of individuals	Diversity Indices		
				Margalef (D_{mg})	Shannon (H')	Pielou (J')
Phytoplankton	I	24	219	2.88 ^{bc}	4.27 ^a	0.65 ^a
	II	16	329	3.18 ^a	2.59 ^d	0.22 ^b
	III	17	239	2.92 ^b	2.83 ^c	0.20 ^{bc}
	IV	21	174	1.88 ^c	3.04 ^b	0.61 ^{ab}
Zooplankton	I	42	376	5.53 ^{bc}	5.74 ^a	0.80 ^a
	II	32	501	6.91 ^a	3.88 ^c	0.55 ^d
	III	36	403	5.95 ^b	4.15 ^{bc}	0.68 ^c
	IV	38	313	4.35 ^c	4.64 ^b	0.72 ^b

For all diversity indices, values within the same column followed by different superscript letters are significantly different ($P < 0.05$) across sampling stations, based on Duncan's Multiple Range Test (DMRT)

habitats (Radhika et al., 2004). Overall, the dominance of Rotifera, particularly *Brachionus* spp., indicates a shift toward an advanced trophic state driven by both nutrient enrichment (bottom-up control) and ecological stress, which is characteristic of urbanized lake ecosystems (Mageed, 2008).

3.3. Assessment of Ecological Diversity Indices

Comparative analysis of ecological indices Margalef richness (D_{mg}), Shannon–Wiener diversity (H'), and Pielou evenness (J') revealed significant spatial variation across the four stations for both phytoplankton and zooplankton (Table 4). Station II exhibited the highest species richness (D_{mg} : 3.18 for phytoplankton; 6.91 for zooplankton), while Station IV recorded the lowest values. In contrast, Station I showed the highest species diversity (H' : 4.27 for phytoplankton and 5.74 for zooplankton), indicating a more stable and heterogeneous community. Despite high species richness and abundance observed at Station II, the lower species diversity (Shannon–Wiener index) values reflect poor species distribution, further supported by reduced species evenness (Pielou Index). Station I also showed the highest species evenness (0.65 and 0.80 phyto-zooplankton, respectively), whereas Stations II and III recorded lower species diversity (H') and evenness (J'), suggesting dominance by a few taxa. Such reduced species evenness typically indicates environmental stress and community imbalance, often associated with bloom-forming or pollution-tolerant species.

The biological diversity indices indicate that Station I maintains the most stable and diverse ecosystem for both phytoplankton and zooplankton. In contrast, Station II, despite its high abundance, exhibited lower Shannon (H') and Pielou (J') values, reflecting dominance by a few opportunistic species and suggesting localized organic enrichment or anthropogenic stress. DMRT analysis confirmed that spatial variations among stations were statistically significant ($P < 0.05$). Plankton communities (phytoplankton and zooplankton) are widely recognized as sensitive indicators of water quality (Singh et al., 2013). Overall, relatively high values of Shannon, Margalef, and Pielou indices across the lake indicate good species diversity; however, localised reductions in H' (Shannon's index) may be attributed to factors such as heavy rainfall and associated environmental fluctuations (Dash, 1996).

4. CONCLUSION

Based on a multi-parametric assessment, Vandiyur Lake is undergoing advanced eutrophication. Although elevated

nutrient levels support high planktonic productivity, the dominance of pollution-tolerant taxa and reduced species evenness indicate ecological imbalance and environmental stress, primarily driven by untreated sewage inflow. Despite this degradation, the lake retains significant biological potential; with effective sewage control and proper screening for contaminants, it could support sustainable inland aquaculture. To restore the ecological integrity of Vandiyur Lake, immediate management interventions are essential, including: (1) Sewage diversion: Implementing infrastructure to prevent the direct ingress of domestic and market effluents into the lake basin. (2) Riparian buffers: Establishing biological filters and green belts along the lake margins to mitigate nutrient-rich surface runoff. (3) Holistic management: Developing a long-term, sustainable conservation plan that balances the lake's ecological restoration with its multi-use functions for the local community.

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CRediT authorship contribution statement

Thangavel Rajagopal: Conceptualization, Data curation, Supervision, and Writing original draft, Review and editing preparation, Formal analysis and validation. **Ponnirul Ponmanickam** Conceptualization, Writing, review and editing. **Selvam Kaviya** Resources, investigation, and methodology, Formal analysis and validation. **Pandiyarajan Seenivasan** Resources, investigation, and methodology.

Conflict of interest

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no artificial intelligence tools were used to write this manuscript.

Data availability statement

All results are published in this article; raw data can be made available from the authors upon reasonable request.

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Mechanizing Small-scale Potato Farming: Development and Performance Evaluation of Power Tiller Operated Potato Planter cum Fertilizer Applicator

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ABSTRACT: Traditional potato planting methods are labour-intensive, time-consuming, and often lead to inconsistent planting, which reducing yield and quality. Animal-operated planters are slow and costly to maintain, while tractor-operated planters are designed for larger farms and are unfeasible to small farmers due to their high cost and technical requirements. A potato planter for use with a 9 hp power tiller has been developed. The performance of the developed planter was evaluated based on five key parameters: draft requirement, missing index, multiple index, effective field capacity, and fuel consumption. Results shows that the minimum draft was 0.79 kN at forward speed of 1.5 km/h and a depth of 80 mm. The lowest missing index (5.33%) and multiple index (4%) occurred at 1.5 km/h and 150 mm depth. The highest effective field capacity (0.124 ha/h) was achieved at 2.5 km/h and 80 mm depth, while the lowest (0.054 ha/h) was at 1.5 km/h and 120-150 mm depth. Fuel consumption was lowest at 13.96 l/ha when operating at 1.5 km/h and 80 mm depth.

Keywords: Potato planter, Fertilizer applicator, Power tiller, Small scale farming.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important horticultural crops in the world and a cost-effective food that provides low-cost energy to the human diet (Pal and Chattopadhyay, 2020). Global potato production in 2022 was 375 million tonnes harvested in approximately 18 million hectares, with China and India are the leading producers, producing 96 million tonnes and 56 million tonnes, respectively (FAO, 2022). The potato production in India during 2023 was approximately 60.14 million tonnes from 2.3 million hectares. Although potatoes are grown in almost all states, major producing regions include Uttar Pradesh, West Bengal, Bihar, Gujarat, Madhya

Pradesh, Punjab, Haryana, Assam, Jharkhand, and Chhattisgarh (Singh and Dutt, 2024). In the year 2022-23, overall potato production in Chhattisgarh was about 655.438 tonnes, contributing about 1.09 % to the country's total potato production. In developed nations, mechanized planting methods have replaced traditional practices, enabling uniform tuber depth, consistent planting, and improved crop yields (Anand et al., 2023). In many developing nations, traditional and semi-mechanized planting practices are still widely adopted (Bovas et al., 2022). India has seen a gradual shift from traditional planting techniques to mechanized methods, especially in major potato-producing regions such as Uttar Pradesh, West Bengal, and Bihar (Mehta et al., 2018). Despite these

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advancements, a significant proportion of Indian agriculture remains characterized by small and marginal farmers, where mechanization is less prevalent (Sarkar, 2020). In Chhattisgarh, almost 80 per cent of farmers fall into the small and marginal group, with average landholdings of 1.6 hectares (Chandrakar et al., 2021). These farmers predominantly rely on manual labour and animal-drawn implements for potato planting (Kosariya and Singh, 2022). Traditional planting involves manually placing tubers in furrows and covering them with soil using a spade, making the operation labour-intensive, time-consuming, and tedious, requiring approximately 1600-1700 man-hours per hectare (Issa et al., 2025). Furthermore, these result in uneven tuber placement and variable soil coverage, which adversely affect crop yields and quality (Mehta et al., 2018). Variations in planting depth may expose tubers to sunlight, leading to greening and increased solanine accumulation, making the potatoes unsuitable for human consumption (Rymuza et al., 2020). The use of animal power is growing increasingly expensive as it is necessary to be maintained throughout the year, even if there is no workaround on the farm. Moreover, the working rate of draft animals is quite slow, causing farm operations to be prolonged (Rajkhowa and Kubik, 2021). Although tractor-operated potato planters are available, their use is largely restricted to medium and large farmers due to high initial investment, limited utilization, and technical constraints (Zheng et al., 2021; Dhillon and Moncur, 2023). Therefore, a power tiller operated potato planter can offer a cost-effective and low maintenance solution for marginal and small farmers.

Moreover, several developed power tiller-operated potato planters were equipped with seed hoppers, metering mechanisms, furrow openers, and covering devices for effective planting. However, the earlier developed planters exhibited constraints in the capacity of the seed hopper. Most of the developed planters use chain and cup type, finger type seed metering mechanisms, that are significantly affected by power tiller vibration, leading to inconsistent seed distribution and reduced planting accuracy, which results in reduced crop yield (Kus, 2021). The earlier planters lacked integrated fertilizer applicators, preventing fertilizer application during planting and requiring extra field operations, which increased operational complexity, labour requirements, and fuel consumption. Therefore, this research aimed to evaluate the performance of the developed single row power tiller operated potato planter to address these challenges.

2. MATERIALS AND METHODS

The design and development of single-row potato planter operated by a power tiller was carried out in the Department of Farm Machinery and Power Engineering, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. The main function of the developed is to place potato tubers into soil furrows at specified intervals and depths. The design and development of the planter's components were based on functional and ergonomic consideration to keep production costs reasonable.

2.1. Development of Potato Planter

The design criteria for the seed and fertilizer hopper were based on examinations and investigations of the physical characteristics of potatoes and fertilizer (Kosariya and Singh, 2022). The characteristics of potatoes for designing of planter hopper were bulk density of potatoes 750 kg/m^3 , angle of repose 37° , seed rate 2000-2500 kg/ha, and the inter-row spacing in potato cultivation typically falls within the interval of 0.50-0.60 m, accompanied by an intra-row spacing of 0.15-0.25 m between individual tubers (Mandloi et al., 2018). Based on reviews and studies of fertilizer physical properties, the average bulk density of these fertilizers was about 800 kg/m^3 , and the angle of repose was 38° , which were used for the design of the fertilizer hopper. Also, the potato crop requires a large amount of fertilizer mixture, varying from 700 to 900 kg/ha (Manikyam et al., 2022).

2.2. Design of Seed Hopper

The capacity of the seed hopper was designed to be sufficient to cover a 100 m length of operation. The area covered for a 100 m length of run with 0.6 m row-to-row spacing was calculated as 60 m^2 , and the quantity of potato tubers required for this area was estimated to be 12 kg. Therefore, the volume of hopper was determined based on the average bulk density of potato tubers using Eq. 1 (Singh et al., 2022).

$$\text{Volume of hopper} = \frac{\text{Weight of potato tubers}}{\text{Bulk density of potato tubers}} = 0.016 \text{ m}^3 \quad (1)$$

The seed hopper was designed as a cylinder with a perpendicular height of 0.16 m from the bottom and a radius of 0.18 m. The hopper with the above dimensions has a total volume of 0.0172 m^3 , sufficient to carry 12 kg of potato tubers.

2.3. Furrow Opener and Transport/drive Wheel

A shoe-type furrow opener was mounted at the bottom of

the seed delivery tube. A mounting plate was welded to the seed delivery tube, with drilled holes for mounting the furrow opener using a suitable nut-and-bolt arrangement. Moreover, the drilled holes in the mounting plate provide a means to adjust the planting depth. The furrow opener has a point of share that cuts the furrow slice and inverts it with the help of wings (mouldboard) provided. Its purpose is to create a furrow so that the tubers can be planted at a specific depth. Two lugged wheels, each with a 270 mm diameter and a 65 mm rim width, serves as both transport and drive wheels, providing drive to the seed and fertilizer metering shafts through suitable gears arrangements.

2.4. Design of Power Transmission Unit for Seed Metering Mechanism

To ensure appropriate metering of potato tuber, a suitable bevel gear arrangement is selected. The driver gear, consisting of 10 teeth, is mounted on the transport/drive wheel shaft, and the driven gear, with 18 teeth, is mounted on the perpendicular shaft to the drive wheel. The gear ratio between driver gear to driven gear is 1:1.8. This indicates that when the drive wheel rotates 1.8 times, the driven gear rotates the seed metering mechanism 1 time and caters to a suitable speed of seed metering plate that places the tubers at a specified distance and plants the tubers appropriately.

2.5. Ridger/Covering Device

A disc-type ridge former is provided for making ridges. The ridger are made from a 2 mm mild steel sheet. The former was mounted on the main frame using a suitable arrangement. Furthermore, there is a provision for adjusting the height of the ridge former according to the height of the furrow opener. In addition to creating the ridge, it also covers the loose soil over the potato tubers placed by the metering unit.

2.6. Design of Fertilizer Hopper

Considering the seed hopper capacity of 12 kg, the fertilizer hopper was designed to feed fertilizer once every two or three times as often as potato tubers are fed. The hopper was refilled after every 200 m of planting with a row-to-row spacing of 0.60 m. Therefore, the area covered in one operation was calculated as 120 m², and thus the fertilizer requirement for calculated area was determined using Eq. 2 (Madhusudan and Preetham, 2020):

$$\text{Fertilizer requirement} = \frac{\text{Area covered (m}^2\text{)} \times \text{Recommended fertilizer rate (kg ha}^{-1}\text{)}}{10000 \text{ m}^2} = 8.4 \text{ kg} \quad (2)$$

An additional 1.6 kg of fertilizer was provided as a buffer stock; therefore, the capacity of fertilizer hopper was kept to 10 kg. To ensure a smooth and continuous flow of fertilizer toward the metering mechanism under gravity, the hopper design was based on the angle of repose. The hopper's total capacity was kept at 10 kg of fertilizer, assuming an average bulk density of 800 kg/m³. Thus, the volume of fertilizer box was calculated using Eq. 3 (Kumar et al., 2017):

$$\text{Volume of fertilizer box} = \frac{\text{Weight of fertilizer, kg}}{\text{Bulk density, kgm}^3} = 0.0125 \text{ m}^3 \quad (3)$$

Therefore, the fertilizer hopper dimensions were selected to accommodate the required storage volume. To ensure smooth material flow toward the centrally located metering device at the bottom, the hopper was designed with a trapezoidal cross-section, a common configuration for granular materials. The hopper geometry consisted of a bottom width of 0.16 m, a top width of 0.28 m, an upper section height of 0.50 m, a lower section height of 0.30 m, and a length of 0.28 m. Based on these dimensions, the total hopper volume was obtained by combining the volumes of the upper and lower sections, resulting in an overall capacity of 0.02232 m³. The hopper was fabricated from 20-gauge mild steel sheet, and the selected dimensions provided sufficient storage capacity for the fertilizer mixture required during potato tuber planting.

2.7. Fertilizer Metering Mechanism

The fertilizer metering device comprised a fluted roller arrangement integrated with a jack-type adjustment mechanism within the fertilizer hopper. The fluted roller shaft and the jack-type mechanism were mounted on a common shaft to ensure uniform and controlled fertilizer distribution. The jack-type mechanism consisted of a nut-and-bolt assembly mounted on the fluted roller shaft through a spring-tensioning system, which regulated the fertilizer application rate by varying the effective opening of the fluted roller. The fertilizer metering mechanism was driven by the transport wheel through a chain and sprocket transmission system. Fertilizer delivery to the furrow was facilitated using a PVC tube with an internal diameter of 30 mm, allowing smooth flow of fertilizer into the furrow formed by the furrow opener.

2.8. Design of Fertilizer Metering Drive Chain

The ground wheel shaft transmitted rotational motion to the 15 mm diameter fertilizer metering shaft through a chain-and-sprocket mechanism. A 19-tooth gear was mounted at

one end of the fertilizer metering shaft. In contrast, the opposite end was fitted with a jack-type lever adjustment that regulated the fertilizer application rate by sliding the shaft along a keyed section. A 13-tooth gear mounted on the transport wheel shaft provided the driving input to the fertilizer metering shaft. The chain length between the transport wheel shaft and the fertilizer metering shaft was determined using Eq. 4 (Yunus et al., 2015):

$$L=2C+1.57 (D_1+D_2) + \frac{(D_1-D_2)^2}{4C} = 1170.25 \text{ mm} \quad (4)$$

Where; C = Centre to centre distance of two sprockets in mm D = Number of teeth of two sprockets. During continuous operation of the machine under undulating field conditions, the chain was subjected to tensile stresses, leading to an elongation of about 20 to 30 mm. Accordingly, the required number of chain links was determined using Eq. 5 (Kumar et al., 2017):

$$m = \frac{2C}{P} + \frac{Z_1+Z_2}{2} + \frac{P(Z_2-Z_1)}{4\pi^2 C} = 90 \text{ links} \quad \dots(5)$$

Where, m represents the number of chain links, C denotes the center-to-center distance between sprockets (560 mm), Z₁ and Z₂ are the numbers of teeth on smaller and larger sprocket (13 and 19, respectively), and P is the chain

pitch of 15 mm. Based on these parameters, it was determined that a chain length of approximately 1200 mm requires about 96 links, providing sufficient allowance for chain elongation and ensuring smooth, reliable power transmission during field operation.

Table 1. Specification of developed single row power tiller operated potato planter

Specification	Values
Seed hopper	Height:160 mm, Dia.:370 mm,
Fertilizer hopper	Top width: 280 mm, Length: 280 mm, Bottom width:160 mm, Height: 350mm
Cell feed type seed metering	Number of cells: 9, Dia.: 360 mm
Seed delivery tube	Dia.: 90 mm, Length: 510 mm
Fertilizer tube	Dia.: 30 mm, Length: 290 mm
Furrow opener	Shoe type
Ridge former	Disc type
Ground wheel	Dia.: 270 mm
Overall dimensions	Length: 706 mm, Height: 850 mm, Width: 685 mm
Weight	45 kg
Power source	Power tiller 9 hp or above

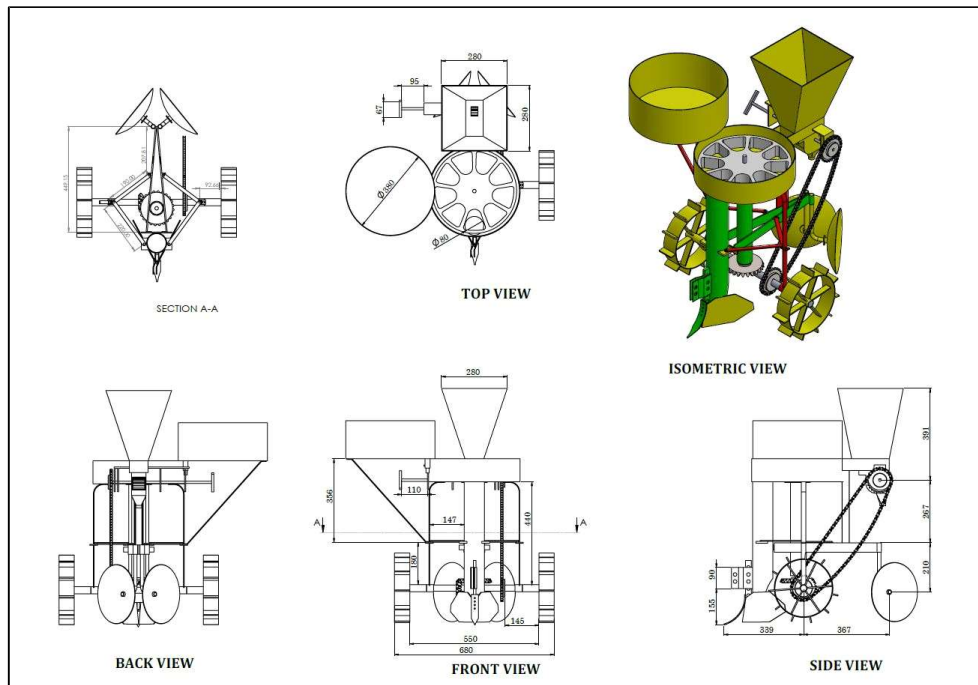


Figure 1. Developed single row power tiller operated potato planter

2.9. Developed Single Row Power Tiller Operated Potato Planter

The developed power tiller operated potato planter consists of six major components: a seed hopper, cell feed type seed metering mechanism, fertilizer hopper, furrow opener, and ridger/covering device (Figure 1). Moreover, it allows uniform planting, ensuring that potato tubers were placed at consistent depths and spacings. The planter incorporates a fertilizer application system, allowing for simultaneous planting and fertilization. Table 1 presents the detailed specifications of the developed single-row power tiller-operated potato planter.

2.10. Experimental design and performance evaluation of developed potato planter

The experiment was laid out in two-factor randomized design for analysis potato planting. The experiments were conducted in the field to evaluate the performance of the potato planter (Figure 2). The statistical analysis was performed using SAS 9.4 Software. The field performance



Figure 2. Field evaluation of power tiller operated potato planter

of the developed planter was assessed using following parameters with 5 replications. The experimental parameters used for the performance evaluation of developed potato planter presents in Table 2.

2.11. Draft Requirement

A spring balancing dynamometer was fastened to the front of the power tiller, to which the planter was attached, for draft measurement. An auxiliary tractor pulled the power tiller (in neutral gear but with the planter in operating position) through the dynamometer over a 30-meter distance. The draft was recorded with and without the planter attached. The difference between these two readings determined the planter's draft. This process was repeated for each speed considered in the study (Hardik, 2014).

2.12. Missing Index

The planter was operated in the field, and the distance between two consecutive tubers was measured in a span of 5 m. If the distance between two consecutive tubers exceeded 1.5 times of the theoretical spacing, then this was considered as missing. A Missing index thus indicates how many times the labour failed to place the tubers in the cell of seed metering plate. The percentage of missing of tubers was calculated by using Eq. 6 (Gautam et al., 2019; Choudhary et al., 2024):

$$\text{Missing index (\%)} = n/N \dots\dots (6)$$

Where, n = No. of spacings greater than 1.5 times the theoretical spacing in the given observation; N = Total number of observations

2.13. Multiple Index

It consists of two or more tubers dropped by the seed metering unit through a single hole in the metering plate. The multiple index indicates that more than one tuber has been dropped within the desired spacing. It is the fraction of the

Table 2. Experimental parameters for performance evaluation of the developed planter

Parameters	Factors	Levels		
		Low (-1)	Middle (0)	High (+1)
Independent parameters	1. Forward speed (km/h)	1.5	2.0	2.5
	2. Planting depth (mm)	80	120	150
Dependent parameters	1. Draft requirement (kN)			
	2. Missing index (%)			
	3. Multiple index (%)			
	4. Effective field capacity (ha/h)			
	5. Fuel consumption (l/ha)			

theoretical spacing that is less than or equal to half, and calculated using Eq. 7 (Thakur et al., 2020; Tsegaye, 2025).

$$\text{Multiple index (\%)} = n_i/N \dots\dots\dots (7)$$

Where, n_i = number of spacings in the region less than or equal to 0.5 times of the considered spacing, N = total number of observations

2.14. Effective Field Capacity

The effective field capacity is the actual average rate of coverage by the planter. Effective field capacity is usually expressed in hectare per hour, and calculated using Eq. 8 (Parihar et al., 2022):

$$\text{Effective field capacity (ha/h)} = \text{Area of plot (ha)}/\text{Time taken (h)} \dots\dots (8)$$

2.15. Fuel Consumption

Fuel consumption was measured in liters per hectare (l/ha) using top fill method (Devojee et al., 2019).

3. RESULTS AND DISCUSSION

3.1. Effect of Forward Speed and Planting Depth on Draft Requirement

The determination of draft is highly important for achieving an economical, energy-efficient potato planting operation. The maximum draft requirement (1.22 kN) occurred at a forward speed of 2.5 km/h and planting depth of 150 mm (Table 3). However, the minimum draft (0.79 kN) was observed at a forward speed of 1.5 km/h and planting depth of 80 mm. Moreover, it was observed that draft requirement increased with both forward speed and planting depth. This might be due to the furrow opener encountering greater soil resistance at greater depth and at higher speed. Aruna et al. (2020) observed that the draft requirement was significantly affected by forward speed and operating depth, as the volume of soil managed by the implement increased with both.

The LS-means of speed and planting depth at three

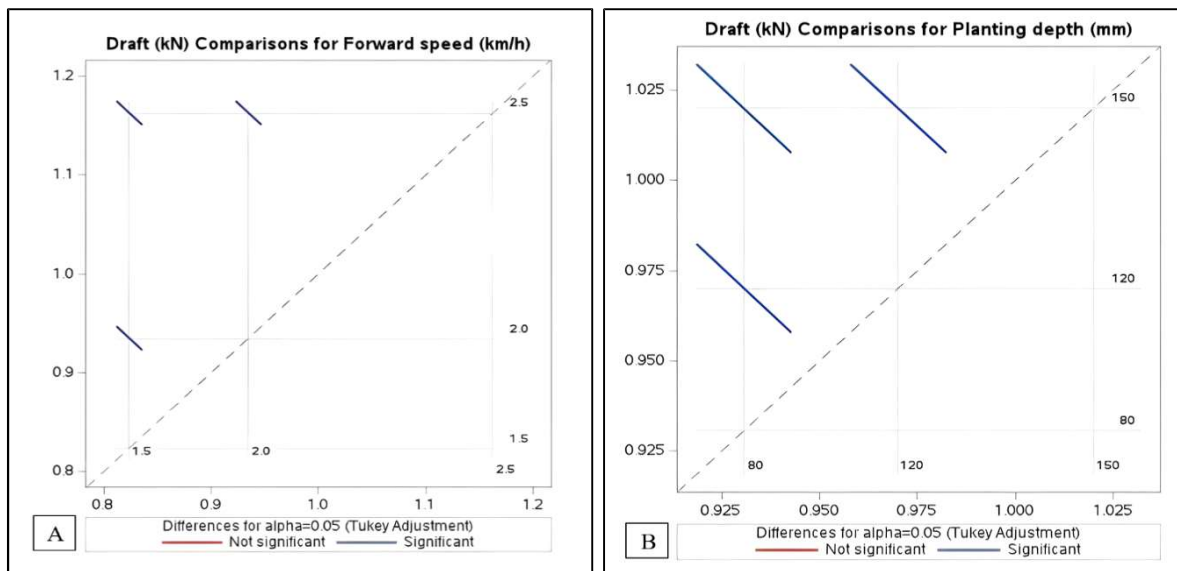


Figure 3. Effect of forward speed and planting depth on draft requirement

Table 3. Effect of forward speed and planting depth on draft requirement

Forward speed, km/h→ Planting depth, (mm)↓	1.5 km/h	2.0 km/h	2.5 km/h	Mean planting depth
80 mm	0.79	0.89	1.11	0.93
120 mm	0.82	0.92	1.15	0.96
150 mm	0.85	0.98	1.22	1.02
Mean Forward Speed	0.82	0.93	1.16	
Factors	Forward speed	Planting depth	Forward speed × Planting depth	
Critical difference (5%)	0.021	0.027	NS	

different levels showed significant effect on the draft requirement (Figure 3A and Figure 3B, respectively). The interactions among operating parameters had non-significant effect on the draft requirement. The critical difference (CD) at the 5% level for draft requirement was 0.021 kN for forward speed and 0.027 kN for planting depth, while their interaction effect was non-significant, indicating that differences exceeding these values were statistically significant (Table 3). Similar trends have been reported by Okoko and Ajav (2020). The study emphasizes that through selecting suitable speeds and planting depths, draft requirements can be reduced, resulting in decreased fuel consumption and less wear on the planter.

3.2. Effect of Forward Speed and Planting Depth on Missing Index

The uniformity of tuber planting was affected by the missing index, which was observed during the performance evaluation of developed potato planter. The maximum value of missing index (12.66%) was observed at a forward speed of 2.5 km/h and planting depth of 80 mm, and the minimum missing (5.33%) was observed at a forward speed 1.5 km/h and planting depth of 150 mm (Table 4). The value of missing index increased with increase in forward speed; however, decreased as planting depth increased from 80 mm to 150 mm. No significant difference observed when

forward speed increased from 1.5 km/h to 2.0 km/h; however, a significant effect on the missing index was observed when forward speed increased 2.0 km/h to 2.5 km/h. At higher forward speeds, the increased rotational speed of the metering plate reduces the time available to place tubers into

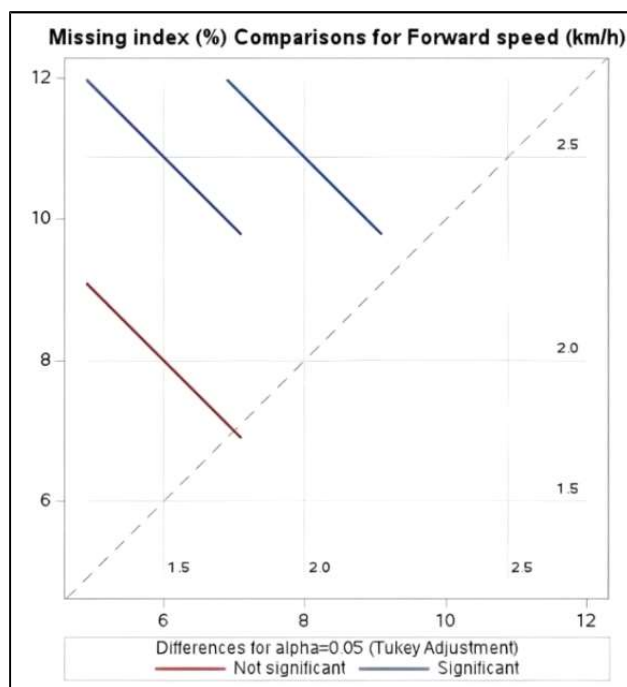


Figure 4. Effect of forward speed on missing index

Table 4. Effect of forward speed and planting depth on missing index

Forward speed, km/h→ Planting depth, (mm)↓	1.5 km/h	2.0 km/h	2.5 km/h	Mean planting depth
80 mm	6.66	8.66	12.66	9.33
120 mm	6	8	10.66	8.22
150 mm	5.33	7.33	9.33	7.33
Mean Forward Speed	6.00	8.00	10.88	
Factors	Forward speed	Planting depth	Forward speed × Planting depth	
Critical difference (5%)	1.876	NS	NS	

Table 5. Effect of forward speed and planting depth on multiple index

Forward speed, km/h→ Planting depth, (mm)↓	1.5 km/h	2.0 km/h	2.5 km/h	Mean planting depth
80 mm	7.33	8.66	9.33	8.44
120 mm	5.33	6.66	7.33	6.44
150 mm	4	5.33	8	5.78
Mean forward speed	5.55	6.88	8.22	
Factors	Forward speed	Planting depth	Forward speed × Planting depth	
Critical difference (5%)	NS	NS	NS	

the cups, leaving some cups unfilled and increasing the missing index. Conversely, greater planting depths increase soil resistance and reduce the planter's effective operating speed, allowing more accurate tuber placement, improved tuber retention, and better placement stability within the furrow. Similar trends have been reported by Hardik (2014) and Khan et al. (2015).

The critical difference (5%) for missing index was 1.876% for forward speed, while planting depth and their

interaction were non-significant (Table 4). The LS-means of forward speed had a significant effect on the missing index (Figure 4). However, neither planting depth nor interactions among operating parameters had a significant effect on the missing index, consistent with the result of Pandey and Sawant (2023). Additionally, the results show that optimizing planting depth and speed not only improves planting uniformity but also enhances overall crop yield.

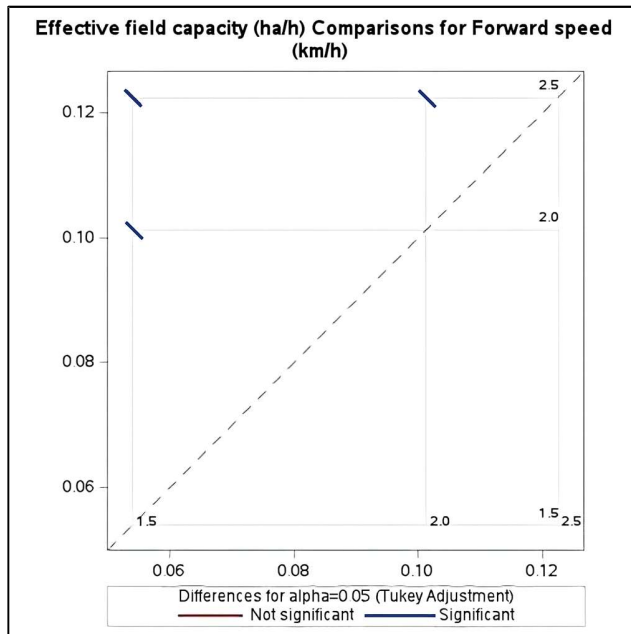


Figure 5. Effect of forward speed on effective field capacity

3.3. Effect of Forward Speed and Planting Depth on Multiple Index

The maximum multiple index (9.33 %) was observed at a forward speed of 2.5 km/h, and planting depth of 80 mm, and minimum multiple index value (4%) was observed at forward speed of 1.5 km/h and planting depth of 150 mm (Table 5). The multiple index increased with forward speed; however, it decreased with increasing planting depth. This was because, as operational speed increased, labourers had less time to fill each cell accurately. This time constraint can lead to more frequent occurrences of multiple tubers or seeds being placed in the same cell, hence the slight increase in the multiple index. A similar result was reported by Hardik (2014) and Kosariya et al. (2023). This finding, highlights the importance of balancing speed and depth in mechanical planting operations to ensure precision and optimize crop yield.

The LS-means for forward speed and planting were non-significant on the multiple index. Furthermore, their

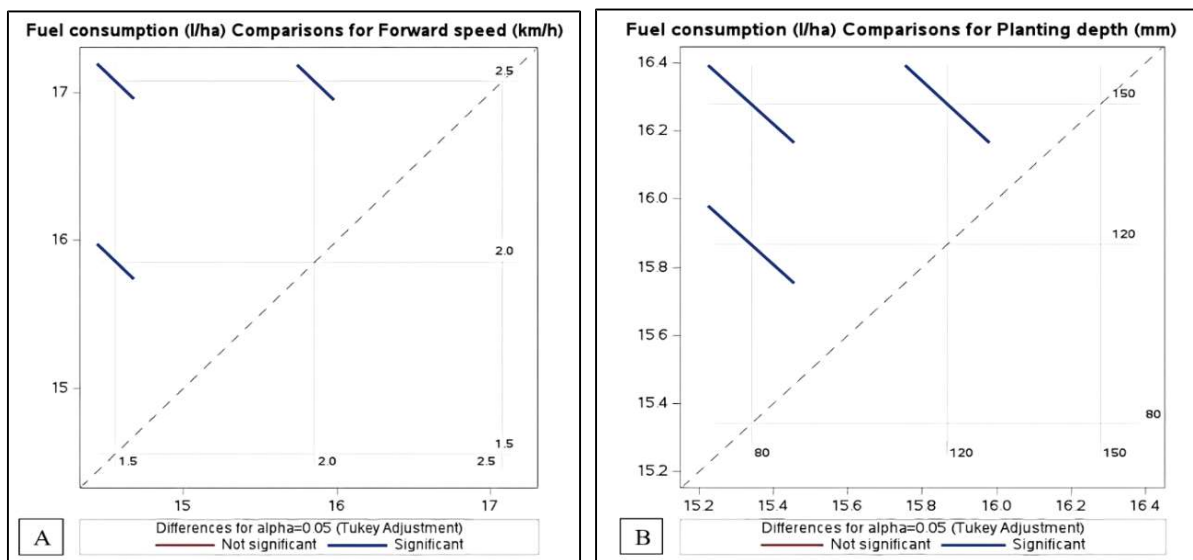


Figure 6. Effect of forward speed and planting depth on fuel consumption

Table 6. Effect of forward speed and planting depth on effective field capacity

Forward speed, km/h→ Planting depth, (mm)↓	1.5 km/h	2.0 km/h	2.5 km/h	Mean planting depth
80 mm	0.055	0.102	0.124	0.094
120 mm	0.054	0.101	0.122	0.092
150 mm	0.054	0.1	0.121	0.092
Mean forward speed	0.054	0.101	0.122	
Factors	Forward speed	Planting depth	Forward speed × Planting depth	
Critical difference (5%)	0.002	NS	NS	

Table 7. Effect of forward speed and planting depth on fuel consumption

Forward speed, km/h→ Planting depth, (mm)↓	1.5 km/h	2.0 km/h	2.5 km/h	Mean planting depth
80 mm	13.96	15.22	16.82	15.33
120 mm	14.76	15.79	17.06	15.87
150 mm	14.96	16.54	17.33	16.28
Mean Forward Speed	14.56	15.85	17.07	
Factors	Forward speed	Planting depth	Forward speed × Planting depth	
Critical difference (5%)	0.192	0.213	0.333	

interactions also had non-significant effect on the multiple index at 5% level of significance. As the effects were statistically non-significant, the observed differences among treatment means were within the critical difference (CD) at the 5% level (Table 5).

3.4. Effect of Forward Speed and Planting Depth on Effective Field Capacity

The minimum effective field capacity (0.054 ha/h) was observed at a forward speed of 1.5 km/h and planting depth of 120 and 150 mm; the maximum (0.124 ha/h) was observed at a forward speed of 2.5 km/h and planting depth of 80 mm (Table 6). The effective field capacity increased with forward speed, but decreased with planting depth. This might be due to as speed increased, the planter covered more area per unit time. The effective field capacity (EFC) was higher in larger fields than in smaller fields and in those with irregular shapes. Moreover, it can be enhanced through intensive operator training in machinery management, which improves operational skills. This finding aligns with the results reported by Kumar et al. (2017). The results accentuate that increasing forward speed can enhance the effective field capacity. However, it is crucial to ensure that the increased speed does not negatively affect the planter's performance.

The LS-means of forward speed had a significant effect on effective field capacity (Figure 5). The critical difference (CD) at the 5% level for forward speed was 0.002 (Table 6), indicating that differences among mean values exceeding this limit were statistically significant. Furthermore, planting depth did not significantly affect the effective field capacity. Furthermore, their interactions also did not significantly affect the effective field capacity.

3.5. Effect of Forward Speed and Planting Depth on Fuel Consumption

The increase in fuel consumption was observed during the field evaluation of developed planter as forward speed and planting depth increased. The maximum fuel consumption (17.33 l/ha) was observed at a forward speed of 2.5 km/h and planting depth of 150 mm. However, the minimum fuel consumption (13.96 l/ha) was observed at a forward speed 1.5 km/h and planting depth 80 mm (Table 7). Fuel consumption increased with increasing speed and planting depth. This is because at higher speeds and depths, it results an increased wheel slippage and a greater draft requirement, which requires more torque; i.e., the engine must exert more effort to sustain the required performance levels, thereby consuming more fuel, further contributing to elevated fuel usage. This result is consistent with the findings of Singh et al. (2024).

Moreover, the results show that maintaining planting depth and forward speed during planting can significantly improve the planter's productivity and operational efficiency.

The LS-means of forward speed and planting depth for three different levels had significant effect on fuel consumption (Figure 6A and 6B, respectively). The interactions also had significant effect on fuel consumption. The critical difference (5%) for fuel consumption was 0.192 l ha⁻¹ for forward speed, 0.213 l ha⁻¹ for planting depth, and 0.333 l ha⁻¹ for their interaction (Table 7). Similar findings have been reported in earlier studies indicate that increases in forward speed and operating depth lead to higher draft requirements and fuel consumption due to increased soil resistance and traction load (Nkakini et al., 2020; Pandey and Sawant, 2023).

4. CONCLUSION

Potato cultivation in Chhattisgarh is largely practiced by small and marginal farmers using traditional, labour-intensive methods. To address these constraints, a single-row power tiller-operated potato planter was developed and evaluated. The planter operated effectively with a 9 hp power tiller, maintaining recommended spacing and planting depth across tuber sizes. Performance evaluation showed that forward speed and planting depth significantly influenced planting quality, draft, and fuel consumption. Optimal performance was achieved at lower forward speed with moderate planting depth, ensuring improved planting uniformity, reduced draft and fuel consumption, and acceptable field capacity. Higher speeds increased field capacity but adversely affected planting precision and energy efficiency. Overall, the developed planter was suitable for small and marginal farmers, offering a balanced compromise between operational efficiency and planting accuracy.

CRedit authorship contribution statement

Conceptualization: S. Jogdand, R. K. Naik, and Sajal Rahangdale. Data curation: S. Jogdand, R. K. Naik, and Sajal Rahangdale. Methodology: S. Jogdand, Gajendra Singh, Khilesh Dewangan, and Sajal Rahangdale. Project administration: S. Jogdand, R. K. Naik. Validation: Sajal Rahangdale, S. Jogdand, R. K. Naik and Gajendra Singh. Writing original draft: Sajal Rahangdale, Gajendra Singh, Khilesh Dewangan. Writing-review and editing: Sajal Rahangdale, Gajendra Singh, Khilesh Dewangan.

Conflicts of interest

The authors declare no conflicts of Interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the conception, design, data collection, analysis, interpretation, or writing of this manuscript.

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Establishment of Soil Quality Critical Limits and Yield Prediction of Two *Musa* Cultivars in *Bambusa tulda* Assisted Jhum and Fallow Systems of Northeastern India

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ABSTRACT: Shifting cultivation (Jhum) landscapes of Northeast India are increasingly affected by shortened fallow cycles, resulting in declining soil fertility and reduced crop productivity. Developing restoration strategies that improve soil quality while sustaining livelihoods is therefore critical for the long-term sustainability of upland agro-ecosystems in the region. The present work investigates the benefits of integrating *Bambusa tulda* and fallow duration in restoring soil productivity and improving banana performance in Jhum and fallow systems of Nagaland, Northeast India. Four land-use conditions—active Jhum land (JL), 3-year fallow (AJL3), bamboo-assisted fallow (AJLB), and 12-year fallow (AJL12)—were assessed using soil quality indicators, critical-limit analysis, and agronomic evaluation of two *Musa* cultivars (Atsu Mungo and Aot Mungo). The findings displayed higher soil organic carbon, nutrient availability, clay content, and moisture at AJLB and AJL12, which corresponded with superior vegetative growth, reduced crop cycle duration, and higher bunch weight and relative yield compared with JL and AJL3. Critical-limit categorization placed most soil indicators of AJLB and AJL12 within the adequate range, while JL and AJL3 remained largely moderate. Stepwise regression depicted that soil moisture and clay content were the strongest predictors of yield across cultivars. This study provides the first critical-limit framework linking soil quality indicators with banana productivity in bamboo-assisted Jhum systems of Northeast India, demonstrating that bamboo-based fallow management can accelerate soil recovery and enhance crop productivity in degraded shifting-cultivation landscapes.

Keywords: Critical limits, *Bambusa tulda*, Banana yield prediction, Jhum.

1. INTRODUCTION

In Northeast India, shifting cultivation (Jhum)-involving slashing, burning, cultivation and fallow-has historically sustained rural livelihoods (Tripathi et al., 2017). However, population pressure has shortened fallow cycles, driving unsustainable practices that alter soil structure, deplete

organic matter and suppress microbial activity, ultimately degrading soil quality (Mishra et al., 2021; Semy et al., 2022). In contrast, the introduction of bamboo (*Bambusa tulda* Roxb.) systems is noted for stabilising soils, curbing erosion and runoff, and enhancing nutrient status and overall soil health (Shanmughavel et al., 2000). Given India's extensive bamboo cover and rapid growth rates, bamboo

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offers a promising nature-based route for restoring degraded jhum lands while contributing to carbon sequestration (Kaushal et al., 2021). Demand for bamboo across dietary, nutraceutical, fuel and timber sectors further strengthens its socio-economic appeal in the Northeast (Basumatary et al., 2017).

Defining locally relevant soil indicators and their critical limits, i.e., concentrations associated with achieving ~80–90% of attainable yield, facilitates interpretation of soil function across land uses (Biswas et al., 2017). In Nagaland, banana is a key livelihood crop, yet hilly terrain, erosion risk and variable water supply under Jhum landscapes hinder

productivity (Nyamamba et al., 2020; Solo and Kikhi 2021). Evidence suggests that shortened fallows reduce productivity across shifting-cultivation systems (Rahman et al., 2014). Bamboo's restorative effects are documented, but potential to enhance agronomic performance on degraded Jhum lands—particularly for banana—remains underexplored in the region (Shilla and Mir 2017; Kaushal et al., 2021). Therefore, the present study was conducted to evaluate agronomic performance of selected *Musa* cultivars under experimental conditions and defined critical limits for key soil indicators to support monitoring and management in Nagaland, North-East India.

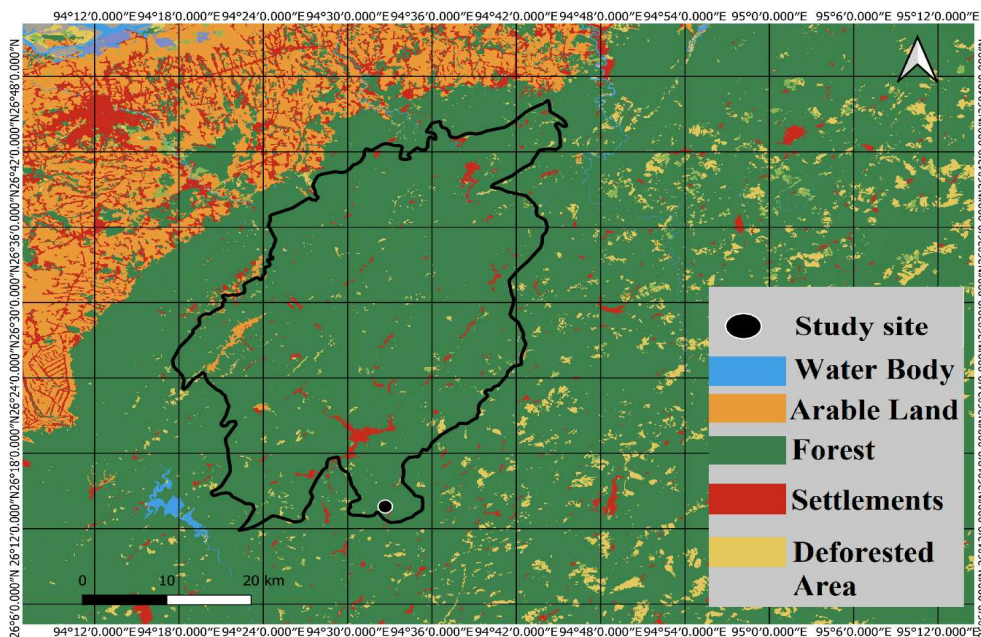


Figure 1. Land use map of study area under Mokokchung district, Nagaland, North-East India (QGIS 4.0)

Table 1. Description of the experimental sites

Site	Cultivation period	Fallow period	Vegetation
Jhum land (JL)	3 years	Not available	3 rd cycle of cassava monocropping
Abandoned Jhum land 3 (AJL3)	3 years	3 rd year of fallow	<i>Ageratum conyzoides</i> , <i>Eupatorium</i> sp., <i>Erigeron Canadensis</i> , <i>Erechtites</i> sp., <i>Ischaemum muticum</i> , <i>Galinsoga</i> sp., <i>Macaranga</i> sp., <i>Mucuna puriens</i> , <i>Mikiania scadens</i> , <i>Poa trivialis</i> , <i>Pteris vittata</i> , <i>Sonchus</i> sp., <i>Thysanolaena maxima</i> , and <i>Thysanolaena</i> sp.
Abandoned Jhum land bamboo (AJLB)	3 years	3 rd year of fallow	Mainly composed of <i>Bambusa tulda</i> with other associated species such as <i>Angiopteris evecta</i> , <i>Ageratum conyzoides</i> , <i>Artemisia vulagris</i> , <i>Eupatorium</i> sp., <i>Spatholobus</i> sp., <i>Thysanolaena maxima</i> , <i>Macaranga peltata</i> , <i>Persea fructifera</i>
Abandoned Jhum land 12 (AJL12)	3 years	12 th year of fallow	<i>Albizia chinensis</i> , <i>Angiopteris</i> sp., <i>Azadirachta indica</i> , <i>Macaranga peltata</i> , <i>Phyllanthus emblica</i> , <i>Polygonum molle</i> , <i>Persea fructifera</i> , <i>Sonchus</i> sp., <i>Schima wallichii</i> , <i>Thysanolaena maxima</i> and <i>Terminalia myriocarpa</i> .

2. MATERIALS AND METHODS

2.1. Description of the Study Site

The experimental sites (Figure 1) were selected under Mokokchung District, Nagaland, India (94°32'39"E and 26°13'44"N, 1093m amsl), which has a humid subtropical climate and ~2,500 mm annual rainfall (Longchar et al., 2023). Shifting cultivation is the main form of agriculture for the indigenous inhabitants of the region.

2.2. Experimental design and treatment

Four sites with contrasting fallow periods were selected to test the effect of fallow and *Bambusa tulda* Roxb. on productivity (Table 1). Two *Musa* cultivars common in the region—Aot Mungo (Figure 2) and Atsu Mungo (Figure 3)—were used, with Mokokchung being the state's second-largest banana producer (Murry and Das 2019). Mother plants were inspected for the presence of pests and diseases before sucker collection (Tumuhimbise and Talengera 2018). Uniform suckers from a single farm received hot-water treatment to reduce weevil and nematode infestation (Uwimana et al., 2020). At each site, eight suckers per cultivar (16 per site; 64 total) were planted at 5 × 5 m spacing. No fertilizers or chemicals were applied post-planting, and all sites were monitored weekly through the

first crop cycle (planting to harvest).

2.3. Soil Analysis

Soil samples (0-30 cm) were collected seasonally from the study sites from March (2020) till the completion of the first plant cycle (i.e., planting to harvest). Bulk density and soil moisture were determined by utilizing fresh soil. All other soil tests were performed by utilizing air-dried samples

Table 2. Soil analysis protocol

Soil parameters	Methods
pH	Digital pH meter
Electrical conductivity (EC)	EC meter
Soil moisture	Gravimetric method (Misra, 1968)
Clay content	Pipette method (Piper, 1942)
Bulk density (BD)	Core sampler method (Allen, 1989)
Soil organic carbon (SOC)	Walkley and Black method (1934)
Available nitrogen (N)	Kjeldahl method (1883)
Available phosphorus (P)	Bray's no. 1 extract method (Bray and Kurtz 1945)
Available potassium (K)	Photometric method (Trivedy and Goel 1986)
Cation exchange capacity (CEC)	Bower et al. (1952)
Total Nitrogen (TN)	Kjeldahl (1883)

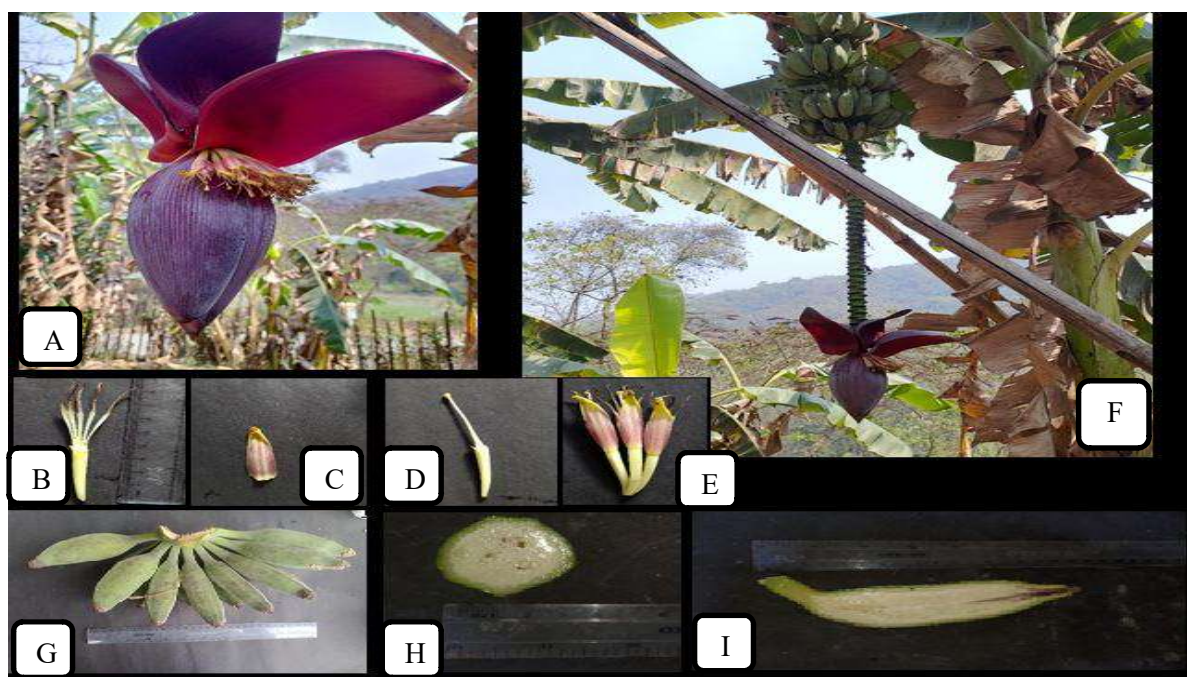


Figure 2. *Musa* sp 1: Aot Mungo (Ao Naga) with various floral parts in picture. A: Banana inflorescence. B: Stamens. C: Tepal. D: Style and stigma. E: Male Flowers. F: Banana flowering. G: Banana bunch. H: Transverse section of fruit. I: Longitudinal section of fruit

sieved through a 2-mm nylon sieve (Table 2). All tests were performed in triplicates and expressed as mean ± standard deviation.

2.4. Agronomic Performance of the *Musa* Cultivars

Suitable agronomic traits were selected from the method proposed by Uwimana et al. (2020), consisting of three broad categories, namely: Vegetative, maturity, and fruit yield (Table 3)

The expected fruit yield (Eq. 1) was reported as per Hauser and Van Asten (2010), and the Relative yield (Eq. 2) was estimated as per Biswas et al. (2017):

$$\text{Expected fruit yield} = \frac{\text{BW} \times 10,000 \times 365}{\text{Spacing} \times \text{PC} \times 1000} \dots (1)$$

$$\text{Relative yield (\%)} = \frac{\text{Yield of a sampling plot}}{\text{Maximum yield of a sampling plot}} \times 100$$

2.5. Critical Limits of Soil Quality Indicators of the Selected *Musa* Cultivars

It is defined as the optimum values required for the normal functioning of soil and its health for sustainable crop production (Biswas et al., 2017). This is obtained by running a linear regression between soil parameters and relative yield for obtaining the 40% and 80% of the maximum possible yield. For this equation, the relative yield (X) = 40 and 80 are recorded and the corresponding value of soil indicators (Y) represents the upper and lower critical values, respectively. Soil values higher than the relative yield of

Table 3. Agronomic traits

Agronomic trait	Parameters	Details
Vegetative	Plant girth (cm)	Measured at 1m, above ground during flowering
	Plant height (cm)	The distance from the ground to the angle made between the bunch stalk and bunch cover leaf
	Number of suckers	Number of suckers produced during flowering
	Number of functional leaves	Counted at flowering, leaves with at least 50% of the green area
Maturity	Plant cycle (days)	Number of days between harvest date and planting date
	Days to flowering	Number of days from planting to flowering
	Flowering to harvest (days)	Number of days from flowering to harvesting
Fruit yield	Bunch weight (kg)	Fruit weight at harvest
	Number of fruits	Number of fruits at harvest
	Number of hands	Number of hands during harvest
	Fruit filling index	Sum of bunch weight divided by the number of days from flowering to harvest

Table 4. Agronomic performance of *Musa* cultivar Atsu Mungo

Agronomic trait	JL	AJL3	AJLB	AJL12	
Vegetative	Plant girth (cm)	42.66 ^a	41.33 ^b	47.3 ^{ab}	52 ^b
	Plant height (cm)	290.3 ^a	345 ^c	316 ^b	329.66 ^{bc}
	Number of suckers	2 ^a	2.33 ^a	3.66 ^{ab}	4.6 ^b
	Number of functional leaves	7.6 ^a	11.66 ^b	10.66 ^b	13.33 ^b
Maturity	Plant cycle (days)	672 ^c	596 ^b	461 ^a	435 ^a
	Days to flowering	520 ^b	503.66 ^b	358.66 ^a	337.66 ^a
	Flowering to harvest (days)	137 ^b	92.33 ^a	102.33 ^a	97.33 ^a
Fruit yield	Bunch weight (kg)	9.68 ^a	11.73 ^b	14.67 ^c	17.33 ^d
	Number of fruits	52.33 ^a	54.00 ^a	67.00 ^a	55.67 ^a
	Number of hands	5.33 ^a	5.33 ^a	5.33 ^a	6.00 ^a
	Fruit filling index	0.0622 ^a	0.1285 ^b	0.1447 ^b	0.178 ^c
	Expected fruit yield (t ha ⁻¹ year ⁻¹)	0.0057 ^a	0.0078 ^b	0.0127 ^c	0.0160 ^d
	Relative yield	55.87 ^a	67.705 ^b	84.63 ^c	100 ^d

Values in the same row with different superscripts are significantly by Duncan’s multiple range test (p<0.05). JL: Jhum land; AJL3: Abandoned Jhum land 3; AJLB: Abandoned Jhum land with bamboo; AJL12: Abandoned Jhum land 12.

80% are considered adequate, 41%-80% moderate, and <40% considered low (Lopes et al., 2013).

2.6. Statistical Analysis and Map Generation

2.6.1. Stepwise regression model

To retain soil variables that contribute to yield, a fitted regression models were constructed to study the effect of the various soil properties on the relative yield of the two selected *Musa* cultivars (Ghosh and Devi, 2019). The general linear regression model used in the analysis can be expressed as (Eq.3):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_n X_n + \epsilon \dots (3)$$

where Y represents the relative yield (%), β_0 is the intercept, β_1 – β_n are regression coefficients, X_1 – X_n represent the independent soil variables (pH, EC, SOC, N, P, K, soil moisture, BD, clay content, CEC, TN), and ϵ is the error term. A stepwise variable selection procedure was applied to determine the most significant predictors influencing yield, where variables were sequentially entered into or removed from the model based on their statistical significance ($p < 0.05$). Statistical analyses were implemented using SPSS version 26.0. Map generated using QGIS (4.0)

3. RESULTS AND DISCUSSION

3.1. Agronomic Performance of *Musa* Cultivars

3.1.1. Atsu mungo

Vegetative and yield traits varied by site (Table 4). Plant height and sucker number were greater under longer

fallow/bamboo sites, while JL consistently showed inferior values. Maturity traits (cycle length, days to flowering) were shortest at AJL12 and longest at JL ($p \leq 0.05$), indicating faster phenology with better soil conditions. Bunch weight and expected yield were highest at AJL12 and lowest at JL. Fruit number and hands were similar, highlighting that source–sink strength (fruit filling index) and biomass allocation, not merely fruit counts, drove yield differences. Relative yield followed the same gradient (AJL12 > AJLB > AJL3 > JL).

3.1.2. Aot Mungo: Most agronomic patterns paralleled Atsu Mungo (Table 5). Height peaked at AJL3, but overall yield attributes (bunch weight, fruit filling index, expected yield, relative yield) were highest at AJL12/AJLB and lowest at JL. Cycle length and days to flowering shortened with improved soils, again indicating that edaphic recovery accelerates phenology and yield formation. Across both cultivars, sites with greater clay and moisture i.e., AJLB and AJL12 (Tables 7 and 8) produced taller plants, more functional leaves and higher bunch weight, aligning with reports that texture-mediated water and nutrient retention supports root development, photosynthetic capacity and yield (Alhaj et al., 2019; Cardone et al., 2020). Conversely, the high BD/low SOC state at JL constrained growth—typical of rapidly growing herbaceous crops under suboptimal environments (Grime 2006).

3.2. Critical Limits and Site Categorization

Critical-limit analysis (Table 6) placed AJLB and AJL12

Table 5. Agronomic performance of *Musa* cultivar Aot Mungo

Agronomic trait		JL	AJL3	AJLB	AJL12
Vegetative	Plant girth (cm)	61.33 ^a	65.00 ^{ab}	66.00 ^b	67.00 ^b
	Plant height (cm)	404.00 ^b	427.00 ^b	320.33 ^a	311.33 ^a
	Number of suckers	2.67 ^a	2.33 ^a	3.67 ^{ab}	4.33 ^b
	Number of functional leaves	9.67 ^a	13.33 ^b	12.67 ^b	14.33 ^b
Maturity	Plant cycle (days)	639.67 ^b	612.67 ^b	491.33 ^a	440.67 ^a
	Planting to flowering	491.00 ^d	482.00 ^{bc}	395.67 ^{ab}	357.00 ^a
	Flowering to harvest(days)	159.75 ^a	130.67 ^a	95.67 ^a	83.67 ^a
Fruit yield	Bunch weight (kg)	14.50 ^a	16.00 ^b	20.37 ^c	22.57 ^d
	Number of fruits	93.33 ^a	151.00 ^a	103.33 ^a	221.33 ^b
	Number of hands	9.33 ^b	10.67 ^{bc}	8.67 ^a	12.33 ^c
	Fruit filling index	0.090 ^a	0.126 ^a	0.243 ^b	0.235 ^b
	Expected fruit yield (t ha ⁻¹ year ⁻¹)	0.0090 ^a	0.01045 ^a	0.0185 ^b	0.0185 ^b
	Relative yield	64.25 ^a	70.90 ^b	90.25 ^c	99.99 ^d

Values in the same row with different superscripts are significantly by Duncan’s multiple range test ($p < 0.05$). JL: Jhum land; AJL3: Abandoned Jhum land 3; AJLB: Abandoned Jhum land with bamboo; AJL12: Abandoned Jhum land 12.

Table 6. Critical limits of soil quality indicators

Soil properties	Relative yield (%)	Musa cultivars			
		Aot Mungo		Atsu Mungo	
pH	40	5.90	r ² = 0.860	5.78	r ² = 0.928
	80	5.26		5.20	
EC (dS m ⁻¹)	40	0.17	r ² = 0.895	0.05	r ² = 0.940
	80	0.42		0.48	
SOC (%)	40	0.42	r ² = 0.919	0.80	r ² = 0.976
	80	2.43		2.62	
N _{av} (Kg ha ⁻¹)	40	137.39	r ² = 0.946	196.09	r ² = 0.900
	80	385.36		407.11	
K _{av} (Kg ha ⁻¹)	40	34.84	r ² = 0.987	62.43	r ² = 0.939
	80	151.43		161.65	
P _{av} (Kg ha ⁻¹)	40	11.15	r ² = 0.945	14.62	r ² = 0.951
	80	27.35		28.80	
Moisture (%)	40	16.80	r ² = 0.986	21.99	r ² = 0.995
	80	41.13		43.31	
BD (g cm ⁻³)	40	2.54	r ² = 0.884	2.31	r ² = 0.890
	80	1.47		1.37	
Clay (%)	40	14.76	r ² = 0.933	17.46	r ² = 0.972
	80	28.22		29.44	
CEC (meq100g ⁻¹)	40	3.77	r ² = 0.956	8.18	r ² = 0.925
	80	22.97		24.66	
TN (%)	40	0.42	r ² = 0.956	0.62	r ² = 0.946
	80	1.32		1.40	
Sand (%)	40	61.00	r ² = 0.987	57.01	r ² = 0.949
	80	43.86		42.35	
Silt (%)	40	19.40	r ² = 0.912	20.95	r ² = 0.927
	80	26.77		27.43	

Table 7. Classification of soil based on the critical limits of soil quality indicators for Aot Mungo

Site	pH	EC (dS m ⁻¹)	SOC (%)	N (Kg ha ⁻¹)	K (Kg ha ⁻¹)	P (Kg ha ⁻¹)	Moisture (%)	BD (g cm ⁻³)	Clay (%)	CEC (meq100g ⁻¹)	TN (%)	Sand (%)	Silt (%)
JL	*5.63 ^c	**0.11 ^a	*1.40 ^a	*274.29 ^a	*106.01 ^a	*19.11 ^a	*31.15 ^a	*2.07 ^c	*21.20 ^a	*14.06 ^a	*0.88 ^a	*50.95 ^d	*22.79 ^a
AJL3	5.12 ^b	*0.39 ^b	*2.25 ^b	*326.50 ^a	*118.34 ^{ab}	*25.11 ^{ab}	*35.86 ^a	*1.58 ^b	*26.90 ^b	*18.96 ^b	*1.16 ^b	*48.25 ^c	*26.00 ^b
AJLB	5.13 ^b	0.57 ^c	2.76 ^c	480.59 ^b	188.00 ^{bc}	32.30 ^{bc}	45.63 ^b	1.08 ^a	31.28 ^c	29.82 ^c	1.61 ^c	38.44 ^b	29.11 ^b
AJL12	4.93 ^a	0.70 ^d	3.51 ^d	484.88 ^b	205.0 ^c	34.49 ^d	54.30 ^c	1.04 ^a	34.84 ^d	30.95 ^c	1.71 ^c	36.09 ^a	29.91 ^c

**=Low, *=Moderate, Na= Adequate

Table 8. Classification of soil based on the critical limits of soil quality indicators for Atsu Mungo

Site	pH	EC (dS m ⁻¹)	SOC (%)	N (Kg ha ⁻¹)	K (Kg ha ⁻¹)	P (Kg ha ⁻¹)	Moisture (%)	BD (g cm ⁻³)	Clay (%)	CEC (meq100g ⁻¹)	TN (%)	Sand (%)	Silt (%)
JL	*5.6 ^c	*0.11 ^a	*1.40 ^a	*274.29 ^a	*106.01 ^a	*19.11 ^a	*31.15 ^a	*2.07 ^c	*21.20 ^a	*14.06 ^a	*0.885 ^a	*50.95 ^d	*22.79 ^a
AJL3	5.12 ^b	*0.39 ^b	*2.25 ^b	*326.50 ^a	*118.34 ^{ab}	*25.11 ^{ab}	*35.86 ^a	*1.58 ^b	26.90 ^b	*18.96 ^b	*1.166 ^b	*48.25 ^c	*26.00 ^b
AJLB	5.13 ^b	0.57 ^c	2.76 ^c	480.59 ^b	188.00 ^{bc}	32.30 ^{bc}	45.63 ^b	1.08 ^a	31.28 ^c	29.82 ^c	1.616 ^c	38.44 ^b	29.11 ^b
AJL12	4.93 ^a	0.70 ^d	3.51 ^d	484.88 ^b	205.0 ^c	34.49 ^d	54.30 ^c	1.04 ^a	34.84 ^d	30.95 ^c	1.710 ^c	36.09 ^a	29.91 ^c

**=Low, *=Moderate, Na= Adequate

in the adequate category for most parameters in both cultivars, consistent with their superior performance. JL frequently fell in moderate (and low EC for Aot), while AJL3 remained mostly moderate except pH (Adequate). These categories mirror the yield gradient and reflect the role of litter inputs, mineralization and SOC build-up under longer fallow and bamboo. Short fallows (<5 years) depress soil nutrients and output–input energy ratios relative to older fallows (Datta and Singh 2012). The bamboo effect is also evident in the present study: AJLB > AJL3 for SOC, N, P and moisture with lower BD matching observations of bamboo plantations enhancing microbial biomass and SOC turnover (Arunachalam and Arunachalam 2000; Mishra et al., 2014;

Shilla and Mir 2017 Shia et al., 2017).

3.3. Soil–yield Relationships (Stepwise regression)

Stepwise regression analysis was implemented to evaluate the impact of soil properties on the relative yield of the Aot Mungo cultivar (Table 11). The fitted regression model was:

$$\text{Yield}_{\text{Aot}} = 7.845 + 1.553 (\text{CLAY}) + 0.687 (\text{MOISTURE})$$

The model was statistically significant ($R^2 = 0.930$, $p = 0.017$). Clay ($\beta = 1.553$, $p = 0.005$) and soil moisture ($\beta = 0.687$, $p = 0.017$) showed positive relationships with relative yield.

For Atsu Mungo, the fitted regression model was:

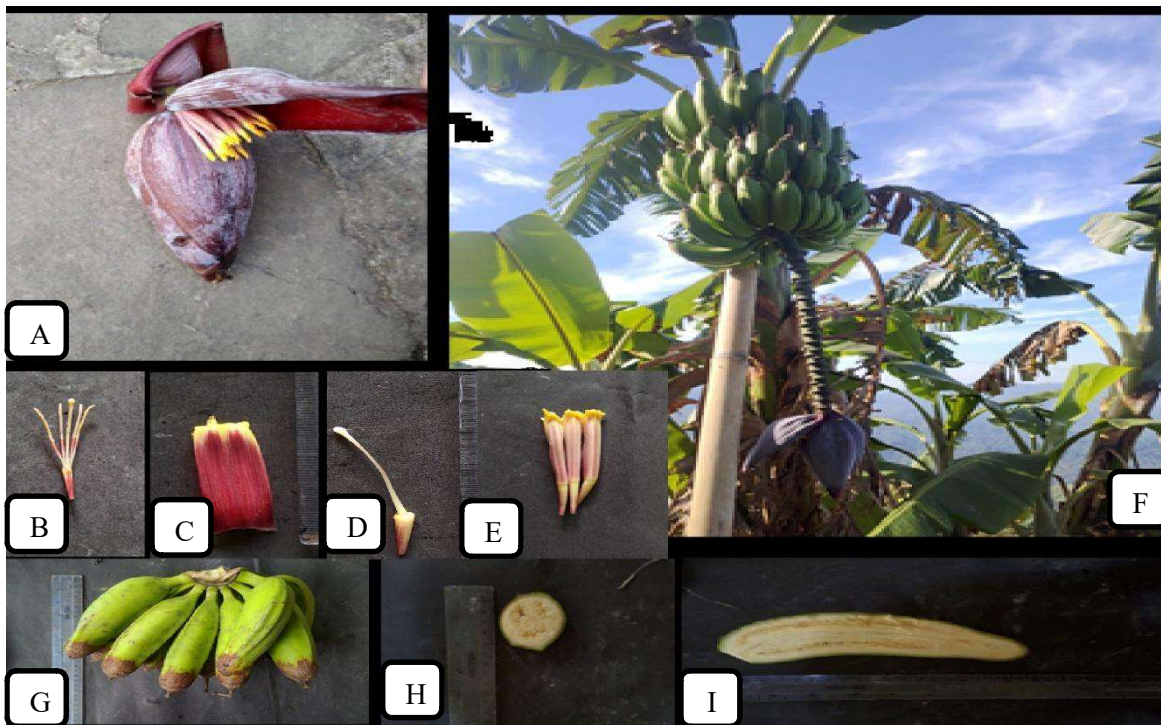


Figure 3. *Musa* cultivar: Atsu Mungo (Ao Naga) with various floral parts in picture. A: Banana inflorescence. B: Stamens. C: Tepal. D: Style and stigma. E: Male Flowers. F: Banana flowering. G: Banana bunch. H: Transverse section of fruit. I: Longitudinal section of fruit

Table 9. Model summary of stepwise regression of soil properties and relative yield

		R	R ²	Adjusted R ²	Std. Error	R ² Change	F Change	p value
Aot Mungo	Model 1: Clay	.943 ^a	.890	.882	5.13175	.890	112.882	<.001
	Model 2: Clay, Moisture	.964 ^b	.930	.919	4.24333	.040	7.476	.017
Atsu Mungo	Model 1: Clay	.959 ^a	.919	.913	5.13222	.919	158.435	<.001
	Model 2: Clay, Moisture	.975 ^b	.951	.944	4.13532	.032	8.564	.012
	Model 3: Clay, Moisture, pH	.983 ^c	.966	.958	3.58596	.015	5.288	.040

Dependent variable: Relative yield; predictors retained at $p < 0.05$

Yield Atsu=58.793+1.254 (clay) + 0.853 (moisture) - 10.137(pH)

The model was statistically significant ($R^2 = 0.966$, $p < 0.001$). Clay ($\beta = 1.254$, $p = 0.025$) and soil moisture ($\beta = 0.853$, $p = 0.004$) positively influenced relative yield, whereas pH showed a negative association ($\beta = -10.137$, $p = 0.040$). All predictors retained in the final model were statistically significant ($p < 0.05$) (Table 9).

Moisture governs canopy development and leaf area, so deficits suppress productivity (Carr 2009; Stevens et al., 2020). This in response may led to increased disease risks and reduce yield (Olivares et al., 2021). Clay content in soil supports structural stability, nutrient retention and buffering (Newman 1984). Hence, these soil factors are crucial for adequate banana growth. In contrast, elevated pH under recent burning can impair nutrient availability (Shahid et al., 2013), explaining the negative pH coefficient. Overall, regression results reinforce the bamboo- and fallow-mediated improvements in clay-related structure and moisture storage that underpin higher yields at AJLB and AJL12.

4. CONCLUSION

This study addresses the impact of fallow practices on the agronomic performance of *Musa* cultivars within the context of land degradation, mitigation, and rehabilitation in terrestrial environments. Establishing soil quality indicators is vital for achieving efficient productivity and sustainable management goals. All soil parameters for both cultivars fell within the "Adequate category" at sites AJLB and AJL12. However, there were variations at other sites, with some parameters falling into the moderate" category. These variations indicate decreased plant performance at certain sites, which can be attributed to reduced soil nutrient levels associated with land degradation. The findings suggest that while a long fallow period (<12 years) facilitates maximum soil regeneration, incorporating *B. tulda* into degraded Jhum soil leads to significant improvements in soil organic carbon, macronutrient levels, clay content, and moisture. This highlights the potential of *B. tulda* plantation to expedite soil recovery in degraded Jhum lands, promoting sustainable land management practices.

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Wati Temjen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing, original draft Writing, review & editing. **Maibam Romeo Singh:** Investigation, Methodology, Supervision, Writing, review & editing. **Tali Ajungla:** Conceptualization, Supervision, Writing, review & editing. **Merenlemla Jamir:** Resources, Software, Validation, Writing, review & editing. **Watitoshi Ao:** Methodology, Software, Validation, Writing, review & editing. **Nuksungmenla Jamir:** Formal analysis, Investigation, Resources, Validation, Writing, review & editing.

Conflict of interest

All Authors declare no conflict of interest.

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Available on request from the corresponding author.

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Biofortification Approaches for Improving Nutritional Quality of Feed and Fodder

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ABSTRACT: Fodder crops are an important component of livestock nutrition, serving as the primary source of food for dairy and meat-producing animals. However, the nutritional quality of fodder crops is lower, leading to suboptimal animal health and productivity. Long-term mineral shortages in animal diets cause nutritional disorders. Micronutrient deficiencies are more difficult to detect because they cause minor functional and structural changes in the animal body. Micronutrient requirements are essential, especially during the three to four weeks before and after parturition, as they affect the health of milch animals, breastfeeding, milk supply, and reproductive performance. Dietary supplementation of micronutrients helps to reduce the risk of infertility, foetal development, metabolic illnesses caused by negative energy balance, lowering somatic cell counts in milk, and reducing the risk of bacterial infections associated with mastitis. Micronutrients like iron, copper, manganese, zinc, selenium, cobalt and iodine are beneficial to high-yielding dairy cows, buffalo, goats, and sheep. Biofortification approaches address nutritional problems in animals worldwide by regularly introducing nutrient-enriched feed and fodder crops. Biofortification of fodder crops holds great promise as a sustainable solution to enhance the overall efficiency and productivity of livestock. Bolstering the nutritional content of fodder crops can contribute to the well-being of both animals directly and humans indirectly, fostering healthier diets, reducing malnutrition in livestock, and supporting the livelihoods of farmers worldwide.

Keywords: Biofortification, Deficiencies, Fodder crops, Micronutrients, Nutritional security.

1. INTRODUCTION

Indian agriculture relies heavily on livestock and agriculture. India has the largest livestock population in the world (Livestock Census, 2019). Micronutrients play a vital role in animal's body, but deficiencies lead to major problems, such as increased risk of disease, infections, and mortality. In dairy cows, micronutrients play a crucial role in regulating the reproductive tract, immune function, oxidative stress, and energy metabolism. Micronutrients act as antioxidants in the immune system, protecting cells from free radicals and maintaining the membrane integrity of cells

involved in milk production (Lean et al., 2013). Zn, Fe, Cu, Co, I, Mn, and Se are essential elements for cattle and human health, and they play a significant role in internal and external development. Micronutrients are involved in several metalloenzymes, which include glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, Cr, and Mn). These enzymes protect the internal cellular structure from oxidative damage (Keshri *et al.*, 2021). The micronutrients contribute as an important part of the immune system's response to numerous illnesses (Dimkpa & Bindraban, 2016). In the absence of micronutrients, many proteins, enzymes, and other bioactive

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substances that regulate internal biochemical processes in livestock and humans are rendered ineffective (Grusak & Cakmak, 2009). Micronutrient deficiencies affect reproduction by depressing the activity of the rumen microflora, decreasing the activity of enzymes involved in energy and protein metabolism and hormone synthesis of hormones, and impairing the integrity of rapidly dividing cells. Similarly, mineral requirements for optimal growth and development in cattle are easily met by nutrient-rich green and dry fodder or concentrated feed. (Kubkomawa, 2019). The availability of high-quality feed and fodder for dairy and meat-producing animals, as well as poultry, increases yield and productivity. The mineral content, total calories, and, more importantly, nutrient bioavailability are essential aspects of the nutritional quality of feed and fodder. These factors highlighted the need of cultivate highly nutritious crops to achieve nutritional security (Dhaliwal *et al.*, 2020). There are several options for providing nutrients through food fortification, dietary diversity, and medicinal supplements. In fact, biofortification is a promising method of reducing malnutrition easily. It enables the addition of certain nutrients in a crop's edible portion, so that crops consumed by both humans and animals (Sheoran *et al.*, 2022; Rangasami *et al.*, 2024). Breeding, agronomic, transgenic methods, and advanced genome-editing tools are the best approaches of biofortification (Whitem & Broadley, 2009). Agronomic biofortification is the process of employing micronutrients in fodder crops with different management practices to increase concentration in various

plant sections, and it is one of the best choices for producing high-quality fodder (Alloway, 2008). Biofortified forage crops fed to animals will improve animal health and production (Capstaff & Miller, 2018). The supplementation of micronutrients in crops increased the concentration of micronutrients in fodder crops. Biofortified feed and fodder crops enriched with nutrients help overcome malnutrition-related health issues in animals. Biofortification of fodder crops improves the concentration of micronutrients such as Fe, Zn, Mn, Se, B, carotenoids, folates, protein, and vitamins. As a result, biofortification with micronutrients has been increasingly popular in recent years (Sheoran *et al.*, 2022). The current state of biofortification in food and fodder crops remains limited, so further improvement is needed to enhance the nutritional security of animals. This review scrutinises the efficacy of multiple and diverse biofortification techniques in fodder crops.

2. Essential Macro and Micronutrient Requirements for an Animal's Growth and Developments

Essential elements are required for the healthy growth of a plant, while the absence of any of these nutrients prevents plants from completing their life cycle. The seventeen major micro-elements were found to be essential for plant growth. C, H, O, N, P, K, Ca, Mg, and S are examples of basic, macronutrients, and Fe, Zn, Mn, Cu, Cl, Mo, B, and Ni are examples of micronutrients (Bhatla & Kathpalia, 2023). The absence of any single element or their excess availability can

Table 1. Functions and the deficiency symptoms of micronutrients in animals

Micro mineral	Function	Deficiency symptom
Cobalt (Co)	Cobalt and rumen microbes are involved in the synthesis of vitamin B12	Poor appetite, anaemia, rough hair coat
Copper (Cu)	Enzyme activation, blood synthesis, nervous system	Change in colour of hair (grey or reddish), diarrhoea, rough hair coat, mastitis (grey or reddish), and an affected immune system
Iodine (I)	Synthesis of thyroxine (hormone)	Big neck in calves, reduced metabolic rate, poor reproductive performance, goiter
Iron (Fe)	Part of the blood haemoglobin, enzyme systems, and the immune system function	Anaemia with pale mucous membranes decreased milk supply.
Manganese (Mn)	Growth, bone formation, enzyme activation	Impaired growth, poor reproduction, skeletal abnormalities
Selenium (Se)	Involved in immune function, enzyme formation (glutathione peroxidase, and cell membrane protection.	Mastitis, reproductive disorders, immune system dysfunction, retained placenta, white-muscle disease
Zinc (Zn)	Repair of damaged tissue, immune system, enzyme activation, and keratin formation	Elevation in somatic cell count, parakeratosis in the skin, hoof dysfunction, elevation in somatic cell count, mastitis, stiff joints

Source: NRC, 2001

cause growth retardation and impair the regular functioning of a living body (Table 1). The requirements of minerals for the animal's daily basis vary according to animal type (cattle, buffalo, goat, sheep, etc.) and the basis for determining mineral demands in cattle differs from humans (Table 2). The intake of minerals is frequently chosen to increase growth (for example, in pigs, broiler chickens, and beef) and output (for example, in laying hens and dairy cattle) (Coleman & Moore, 2003). As a result, commercial feed often contains additional minerals to meet the mineral requirements of particular livestock (Pond et al., 2004). Conventional breeding and biotechnological interventions in animals' manipulated output potential and nutritional requirements. In certain aspects, the plant matter given to cattle varies from that ingested by humans. Although animals are fed forage, particularly for ruminants, cereals, legumes, and grasses are essential components of their diet (Pond et al., 2004; Reddy et al., 2003). The monocots (e.g., *Pennisetum spp.*, *Lolium spp.*) and dicots (e.g., *Trifolium spp.*, *Medicago sativa*) both serve as pasture; hence, most of the plant scientists should consider these plant species' nutrient-rich crops. Some strategies to improve nutrient concentration in beneficial forage crops, but ensuring an optimal mineral balance would be equally vital.

Elements such as Zn, Fe, Cu, Co, I, Mn, and Se are

important for animals and humans and enter the plant system via non-selective pathways (roots). while micronutrients reach livestock and the human body through the food supply. Most of the forage crops are grasses belonging to the family Poaceae, legume crops of Leguminosae, and some trees like mulga (*Acacia aneura*) and subabul (*Leucaena leucocephala*) are grown in dryland areas (Muir et al., 2011). These crops can be consumed by cattle directly after partial or full drying. The concentrations of micronutrients, proteins, and lipids define the nutritional status and digestibility of fodder crops (Capstaff and Miller 2018). Crops are the main source of minerals in animal diets (Suttle, 2022). Several factors, including plant species and soil conditions, determine the elemental profile of forage crops. Plants absorb major and micronutrients from the soil because these nutrients are abundant in soil, but only a small amount of micronutrients is available to plants (Marijanušić et al., 2017).

3. Biofortification in Fodder Crops

Biofortification is the process of using agronomic treatments, conventional plant breeding, and genetic engineering to improve the nutritional profile of plant-based meals (Figure 1). An integrated approach of nutrient management in crops enhances the micronutrient

Table 2. Quantity of nutrients required for cattle at different ages

Category	Cows						Heifers (age in months)			
	Dry (far off)	Dry (close up)	Fresh	Early	Mid	Late	6	12	18	24 (close up)
Macro-elements required in percent of dry matter										
Calcium	0.44	0.48	0.79	0.60	0.61	0.62	0.47	0.41	0.44	0.40
Phosphorus	0.22	0.26	0.42	0.38	0.35	0.32	0.25	0.23	0.18	0.23
Magnesium	0.11	0.20	0.29	0.21	0.19	0.18	0.11	0.11	0.08	0.14
Chlorine	0.13	0.20	0.20	0.29	0.26	0.24	0.11	0.12	0.10	0.16
Sodium	1.10	0.14	0.34	0.22	0.23	0.22	0.08	0.07	0.12	0.10
Potassium	0.51	0.62	1.24	1.07	1.04	1.07	0.47	0.48	0.46	0.55
Sulfur	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Micro-elements in mg per kg dry matter(for cow and heifer's)										
Iron	Cobalt	Copper	Manganese	Zinc	Iodine	Selenium	Fluorine	Molybdenum		
13-30	0.11	10-16	14-24	22-70	0.3-0.4	0.30	Upto 30	Upto 6		
Vitamins in IU per day										
Vit. A (IU)	58000	60600	75000	75000	75000	75000	16000	24000	36000	60100
Vit. D (IU)	11700	12100	21000	21000	21000	21000	6000	9000	13500	10000
Vit. E (IU)	1168	1211	545	545	545	545	160	240	360	1202

Source: NRC, 2001

concentration (Hussain, 2022). The biofortification of fodder crops depends on improved absorption of nutrients from the soil. However, if micronutrients are present in low concentrations in the soil, they must be supplied as fertilisers. The current need to focus on the bioavailability of these micronutrients in the rhizosphere, their absorption, and the uniform distribution into edible parts of the crop plants (Whitem & Broadley, 2009).

4. Agronomic Biofortification

To improve the concentration of micronutrients in edible plant tissues, agronomic biofortification uses mineral fertilisers to increase the mobilisation and solubility of micronutrients already present in the soil (Chugh et al., 2022). Soluble inorganic fertilisers are applied to the leaves and roots in a targeted manner when plants are grown in soil deficient in microelements. Soluble inorganic fertilisers are administered through the leaves when micronutrients are difficult to translocate into edible tissues. Agronomic

biofortification increases the micronutrient concentration in the crops through agronomic practices (Mandi et al., 2022) (Table 3). Agronomic biofortification practice to increase Zn and Fe contents in edible plant portions is very useful for addressing malnutrition (Shukla & Mishra, 2018).

4.1. Seed priming/coating

The application of nano-zinc oxide (nZnO) in the form of priming of the seed treatment increased the vegetative growth, yield, and quality of fibre fodder maize variety (J-1006). Additionally, nano-zinc oxide (nZnO) priming treatments were shown to be more successful at increasing zinc micronutrient availability in field-grown fodder maize. As a consequence, seed treatments with a small amount of nano-zinc oxide (nZnO) fertiliser can help minimise zinc fertiliser application dosages and waste. Zn-based fertilisers and nano-zinc oxide (nZnO) particles can also help improve Zn nutrient use efficiency in fodder crops and their content in crops, particularly fodder maize (Tondey et al., 2021). The

Table 3. Application of micronutrients in fodder crops for agronomic ferti-fortification

Micronutrient	Crop	Source
Seed coating/priming		
Nano-Zn	Maize	Tondey et al. (2021)
Nano-Zn and Cu	Cowpea	Srinivasan et al. 2017
Foliar application		
B	Berseem	Arora (2007)
Mo + B	Berseem	Dhaliwal et al. (2008)
Fe + Zn + Mn	Sorghum	Soleymani et. al. (2012)
Fe	Teosinte	Kumar et al. (2016)
Zn + Cu	Barley	Iahaq et al. (2018)
Cu	Oats	Sandhu et al. (2020)
Zn	Cowpea	Kumar and Dhaliwal (2021)
Soil application		
Cu	Oats	Kaur et al. (2015)
Zn	Berseem and lucerne	Kumari, (2017)
Zn + Fe + Se	Sorghum	Quereshi et al. (2021)
N + Zn + Se	Maize	Petković et al. (2022)
Soil + foliar application		
Fe + Zn and Mn + Cu	Barley	Booboori et al. (2012)
B + Mn	Lucerne	Kherikhah et al. (2016)
Zn	Maize	Kumar et al. (2017)
Zn	Pearlmillet	Shekhawat and Kumawat (2017)
Zn	Oats	Dhaliwal et al. (2020)
Zn	Sorghum	Kumar and Ram (2021)

seed treatments of cowpea with different levels of nano-copper oxide (nCuO; 25, 50, 100, 200 mg kg⁻¹) and nano-zinc oxide (nZnO; 250, 500, 750 mg kg⁻¹) recorded the highest growth and micronutrient concentration. The concentration of copper (17.7 mg kg⁻¹) increases up to the application of nCuO 100 mg kg⁻¹ but the highest zinc availability (45.4 mg kg⁻¹) in plant dry matter was recorded with nCuO 25 mg kg⁻¹. Nano-zinc treatments also recorded an increment of copper and zinc in plant dry matter. The maximum concentration of zinc (50.4 mg kg⁻¹) was recorded with nZnO 700 mg kg⁻¹ (Srinivasan et al., 2017).

4.2. Foliar application of micronutrients

In berseem crop applied boron (B) 2.0 mg kg⁻¹ to increase the dry matter yield by 6.3% and 8.5% in both first and second cuttings over the control, respectively Arora, (2007). Berseem crop mean boron content and absorption both depend on boron application rates significantly. The total B concentration in berseem plants increased by 10.7% and 20% over the control at first cutting when B was applied at 1.0 and 2.0 mg kg⁻¹, respectively. At the first and second cuttings, the effect of B application of 2.0 mg kg⁻¹ in the berseem crop was a 23.1% and 24.4% increase in B uptake over the control, respectively. Seed production of berseem is

strongly affected by B foliar spray, followed by Zn and Mo spray. (Dhaliwal et al., 2008). The maximum fresh forage yield of sorghum and quality forage was achieved with foliar application of Fe, Zn, and Mn together in comparison to the rest of the other treatments applied in sorghum fodder crops (Soleymani & Shahrajabian, 2012). Applying Fe to the teosinte fodder crop as a foliar increased yield, Fe content, and digestibility of teosinte (Kumar et al., 2016). Ishaq et al. (2018) found that foliar application of Zn and Cu in barley improves grain yield and quality. Sandhu et al., (2020) investigated the effect of Cu on oats fodder crops and recorded that Cu absorption, content, yield, and protein content were all considerably improved by foliar application of Cu (0.2%) at 60 and 90 DAS (days after sowing). The higher productivity and quality of dual-purpose cowpea were achieved with 16 kg ha⁻¹ of zinc sulfate applied at sowing, and after 0.3% zinc sulfate, two foliar sprays were applied at the early bloom and full bloom stages (Kumar & Dhaliwal, 2021).

4.3. Soil application of micronutrients

The application of Cu 6 kg ha⁻¹ increased oats fodder output, Cu content, crude protein (CP), and other nutrients. Cu application increased the fodder quality of oats by

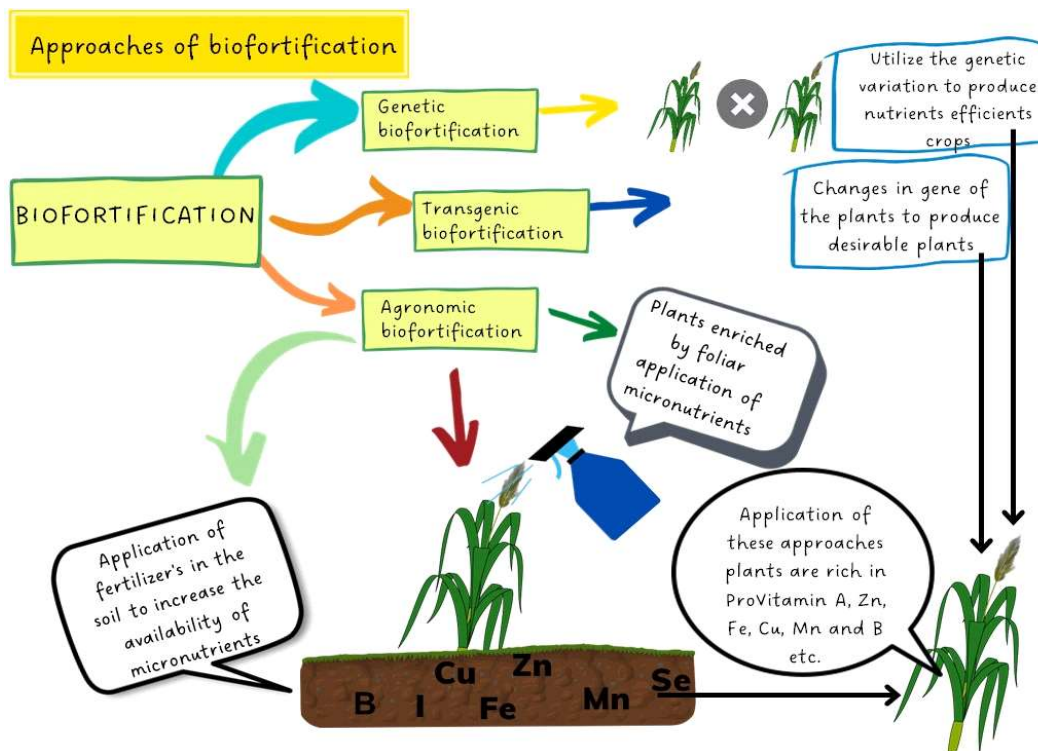


Figure 1. Different approaches to biofortification

lowering total ash content, NDF (Neutral Detergent Fibre), ADF (Acid Detergent Fibre), and cellulose content. The concentrations of Fe, Zn, and Mn in oats fodder were not affected by Cu treatment, but the bioavailability of nutrients in fodder oats increased due to the soil application of Cu 6 kg ha⁻¹ (Kaur et al., 2015). Soil application of chelated Zn in fodder berseem and lucerne enhances crop quality, including increased CP content and Zn uptake (Kumari, 2017). Zinc treatment in the soil is an efficient technique to increase Zn uptake by plants in zinc-deficient soils, and has been shown to inhibit phosphorus uptake and phytate-P buildup in plants. Sorghum crop biofortification with Fe, Zn, and Se had a positive effect on all the sorghum cultivars studied. Sorghum cultivars achieve maximum values for all growth and quality parameters with the application of Fe, Zn, and Se (Qureshi et al., 2021). The application of nitrogen with Zn and Se for biofortification of fodder maize increased yield and nutrient concentration (Zn and Se) in the crop (Petković et al., 2022).

4.4. Soil and foliar application of micronutrients

Soil and foliar Zn application improved Zn contents in fodder crops, CP contents, biomass, total biomass yield, and quality of fodder. Zn application in the soil and foliar spray increased nutrient absorption and the speed of transport to storage organs. Zinc enhanced the rate of protein synthesis by the buildup of amino acids. The application of zinc in the form of foliar and soil applications increased crude protein content and enhanced nitrogen metabolism in plants (Alloway, 2008). The soil application of Zn, after the zinc foliar spray in the cowpea crop, reported maximum grain yield and quality parameters. The highest CP, ash content and ether extract (EE), and yield were also reported with 20 kg ha⁻¹ ZnSO₄ application in maize fodder crop, followed by combined fertilisation soil application of 10 kg ha⁻¹ ZnSO₄ and one foliar spray of 0.5 per cent ZnSO₄ and two foliar sprays of 0.5 per cent ZnSO₄ (Kumar et al., 2017). Kheirkhah et al. (2016) investigated that the individual application of PSB, Mn, and B obtained the maximum fodder of lucerne and improved fodder quality when the foliar application of B was applied. Shekhawat & Kumawat (2017) found that using 20 kg ha⁻¹ ZnSO₄ in soil and four foliar sprays of 0.5 percent in pearl millet increased Zn and CP content, and overall quality. Zn is more readily available in the rhizosphere when applied to the soil, whereas foliar application increases absorption and fast translocation in plant systems. The elevated Zn level (foliar spray of zinc)

was attributed to improved nitrogen metabolism, which increased the speed of protein synthesis and the formation of amino acids, increasing the CP content in fodder oats (Dhaliwal et al., 2020). In sorghum crop, soil and double foliar ZnSO₄ fertilization improved digestibility parameters such as total digestible nutrients, digestible crude protein, digestible dry matter, dry matter intake, net energy for lactation, relative feed value, digestible feed energy as well as relative fodder quality and decrease in fibre fraction (Kumar & Ram, 2021).

4.5. Soil amendments application

The application of cattle dung with forest leaf litter and Zn-rich fertiliser in the soil resulted in significantly higher grain yield and Zn content in maize grain. Organic matter's long-term application in the soil increases the overall fraction of labile zinc and zinc content in the soil. Labile zinc is easily absorbed by plant roots and easily translocated in the plant system (Manzeke et al., 2017). Alkaline soil is often calcareous with a high concentration of carbonates. Deep ploughing increases the amount of carbonate in the top layers, raising the pH of the soil. Furthermore, higher soil pH reduces Fe and Mn solubility in the soil, the excess Ca and Mg in calcareous soils cause multiple nutrient deficiencies in the soil. To reduce the pH of sodic and saline-sodic soils, the use of acid-producing fertilisers and additives such as elemental sulfur and gypsum has long been applied (Tavakkoli et al., 2022).

5. Genetic Biofortification

Increasing the micronutrient content in plant tissue by the use of mineral fertilisers can help grow crops with a better ability to absorb and accumulate these elements in their edible portions. The identified genetic variation found in plant species defines Fe, Zn, Cu, and Se concentrations in edible tissues of crop plants. According to present and past findings, changes in micronutrient concentration in plant tissue are significantly less than those of the above-mentioned micronutrients (Yadava et al., 2017; Earagariyanna et al., 2017). The *Lolium spp.*, *Medicagospp.*, and *Festuca spp.* are some of the fodder crops improved through breeding. Individual plants have been discovered with high genotypic and phenotypic diversity, as well as polyploidy, which is increased by inbreeding in a variety of grasses and the association of a few agronomic factors with unique genes. (Collins et al., 2012; Blackmore et al., 2016). Pearl millet is the cheapest source of Fe and Zn; pearl millet has a wide range of genotypes for these minerals (Velu et al.,

2007). ICRISAT and Harvestplus launched the biofortified variety of pearl millet, which is rich in Fe and Zn, "Dhanashakti" and a hybrid ICMH 1201 (Shakti-1201) in India in 2014. Several commercial cultivars with high Fe and Zn content in grain have been identified, along with their progenies and hybrids (Rai et al., 2012). Sorghum cultivars with high mineral, protein, lutein, zeaxanthin, and beta-carotene content have been examined. For Fe and Zn content, sorghum germplasm has demonstrated a lot of diversity and genetic heritability (Kumar et al., 2013). ICRISAT has developed and released biofortified Fe sorghum varieties ICSR 14001, ICSR 14002, and hybrids ICSA 318, ICSA 336, ICSA 661, ICSR 94, ICSR 196, and IS 3760. Cowpea has been biofortified through breeding to increase Fe content. G. B. Pant University of Agriculture and Technology, Pantnagar, in partnership with HarvestPlus, in India, has released the varieties of cowpea Pant Lobia-1, Pant Lobia-2, Pant Lobia-3, Pant Lobia-4, and Pant Lobia-7. These varieties are rich in Fe content. An experimental trial was conducted to feed Zn-biofortified sorghum stover of sorghum to sheep to ameliorate Zn deficiency (Giridhar et al., 2021). So, we can use biofortified crop varieties by-products and grain for animal feeding developed by conventional breeding.

6. Transgenic Biofortification

Genetically modified alfalfa crops increase phytase activity that eliminates the needs of phosphorus supplementation in animal feed used for livestock, fish, and poultry (Austin-Phillips et al., 2001). Barley has been targeted for improvement in nutritional quality because it is an ideal cereal and fodder crop. Its Zn level has increased due to the overexpression of zinc transporters. Activation of the phytase gene [HvPAPhy] has increased phytase activity in barley seeds, thereby increasing Fe and Zn bioavailability (Holme et al., 2017). Improvements in feed digestibility have also been studied since they are linked to animal

performance. Three distinct cytochrome P450 enzymes were targeted for antisense-mediated expression reduction to establish modified alfalfa cultivars with substantially lower lignin concentrations (Reddy et al., 2005). Agronomic and genetic biofortification are combined in transgenic biofortification to promote micronutrient phytoavailability inside the soil rhizosphere and absorption translocation into smaller parts of the crop plant, then storage in consumable organs (Davies, 2007). Methionine is an amino acid that is used to make proteins and also plays a key role in many other biological functions. The Dzs10 cis-acting location was added to increase its concentration in maize. The amino acid equilibrium of maize has been improved to generate milk protein lactalbumin in the milch animals (Chen et al., 2008). In addition, the intention of transgenic biofortification seems to reduce antinutrient concentrations while raising the level of enhancer chemicals, which facilitate microelement assimilation inside the gut (Whitem & Broadley, 2009). Sorghum is one of the most important staple foods for millions of poor rural people. The Improvement of provitamin A levels by releasing Homo188-A (beta-carotene). Sorghum's concentration of the essential amino acid lysine was raised after a high-lysine protein [HT12] was inserted (Zhao et al., 2003; Lipkie et al., 2013). Several stress-tolerant forage crop gene editing lines have been produced to aid biomass growth and nutritional quality. CRISPR/Cas9 was used to effectively alter the Squmaosa promoter binding (SPL9) gene in *Medicago sativa* (Gao et al., 2018). The identification of genes or quantitative trait loci (QTL) connected to micro-elements like carotenoids, Fe and Zn and important amino acids in pearl millet germplasm lines is aided by molecular segmentation of germplasm types. Varietals having better nutrient quality have been generated thanks to the evolutionary divergence of these genes, also known as quantitative trait loci (QTL) (Boncompagni et al., 2018). In sorghum (*Sorghum bicolor*), recent advances in biotechnological technologies have

Table 4. Application of transgenic biofortification techniques in fodder crops

Crop	Gene/protein/enzyme	Targeted nutrient	Gene/protein expression	Source
Alfalfa	phytase	Phosphorus	Eliminates the needs of phosphorus supplementation in animal feed	(Austin-Phillips et al., 2001)
Barley	phytase gene [HvPAPhy]	Zn	Increasing Fe and Zn bioavailability	(Holme et al., 2017)
Alfalfa	Cytochrome P450 enzymes	Lignin	Improvements in feed digestibility reduce lignin content	(Reddy et al., 2005)
Sorghum	Homo188-A, protein [HT12]	Provitamin-A amino acid lysine	Improvement of provitamin-A and lysine content	(Zhao et al., 2003; Lipkie et al., 2013)

allowed improving the important minerals, micronutrients, amino acids, and fatty acids, through genetic engineering (Reddy et al., 2005; Elkonin et al., 2018). Using genetic manipulation specifically increased the bioavailability of lysine, vitamin A, iron, and zinc in the sorghum crop (Zhao et al., 2019) (Table 4). Therefore, this method makes it possible to generate multiple nutrient-rich crop cultivars in a single phase and provides new opportunities. By developing methods to jointly target multiple micronutrients while accounting for their stability, metabolic engineering applications will make significant progress in this direction.

7. Conclusion

To solve the nutritional problem in animals, biofortification is a sustainable and cost-effective way to enrich feed and fodder crops. Biofortified material improves the health of malnourished animals. Different approaches of biofortification to increase the bioavailability of essential micronutrients in the feed and fodder crops eradicate all forms of nutritional problems in the animals. To overcome micronutrient insufficiency in the animal population, agronomic biofortification by fertilisation appears to be a viable and cost-effective strategy. The inorganic and organic sources of inputs might be used as direct sources of nutrients to help in the adequate availability of micronutrients to the animals. Multi-biofortified feed and fodder crops are given more weightage to produce quality feed and fodder. Feeding biofortified material to the animals increases the milk yield, growth, and overall performance. Optimising quality enhances the capacity of fodder utilisation, reduces crop wastage, and helps to maintain a sustainable dairy industry. As a result, the knowledge presented here will help to address future nutritional security and to increase the bioavailability of micronutrients within forage crops to the animals.

CRedit authorship contribution statement

Brijesh Kumar: Conceptualization, review collection, Writing-original draft, Supervision, **Magan Singh:** Reviewing and editing, **Deepak Kumar:** Reviewing and editing, **Sandeep Kumar:** Reviewing and editing, **Rekha Rana:** Visualization and tabulation.

Conflicts of interest

There is no conflict of interest in personal and financial interests.

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Data availability statement

The published data will be available as per the user's request.

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Influence of Feeding Regimes on Survival, Growth Performance and Production Efficiency of Indian Major Carps Under Semi-Intensive Polyculture System

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ABSTRACT: This study evaluated the effect of different feeding regimes on survival, growth, and production performance of Indian major carps (*Labeo catla*, *Labeo rohita*, and *Cirrhinus mrigala*) in a semi-intensive polyculture system. Fingerlings were stocked in 80 m² cemented outdoor tanks at the rate of 1 fish m⁻². The experiment was conducted by following a Completely Randomised Design: FR1 (control) with dough feed twice daily at the rate of 2% body weight; FR2, FR3, FR4, and FR5 with pelleted feed @ 2% twice daily, 2% thrice daily, 3% twice daily, and 3% thrice daily, respectively. The water quality parameters remained within optimal throughout the study. FR5 exhibited the highest survival (91.43%), followed by FR4, FR2 and FR3. Maximum net weight gain and specific growth rate were observed in FR5 for catla (33.96 g; 2.28%), followed by rohu (24.70 g; 1.67%). Feed conversion ratio (FCR) and protein efficiency ratio (PER) improved significantly in FR2 and FR3, whereas net fish biomass production was significantly higher in FR3, FR4, and FR5. From the results, we concluded that pelleted feed administered at 2% body weight thrice daily (FR3) represents the optimal feeding strategy for maximising growth performance and production efficiency in semi-intensive carp polyculture systems, balancing both growth outcomes and feed utilisation efficiency.

Keywords: Indian major carps, Polyculture system, *Labeo catla*, *Labeo rohita*, *Cirrhinus mrigala*.

1. INTRODUCTION

Global food security, nutrition, and livelihoods are greatly enhanced by fisheries and aquaculture, especially in areas where aquatic resources are essential. With the capacity to meet the growing demand for fish and the ability to efficiently utilize aquatic habitats, aquaculture is now the

most important food production industry with the highest rate of growth (Dhala et al., 2023). Inland fisheries produce over 75% of India's total fish production, ranking it third in the world for fish production and second for aquaculture (Handbook on Fisheries Statistics, 2023). Freshwater aquaculture is dominated by Indian main carps, such as catla, rohu, and mrigal, because of their market demand,

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adaptability and ease of breeding. Aquaculture, especially in underdeveloped nations, is an important strategy for enhancing food security, since it produces more efficiently on a comparatively smaller area of land or water than traditional agriculture (Dhala et al., 2023). Fish productivity and ecosystem health are improved by polyculture systems, which use several compatible species and have been shown to be more environment friendly and productive than monoculture (Prakash et al., 2018). Further, the most common method of fish production in India is carp polyculture, which includes the use of manures/fertilisers and supplementary feeding to achieve economical production, thereby supporting higher stocking densities and better fish growth performance. Since, supplementary feed accounts for 50-60 of production costs, effective feed management is essential (Sharma et al., 2022). Growth performance, feed utilisation, disease resistance, and overall profitability can all be greatly enhanced by optimising the feeding schedule (feed types, forms, rates, and frequency), which significantly impacts fish growth, survival, feed utilisation efficiency, and the economics of the entire production process. To maximize output and reduce environmental effects, the present study addresses how feeding management influences ecosystem balance, nutrient dynamics, and sustainable productivity in semi-intensive carp polyculture systems. It is important to develop an ideal feeding strategy for the species based on its nutritional needs and culture conditions. Although previous studies have explored various aspects of feeding strategies in carps (Das et al., 2000; Sultana et al., 2001; Abid & Ahmed, 2009; Baruah et al., 2020; Das et al., 2021), a limited comprehensive literature is available on the complete

package of practices for the feeding regime for the rearing of carps under a semi-intensive polyculture system. With this background, the present study was undertaken to assess how different feeding regimes, such as feed types, feeding rates, and feeding frequencies, will affect the survival, growth, and productivity of Indian major carps produced in semi-intensive carp polyculture.

2. MATERIALS AND METHODS

2.1. Experimental Design

The experiment was conducted for 120 days at the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in outdoor cemented tanks (80 m²) with five treatments (FR1/C to FR5) by following a completely randomised design (Table 1).

2.2. Preparation of Experimental Feed

Both dough and pelleted feed were prepared using locally available ingredients, viz. rice bran (49%), mustard meal (49%), fish-specific mineral mixture prepared by Guru Angad Dev Veterinary and Animal Sciences University (1.5%) and common salt (0.5%) (Kumar et al., 2019). Dough feed was prepared daily, whereas pelleted feeds were prepared by thoroughly mixing all ingredients and using a mechanical pelletizer, then stored in an airtight container for use during the experimental period.

2.3. Proximate Analysis of Feed Ingredients and Experimental Feed

The proximate analysis (% DM basis) with respect to crude protein (CP), crude fibre (CF), Ether extract (EE), ash, moisture and nitrogen free extract (NFE) of different feed

Table 1. Treatments with respect to feeding regimes

Treatments	FR1/C	FR2	FR3	FR4	FR5
Feed form*	D1	D2	D2	D2	D2
Feeding rate (% BW)	2%	2%	2%	3%	3%
Feeding frequency day ⁻¹	Twice	Twice	Thrice	Twice	Thrice

*Feed D1: Dough feed, Feed D2: Pelleted feed

Table 2. Proximate composition of feed ingredients and experimental feeds (on % dry matter basis)

Ingredients/ experimental diets	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	Nitrogen free extract (%)
Rice bran*	12.24	1.44	15.06	11.19	60.05
Mustard meal*	38.69	2.60	14.80	8.10	35.80
Dough & pelleted feed	27.24	1.52	17.53	10.92	42.78

*deoiled

ingredients and experimental feed (Table 2) was done, by following the standard methods of (AOAC, 2005).

2.4. Preparation of Experimental Tanks

All the experimental tanks were cleaned, dried and layered with a 4-inch soil layer. Liming was carried out by using limestone (CaCO_3) @ 250kg ha^{-1} . The water depth was maintained at 105 ± 5.0 cm. Experimental tanks were manured with farmyard manure @ 5,000 kg ha^{-1} (40 kg tank^{-1}) a fortnight before fish stocking of fish, and then @ 1,250 kg ha^{-1} (10 kg tank^{-1}) at monthly intervals to maintain nutrient availability and plankton production. The experimental tanks were maintained for 15 days after initial manuring.

2.5. Stocking of Fish

Fish fingerlings were stocked @ stocking ratio of 3:4:3 [Surface (SF): column (CF): bottom feeder (BF)] in all the treatment groups @ 80 fish fingerlings tank^{-1} (10,000 fish fingerlings ha^{-1}), after proper acclimatisation.

2.6. Feeding of Fish

Fish were fed with dough and pelleted supplemental feeds. The amount of feed was adjusted after each monthly sampling in accordance with the increase in the weight of the fish.

2.7. Water Quality Parameters

Water quality parameters, i.e., Temperature, pH, Dissolved oxygen (DO), Total alkalinity (TA), Total hardness (TH), Ammonical-nitrogen ($\text{NH}_3\text{-N}$), Nitrate-nitrogen ($\text{NO}_3\text{-N}$) and Orthophosphate, were analysed

monthly as per standard procedures of APHA (2012).

2.8. Fish Survival and Growth

Fish growth was studied at monthly intervals in terms of total length gain (TLG), net weight gain (NWG), net fish biomass production (g tank^{-1}), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR) and survival (%) were calculated after the completion of experiment.

2.9. Blood Metabolic Profile and Haematological

Parameters

The blood metabolic profile parameters, i.e., glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). However, the haematological parameters, i.e., total erythrocyte Count (TEC)/ total red blood cell count (RBC), total leukocyte count (TLC)/ total white blood cell Count (WBCs), haemoglobin (Hb) and haematocrit value or packed cell volume (PCV) were recorded at the termination of the experiment.

2.10. Statistical Analysis

The statistical package (SPSS 16.0 for Windows, SPS Inc., Richmond, CA, USA) was used for statistical analysis of the data. The data were statistically analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) to establish the influence of feeding regimes on water quality, fish survival, growth, biochemical and haematological parameters with respect to significant difference ($p < 0.05$) among the treatment group means. Data are presented as mean \pm standard error (SE).

Table 3. Water quality parameters in different treatments

Parameters	FR1/C	FR2	FR3	FR4	FR5
Temperature ($^{\circ}\text{C}$)	21.08 ^{bc} \pm 0.12	21.52 ^a \pm 0.05	21.32 ^{ab} \pm 0.02	21.12 ^{bc} \pm 0.01	20.96 ^c \pm 0.15
pH	7.75 ^a \pm 0.01	7.74 ^a \pm 0.01	7.77 ^a \pm 0.01	7.78 ^a \pm 0.01	7.77 ^a \pm 0.01
D.O (mg L^{-1})	8.49 ^a \pm 0.17	8.83 ^a \pm 0.25	8.77 ^a \pm 0.24	8.07 ^a \pm 0.11	9.12 ^a \pm 0.17
Total alkalinity (CaCO_3 mg L^{-1})	175.73 ^{ab} \pm 1.25	186.40 ^a \pm 4.46	166.27 ^{ab} \pm 1.25	159.33 ^{ab} \pm 5.54	154.40 ^b \pm 6.80
Total hardness (CaCO_3 mg L^{-1})	191.47 ^a \pm 1.24	200.80 ^a \pm 1.09	168.93 ^a \pm 1.14	174.73 ^a \pm 9.88	176.60 ^a \pm 6.90
$\text{NH}_3\text{-N}$ (mg L^{-1})	0.047 ^a \pm 0.00	0.045 ^a \pm 0.00	0.043 ^a \pm 0.00	0.044 ^a \pm 0.00	0.044 ^a \pm 0.00
$\text{NO}_3\text{-N}$ (mg L^{-1})	0.286 ^a \pm 0.03	0.311 ^a \pm 0.01	0.270 ^a \pm 0.05	0.263 ^a \pm 0.01	0.259 ^a \pm 0.01
Ortho-phosphate (mg L^{-1})	0.012 ^a \pm 0.00	0.010 ^{ab} \pm 0.00	0.010 ^{ab} \pm 0.00	0.008 ^b \pm 0.00	0.010 ^{ab} \pm 0.00

Data are presented as mean \pm SE; n=3

Mean values with different superscripts across the rows indicate significant differences ($p < 0.05$), as assessed by one-way ANOVA and Duncan's multiple range test

FR1/C= D1, 2% & twice, FR2= D2, 2% & twice, FR3= D2, 2% & thrice, FR4= D2, 3% & twice and FR5= D2, 3% & thrice

3. RESULTS AND DISCUSSION

3.1. Water Quality

The water quality parameters range viz. water temperature pH, Dissolved oxygen (DO), total hardness (TH), total alkalinity (TA), $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, Ortho-Phosphate was 14.36 to 26.93°C, 7.33 to 8.40, 8.00 to 9.66 mg L^{-1} , 151.33 to 235.33 $\text{CaCO}_3 \text{ mg L}^{-1}$, 140.00 to 214.00 $\text{CaCO}_3 \text{ mg L}^{-1}$, 0.035 to 0.051 mg L^{-1} , 0.200 to 0.495 mg L^{-1} and 0.005 to 0.017 mg L^{-1} (Table 3). Water quality was not impacted by feeding fish with varying feeding schedules. Feeding frequency and rate are essential elements in semi-intensive fish culture for improved growth and increased yield. Increased feeding frequency may lead to increased growth and production rate. The water quality parameters remained within the suitable range for carps culture and were in similar trend as demonstrated by different researchers with respect to the water temperature (Seetha & Chandran, 2020), pH (Bhatnagar & Devi 2019), Dissolved Oxygen (Boyd & Tucker, 1998), total alkalinity (Ayyappan et al., 2011; Bhatnagar & Devi 2019), total hardness (Ayyappan et al., 2011; Purnamawati, 2019), ammonia (Ayyappan et al., 2011), nitrate (Santhosh & Singh, 2007) and ortho-phosphate (Boyd & Tucker, 1998).

3.2. Fish Survival and Growth

Fish's ability to adapt to changes in the environment is essential to their survival (Koedijk et al., 2012). The highest species survival rate was 94.44, 90.28, and 89.58% for mrigal, catla and rohu, respectively. Overall, FR5 (fish fed with pelleted feed @ 3% BW & three times a day) recorded the highest average fish survival rate (91.43%), while FR1 recorded the lowest (83.56%) (Table 4). Garg and Kalla (2018) also reported that, with an increase in feeding frequency from one to four times a day, both survival rate

and growth increased significantly in Indian major carps. These results suggest that a feeding frequency of thrice a day is sufficient for achieving better survival and optimal growth performance of Indian major carps under intensive culture conditions, as it ensures adequate nutrient availability throughout the day while minimising metabolic stress and maintaining stable water quality parameters.

Total length gain in catla (*Labeo catla*) ranged from 5.73 cm (FR1/C) to 8.71 cm (FR3), showing significant variation among treatments. Net weight gain ranged from 19.92 g (FR1/C) to 33.96 g (FR5), with significantly higher values in FR3–FR5 than in the control. The specific growth rate ranged from 1.87% (FR1/C) to 2.28% (FR5), showing a significant increase across treatments. The condition factor (K) ranged from 1.02 (FR3) to 1.52 (FR4 and FR5) with significant difference between FR1/C and FR3, but non-significant differences among FR2, FR4, and FR5. Net biomass production ranged from 360.51 g (FR1/C) to 730.27 g (FR5), with significantly higher yields in FR3–FR5 compared to FR1/C and FR2.

Total length gain in rohu (*Labeo rohita*) ranged from 6.14 cm (FR1/C and FR2) to 7.58 cm (FR5), showing significant variation among treatments. Net weight gain ranged from 16.63 g (FR1/C) to 24.70 g (FR5), with significant differences observed between FR1/C and FR3 and non-significant differences between FR4 and FR5. Specific growth rate ranged from 1.42% (FR1/C) to 1.67% (FR5), with significant differences among treatments. The condition factor (K) ranged from 1.03 (FR1/C) to 1.20 (FR4), with no significant differences among FR2, FR3, and FR4 but a significant difference between FR1/C and FR5 ($P \leq 0.05$). Net biomass production ranged from 437.23 g (FR1/C) to 695.17 g (FR5), with values significantly higher in FR3–FR5 than in FR1/C and FR2.

Table 4. Survival (%) of fish in different treatments at the end of the experiment

Species	Treatments				
	FR1/C	FR2	FR3	FR4	FR5
Catla	80.55 ^a ±3.67	87.50 ^a ±6.36	86.11 ^a ±5.00	88.89 ^a ±1.39	90.28 ^a ±1.39
Rohu	85.42 ^a ±3.75	89.58 ^a ±7.29	88.54 ^a ±4.54	87.50 ^a ±1.80	89.58 ^a ±1.04
Mrigal	84.72 ^a ±3.67	91.66 ^a ±6.36	90.27 ^a ±2.77	93.05 ^a ±1.38	94.44 ^a ±1.38
Average survival	83.56 ^a ±3.33	89.58 ^a ±6.59	88.31 ^a ±4.06	89.81 ^a ±1.02	91.43 ^a ±0.70

See Table 3 for legends

(Data are presented as mean ± SE; n=3)

Mean values with different superscripts across the rows indicate significant differences ($p < 0.05$), as assessed by one-way ANOVA and Duncan's multiple range test.

FR1/C= D1, 2% & twice, FR2= D2, 2% & twice, FR3= D2, 2% & thrice, FR4= D2, 3% & twice and FR5= D2, 3% & thrice)

Total length gain in mrigal (*Cirrhinus mrigala*) ranged from 4.89 cm (FR1/C) to 6.13 cm (FR5), with significant differences among FR1/C–FR3 ($P \leq 0.05$) and non-significant differences between FR4 and FR5 ($P \leq 0.05$). Net weight gain ranged from 16.86 g (FR1/C) to 22.86 g (FR4), indicating significant differences among treatments. Specific growth rate ranged from 1.16% (FR1/C) to 1.35% (FR4), with significant differences among FR1/C, FR2, and FR3. The condition factor (K) ranged from 0.96 (FR5) to 1.07 (FR1/C) with non-significant differences among FR1/C, FR2, and FR3 but significant differences in FR4 and FR5. Net biomass production ranged from 290.67 g (FR1/C) to 501.37 g (FR4), with significant differences among

treatments. The maximum net weight gain for mrigal was observed in FR4 (22.86 g), while for catla (33.96 g) and rohu (24.70 g) was in FR5 (Table 5).

The best growth performance observed with pelleted feed in the present study is consistent with the findings of Das et al. (2021), who reported better growth performance when fish were fed pelleted feed compared to dough feed. Garg and Kalla (2018) reported that higher feeding frequencies were associated with higher SGR in *Cirrhinus mrigala*. Aga et al. (2017) also observed higher SGR and body weight gain in rohu fed a supplementary diet at a 4% feeding rate twice daily. Sultana et al. (2001) also observed that common carp fry fed pelleted feed at a 5% feeding rate,

Table 5. Growth parameters of catla (*L. catla*), rohu (*L. rohita*) and mrigal (*C. mrigala*)

Parameters	Treatments				
	FR1/C	FR2	FR3	FR4	FR5
Catla					
TLG (cm)	5.73 ^c ±0.91	6.20 ^c ±0.97	8.71 ^a ±0.11	6.81 ^b ±0.13	6.93 ^b ±0.26
NWG (g)	19.92 ^d ±0.26	25.93 ^c ±0.23	32.90 ^b ±0.30	32.90 ^b ±0.15	33.96 ^a ±0.23
SGR (%)	1.87 ^d ±0.03	2.03 ^c ±0.02	2.20 ^b ±0.02	2.22 ^{ab} ±0.00	2.28 ^a ±0.01
Condition factor (K-value)	1.24 ^b ±0.03	1.42 ^a ±0.03	1.02 ^c ±0.01	1.52 ^a ±0.04	1.52 ^a ±0.08
Net fish biomass production (g tank ⁻¹)	360.51 ^a ±1.28	537.90 ^b ±4.63	671.13 ^a ±3.84	695.20 ^a ±9.99	730.27 ^a ±7.78
Rohu					
TLG (cm)	6.14 ^c ±0.16	6.14 ^c ±0.16	6.60 ^b ±0.03	6.86 ^b ±0.08	7.58 ^a ±0.05
NWG (g)	16.63 ^d ±0.16	19.60 ^c ±0.05	21.96 ^b ±0.24	24.36 ^a ±0.14	24.70 ^a ±0.32
SGR (%)	1.42 ^d ±0.01	1.50 ^c ±0.00	1.61 ^b ±0.01	1.66 ^a ±0.01	1.67 ^a ±0.02
Condition factor (K-value)	1.03 ^b ±0.03	1.18 ^a ±0.04	1.15 ^a ±0.01	1.20 ^a ±0.02	1.04 ^b ±0.01
Net fish biomass production (g tank ⁻¹)	437.23 ^c ±2.12	548.67 ^b ±5.35	608.20 ^{ab} ±3.02	667.00 ^a ±1.90	695.17 ^a ±6.25
Mrigal					
TLG (cm)	4.89 ^b ±0.18	5.04 ^b ±0.04	5.20 ^b ±0.07	6.05 ^a ±0.05	6.13 ^a ±0.07
NWG (g)	16.86 ^d ±1.00	17.93 ^{cd} ±0.16	18.50 ^c ±0.05	22.86 ^a ±0.14	20.96 ^b ±0.17
SGR (%)	1.16 ^c ±0.03	1.19 ^{bc} ±0.00	1.23 ^b ±0.01	1.35 ^a ±0.00	1.31 ^a ±0.00
Condition factor (K-value)	1.07 ^a ±0.01	1.06 ^a ±0.00	1.06 ^a ±0.00	1.03 ^b ±0.00	0.96 ^c ±0.01
Net fish biomass production (g tank ⁻¹)	290.67 ^d ±54.52	317.57 ^{cd} ±27.04	388.03 ^{bc} ±16.09	501.37 ^a ±1.03	468.00 ^{ab} ±1.02
FCR	1.80 ^{bc} ±0.18	1.62 ^c ±0.20	1.61 ^c ±0.18	2.45 ^{ab} ±0.18	2.68 ^a ±0.27
PER	2.08 ^{ab} ±0.19	2.33 ^a ±0.30	2.32 ^a ±0.24	1.51 ^b ±0.11	1.39 ^b ±0.14
Total initial biomass (g tank ⁻¹)	308.00 ^b ±0.46	317.33 ^a ±2.82	309.60 ^{ab} ±2.01	316.27 ^a ±3.00	310.67 ^{ab} ±2.2
Total final biomass (g tank ⁻¹)	1396.40 ^c ±8.52	1721.50 ^b ±1.27	1977.00 ^a ±8.33	2179.80 ^a ±2.67	2204.10 ^a ±1.29
Net fish biomass (g tank ⁻¹)	1088.40 ^c ±8.49 (10.88 Kg)	1404.10 ^b ±1.24 (14.04 Kg)	1667.40 ^a ±8.37 (16.67 Kg)	1863.60 ^a ±2.87 (18.63 Kg)	1893.40 ^a ±1.0 (18.93 Kg)

See Table 3 for legends

(Data are presented as mean ± SE; n=3)

Mean values with different superscripts across the rows indicate significant differences ($p < 0.05$), as assessed by one-way ANOVA and Duncan's multiple range test.

FR1/C= D1, 2% & twice, FR2= D2, 2% & twice, FR3= D2, 2% & thrice, FR4= D2, 3% & twice and FR5= D2, 3% & thrice)

four times/day, exhibited the best growth performance in terms of WG and SGR. Baruah et al. (2020) observed that feeding regimes with lower feeding rates (1% BW day⁻¹) resulted in poorer growth performance than feeding rates of 3% BW day⁻¹ and 6% BW day⁻¹. Ahmed (2007) reported greater weight gain and SGR in rohu at feeding rates of 6-8% body weight. Khan (2004) reported increased daily body weight gain in mrigal when feeding rates increased from 2% to 6%. The direct relationship between feeding frequency and growth in *L. rohita* fingerlings was observed by Choudhury et al. (2002). Abid and Ahmed (2009) further support the findings of the present study. Das et al. (2000) observed a decrease in FCR with an increase in feeding frequency from once to twice per day for catla and rohu, which is in accordance with the present study, collectively suggesting that optimised feeding frequency and rate, along with feed form, are critical determinants of growth performance and feed utilisation efficiency in Indian major carps. At the end of the experiment, FCR and PER values ranged from 1.62 (FR3) to 2.68 (FR5) and from 1.39 (FR5) to 2.33 (FR2), respectively, indicating significant differences among the treatments (Table 4). However, FR2 (1.62 and 2.33) and FR3 (1.61 and 2.32) exhibited the highest FCR and PER values among the treatments.

The present study demonstrated improved growth performance with increasing feeding frequency, consistent

with previous reports in carps. Abid and Ahmed (2009) observed that increasing feeding frequency from 1 to 3 times per day significantly increased final weight in rohu fingerlings, supporting the current findings. Similarly, Sultana et al. (2001) reported superior growth performance, including higher NWG, SGR, and PER, in common carp fry fed four times per day compared with lower frequencies. Das et al. (2000) found optimal growth and feed utilisation in catla and rohu fingerlings at twice-daily feeding, although they suggested three times feeding as adequate under intensive culture conditions. Honnananda et al. (2019) further confirmed that increased feeding rates improved survival, growth, SGR, and FCR in Indian major carps. However, Sultana et al. (2001) reported improved PER. They reduced FCR with higher feeding frequency, which contrasts with the present results and suggests that feed utilisation responses to feeding frequency may vary with species, developmental stage, feeding rate, and culture conditions. At the end of the experiment, net fish biomass ranged from 10.88 Kg (FR1/C) to 18.93 Kg (FR5), showing significant differences ($P \leq 0.05$) among treatments. FR3 (16.67 Kg) had the highest net biomass, followed by FR4 (18.63 Kg) and FR5 (18.93 Kg) (Table 5).

3.3. Biochemical Parameters

Glucose level (mg dL⁻¹) of catla ranged from 80.75

Table 6. Biochemical parameters of blood serum of different fish species

Parameters	Treatments				
	FR1/C	FR2	FR3	FR4	FR5
Catla					
Glucose (mg dL ⁻¹)	80.75 ^c ±4.24	83.54 ^{bc} ±1.23	89.76 ^{abc} ±4.57	95.86 ^{ab} ±3.14	99.86 ^a ±4.95
Aspartate aminotransferase (AST) (U L ⁻¹)	154.58 ^a ±5.36	133.63 ^a ±1.44	130.68 ^a ±1.76	147.86 ^a ±2.50	156.56 ^a ±2.44
Alanine aminotransferase (ALT) (U L ⁻¹)	17.88 ^b ±0.31	21.99 ^a ±0.44	20.97 ^{ab} ±0.76	22.44 ^a ±1.87	21.83 ^a ±1.03
Rohu					
Glucose (mg dL ⁻¹)	82.83 ^a ±9.55	83.14 ^a ±7.96	93.14 ^a ±5.77	95.39 ^a ±12.54	93.05 ^a ±28.46
Aspartate aminotransferase (AST) (U L ⁻¹)	103.30 ^a ±7.31	126.34 ^a ±1.47	121.93 ^a ±2.00	131.71 ^a ±2.15	120.62 ^a ±5.56
Alanine aminotransferase (ALT) (U L ⁻¹)	23.15 ^a ±1.49	24.89 ^a ±3.06	24.88 ^a ±2.22	23.68 ^a ±2.11	22.16 ^a ±1.50
Mrigal					
Glucose (mg dL ⁻¹)	81.44 ^a ±4.39	86.17 ^a ±0.99	82.47 ^a ±1.80	90.42 ^a ±11.35	84.07 ^a ±12.95
Aspartate aminotransferase (AST) (U L ⁻¹)	110.04 ^a ±1.44	97.05 ^a ±0.76	110.03 ^a ±8.14	118.25 ^a ±2.43	108.78 ^a ±1.62
Alanine aminotransferase (ALT) (U L ⁻¹)	21.52 ^a ±0.78	20.17 ^a ±0.90	20.45 ^a ±0.60	22.45 ^a ±1.42	23.11 ^a ±3.26

See Table 3 for legends

(Data are presented as mean ± SE; n=3)

Mean values with different superscripts across the rows indicate significant differences ($p < 0.05$), as assessed by one-way ANOVA and Duncan's multiple range test.

FR1/C= D1, 2% & twice, FR2= D2, 2% & twice, FR3= D2, 2% & thrice, FR4= D2, 3% & twice and FR5= D2, 3% & thrice)

Table 7. Biochemical parameters of blood serum of different fish species

Parameters	Treatments				
	FR1/C	FR2	FR3	FR4	FR5
Catla					
TLC ($\times 10^3 \text{mm}^{-3}$)	1.56 ^b ±0.26	2.30 ^a ±0.18	2.21 ^a ±0.06	2.46 ^a ±0.14	2.46 ^a ±0.15
TEC ($\times 10^6 \text{mm}^{-3}$)	1.56 ^b ±0.21	2.06 ^{ab} ±0.12	2.10 ^{ab} ±0.07	1.97 ^{ab} ±0.29	2.23 ^a ±0.03
Hb (g%)	5.53 ^a ±0.43	6.40 ^a ±0.60	6.36 ^a ±0.58	6.06 ^a ±0.06	6.76 ^a ±0.33
PCV/Hct (%)	22.83 ^a ±2.03	25.73 ^a ±0.83	27.16 ^a ±1.05	23.10 ^a ±1.68	25.63 ^a ±0.29
Rohu					
TLC ($\times 10^3 \text{mm}^{-3}$)	1.63 ^a ±0.17	1.92 ^a ±0.15	1.72 ^a ±0.06	1.92 ^a ±0.09	1.96 ^a ±0.15
TEC ($\times 10^6 \text{mm}^{-3}$)	1.70 ^a ±0.30	1.35 ^a ±0.15	1.52 ^a ±0.20	1.75 ^a ±0.16	1.67 ^a ±0.23
Hb (g %)	5.26 ^b ±0.43	6.80 ^a ±0.37	6.86 ^a ±0.49	6.23 ^{ab} ±0.08	6.03 ^{ab} ±0.23
PCV/Hct (%)	22.93 ^a ±2.03	23.13 ^a ±0.74	25.06 ^a ±1.61	26.33 ^a ±0.86	23.73 ^a ±0.67
Mrigal					
TLC ($\times 10^3 \text{mm}^{-3}$)	1.48 ^a ±0.27	1.88 ^a ±0.09	1.76 ^a ±0.09	1.92 ^a ±0.06	1.66 ^a ±0.19
TEC ($\times 10^6 \text{mm}^{-3}$)	1.19 ^a ±0.01	1.53 ^a ±0.22	1.43 ^a ±0.13	1.82 ^a ±0.03	1.62 ^a ±0.42
Hb (g%)	5.56 ^a ±0.18	6.26 ^a ±0.63	6.56 ^a ±0.16	5.83 ^a ±0.23	6.90 ^a ±0.75
PCV/Hct (%)	21.30 ^a ±2.01	25.70 ^a ±2.90	24.56 ^a ±1.09	26.03 ^a ±0.92	25.00 ^a ±1.11

See Table 3 for legends

(Data are presented as mean \pm SE; n=3)

Mean values with different superscripts across the rows indicate significant differences ($p < 0.05$), as assessed by one-way ANOVA and Duncan's multiple range test.

(FR1/C) to 99.86 (FR5), showing significant differences among treatments. Rohu ranged from 82.83 (FR1/C) to 95.39 (FR4), differences were nonsignificant, and *mrigal* ranged from 81.44 (FR1/C) to 90.42 (FR4), differences were not significant. *AST values* (U L^{-1}) of *catla* ranged from 130.68 (FR3) to 156.56 (FR5), *rohu* ranged from 103.30 (FR1/C) to 131.71 (FR4), and *mrigal* ranged from 97.05 (FR2) to 118.25 (FR4). Differences in AST were not significant across treatments for any species. *ALT values of catla* ranged from 17.88 (FR1/C) to 22.44 (FR4), with significant differences; *rohu* ranged from 22.16 (FR5) to 24.89 (FR2), with insignificant differences; and *mrigal* ranged from 20.17 (FR2) to 23.11 (FR5), with insignificant differences. Overall, glucose and ALT levels showed significant variation among treatments only in *catla*, whereas AST levels did not differ significantly across species (Table 6).

Fish growth is related to feed intake; higher feed intake results in greater weight gain, but excessive feeding increases the feed conversion ratio, water pollution, and other disorders (Zhang et al., 2019). Fish fed less frequently had lower glucose concentrations than fish fed regularly, suggesting that feeding schedules may affect carbohydrate metabolism of carbohydrates and lead to behavioural

changes, such as compensatory feeding (Dametto et al., 2018). Fish blood glucose is an extremely sensitive indicator of environmental stress. Fish blood glucose is an extremely sensitive indicator of environmental stress. Nekoubin et al. (2013) found that grass carp fed pelleted meals with higher protein content (25% to 35%) at 5% body weight, three times a day, had higher glucose levels, consistent with the present study. Wu et al. (2021) observed that feeding more frequently (1–4 times per day) was associated with noticeably higher blood glucose levels. The glucose levels in *catla*, *rohu*, and *mrigal* increased in tandem with feeding frequency (2 to 3 times/day).

3.4. Haematological Parameters

The minimum TLC was observed in FR1/C, while the maximum was in FR4 (*catla* and *mrigal*) and FR5 (*rohu*). Significant differences were observed only between the control (FR1/C) and treatments in *catla*, whereas differences were non-significant in *rohu* and *mrigal* across all treatments. The minimum TEC was observed in FR1/C and FR2, while the maximum was in FR5 (*catla*) and FR4 (*rohu* and *mrigal*). Significant differences were observed among treatments for *catla*, whereas differences were non-

significant for *rohu* and *mrigal*. The minimum Hb concentration was consistently observed in the control (FR1/C) across all species, whereas the maximum was observed in FR5 (*catla* and *mrigal*) and FR3 (*rohu*). Significant differences were observed among treatments for *rohu*, whereas differences were non-significant for *catla* and *mrigal*. The minimum haematocrit was recorded in the control (FR1/C) for all species, while the maximum was recorded in FR3 (*catla*) and FR4 (*rohu* and *mrigal*). Differences among treatments were insignificant for all three species (Table 7). Fish health and physiological status can be evaluated using haematology as a major indicator (Silva & Chamul, 2000; Fazio et al., 2019). Nekoubin et al. (2013) observed that grass carp fed pelleted diets at 5% BW with thrice-daily feeding had haematocrit values and RBC counts comparable to those of the present study.

4. CONCLUSION

The overall results demonstrate that the feeding regime significantly influences the survival, growth, and production performance of Indian major carps under semi-intensive polyculture. Although higher feeding rates and frequencies enhanced survival and individual growth. However, administration of pelleted feed at 2% body weight, three times per day, results in the best balance between growth, feed utilisation, and biomass production with improved feed conversion and protein efficiency, coupled with high net yield, indicating the most efficient and economically viable feeding strategy for sustainable carp polyculture systems.

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CRedit authorship contribution statement

Rubalpreet Kaur: Conducted the experiment, Analysed the data, Software, and wrote the original draft. **Khushvir Singh:** Conceptualisation, Supervision, Data curation, Critical evaluation of data, Writing - review & editing. **Amit Mandal:** Reviewing and editing. **Chanchal Singh:** Formal analysis, Reviewing and editing. **Abhishek Srivastava:** Reviewing and editing. **Jaspal Singh Hundal:** Data curation, Reviewing and editing

Conflict of interest

The authors declared that they have no conflicts of interest among themselves or with the institute conducting

the experiment.

Data availability statement

Data will be made available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the author(s) have not used an AI tool.

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Traditional Fishing Gears and Craft of Dal Lake: Insights into Indigenous Fishing Practices in the Kashmir Valley, India

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ABSTRACT: The present study documents indigenous fishing gear and crafts traditionally used in Dal Lake, the Kashmir Himalaya, with emphasis on their design, specifications, and operational features. A variety of gears, including the cast net, gill net, pole-and-line (*Bislia*), hook-and-line, multiple-head spear, and double-pronged spear, were systematically described and standardized in terms of dimensions, materials, mesh sizes, and target species. Cast nets, identified as the dominant lacustrine gear across the valley, are primarily used to capture *Cyprinids* such as *Schizothorax* spp. and *Cyprinus carpio*. Gill nets (*Patij*) and pole & line (*Bislia*) also play a crucial role in selective fishing, targeting commercially important species. Spears and other traditional gears highlight the adaptive strategies of local fishers in shallow, weedy zones of the lake. Traditional crafts (*Naav*), largely plank-built from durable deodar wood, remain the primary means of operating gear and transporting fish. This study provides baseline technical specifications of the gears and crafts, reflecting the ecological adaptation and socio-cultural heritage of Dal Lake fishers and serving as an important reference for sustainable fisheries management, the conservation of indigenous practices, and future aquaculture engineering innovations in the Himalayan region.

Keywords: Dal Lake, Fishing craft, Traditional fishing, Gears.

1. INTRODUCTION

Kashmir is endowed with diverse inland water resources, with lakes constituting the primary water bodies, followed by rivers, streams, and springs. These inland fisheries resources play a pivotal role in the regional economy, particularly in sustaining the livelihoods of the fishing communities (Shakire et al., 2023). Among the prominent fisheries, Dal Lake is a major contributor to the total fish catch of Jammu and Kashmir. The lake covers an area of 11.56 km² and is located at 34°05' N, 74°09' E (Bhat et al., 2024).

Despite the ecological and socio-economic importance of Dal Lake, there is a significant gap in comprehensive

documentation of the fishing techniques employed in the lake, which poses challenges for effective resource management and the promotion of sustainable fisheries (Qayoom et al., 2016; 2019; Bhat et al., 2025). The present study investigates and characterizes the traditional fishing gear and techniques used by local fishers, thereby generating insights essential for both conservation planning and the sustainable development of fisheries in Jammu and Kashmir.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Dal Lake, one of the largest freshwater lakes in Jammu and Kashmir, from June 2021 to May 2022. To ensure adequate spatial coverage, five fishing

villages and landing centers situated along the lake, Hazratbal, Dal Gate, Fishermen Colony, Moti Mohalla, and Dhobi Ghat, were selected as representative sites. These locations were chosen based on their high fishing activity and the dependence of local communities on capture fisheries.

2.2. Sampling Design and Data Collection

Data were collected at bimonthly intervals over a period of one year, enabling the assessment of short-term temporal variations in fish species composition, catch abundance, and fishing practices. A combination of qualitative and quantitative approaches was employed.

2.3. Field Observations

Direct monitoring of fishing operations, including deployment and retrieval of gears, types of craft employed, and catch composition.

2.4. Physical Measurements

Standard measurements of fishing gears (mesh size, length, depth) and crafts (length, breadth, construction material).

2.5. Structured Interviews

Semi-structured questionnaires and conversations with local fishers to document indigenous knowledge, fishing techniques, seasonal preferences, and socio-economic aspects. Respondents were approached at multiple points, including landing centers, individual households, and the fisheries cooperative society, to obtain a broad representation of fishing communities. Secondary information on fish landings, fishing practices, and management frameworks was collected from the official records of the Department of Fisheries, Jammu and Kashmir. These data sources were used to complement and validate field-based observations.

2.6. Data Analysis

Information from primary and secondary sources was compiled, tabulated, and categorized according to gear type, mode of operation, and target species. A comparative analysis across sites and time intervals was conducted to identify patterns in gear use, fishing effort, and resource dependence. Descriptive statistics were applied to summarize observations, and qualitative data from the fishermens' interviews were thematically analyzed to highlight indigenous knowledge and traditional practices.

3. RESULTS AND DISCUSSION

3.1. Fishing Crafts (NAAV)

The construction and design of fishing crafts in Dal Lake are largely determined by the availability of local materials, durability requirements, and functional adaptability to different fishing practices. The majority of boats on the lake are plank-built, locally referred to as Naav, which remains the predominant mode of water transport and fishing in the region. Wood is the primary raw material used, and among locally available timber species, Deodar (*Cedrus deodara*) is preferred for its high strength, water resistance, and long durability, making it particularly suitable for boat construction in freshwater ecosystems. The *Naav* exhibits significant variability in size and structural dimensions, reflecting both ecological conditions and the intended fishing operation. Based on overall length (OAL), the crafts in Dal Lake can be broadly categorized into three groups. Fishing crafts operating in the lake can be broadly classified into three categories based on size. Large-sized crafts generally measure 7 to 10 meters in overall length (OAL), with a breadth of 1.0 to 1.5 meters and a depth of 0.2 to 0.6 meters. Medium-sized crafts are relatively small, with an overall length of 6 to 8 meters, a breadth of 0.7 to 1.0 meters, and a depth of 0.2 to 0.5 meters. The small-sized crafts are the most compact type, usually measuring 4 to 5 meters in length, 0.5 to 0.7 meters in breadth, and 0.2 to 0.3 meters in depth.

The OAL represents the total length of the fishing craft from the bow's foremost point to the stern's aft-most point. Plank-built construction involves joining wooden planks edge-to-edge and fastening them to form the hull, which is often designed with a slightly curved or V-shaped bottom. Such a shape enhances hydrodynamic efficiency, reducing drag and improving stability during fishing operations, particularly when nets are deployed.

These crafts are primarily used to operate cast nets, gillnets, and scoop nets. Propulsion is traditionally achieved with oars, locally called *Khoor*, crafted from wood. Depending on the craft's size, 2–3 oars are used, allowing the fishers and anglers to row in either direction and providing high manoeuvrability. In shallow zones of the lake, bamboo poles are also employed for navigation. The majority of *Naav* are open-decked, providing sufficient working space for fishers to manage gear, sort catches, and execute fishing operations efficiently. The classification of fishing crafts in Dal Lake aligns with similar observations from other inland water bodies. Syed et al. (2021) categorized fishing crafts of Wular Lake into three length-based groups (2–5 m, 5–8 m,

and 8–12 m), while Manna et al. (2011) reported the prevalence of plank-built boats measuring 8–10 m in length and 1.0–1.5 m in breadth in the Krishna River. These comparative findings highlight regional similarities in plank-built boat construction while also reflecting adaptations to local ecological conditions and fishing requirements (Table 1).

3.2. Fishing Gears

3.2.1 Cast net: The cast net, locally known as *Zaal*, is one of the most commonly used fishing gears in Dal Lake, characterized by its circular design and efficient encircling

Table 1. Technical and structural specifications of fishing craft (*NAAV*) operating in the Dal Lake

Specifications	Average
Local name	<i>Naav</i>
Length overall (m)	7.82 ±1.24
Breadth at midship (m)	1.12 ±0.30
Breadth at stern (m)	0.28± 0.10
Depth of vessel (m)	0.35±0.10
Freeboard (cm)	22.02 ±1.70
Draught (cm)	12.96 ±1.20
Material	<i>Cedrus</i> wood
Weight of vessel (kg)	92.10 ± 4.70
Length (m) of fish hold	0.86 ± 0.10
Breadth (m) of the fish hold	0.69 ± 0.07
Depth (m) of fish hold	0.32 ±0.10
Fish holding capacity (kg)	47.13 ±3.20

Table 2. Cast Net: Structural and operational specifications in the Dal Lake

Local name of the Gear	Cast Net
Local Name	<i>Zaal</i>
Total length of net (m)	4.55±0.21
Material of webbing	P.A multifilament
Specification of webbing	210D×2×2×210D×4×3
Mesh size of webbing (mm)	42±1.50
Colour of webbing (mm)	White
Selvedge mesh size (mm)	48.50±6.11
Number of sinkers	336±3.22
Materials of sinker	Lead/Iron
Weight of sinkers (g)	30.5±4.95
Diameter of sinkers (mm)	11.25±1.35

mechanism. The average total length of the net is 4.55 m, with the webbing fabricated from polyamide (P.A.) multifilament yarn, ensuring both flexibility and durability in aquatic environments. The webbing is constructed with a specification of 210D×2×2×210D×4×3, with an average mesh size of 42 mm, designed to target medium-sized lacustrine fish species.

The webbing is white, with a selvedge mesh size of 48.50 mm, reinforcing the gear's periphery's structural strength. The net is weighted with an average of 336 sinkers, made primarily of lead or iron, each weighing 30.5 g and having a diameter of 11.25 mm. These sinkers enable rapid sinking of the gear, ensuring effective encirclement and capture of fish (Table 2). The present study highlights the technical specifications of the *Zaal*, which are consistent with the typical design of cast nets used in other lacustrine systems of the Himalayan region. Dar et al. (2024 & 2025) documented the cast net as the most dominant fishing gear in the Kashmir valley owing to its simplicity, efficiency, and adaptability to shallow waters. The average mesh size of 42 mm observed in Dal Lake closely matches the ranges reported by Shakir et al. (2023) for other Kashmir lakes, indicating a standardized mesh size suitable for capturing native *Schizothorax* spp. and *Cyprinus* species. Furthermore, the number and weight of sinkers (average 336 units, 30.5 g each) recorded in this study are slightly higher than the observations of Bhat et al. (2024), where sinker counts between 280–320 per net were observed in Wular Lake. The additional sinkers in the Dal Lake cast nets likely increase sinking velocity, thereby improving catch efficiency in the lake's deeper, more vegetated habitats of the lake. The use of polyamide multifilament as the primary material is also consistent with studies by Megalhaes (2023), which emphasized its high tensile strength, low water absorption, and abrasion resistance, making it a preferred choice among traditional fishers.

Overall, the present findings reaffirm that the *Zaal* cast net in Dal Lake is an efficient and adaptive fishing gear, optimized for the lake's ecological conditions while reflecting both traditional knowledge and modern material preferences.

3.3. Gill Net

The gill net, locally referred to as *Patij*, is an important passive fishing gear employed in Dal Lake for the capture of medium- to large-sized fish species. The net is operated at an average depth of 4.5 m, with a total length of 4.12 mand a depth of 2.12 m, making it suitable for lacustrine

environments with moderate water depths. The webbing is fabricated from polyamide monofilament, with a mean mesh size of 67 mm, optimized for selective entanglement of species such as *Schizothorax* spp. and *Cyprinus carpio*.

The selvedge is constructed from polyamide multifilament, specified as 210D×2×2, with a mean mesh size of 36.5 mm and approximately 4.4 meshes in depth, ensuring structural reinforcement and durability. The net is supported by an average of 10 cylindrical plastic floats, which provide buoyancy and maintain the gear's vertical orientation of the gear during operation. For sinking, the net is weighted with 17 oval lead sinkers, each measuring 48 × 20 mm and weighing 12.4 g. The sinkers are distributed along the footrope at an average spacing of 284 mm, enabling uniform sinking of the gear. Both head and foot ropes are fabricated from polyethylene (PE) with diameters of 5–7 mm, providing adequate tensile strength for field operations (Table 3).

The specifications of the *Patij* gill net in the Dal lake closely resemble those of traditional lacustrine gill nets

described from other parts of Kashmir. The average mesh size (67 mm) recorded in this study is comparable to the findings of Dar et al. (2019) and Shakir et al. (2023), with mesh sizes ranging from 60–70 mm in Wular and Manasbal lakes, targeting cyprinids and schizothoracines. Larger mesh sizes have been associated with higher selectivity, thereby reducing the capture of juveniles, which aligns with the ecological conservation practices in the valley. The number of floats (10 per net) and their cylindrical plastic design correspond with the specifications reported by Zarka et al. (2023), who emphasized the transition from traditional wooden floats to synthetic plastic floats for improved durability and buoyancy. The distribution of lead sinkers (average spacing 284 mm) in Dal Lake nets is wider than the 200–250 mm spacing in Wular Lake reported by Bhat et al. (2024). This wider spacing may reduce net tension, thereby improving entanglement efficiency in the relatively calm waters of Dal Lake.

The adoption of polyamide monofilament webbing and multifilament selvedge is consistent with trends noted across Himalayan fisheries (Ali et al., 2014). Monofilament nets provide transparency in water, thereby increasing catch efficiency, while multifilament selvedge strengthens the margins of the gear, reducing breakage during retrieval. Overall, the design and operational specifications of the *Patij* gillnet highlight its functional efficiency in Dal Lake fisheries. The moderate dimensions, combined with selective mesh sizes and durable synthetic materials, make it a highly effective gear for artisanal fishers, while still aligning with sustainability considerations documented in previous studies.

3.4. Pole and Line (*Bislia*)

The pole and line, locally known as *Bislia*, is a traditional active fishing gear widely operated in Dal Lake for targeting *Cyprinus carpio* and *Schizothorax* species. The gear is generally operated at an average depth of 2.12 m, making it suitable for nearshore and shallow-water habitats where these species aggregate.

The line is fabricated from polyamide multifilament, specified as 210D×6×3, with an average length of 4.2 m, providing adequate strength and flexibility for repeated casting and retrieval. The pole is constructed from locally available wood, measuring 2.63 m in length and 20 mm in diameter, offering durability and ease of handling by a single fisher. The hook, locally called *Wool*, is made of stainless steel and is of the barbed type, ensuring better retention of fish upon hooking. Commonly used hook sizes include No.

Table 3. Gillnet in the Dal Lake: Design and operational specifications

Name of the Gear	Gill net
Local name	<i>Patij</i>
Depth of operation (m)	4.5±1.00
Mesh size of webbing (mm)	67±24
Twine type of webbing	P. A monofilament
Total length (m)	4.12±0.61
Total depth (m)	2.12±0.26
Twine type of selvedge	P.A multifilament
Specification of selvedge	210D×2×2
Mesh size (mm) of selvedge	36.5±2.30
No. of meshes in depth in selvedge	4.4±2.70
No. of floats	10±0.56
Material of floats	plastic
Shape of floats	Cylindrical
Dimension of sinkers (mm)	48×20
Number of sinker	17±4.03
Material of sinker	Lead
Weight (g) of sinker	12.4 ±1.42
Shape of sinker	Oval
Distance between two sinkers (mm)	284 ±113.30
Material of Head rope/ foot rope	PE
Diameter of head (mm)	5-7±1.10

3 and No. 5, with an average hook gap of 8.7 mm and a total hook length of 26 mm. Each line typically carries a single hook (1.00), allowing for selective, controlled fishing operations. A single sinker, made of iron, lead, or occasionally pebbles, is attached to the line. The sinker is cylindrical, with an average length of 16 mm, and is used to maintain the baited hook at the desired fishing depth (Table 4).

The pole-and-line (*Bislia*) represents one of the simplest yet most selective gears in Dal Lake fisheries. Its reliance on a single barbed hook (No. 3–5) ensures high species selectivity and reduces bycatch, which aligns with the sustainability considerations highlighted by Shakir et al. (2023) in their documentation of artisanal fishing gears in the Kashmir valley. The average line length (4.2 m) and pole dimensions observed in this study correspond with the traditional pole-and-line methods described by Dar et al. (2015) for riverine fisheries in Kashmir, emphasizing the adaptability of this method across different aquatic ecosystems. The use of polyamide multifilament lines confirms a broader trend in artisanal fisheries, where synthetic fibers have replaced natural fibers due to their superior tensile strength, knot-holding capacity, and

resistance to wear (Yousuf et al., 2020). Similarly, the adoption of stainless-steel barbed hooks aligns with the observations of Bhat et al. (2024), indicating their durability and efficiency in enhancing hook retention compared to traditional iron hooks.

The single cylindrical sinker recorded in this study (average length 16 mm) differs from the multiple small sinkers sometimes employed in riverine pole-and-line fisheries in the Himalayan region (Ali et al., 2022). The use of a single, heavier sinker in Dal Lake may reflect local adaptations to calm lacustrine waters, ensuring quick sinking of the bait without excessive line tension. Overall, the pole and line (*Bislia*) exemplifies a highly selective, low-cost, and eco-friendly gear that continues to play an important role in the subsistence fisheries of Dal Lake. Its structural simplicity, combined with durability and efficiency, demonstrates the balance between traditional fishing knowledge and modern material innovations.

Table 4. Pole & line (*Bislia*) operated in the Dal Lake: typical specifications

Name of Gear	Pole & Line
Local Name	<i>Bislia</i>
Depth of operation	2.12±0.42
Main species caught	<i>Cyprinus</i> & <i>Schizothorax</i>
Material of the line	PA multifilament
Specification of the line	210D×6×3
Length of line (m)	4.2±0.36
Material of the pole	Wooden
Length of pole	2.63±0.32
Diameter of pole (mm)	20±0.00
Material of Hook (Woal)	Stainless steel
Type of Hook	Barbed
Hook No.	No.3, No.5
Hook Gap	8.7 ±0.54
Length of Hook (mm)	26±1.44
No. Hooks	1±0.00
Material of sinker	Iron/ lead/ pebble
Shape	Cylindrical
Length of Sinker	16±2.01
No. of sinker	1±0.00

3.5. Long Line

The long line, locally referred to as *WaelRaez*, is a traditional fishing gear employed in Dal Lake for catching *Cyprinus* spp. The operation of this gear typically requires two fishermen, reflecting the labor-intensive nature of its setting and retrieval. The main line is fabricated from polyamide (P.A.) multifilament, specified as 210D×8×3, with an average length of 1000 ± 125 m, allowing coverage of a considerable fishing area. Branch lines, also made of

Table 5. Design and specification of long line

Local name of the Gear	Long line
Local Name	<i>WaelRaez</i>
Main species caught	<i>Cyprinus Spp.</i>
No. of fishermen	2
Material of main line	PA multifilament
Specification of main line	210D×8×3
Length of main line (m)	1000±125
Material of branch line	PA Multifilament
Specification of branch line	210D×3×2
Length of branch line (mm)	55±1.80
Distance between two branch lines (m)	2.75 ±1.85
Type of Hook	Barbed
Hook No.	No. 5, No. 6
No. of Hooks	1000±175
No. of sinkers	329±41.92
Distance between sinkers	3±0

P.A. multifilament (210D×3×2), measure 55 in length and are attached to the main line at an average spacing of 2.75 m.

Hooks used are of the barbed, typically sizes No. 5 and No. 6, with an average of 1000 ± 175 hooks deployed per line. To ensure proper sinking, the gear is weighted with an average of 329 ± 41.92 sinkers, placed at intervals of 3 m along the line. This arrangement ensures hooks remain at the desired depth, increasing the likelihood of successful fish capture. The technical specification of the long line is given in Table 5.

The long line (*Wael Raez*) represents one of the more extensive passive fishing gears operated in Dal Lake, designed to target larger populations of *Cyprinus* spp. The deployment of up to 1000 hooks per operation reflects its high catching efficiency, consistent with descriptions of longline gears in other inland fisheries. Similar large-scale hook-line operations have been reported by Dar et al. (2019) in Wular Lake, Dar et al. (2015) in Manasbal Lake, and by Shakir et al. (2023) in the Jhelum River system, where hook numbers typically range between 800–1200 per gear.

The use of polyamide multifilament for both main and branch lines corresponds with trends in modern artisanal fisheries, replacing natural fibers due to their durability and low water absorption, as also emphasized by Monda et al. (2019). The line specifications in Dal Lake (210D×8×3 for the main line and 210D×3×2 for the branch line) closely match those reported in Himalayan riverine fisheries by Bhat et al. (2024), indicating a standardized material preference across the region.

The average branch line spacing (2.75 m) and hook size (No. 5–6) are comparable to those reported of Ali et al. (2022), who noted that these dimensions optimize both hooking probability and species selectivity. The high number of sinkers (329 units) at a short spacing (3 m) ensures uniform line sinking, particularly critical in still-water habitats like Dal Lake, where water movement is minimal. This design adaptation likely enhances bait presentation and hooking efficiency compared to riverine environments.

Overall, the *Wael Raez* long line is a highly efficient fishing gear in Dal Lake, designed for large-scale harvesting of *Cyprinus* spp. Its reliance on durable synthetic materials, standardized hook sizes, and dense sinker arrangement reflects both traditional fishing knowledge and practical innovations suited to lacustrine conditions.

3.6. Hand Net/Dip Method

The scoop net, locally known as *Khurizal*, is one of the

traditional hand-operated fishing gears practiced in Dal Lake. This gear is primarily used to target *Schizothorax* spp. and *Cyprinus carpio*, particularly in shallow near-shore areas where fish shoals congregate. The scoop net consists of a wooden central bisecting pole with an average length of 2.94 ± 0.90 m and a diameter of 42 ± 1.5 mm, providing sufficient strength for repeated handling. Attached to this is a semicircular wooden frame, measuring 5.35 ± 0.70 m in length with a diameter of 28 ± 2.00 mm, which supports the netting material.

The webbing is constructed of polyamide (P.A.) multifilament, specified as 210D×3×3, with an average mesh size of 32 ± 1.20 mm. This mesh dimension is suitable for capturing medium-sized lacustrine fish. The webbing depth is 1.47 m (range 0.81 m), enabling effective scooping action when the gear is submerged and dragged through fish-concentrated areas. The technical specifications of the Hand net/dip net are given in Table 6.

The structural design of *Khurizal* strikes a balance between simplicity and efficiency, making it widely adopted among subsistence fishers in Dal Lake. The recorded mesh size (32 mm) is consistent with scoop nets documented by Dar et al. (2019) and Dar et al. (2015), and Shakir et al. (2023) in other lakes of Kashmir, where similar dimensions were reported for targeting *Schizothorax* spp. This moderate mesh size ensures that the gear captures marketable fish while allowing smaller juveniles to escape, thus contributing to sustainable harvesting.

The use of polyamide multifilaments aligns with the findings of Mondal et al. (2019), who noted that multifilament yarn offers high strength, knot stability, and

Table 6. Design and specification of hand net/ dip method

Local name of the Gear	Scoop net/ Dip net/ Hand net
Local Name	<i>Khurizal</i>
Species Caught	<i>Schizothorax</i> and <i>Cyprinus sp.</i>
Material of Pole	Wood
Material of webbing	PA multifilament
Specification of webbing	210D×3×3
Mesh size of webbing (mm)	32±1.20
Length of central bisecting pole (m)	2.94 ±0.90
Diameter (mm)	42±1.5
Length of semicircular (m)	5.35 ± 0.70
Diameter (mm)	28 ±2.00
Depth of webbing (m)	1.47 (0.81)

greater abrasion resistance than traditional cotton or hemp nets. The semicircular wooden frame and central bisecting pole described in this study are also similar to designs reported by Bhat et al. (2024) for scoop nets in Wular Lake, although frame lengths in Wular were slightly shorter (4.8–5.0 m), indicating a possible adaptation of Dal Lake scoop nets to deeper or more turbid habitats.

The average webbing depth (1.47 m) suggests that the *Khurizal* is designed for efficient operation in shallow waters, contrasting with riverine dip nets (Ali et al., 2022), which typically employ greater net depths to counter stronger currents. The Dal Lake scoop net thus represents a locally optimized gear type that combines traditional

craftsmanship with material innovations to adapt to the lake's ecological conditions of the lake.

3.7. Spears

Spearing is one of the oldest traditional fishing methods practiced in Kashmir and is still observed in Dal Lake. It is classified as a wounding gear, as fish are captured by direct impalement rather than entanglement or enclosure. Although less commonly used today, this method continues to be practiced on a small scale by skilled fishers (Tables 7 & 8).

Design and types: Multiple-head spears (*Panzri*) consist of several pointed prongs fixed to a single handle, enabling the fisher to strike with a broader surface area and increasing the chances of impaling a fish. The *Panzri* is a traditional impaling gear widely employed in Dal Lake for capturing bottom-dwelling and mid-water species, particularly *Schizothorax* spp. and *Cyprinus carpio*. The spear is manually operated and designed for the precise targeting of fish in shallow areas. The gear consists of a wooden pole measuring 3.32 m in length, with a diameter of 25 mm, providing sufficient strength and maneuverability. The total length of the gear is 3.54 m, including the pronged metallic head. The depth of operation is relatively shallow, averaging 0.95 m, which makes it suitable for use in clear, shallow stretches of Dal Lake.

The spearhead comprises 12 prongs made of durable steel, each measuring 0.33 m in length. Each prong is equipped with two sharp barbs, with an average barb length of 9.95 mm, ensuring efficient impalement and preventing escape of the captured fish. The combination of multiple prongs with barbed ends maximizes the probability of successful strikes during manual thrusting operations.

The *Panzri* spear represents one of the simplest yet effective traditional gears of Dal Lake fisheries. Its reliance on manual thrusting in shallow waters aligns with observations of similar spearing gears in Himalayan freshwater ecosystems (Azhar et al., 2020; Bhat et al., 2024; and Dar et al., 2025). The shallow operational depth (0.95 m) is consistent with its use in littoral zones, where *Schizothorax* and carp are most abundant. The use of 12 steel prongs is relatively higher than those reported in riverine spear designs of central India, which typically employ 4–8 prongs (Ali et al., 2021). This higher number of prongs enhances efficiency by increasing the strike surface area, which is advantageous in the relatively turbid and vegetated zones of Dal Lake. The presence of barbs (length 9.95 mm) also distinguishes the *Panzri* from simpler single- or double-

Table 7. Multiple head spear in the Dal Lake: Technical specifications

Name of the Gear	Multiple Head Spear
Local Name	<i>Panzri</i>
Main species caught	<i>Schizothorax</i> and <i>Cyprinus</i> Sp.
Depth of operation (m)	0.95
Total length (m)	3.54
Material pole	Wood
Length (m) of pole	3.32
Diameter (mm) of pole	25
Material of prongs	Steel
Length of prongs (m)	0.33
Number of prongs	12
Number of barbs	2
Barb length (mm)	9.95

Table 8. Double pronged Spear in Dal Lake: Technical specifications

Name of the gear	Double pronged gear
Local name	<i>Narchoo</i>
Main species caught	<i>Schizothorax</i> and <i>Cyprinus</i>
Depth of operation (m)	0.92
Total length (m)	2.66
Material of pole	Wood
Diameter (mm) of pole	2.51
Material of prongs	25
Length of prongs (m)	Steel
Number of prongs	0.10
Number of barbs	7
Barb length (mm)	2(14)
Barb length (mm)	9.93

pronged spears used in smaller water bodies, as reported by Bhat et al. (2024), thereby reducing fish escape rates. From a socio-cultural perspective, *Panzri* fishing is practiced by skilled individuals. However, being a selective gear with low catch-per-unit-effort, its modern use is declining, echoing patterns observed across South Asian inland fisheries, where traditional impaling gears are gradually being replaced by nets. Comparable double-pronged spears have been reported from Himalayan and South Asian inland fisheries, though variations in pole length and barb design are common (Dar et al., 2019; Ali et al., 2021). The presence of multiple barbs on each prong enhances retention efficiency, a design modification tailored to the turbid and vegetated conditions of Dal Lake. Similar observations have been made in Nepalese, Bhutanese, and inland fisheries, where spears with multiple barbs were preferred in shallow streams (Zarka et al., 2023; Bhat et al., 2024).

4. CONCLUSION

The documentation of fishing crafts and gears operated in Dal Lake provides a comprehensive understanding of the traditional fishery practices sustained by local communities. The classification of fishing crafts into large, medium, and small categories clearly demonstrates the adaptability of fishers to the lake's ecological characteristics and fishing requirements. Large-sized crafts, with greater length and breadth, are suitable for extensive fishing operations and transportation of catch, whereas medium-sized crafts are more versatile, often employed for routine fishing activities within moderate depths of the lake. Small-sized crafts, being compact and lightweight, offer easy manoeuvrability in shallow, confined areas, underscoring their importance for localized fishing operations. Similarly, the specifications of fishing gears reflect the ingenuity and indigenous knowledge of the fisherfolk. Tools such as multi-pronged and double-pronged spears, and pole & line gear are meticulously designed to target specific fish species, such as *Schizothorax* and *Cyprinus*, which dominate the lake's ichthyofauna. The use of nets, both cast and gill nets, further illustrates a strategic approach to maximize efficiency in fish capture while minimizing damage to the aquatic ecosystem. The variations in material, dimensions, and operational depth of these gears emphasize the harmonious relationship between traditional practices and ecological conditions. Preserving and documenting these practices is crucial, not only for sustaining the livelihoods of local fishers but also for maintaining the biodiversity and ecological integrity of the lake. Moreover, such traditional knowledge offers

valuable insights for developing sustainable fisheries management strategies in inland water bodies facing increasing anthropogenic pressures.

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CRedit authorship contribution statement

Shabir Ahmad Dar: Conceptualization, Data collection, Methodology, soft ware, writing- original draft, supervision, formal analysis. **Gohar Bilal Wani:** Reviewing and editing. **Ashfaq F. Aga:** Visualization. **Imtiyaz Qayoom:** Tabulation, Data curation

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data that support the findings of this study are available from the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that artificial intelligence (AI) tools were used only for language editing and improving the clarity of the manuscript. The authors take full responsibility for the content, accuracy, and originality of the work.

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Efficacy of Plant Growth Regulator and Soil Media on Rooting, Growth and Survival of Stem Cuttings of Punjab Baramasi Lemon [*Citrus limon* (L.) Burm] in Sub-mountainous Zone of Punjab, India

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ABSTRACT: Lemon [*Citrus limon* (L.) burm] is one of the most popular fruit of the citrus group in India and around the world as well. Mostly, lemons are propagated through air layering and cutting methods of vegetative propagation. But the study on the effect of plant growth regulators and soil media on stem cuttings of Punjab Baramasi lemon, in particular, are scarce, especially under sub-mountainous agro-climatic conditions. Therefore, the present study was planned to find the efficacy of plant growth regulator and soil media on rooting, growth and survival of stem cuttings of Punjab Baramasi lemon [*Citrus limon* (L.) Burm] in the sub-mountainous zone of Punjab, India and to develop a protocol for the optimum rooting hormone concentration and growth media for faster multiplication and early production of true-to-type nursery plants to meet the increasing demand of fruit growers/farmers of the region. The healthy and disease-free hardwood stem cuttings were obtained from one-year-old shoots of 5-6-years-old mother plants of Punjab Baramasi lemon in the month of February. The basal portion of the cuttings was dipped for one minute in different doses (500ppm, 1000ppm, 1500ppm and control) of indole-3-butyric acid (IBA) plant growth regulator to induce rootings and planted in different growth media (garden soil, sand, farmyard manure, vermicompost, cocopeat) in polythene bags with different treatment combinations and control (without IBA+ garden soil). Stem cuttings of Punjab Baramasi lemon treated with IBA 1500ppm and planted in garden soil + sand+vermicompost+cocopeat (1:1:1:1) growth media in the month of February were found best for rooting, vegetative growth, and survival percentage in the Gurdaspur sub-mountainous zone of Punjab.

Keywords: Growth media, Hardwood cutting, Indole-3-butyric acid, Punjab Baramasi lemon, Rooting, Survival, Vegetative growth.

1. INTRODUCTION

Lemon [*Citrus limon* (L.) Burm] is the most important citrus fruit of India and around the world as well, belongs to the family Rutaceae, sub-family Aurantioideae having chromosome number $2n=18$ and is native to East Asia (Salaria, 2004). In Punjab, citrus is cultivated in 56492 hectare area with 1380867 metric tonnes production, among which lemon and lime contribute about 3252 hectare area with 26278 metric tonnes production (PAU, 2024). Its

propagation through seeds cause problem of non-uniformity of progeny and high chance of viral disease contamination (Babu, 2001). So, it is not advisable to use seeds for commercial planting because these seeds do not produce true fruits. For overcoming this problem, the vegetative propagation is vital to produce citrus plants having desirable characters as mother plant and they are propagated true-to-type from cuttings, budding, grafting, layering, etc. (Seran and Umadevi, 2011). To obtain ideal, true-to-type and good quality planting material through vegetative propagation,

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cutting is the cheapest, rapid and simplest method of multiplication to meet the demand of fruit growers. Such plants lead to uniform growth and a plant canopy which comes into bearing earlier than the seedling plants. The success of cuttings depend upon the many factors, associated with plants i.e. age of the mother plant, parts used of tree, time of planting, rainfall, humidity, temperature, rooting media and after care (Frey et al., 2006). Plant growth regulators, such as indole-3-butyric acid (IBA), naphthalene acetic acid (NAA) and 6-Benzylaminopurine (BAP), are frequently used to promote shooting and rooting in cuttings (Hartmann et al., 2002; Sharma et al., 2013; Verma, 2013). Similarly, rooting media also plays an important role in better root formation and development. The nature of roots arising from the cuttings is also influenced by the type of rooting medium, e.g., cuttings when planted in pure sand produce long, unbranched, coarse and brittle roots, but those planted in a mixture of sand, soil and peat produce well-developed branches (Chattopadhyay, 1994). Different growing media that are well aerated, loose, porous, create optimum conditions for respiration and maintain temperature, well-drained and with good water retention are used in establishing crop plantlets (Rymbai and Reddy, 2010; Bhosale et al., 2014).

Pomegranate cultivar Bhagwa cuttings planted in the month of February after giving IBA 2000 ppm treatment and planted in the media combination of cocopeat, perlite and vermicompost in ratio of 4:1:1 resulted in minimum number of days taken for sprouting with highest sprouts per cutting, sprouted percentage, survival percentage, plant height, stem diameter, number of leaves, shoot fresh weight and shoot dry weight (Saini et al., 2022). Similarly, Kumar and Kumar (2022) reported that 800ppm IBA + garden soil + sand + vermicompost (1:1:1) gave significant results on rooting of stem cuttings and survival percentage of lemon cultivar Pant Lemon-1. Although the studies on the effect of plant growth regulators on rooting of stem cuttings in citrus have been investigated over the years, but the studies on the effect of plant growth regulators and soil media on stem cuttings in citrus, especially in Punjab Baramasi lemon, in particular, are scarce, particularly under sub-mountainous agroclimatic conditions. Therefore, keeping in view the above points, the present study was undertaken to determine the optimum concentration of rooting hormone (IBA) and suitable growth/soil media for faster rooting and survival of Punjab Baramasi lemon cuttings in the sub-mountainous zone of Punjab.

2. MATERIALS AND METHODS

2.1. Experimental Site

The present investigation on “Efficacy of plant growth regulator and soil media on rooting, growth and survival of stem cuttings of Punjab Baramasi lemon [*Citrus limon* (L.) Burm] in sub-mountainous zone of Punjab, India” was conducted during the years 2022-23 and 2023-24 at Punjab Agricultural University, Regional Research Station, Gurdaspur in sub-mountainous region of Punjab which is situated between 32°3' N latitude, 75°22' E longitude and has an altitude of about 257 m from mean sea level having humid subtropical and dry winter climate. The submountain zone of Punjab (Kandi region) features hilly topography with Shiwalik foothills, undulating terrains, and deep, coarse-textured sandy loam to silt loam coarse-textured soils.

2.2. Planting Material

Punjab Baramasi lemon (5-6 years old plants) plants grown in the orchard of Punjab Agricultural University, Regional Research Station, Gurdaspur, were selected for this experiment on the basis of their uniformity in appearance, growth habit, and being free from visual symptoms of pests and disease occurrence.

2.3. Experimental Design

The experiment was performed in a two-way analysis of variance in Completely Randomised Block Design (CRBD) with three replications.

2.4. Preparation of Stem Cuttings and Growth Media

Healthy and mature shoots, preferably one year old, having a thickness of about 1.0 cm, were selected in the month of February before sprouting from the central and basal part of the branch of the Baramasi lemon plant, and cuttings were made of about 15-20 cm in length, possessing 6-8 buds. Leaves were completely removed from the cuttings with the help of secateurs to reduce the transpiration loss. A slanting cut was given at the upper side, and a slight slanting cut was also given at the lower end to provide a large surface area to encourage rooting in cuttings. The basal parts (2-3cm) of all the cuttings were dipped in different concentrations of IBA 500ppm (dissolve 500mg IBA in one litre of water), 1000ppm (dissolve 1000mg IBA in one litre of water), and 1500 ppm (dissolve 1500mg IBA in one litre of water) for one minute dip along with control (Hartmann et al., 2002). After this, these treated and un-treated cutting

Treatment number	Treatment detail
T ₁	500ppm IBA+ garden soil + sand (1:1)
T ₂	500ppm IBA+ garden soil + sand + FYM (1:1:1)
T ₃	500ppm IBA +garden soil + sand + vermicompost (1:1:1)
T ₄	500ppm IBA + garden soil + sand+ FYM+cocopeat (1:1:1:1)
T ₅	500ppm IBA + garden soil + sand+ vermicompost +cocopeat (1:1:1:1)
T ₆	1000ppm IBA+ garden soil + sand (1:1)
T ₇	1000ppm IBA+ garden soil + sand + FYM (1:1:1)
T ₈	1000ppm IBA +garden soil + sand + vermicompost (1:1:1)
T ₉	1000ppm IBA + garden soil + sand + FYM+cocopeat((1:1:1:1)
T ₁₀	1000ppm IBA + garden soil + sand +vermicompost +cocopeat (1:1:1:1)
T ₁₁	1500ppm IBA+ garden soil + sand (1:1)
T ₁₂	1500ppm IBA+ garden soil + sand + FYM (1:1:1)
T ₁₃	1500ppm IBA +garden soil + sand + vermicompost (1:1:1)
T ₁₄	1500ppm IBA + garden soil + sand + FYM+cocopeat (1:1:1:1)
T ₁₅	1500ppm IBA + garden soil + sand+ vermicompost +cocopeat (1:1:1:1)
T ₁₆	Control (without IBA+garden soil)

were planted in different growth/soil media with a total of sixteen treatment combinations as follows:

The treated cuttings were planted in the polythene bags by incorporating a different media mixture and kept under the shade of a tree. The medium was prepared well and drenched with chlorpyrifos 20EC solution to avoid the attack of termites. The holes for planting the cuttings were made in the poly bags with the help of iron rod so as to avoid any damage to cuttings. While planting, about 2/3rd portion of the cuttings were buried in the rooting medium, leaving 1/3rd portion exposed to the environment.

2.5. Observations Recorded

The various observation i.e. days to first sprout, number of sprouts, sprouting percentage, plant height(cm), number of shoot, shoot length(cm), shoot diameter(mm), number of leaves, leaf length(cm), leaf breadth(cm), rooting (%), number of primary roots, longest root length(cm), thick root diameter(mm), fresh root weight(gm), dry root weight(gm), fresh shoot weight(gm), dry shoot weight(gm), root spread

(cm) and survival (%) were recorded after planting of cutting.

2.6. Statistical Analysis

The recorded data were statistically analysed by using two-way analysis of variance (ANOVA) with a Completely Randomised Design (CRBD) as suggested by Gomez and Gomez (2010). Experimental data were statistically analysed using SPSS-30 software.

3. RESULTS AND DISCUSSION

3.1. Number of Days Taken for Sprouting

Punjab Baramasi lemon cuttings treated with IBA 1500 ppm and planted in garden soil+sand+vermicompost :cocopeat (1:1:1:1) medium recorded the minimum days for sprouting (5.02 days) (Table1). The longest days for sprouting (28.50 days) were observed in control treatment. Rajangam et al. (2022) also reported that acid lime cutting treated with IBA 2000 ppm recorded early sprouting as compared to untreated ones. It might be due to wood maturity of cutting which probably reserves high starch and sugar (Singh et al., 2013). Lalhrwaitluanga et al. (2022) also reported the minimum number of days taken to sprout in Assam lemon stem cuttings under IBA 800ppm+soil + sand + vermicompost+ cocopeat (1:1:1:1) treatment. This might be due to presence of endogenous auxins in cuttings might have brought early breakage of bud dormancy and results in early bud sprouting (Iqbal et al., 1999). Chandramouli (2001) observed that the increase in the concentration of IBA significantly decreased the number of days to first sprouting of cuttings and earliness in sprouting might be due to better utilization of stored carbohydrates, nitrogen and other factors with the help of growth regulators. The earliest number of days taken to first bud sprouting may be caused by the downward transfer of carbohydrates and auxin build-up inside of cuttings for the completion of physiological processes. Similar findings were also reported by Patel et al. (2018), Malakar et al. (2019), Kumar and Singh (2020) and Patel et al. (2021). Kumar et al. (2015) also reported minimum days were taken to sprouting in lemon cultivar Pant Lemon-1 cuttings treated with 800ppm IBA and planted in garden soil + sand + vermicompost (1:1:1). Likewise, Saini et al. (2022) noted that pomegranate cultivar Bhagwa stem cuttings treated with IBA 2000 ppm and planted in the combination of cocopeat, perlite and vermicompost growth media in ratio of 4:1:1 resulted in minimum number of days taken for sprouting. Amrutha and

Table 1. Effect of plant growth regulator and soil media on shoot growth parameters of Punjab Baramasi lemon [*Citrus limon* (L.) Burm]

Indole-3-butyric acid (IBA)	Soil media combinations					
	Garden soil+sand (1:1)	Garden soil+sand+farmyard manure (1:1:1)	Garden soil + sand + vermicompost (1:1:1)	Garden soil+sand+farmyard manure + cocopeat (1:1:1:1)	Garden soil +sand+vermicompost+cocopeat (1:1:1:1)	
Days taken to sprout						
500ppm	-	19.65	18.35	17.48	15.56	14.42
1000ppm	-	13.05	12.35	11.26	10.75	9.62
1500ppm	-	9.10	8.50	8.02	7.52	5.02
Control	28.50	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.97		Soil media (B): 1.08	(A x B) :0.01	
Number of sprouts						
500ppm	-	17.50	18.30	18.74	17.35	17.82
1000ppm	-	18.10	19.21	20.16	21.35	22.12
1500ppm	-	22.75	23.52	24.70	26.25	30.52
Control	10.10	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.76		Soil media (B): 0.85	(A x B) :1.70	
Sprouting (%)						
500ppm	-	50.32	52.18	53.10	55.52	58.45
1000ppm	-	60.15	62.36	65.28	68.05	71.56
1500ppm	-	75.10	78.65	82.15	85.62	95.56
Control	30.54	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.46		Soil media (B): 1.63	(A x B) :3.25	
Plant height(cm)						
500ppm	-	50.52	52.45	53.30	54.52	55.12
1000ppm	-	56.06	58.10	58.85	59.12	59.56
1500ppm	-	60.36	62.28	63.65	65.42	70.72
Control	45.52	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.44		Soil media (B): 1.61	(A x B) :3.22	
Number of shoots						
500ppm	-	6.32	7.85	8.42	8.91	9.54
1000ppm	-	10.10	10.52	11.16	11.41	11.66
1500ppm	-	12.28	12.65	13.16	13.72	16.85
Control	3.50	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.75		Soil media (B): 0.34	(A x B) :1.67	
Shoot length(cm)						
500ppm	-	19.50	20.21	20.60	21.10	21.56
1000ppm	-	22.22	22.75	23.35	25.36	26.15
1500ppm	-	27.02	27.65	29.06	31.10	36.52
Control	15.02	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.17		Soil media (B): 1.31	(A x B) :2.62	
Shoot diameter (mm)						
500ppm	-	4.12	4.51	4.86	5.24	5.61
1000ppm	-	6.02	6.30	6.65	7.21	7.40
1500ppm	-	7.82	8.35	8.60	10.25	14.05
Control	2.50	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.63		Soil media (B): 0.71	(A x B) :1.42	

Patel (2025) also noted that exogenous application of IBA 1500 ppm to dragon fruit stem cuttings with a media combination of soil + sand + cocopeat significantly reduced the minimum days for initiation of sprouts. Likewise, Mehra et al. (2019) also noted that persimmon cultivar Fuyu cuttings treated with IBA 6000ppm and planted in soil+FYM(1:2) rooting media taken minimum number of days to sprouting with maximum number of sprouts.

3.2. Number of Sprouts per Cutting and Percent Sprouting

Punjab Baramasi lemon cuttings treated with IBA 1500ppm and planted in garden soil+sand+vermicompost:cocopeat(1:1:1:1) medium recorded maximum number of sprouts per cutting (30.52) & sprouting percentage (95.56), and the control treatment was recorded minimum sprouts(10.10) & sprouting percentage (30.54) per cutting (Table 1). Moisture holding capacity of cocopeat might have improved the sprouting of shoots and might be due to better aeration, nutrient availability, enzymatic activity and moisture retention capacity of substrate. This might be attributed to auxin-stimulated cell division at sprout union initiation (Seiar, 2017). Hydrolysis and translocation of carbohydrates and nitrogenous substances result in the accelerated cell growth and division, which might be triggered by the use of auxins. It also tends to promote the histological features like formation of callus and tissues and then further differentiation of vascular tissues (Singh, 2017). Similarly, Rajangam et al. (2022) observed highest number of sprouts in acid lime stem cuttings cultivar PKM1 treated with IBA 2000 ppm and the lowest number of sprouts was found in control. It might be due to a high accumulation of callus formation in cuttings with an optimum dose of auxin, resulting in the highest percentage of sprouted cuttings and leading to an increased percentage of branches per shoot. It is a fact that IBA directly affects the number of root and root growth and indirectly affects shoot length, which may result in a high number of branches per shoot (Stefancic et al., 2005). The more number of sprout formation with the growth regulator might be due to the vigorous root system which increased nutrient uptake under the combined influence of IBA application. Similarly, highest number of sprouts were reported in IBA 500 ppm treated cuttings of acid lime cultivar Kagzi (Malakar et al., 2019). Kumar et al. (2015) also reported maximum percentage of sprouting in Pant Lemon-1 cuttings with 800ppm IBA + garden soil + sand + vermicompost (1:1:1) treatment. Similarly, shoot

parameters like number of sprouts and sprouted percentage were significantly highest in IBA 2000ppm treated stem cuttings of pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost(4:1:1) growth media as reported by Saini et al.(2022). Amrutha and Patel (2025) also noted that exogenous application of IBA 1500 ppm to dragon fruit stem cuttings with a media combination of soil + sand + cocopeat significantly increased sprout length and number of sprouts per cutting.

3.3. Plant Height

From Table 1, it has been noted that the maximum plant height (70.72cm) was recorded in Punjab Baramasi lemon cuttings treated with 1500ppm IBA and planted in soil+sand+vermicompost:cocopeat (1:1:1:1) medium and the minimum plant height(45.52cm) was noted in the control treatment. Optimum dose of IBA on plant height might be attributed to the fact that cuttings treated with this concentration had an improved root system, thus absorbed more nutrients, which helped in better stem growth and plant height. A similar explanation had been suggested by Chauhan and Maheshwari (1970). Likewise, plant height was highest in IBA 2000ppm treated stem cuttings of pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost (4:1:1) as noted by Saini et al. (2022). Similarly, Rajangam et al. (2022) observed that in acid lime cultivar PKM1 stem cuttings, the highest plant height was recorded in the IBA 2000 ppm treatment and the lowest plant height was noticed in the control. Singh and Rattanpal (2017) also reported that IBA is the active inhibitor of axillary bud break on developing shoots, and it stimulates shoot initiation. Indole-3-butyric acid treatment enhanced rooting, plant growth and produced taller and healthy plants (Umrao, 1999). Lalhrualtuanga et al. (2022) also reported maximum plant height of assam lemon stem cuttings in IBA 800ppm + soil + sand+ vermicompost + cocopeat (1:1:1:1) treatment. Similar results of effect of IBA and growing media in lemon stem cuttings were also observed by Patel et al. (2018).

3.4. Number of Shoots

It has been observed that Punjab Baramasi lemon stem cutting treated with 1500ppm IBA and planted in soil+sand+vermicompost:cocopeat (1:1:1:1) medium produced maximum(16.85) number of shoots per plant, and the minimum (3.50) number of shoots per plant was recorded in the control treatment (Table 1). Similarly, Lalramhluna and Prasad (2016) reported the maximum

number of branches in air-layered IBA 2000 ppm-treated assam lemon and the minimum number of branches in the control treatment. Likewise, Lalhrualtuanga et al. (2022) noted maximum number of branches in IBA 800ppm treated assam lemon cuttings planted in soil + sand + vermicompost+ cocopeat (1:1:1:1). The maximum number of branches due to higher number of roots, if there will be more number of food materials and other necessary minerals will be higher, in this way plant will take higher growth which results more number of branches. These findings are in accordance with the results reported in IBA treatment in air-layered guava by Maurya et al. (2022). An increase in the number of shoots per plant might be due to the activation of auxin in vegetative parts by using auxin. Similar results were reported earlier in IBA-treated Kagzi lime by Kumar and Singh (2020). Kumar et al. (2015) also reported that stem cutting of lemon treated with 1800ppm IBA and planted in garden soil + sand + vermicompost (1:1:1) produced maximum number of shoots per cutting, while minimum number of shoots were recorded per cutting was recorded under control treatment. Likewise, ficus cuttings treated with 2000 mg/litre IBA and planted in growing media of sand + cocopeat + vermicompost (1:1:1) recorded a significantly maximum number of shoots (Zala and Masu, 2025).

3.5. Shoot Length and Shoot Diameter

From Table 1, Punjab Baramasi lemon stem cuttings treated with 1500ppm IBA and planted in soil+sand +vermicompost:cocopeat (1:1:1:1) medium produced the longest shoot length (36.52cm) with highest shoot diameter (14.05mm) and the control treatment cuttings retained the shortest shoot length(15.02cm) with the lowest shoot diameter(2.50mm). Rajangam et al. (2022) also reported that IBA 2000ppm treated acid lime stem cuttings cultivar PKM1 showed the highest shoot length, while the minimum shoot length was recorded in the control . Similarly, maximum shoot length was reported in IBA 800ppm treated cuttings of assam lemon and planted in soil + sand + vermicompost + cocopeat (1:1:1:1) growing media (Lalhrualtuanga et al., 2022). Likewise, Lalramhluna and Prasad (2016) observed the maximum length of branches in the IBA 2000ppm treated air-layered assam lemon, which was statistically superior to the control. As a growth promoter IBA promotes cell division, which results in early rooting leading towards efficient absorption of mineral nutrients and hence maximizes shoot length. This might be due to increased concentrations and activity of IBA, which

causes hydrolysis and translocation of carbohydrates and nitrogenous substances at the cellular level at the base of cuttings, resulting in accelerated cell elongation and cell division, causing extension in shoot length under favourable environmental conditions (Singh and Singh, 2011). Mehra et al. (2019) also reported that persimmon cultivar Fuyu cuttings treated with IBA 6000ppm and planted in soil+FYM (1:2) rooting media resulted in the highest length and diameter of sprouts. Likewise, ficus cuttings treated with IBA 2000 mg/litre and planted in growing media of sand + cocopeat + vermicompost (1:1:1) recorded a significantly maximum length of the longest shoot (Zala and Masu, 2025). It has been noted that different treatments of growth regulator and soil media significantly affect the shoot diameter (Table1). Punjab Baramasi lemon cuttings treated with IBA 1500 ppm and planted in soil+sand+vermicompost:cocopeat (1:1:1:1) growth media had the highest shoot diameter(14.05mm), and control treatment cuttings had the lowest shoot diameter(2.50mm). This might be due to the frequency at which the vascular bundles absorb water and nutrients is closely correlated with the increase in plant height and diameter. The greater rooting percentage in IBA treated plants enable the plant to absorb more nutrients, increasing plant height and shoot diameter (Siddiqui and Hussain, 2007 ; Kasim et al., 2009). This finding is also accordance with Maniriho et al. (2021) that reported thicker stems in peach by hard wood cuttings treated with IBA. Similarly, Kumar et al. (2024) reported that maximum shoot diameter in fig cuttings treated with IBA 2000ppm. Similarly, maximum stem diameter was reported in IBA 800 ppm-treated cuttings of Assam lemon and planted in soil + sand + vermicompost + cocopeat (1:1:1:1) growing media (Lalhrualtuanga et al., 2022). The girth of the plants was also much bigger in the stem cuttings of peach treated with IBA 2400 ppm (Pathlan et al., 2022). Similar results were also recorded in IBA treated fig cuttings by Singh and Rattanpal (2017). Similarly, shoot diameter was highest in IBA 2000ppm treated stem cuttings of pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost (4:1:1) growth media as observed by Saini et al.(2022).

3.6. Number of Leaves, Leaf Length and Leaf Breadth

The maximum number of leaves (143.65) was observed in Punjab Baramasi lemon cuttings treated with IBA 1500ppm grown in soil+sand+vermicompost:cocopeat (1:1:1:1) media as compared to the rest of the treatments (Table 2). Similarly, the maximum number of leaves per

Table 2. Effect of plant growth regulator and soil media on leaf and root growth parameters of Punjab Baramasi lemon [*Citrus limon* (L.) Burm]

Indole-3-butyric acid (IBA)		Soil media combinations				
		Garden soil+sand (1:1)	Garden soil+sand+farmyard manure (1:1:1)	Garden soil + sand + vermicompost (1:1:1)	Garden soil+sand+farmyard manure + cocopeat (1:1:1:1)	Garden soil +sand+vermicompost+cocopeat (1:1:1:1)
Number of leaves						
500ppm	-	80.48	83.11	85.08	88.28	90.42
1000ppm	-	92.18	95.21	101.62	105.16	110.11
1500ppm	-	117.28	123.05	128.32	133.12	143.65
Control	65.10	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.49		Soil media (B): 1.67	(A x B) : 3.33	
Leaf length (cm)						
500ppm	-	9.05	9.30	9.48	9.71	10.05
1000ppm	-	10.60	11.05	11.31	11.50	11.76
1500ppm	-	12.15	12.40	12.72	13.25	16.56
Control	7.02	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.70		Soil media (B): 0.78	(A x B) : 1.55	
Leaf breadth (cm)						
500ppm	-	5.50	5.72	6.05	6.41	6.65
1000ppm	-	6.81	6.96	7.35	7.48	7.61
1500ppm	-	7.83	8.05	8.36	8.51	10.46
Control	4.50	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.78		Soil media (B): 0.01	(A x B) :0.01	
Rooting (%)						
500ppm	-	51.18	53.10	56.13	58.45	60.25
1000ppm	-	63.38	65.05	67.51	70.22	74.52
1500ppm	-	77.76	82.65	86.06	90.52	96.10
Control	35.25	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.76		Soil media (B): 1.97	(A x B) : 3.94	
Number of primary roots						
500ppm	-	20.36	22.22	23.62	25.58	26.08
1000ppm	-	28.62	30.38	32.35	35.20	38.45
1500ppm	-	40.56	43.10	45.18	47.51	55.16
Control	13.52	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.75		Soil media (B): 1.95	(A x B) :3.91	
Longest root length (cm)						
500ppm	-	18.36	19.10	19.50	20.08	20.74
1000ppm	-	21.28	23.45	24.23	26.68	27.05
1500ppm	-	29.10	31.65	33.42	34.65	40.56
Control	13.02	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.15		Soil media (B): 1.29	(A x B) :2.58	
Thick root diameter (mm)						
500ppm	-	0.65	0.82	1.05	1.42	1.73
1000ppm	-	1.95	2.16	2.41	2.70	3.02
1500ppm	-	3.32	3.55	3.86	4.05	6.15
Control	0.30	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.20		Soil media (B): 0.22	(A x B) :0.45	

cutting was observed in the IBA 1000 ppm treatment in the pomegranate cultivar Bhagwa, as reported by Satnam et al. (2022). Indole-3-butyric acid treated cuttings resulted in longer, healthier roots that aid in the absorption of water and nutrients, significantly impacting the cuttings ability to produce more leaves. There may be more roots, branches and plant height with IBA treatment which results in more leaves per cutting (Bowden et al., 2022). Similarly, stem cuttings treated with IBA produced more number of leaves in lemon (Maurya et al., 2022) and pomegranate (Kaushik et al., 2020). Similarly, the maximum number of leaves was reported in IBA 800 ppm-treated cuttings of assam lemon and planted in soil + sand + vermicompost + cocopeat (1:1:1:1) growing media (Lalhruaitluanga et al., 2022). Number of leaves was also highest in IBA 2000ppm treated stem cuttings of pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost(4:1:1) growth media as observed by Saini et al.(2022). Mehra et al. (2019) also noted that persimmon cultivar fuyu cuttings treated with IBA 6000ppm and planted in soil+FYM(1:2) growth media resulted highest number of leaves. Likewise, ficus cuttings treated with IBA 2000 mg/litre and planted in growing media of sand + cocopeat + vermicompost (1:1:1) recorded a significantly maximum number of leaves (Zala and Masu, 2025). It has been noted that leaf length and breadth of Punjab Baramasi lemon were varied significantly among different IBA and soil media treatments (Table2). Maximum mean leaf length and breadth (16.56cm & 10.46cm respectively) were recorded in treatment IBA 1500 ppm+ soil+sand+vermicompost: cocopeat (1:1:1:1) and lowest leaf length and breadth (7.02cm & 4.50cm respectively) were observed in control. Similarly, Singh and Rattanpal (2017) noted maximum leaf length and breadth in fig cuttings treated with IBA 1250 ppm as compared to control. Likewise, Ausari et al. (2023) reported maximum leaf area in grape cultivar Pusa Navrang cuttings treated with IBA 6000 ppm. Likewise, highest leaf length and leaf breadth were reported in IBA 3000 ppm treated Flordaguard peach cuttings (Kaur, 2017). This may be due to IBA, which produced healthier, longer roots and hence absorbed more nutrients and water content, which has a great influence on leaf growth. Treatment of IBA 1000 ppm to grape cuttings had a maximum mean leaf area; however, a minimum mean leaf area was noticed in the control(Ghangale et al., 2021).The relative humidity and optimum light intensity, which are the important factors in the development of leaves, coupled with auxin content, activated the synthesis of more carbohydrates in the leaves,

which might have resulted in the elongation of leaves through cell division and cell elongation, resulting in more leaf area per cutting when compared to all other treatments. Similar results were reported by Shao et al. (2018) in Chinese jujube and by Abhinav et al. (2016) in grapes. Mehra et al. (2019) also noted that persimmon cultivar fuyu cuttings treated with IBA 6000ppm and planted in soil+FYM(1:2)rooting media resulted highest leaf area.

3.7. Dry and Fresh Weight of Shoot

Maximum shoot fresh weight (33.10gm) and dry weight (21.65gm) were reported in the Punjab Baramasi lemon cuttings treated with IBA 1500 ppm and planted in soil+sand+vermicompost:cocopeat (1:1:1:1) growth media as compared to other treatments(Table 2). Samim et al. (2018) observed that IBA 5000 ppm treated stem cuttings of barbados cherry showed maximum fresh and dry weight of shoot. Indole-3-butyric acid treatment might have increased the number of shoots, resulting in increased accumulation of fresh and dry weight in shoots. Likewise, shoot fresh weight and shoot dry weight were highest in IBA 2000ppm treated stem cuttings of pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost (4:1:1) growth media as noted by Saini et al.(2022). Ficus cuttings treated with IBA 2000 mg/litre and planted in growing media of sand + cocopeat + vermicompost (1:1:1) recorded the highest fresh and dry weight of shoot (Zala and Masu, 2025).

3.8. Rooting Percentage, Number of Primary Roots, Longest Root Length, Thick Root Diameter, Fresh Root Weight, Dry Root Weight and Root Spread

From Table 2 and 3, it was observed that maximum rooting (96.10%), number of primary roots (55.16), longest root length (40.56cm), thick root diameter (6.15mm), fresh root weight (16.45gm), dry root weight (11.56gm) and root spread(32.65cm) were noted in Punjab Baramasi lemon cuttings treated with IBA 1500 ppm and planted in soil+sand+vermicompost:cocopeat (1:1:1:1) growth media as compared to rest of treatments. Similarly, Kaur and Singh (2022) observed that per cent rooting, number of roots per cutting, root length, fresh weight of roots and dry weight of roots were significantly highest in pomegranate cultivar Bhagwa cuttings treated with 1000 ppm IBA. The improved rooting in cuttings treated with auxin might be due to enhanced hydrolytic activity, which favours the formation of high carbohydrate levels and low nitrogen levels, leading to

Table 3. Effect of plant growth regulator and soil media on root and shoot growth parameters of Punjab Baramasi lemon [*Citrus limon* (L.) Burm]

Indole-3-butyrac acid (IBA)	Soil media combinations					
	Garden soil+ sand (1:1)	Garden soil+ sand+farmyard manure (1:1:1)	Garden soil + sand + vermicompost (1:1:1)	Garden soil+sand+ farmyard manure + cocopeat (1:1:1:1)	Garden soil +sand+ vermicompost+ cocopeat (1:1:1:1)	
Root spread (cm)						
500ppm	-	20.38	20.73	21.14	21.38	21.72
1000ppm	-	22.10	22.31	23.42	24.10	24.62
1500ppm	-	25.15	25.52	25.86	27.22	32.65
Control	15.20	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.93		Soil media (B): 1.03		(A x B) :2.07
Fresh weight of roots (gm)						
500ppm	-	8.18	9.10	9.32	9.65	10.12
1000ppm	-	10.56	11.02	11.25	11.76	12.25
1500ppm	-	12.76	13.10	13.34	13.70	16.45
Control	5.53	-	-	-	-	-
CD (5%)		Plant growth regulator (A):0.79		Soil media (B): 0.89		(A x B) :0.01
Dry weight of roots (gm)						
500ppm	-	3.50	3.76	4.05	4.30	4.60
1000ppm	-	5.10	5.55	5.82	6.12	6.34
1500ppm	-	6.60	6.86	7.60	8.71	11.56
Control	1.50	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.60		Soil media (B): 0.70		(A x B) :1.40
Fresh weight of shoot(gm)						
500ppm	-	19.10	19.64	20.35	21.45	22.11
1000ppm	-	22.65	23.08	23.40	23.85	24.46
1500ppm	-	24.95	25.61	27.12	29.05	33.10
Control	14.21	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.81		Soil media (B): 0.91		(A x B) :1.82
Dry weight of shoot (gm)						
500ppm	-	11.12	12.18	12.45	13.76	14.28
1000ppm	-	14.60	15.06	15.36	15.68	16.04
1500ppm	-	16.46	16.72	17.26	18.71	21.65
Control	7.10	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 0.68		Soil media (B): 0.76		(A x B) :1.52
Survival (%)						
500ppm	-	40.12	43.14	45.65	47.56	50.22
1000ppm	-	52.10	55.62	60.46	63.05	67.15
1500ppm	-	71.05	75.12	80.18	86.30	97.25
Control	25.10	-	-	-	-	-
CD (5%)		Plant growth regulator (A): 1.67		Soil media (B): 1.87		(A x B) :3.74

increased root formation (Narula, 2018). The production of a good amount of roots in auxin-treated cuttings pertains to the fact that the auxins promoted cell division and their elongation which led to differentiation of cambial initials into root primordia and in the mobilization of reserve food material to sites of root initiation, thereby giving a higher number of roots per cutting (Sharma, 1999). Auxins might have helped in amplifying rooting and boosting the length of roots, as the root elongation stage is very responsive to auxin concentrations (Hartmann et al., 2002). The availability of carbohydrates is often considered solely as an energy source and a carbon skeleton for driving root development (Correa et al., 2005). The increase in root weight might be due to the reserved food materials in the cuttings; the translocation of reserved carbohydrates may have also helped in better root growth and weight. Maximum root formation zone and rooting percentage, which might have been due to increased cell division and their differentiation under the influence of rooting chemicals, enhanced hydrolysis of nutritional reserves resulting into increased root formation zone (Singh, 2014). Likewise, Lalhruitluanga et al. (2022) reported that IBA 800ppm treated cuttings of assam lemon and planted in soil + sand + vermicompost + cocopeat (1:1:1:1) growing media showed maximum values of length of tap root, root spread, fresh and dry weight of root. Samim et al. (2018) also reported that IBA 5000 ppm treated stem cuttings of barbados cherry showed maximum values of percentage of rooting, number of root, average root length, length of longest root, number of primary and secondary roots, fresh and dry weight of root and root volume. Kumar and Kumar (2022) also reported that the significantly maximum length and diameter of primary root were recorded with the treatment of 800 ppm IBA + garden soil + sand + vermicompost (1:1:1) in stem cutting of lemon cultivar Pant Lemon-1. Likewise, Amrutha and Patel (2025) also noted that exogenous application of IBA1500 ppm to dragon fruit stem cuttings with media combination of soil + sand + cocopeat significantly increased the length of roots and rooting percentage. Mehra et al. (2019) also noted that persimmon cultivar Fuyu cuttings treated with IBA 6000 ppm and planted in soil + FYM(1:2) rooting media resulted in maximum values of number of rooted cuttings, root length and survival of cuttings. Likewise, ficus cuttings treated with IBA 2000 mg/litre and planted in growing media of sand + cocopeat + vermicompost (1:1:1) was recorded significantly maximum length of longest root as well as fresh and dry weight of root (Zala and Masu, 2025).

3.9. Survival Percentage

It is evident from the data shown in Table 2 that growth regulator and soil media had significant effect on survival percentage of cuttings. The highest survival percentage (97.25) of cuttings of Punjab Baramasi lemon was found in the cuttings treated with 1500 ppm IBA and planted in garden soil+sand+vermicompost+cocopeat(1:1:1:1) growth medium, while the minimum survival percentage (25.10) of cuttings was observed in the cuttings planted under the control treatment. Significant effect of growth regulators on survival percentage was due to root induction capacity of auxin and better root growth in rooting media which help in production of adventitious roots and thereby increase in the survival of plants. It also might be probably due to root and root length at this concentration which could have caused the absorption of nutrients and the fact that rooting co-factors and their balance with auxin and nutritive substances. Likewise, the maximum survival percentage of assam lemon stem cuttings was recorded under the IBA 800 ppm + soil + sand + vermicompost + cocopeat (1:1:1:1) treatment, as reported by Lalhruitluanga et al. (2022). In addition to that, when using growth regulators, boosting the rooting can be attributed to the favourable environmental conditions with higher photosynthetic activity, which promoted better rooting in cutting and survival percentage. The facilitated absorption of nutrients and moisture from soil and better growth might have developed capacity to withstand for a longer period (Ram et al., 2005). Auxin may have induced favourable environment for root and shoot development and enhanced survival (Constanzi et al., 1988). Similarly, the survival percentage was highest in IBA 2000 ppm-treated stem cuttings of the Pomegranate cultivar Bhagwa planted in cocopeat, perlite and vermicompost (4:1:1) growth media, as reported by Saini et al. (2022). This could be due to the availability of good amount of nutrients in vermicompost growth media. Amrutha and Patel (2025) also noted that exogenous application of IBA1500 ppm to dragon fruit stem cuttings and planted in media combination of soil + sand + cocopeat significantly increased the survival percentage.

4. CONCLUSION

The treatment combination of 1500 ppm IBA + garden soil + sand + vermicompost + cocopeat (1:1:1:1) for stem cuttings of Punjab Baramasi lemon the month of February was found to be best for rooting, growth, and survival percentage in the Gurdaspur sub-mountainous zone of

Punjab. This treatment combination gave the significant response with respect to all the shoot and root growth parameters. Moreover, the above mentioned treatment combination promoted faster multiplication and early production of nursery plants and also provides true-to-type and elite planting material of Punjab Baramasi lemon to the fruit growers of the sub-mountainous regions of Punjab.

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CRediT authorship contribution statement

Sukhjit Kaur: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation Visualization, Writing – original draft, Writing - review and editing

Conflict of interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

Authors declare that they have not used any generative AI and AI-assisted technologies in the writing process, figures/ images and artwork.

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Botanical Biopesticides for Climate Resilient Agriculture: From Phytochemicals to Agroecosystems-A Review

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ABSTRACT: Global agricultural systems face mounting pressure from climate change and insect pest damage, which destroys 20–40% of global crop production annually. Synthetic chemical pesticides, the conventional response, have incurred high ecological costs through environmental contamination, toxicity to non-target organisms and the rapid evolution of resistance in pest populations. Botanical biopesticides, derived from the rich phytochemical arsenals of medicinal plants, offer a scientifically validated alternative for building climate-resilient agricultural systems. This review synthesises current knowledge on three critical dimensions: (1) the chemical complexity of plant secondary metabolites, including terpenoids, alkaloids and phenolics, which confer multi-target mechanisms of action against pests; (2) advanced formulation technologies such as microencapsulation and nano-formulations that enhance field persistence and climate resilience; and (3) strategic integration into integrated pest management (IPM) systems for insecticide resistance management. Evidence demonstrates that botanical biopesticides, when formulated and deployed strategically, provide broad-spectrum efficacy across insect pests, fungal pathogens and nematodes. Significant barriers, including climate-driven variability in phytochemical composition, inherent instability under environmental stressors and regulatory frameworks designed for single-molecule synthetics, currently limit widespread adoption. A coordinated advance across molecular biology, formulation science and regulatory reform is essential to realise the full potential of botanical biopesticides in sustainable, climate-adaptive agriculture.

Keywords: Biopesticides, Climate change, Formulation technology, Insecticide resistance management, Integrated Pest Management, Phytochemicals.

1. INTRODUCTION

1.1. The Imperative for Sustainable Pest Management in a Changing Climate

Global agricultural systems confront an unprecedented crisis, namely ensuring food security for a growing population while adapting to the profound impacts of climate change (Dhillon & Gujar, 2010; Harvey et al., 2023). Insect pests represent a persistent and significant biotic threat, destroying an estimated 20–40% of global crop production annually (Skendi et al., 2021). Rising global

mean temperatures are expected to increase crop losses by an additional 10–25% per degree Celsius for staple crops including rice, maize and wheat (Furlong & Zalucki, 2017). As ectothermic organisms, insects respond to climate warming through accelerated development rates, increased voltinism (number of generations per season), poleward range expansion and extended activity periods (Furlong & Zalucki, 2017 and Harvey et al., 2023).

For over five decades, intensive application of synthetic chemical pesticides has been the primary response to pest threats. While delivering short-term gains in crop protection,

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this paradigm has incurred severe environmental costs: soil and water contamination, toxicity to non-target organisms, including vital pollinators and natural enemies, and direct health risks to agricultural workers and consumers (Stehle & Schulz, 2015; Rezende-Teixeira et al., 2022). Furthermore, the relentless selection pressure exerted by single-target synthetic pesticides has driven the rapid evolution of resistance in hundreds of pest species (Hawkins et al., 2019). This chemical-dependent paradigm is itself vulnerable to climate change. Warmer temperatures and altered precipitation patterns accelerate volatilisation, photodegradation and wash-off of synthetic pesticides, reducing persistence and necessitating more frequent or higher-dose applications (Chen & McCarl, 2001; Koleva & Schneider, 2009; Delcour et al., 2015).

1.2. Botanical Biopesticides: A Scientifically Grounded Alternative

These concerns have catalysed global demand for safer, more sustainable alternatives, creating fertile ground for renewed scientific interest in botanical insecticides derived

from medicinal and aromatic plants (Isman, 2006). Medicinal plants have evolved over millennia to produce complex arsenals of secondary metabolites, specifically terpenoids, alkaloids and phenolics, as natural defences against herbivory and pathogens (Isman & Grieneisen, 2014). The fundamental advantage of botanical agents lies in their inherent chemical complexity. Unlike single-molecule synthetics, crude plant extracts or essential oils contain a variety of bioactive compounds that act on multiple physiological and behavioural targets in pests simultaneously (Koul et al., 2008). This multimodal action significantly reduces the likelihood of resistance development, a crucial attribute given that shorter pest generation times may accelerate resistance evolution in warming scenarios (Matzrafi, 2019). The diverse biological activities of selected botanical agents are illustrated in Table 1, and field-level practices of botanical biopesticide application are shown in Figure 1.

The novelty of this review lies in its integrative, climate-focused perspective. Unlike earlier reviews that addressed botanical biopesticides in isolation, the present work

Table 1. Major classes of phytochemicals in botanical biopesticides with representative examples and primary modes of action

Compound class	Representative example	Source plant	Primary mode of action	Target insect pest/Pathogen
Terpenoids	Azadirachtin	<i>Azadirachta indica</i> A. Juss.	Insect growth regulator; antifeedant; endocrine disruption (ecdysone and JH antagonism)	Broad-spectrum insects (aphids, caterpillars, beetles)
	Pyrethrins	<i>Chrysanthemum cinerariifolium</i> (Trevir.) Bocc.	Voltage-gated sodium channel modulation; rapid knockdown	Aphids, mosquitoes, stored product pests
	1,8-Cineole	<i>Eucalyptus</i> spp. L.	Fumigant; membrane disruption; ROS induction in fungi	Stored product weevils; <i>Alternaria</i> spp.; fungi
	Menthol/ Menthone	<i>Mentha</i> spp. L.	Contact neurotoxin; GABA receptor modulation; repellent	Diverse insect orders
	Toosendanin	<i>Melia azedarach</i> L.	Antifeedant; insect growth disruption	Lepidopteran pests
Alkaloids	Nicotine	<i>Nicotiana tabacum</i> L.	nAChR agonist; continuous depolarisation; paralysis	Soft-bodied insects; aphids
	Phytolacca alkaloids	<i>Phytolacca dodecandra</i> L'Her.	Contact and ingestion toxicity	Termites; molluscs
Phenolic Compounds	Eugenol	<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	AChE inhibition; mitochondrial Complex I inhibition; fungal membrane disruption	Mites; fungi; insects
	Flavonoids	Various medicinal plants	Antifeedant; digestion inhibitor; antioxidant interference	Herbivorous insects
	Tannins	Various plant families	Protein precipitation; digestive enzyme inactivation; nutrient absorption reduction	Herbivorous insects; nematodes

specifically examines how climate change simultaneously increases the need for botanicals and complicates their deployment through phytochemical plasticity and formulation challenges. It bridges the gap between molecular mechanisms, advanced delivery technologies and agroecosystem-based IPM strategies, offering a comprehensive roadmap that is particularly relevant to tropical and subtropical agricultural contexts such as India, where climate variability and biodiversity of medicinal plants converge.

1.3. Scope and Structure of This Review

However, the transition from promising laboratory results to widespread field adoption is fraught with challenges: variability in chemical composition driven by climate change, rapid environmental degradation and regulatory frameworks designed for single chemical entities have historically limited commercial success (Isman & Grieneisen, 2014). This review critically examines the state of the art of botanical biopesticides within the context of climate change, providing deep mechanistic understanding of their potential while addressing the scientific, technological and regulatory dimensions essential for mainstream adoption.

Key hypotheses addressed: (1) Complex phytochemistry provides intrinsic advantages for resistance management; (2) Advanced formulation technologies can adequately address environmental instability; and (3) Strategic integration into IPM systems represents the pathway to climate-resilient pest management.

2. METHODOLOGY

This review was conducted following the broad principles of systematic and narrative review methodology as given by PRISMA (Figure 2).

Review articles were consulted for contextual background, but primary research studies, book chapters and technical reports were preferentially used for specific data and mechanistic claims. The review is organised thematically, covering phytochemical diversity (Section 3), molecular mechanisms of action (Section 4), broad-spectrum efficacy and climate effects (Section 5), formulation technology (Section 6), IPM integration (Section 7) and regulatory and commercial dimensions (Section 8).

3. THE PHYTOCHEMICAL PROPERTIES OF PLANTS

Plants, through a co-evolutionary arms race with



Figure 1. Integrated sustainable agriculture: Bio-Pesticide preparation, field application, agroforestry, and crop cultivation systems. The composite image illustrates the complete workflow of an eco-friendly farming system, beginning with the step-by-step extraction of natural neem leaf bio-pesticides (top centre) and their manual application in the field using protective gear (top right). It showcases the practical integration of sustainable land management through agroforestry plantations (top left) alongside diverse crop cultivation practices, including well-maintained field crops (bottom left) and organized row cropping near greenhouse facilities (bottom right)

herbivores and pathogens, have developed a capacity for chemical defence mediated by secondary metabolites that are not directly involved in primary growth and reproduction but are crucial for environmental interactions. This phytochemical arsenal comprises tens of thousands of unique structures, representing a rich, largely untapped reservoir for the discovery of novel biopesticides (Isman & Grieneisen, 2014). The major classes of these compounds, along with

representative examples, are summarised in Table 1.

3.1. Terpenoids

Terpenoids, derived from the five-carbon isoprene unit, constitute the largest and most diverse class of plant secondary metabolites, with structural diversity spanning physicochemical properties and biological functions (Isman, 2006; Bakkali et al., 2008).

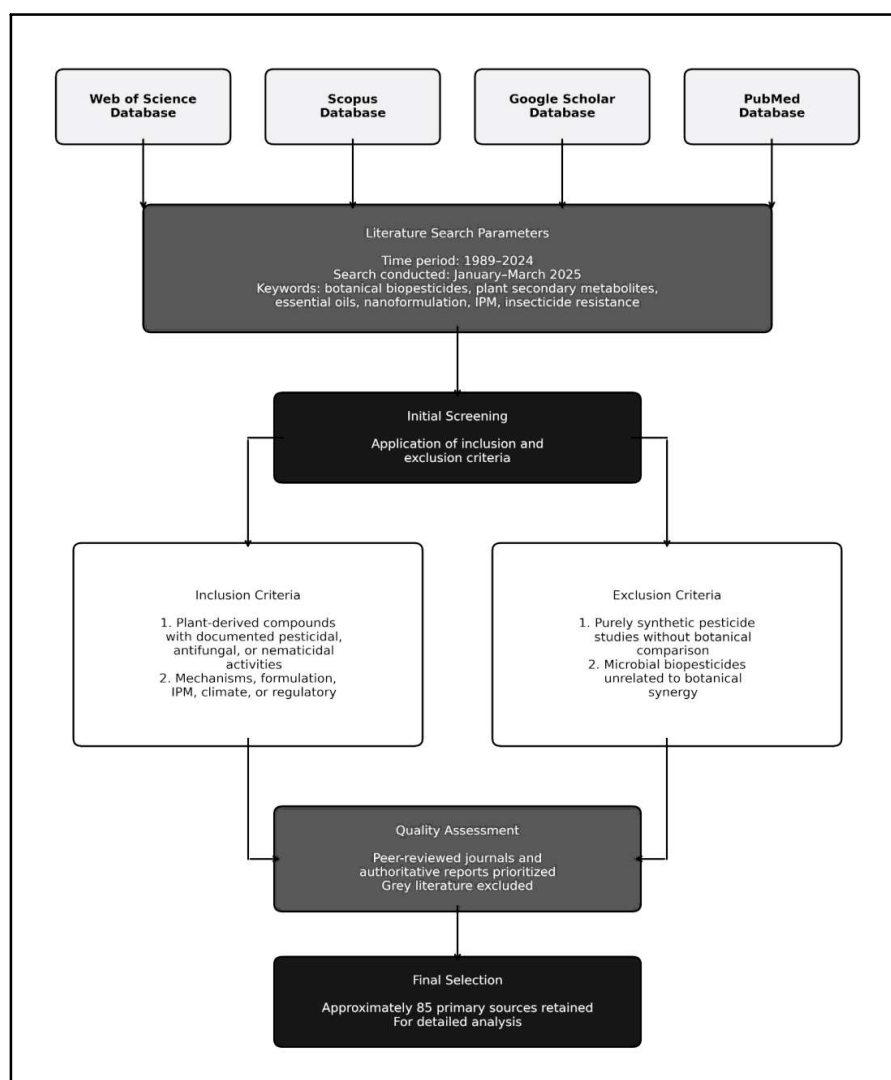


Figure 2. Flowchart of the systematic literature search and selection methodology. Primary databases (Web of Science, Scopus, Google Scholar, and PubMed) were queried using predefined parameters and keywords for the period 1989–2024. The retrieved literature was systematically refined through rigorous inclusion and exclusion criteria, followed by a quality assessment to prioritise peer-reviewed and authoritative reports, ultimately yielding approximately 85 primary sources for detailed analysis

Volatile monoterpenes and sesquiterpenes comprise primary constituents of essential oils. Menthol and menthone from *Mentha* spp. L. (Lamiaceae), 1,8-cineole from *Eucalyptus* spp. L. (Myrtaceae) and citral from *Cymbopogon* spp. Spreng. (Poaceae) are characterised by low molecular weight and high vapour pressure, making them effective fumigants, repellents and contact toxins (Bakkali et al., 2008). Their volatility, beneficial for enclosed space fumigation, contributes to short residual activity in open applications, a challenge exacerbated by rising temperatures.

Non-volatile terpenoids, particularly limonoids (tetranortriterpenoids) abundant in the Meliaceae family, function as potent antifeedants and insect growth regulators. The most studied limonoid is azadirachtin from *Azadirachta indica* A. Juss. (Meliaceae), which acts as a powerful feeding deterrent and disrupts insect endocrine systems through interference with moulting and development (Mordue Luntz & Blackwell, 1993). Other limonoids, such as toosendanin from *Melia azedarach* L. (Meliaceae), also exhibit significant antifeedant and growth regulatory effects (Chen et al., 2005).

3.2. Alkaloids

Alkaloids are structurally diverse nitrogen-containing compounds derived from amino acids that exhibit broad-spectrum pharmacological and toxicological effects (Koul et al., 2008). Nicotine from *Nicotiana tabacum* L. (Solanaceae), historically used since the 17th century, acts as an agonist of the nicotinic acetylcholine receptor in insect nervous systems, causing paralysis and death (Soderlund & Bloomquist, 1989 and Jansen et al., 2010). Its mode of action inspired the development of synthetic neonicotinoids, which are among the most widely used modern insecticides (Bai et al., 1991). Alkaloids from *Phytolacca dodecandra* L'Her. (Phytolaccaceae), known as endod or soapberry, demonstrates potent antitermitic and molluscicidal properties.

3.3. Phenolic Compounds

Phenolic compounds, characterised by hydroxyl groups attached to aromatic rings, include simple phenols, flavonoids and complex tannin polymers with multifaceted defence roles (El-Wakeil, 2013). Eugenol from *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae) and *Ocimum sanctum* L. (Lamiaceae), key essential oil components, possess strong insecticidal, antifungal and neurotoxic properties (Bakkali et al., 2008). Flavonoids act as feeding deterrents, digestive inhibitors, and antioxidants,

thereby reducing herbivore performance. Tannins (high molecular weight polyphenols) bind and precipitate proteins; when ingested, they inactivate digestive enzymes and reduce nutrient absorption, impairing herbivore growth and development.

3.4. Phytochemical Plasticity: How Climate Change

Alters Plant Defences

The phytochemical bioactivity of plants is not static but highly plastic, responding dynamically to environmental cues. Rising atmospheric CO₂, elevated temperatures and altered precipitation patterns modulate plant secondary metabolism, with profound implications for the consistency and efficacy of botanical biopesticides (Zvereva & Kozlov, 2006 and Gherlenda et al., 2016).

Elevated atmospheric CO₂ often stimulates photosynthesis in C3 plants, increasing carbon availability relative to nitrogen. According to the carbon-nutrient balance hypothesis, this excess carbon is allocated to carbon-based secondary metabolites (phenolics and terpenes) (Ibrahim et al., 2011). Temperature and water availability exert powerful control over phytochemical production; plants respond to heat stress and drought by synthesising protective compounds (antioxidants and osmoprotectants), thereby altering overall metabolic profiles and disrupting normal growth cycles and flowering times (Zvereva & Kozlov, 2006).

This climate-induced phytochemical plasticity presents a fundamental paradox. The environmental drivers that make botanicals more necessary simultaneously introduce variability and unpredictability into their chemical composition (Gherlenda et al., 2016; Applequist et al., 2020). Standardisation of extracts becomes complicated, quality control is compromised and regulatory agencies accustomed to consistent active ingredients face evaluation challenges, affecting the entire value chain from cultivation to commercial formulation.

4. MOLECULAR MECHANISMS

An advantage of botanical biopesticides is their ability to engage multiple molecular targets and physiological pathways, making resistance development significantly less likely than with single-target synthetics (Sparks & Nauen, 2015). Understanding these mechanisms at molecular and cellular levels is crucial for optimising their use, guiding the discovery of new agents and designing effective insecticide resistance management (IRM) strategies (Sparks et al., 2019).

4.1. Neurotoxic Disruption

Many fast-acting botanical insecticides function as neurotoxins, interfering with nerve impulse transmission and leading to paralysis and death.

4.1.1. Pyrethrins and voltage gated sodium channels

Extracted from flowers of *Chrysanthemum cinerariifolium* (Trevir.) Bocc. (Asteraceae), Pyrethrins and their synthetic analogues (pyrethroids) are potent neurotoxins targeting voltage-gated sodium channels in nerve cell membranes (Jansen et al., 2010 and Sparks et al., 2019). These channels are essential for action potential generation and propagation. Pyrethrins slow both activation (opening) and inactivation (closing) kinetics, causing prolonged sodium ion influx during nerve excitation, resulting in membrane depolarisation, repetitive hyperexcitability and nerve block leading to paralysis and death (Soderlund & Bloomquist, 1989 and Mordue Luntz & Blackwell, 1993).

4.1.2. Nicotine and nicotinic acetylcholine receptors

Nicotine from *Nicotiana tabacum* acts as a potent agonist of insect central nervous system nicotinic acetylcholine receptors (nAChRs) (Jansen et al., 2010 and Sparks et al., 2019). By mimicking acetylcholine, nicotine induces continuous depolarisation and permanent excitation, leading to muscular paralysis and death.

4.1.3. Essential oils and multiple neurological targets

Essential oil components exhibit neurotoxic effects through diverse mechanisms. Eugenol inhibits acetylcholinesterase (AChE), the enzyme degrading acetylcholine in synaptic clefts. AChE inhibition causes acetylcholine accumulation, leading to nervous system hyperexcitation and paralysis, similar to the mechanisms of organophosphates and carbamates (Koul et al., 2008; Sparks et al., 2019). Monoterpenoids act as antagonists or allosteric modulators of GABA-gated chloride channels and octopamine receptors, contributing to broad neurotoxic profiles (Tong & Coats, 2010).

4.2. Endocrine Disruption and Growth Regulation

Many botanical compounds function as insect growth regulators (IGRs), interfering with endocrine systems governing moulting and metamorphosis (Isman & Grieneisen, 2014).

4.2.1. Azadirachtin

Azadirachtin from neem represents the archetypal botanical IGR, exerting effects multi pronged disruption of insect endocrine systems. Its primary action involves antagonism of key developmental hormones (ecdysone and

juvenile hormone). Azadirachtin's structure mimics the steroid moulting hormone ecdysone, interfering at multiple levels of signalling. Centrally, it blocks the release of prothoracicotropic hormone (PTTH) from the corpus cardiacum complex (Mordue Luntz & Blackwell, 1993). PTTH is the neuropeptide that signals the prothoracic gland to synthesise and release ecdysone; by inhibiting PTTH release, azadirachtin effectively halts ecdysone production, preventing moulting and trapping insects in larval stages or causing fatal moulting defects. Azadirachtin also interferes with juvenile hormone through blockade of allatotropin release; this disruption creates developmental chaos, resulting in malformed pupae and often sterile, non-viable adults.

4.3. Metabolic and Cellular Disruption

Botanical compounds target fundamental cellular processes, including energy metabolism and membrane integrity. Beyond its neurotoxic effects, eugenol is a potent metabolic poison. A key molecular target in mites is NADH-ubiquinone oxidoreductase. Eugenol binds the MTND2 subunit, inhibiting function, disrupting electron flow, halting ATP production and leading to cellular energy failure and death. Molecular docking studies show eugenol binds effectively to mite MTND2 but not human homologues, providing a molecular basis for selective toxicity. Rotenone, another botanical insecticide, inhibits respiratory enzymes by disrupting electron transport between NAD and coenzyme Q (Mordue Luntz & Blackwell, 1993).

The monoterpene 1,8-cineole, the major eucalyptus oil component, powerfully compromises fungal cell structural and functional integrity. Its lipophilic nature allows it to intercalate into fungal plasma membranes, disrupting fluidity and permeability and causing leakage of essential ions and metabolites. Concurrently, 1,8-cineole induces massive reactive oxygen species (ROS) bursts within fungal cells, creating severe oxidative stress and widespread cellular damage, while downregulating ergosterol biosynthesis genes; ergosterol is a fungi specific sterol essential for membrane function.

4.4. Behavioural Modification

Many botanical compounds protect plants by modifying pest behaviour rather than directly killing pests (Isman & Grieneisen, 2014). Azadirachtin exhibits potent antifeedant activity through both immediate primary and delayed secondary mechanisms. Primary antifeedancy is immediate and taste mediated, where azadirachtin stimulates specialised deterrent chemoreceptors on mouthparts.

Secondary antifeedancy is delayed and post-ingestive, where sublethal consumption causes physiological distress and learned taste aversion. This dual-pronged behavioural modification provides robust crop protection by preventing feeding damage from initiation.

The power of botanical biopesticides lies not in single modes of action but in synergistic disruption portfolios. Single applications simultaneously launch multi-pronged assaults with neurotoxins, metabolic poisons, repellents, and antifeedants, representing an evolutionarily robust, integrated strategy that poses formidable adaptation challenges for pests and constitutes a cornerstone of sustainable resistance management.

5. BROAD-SPECTRUM EFFICACY AND ENVIRONMENTAL VARIABLES

The chemical diversity of secondary metabolites translates into exceptionally broad-spectrum biological activities, making medicinal plants versatile pest management tools (Isman, 2006 and Bakkali et al., 2008). Utility extends beyond direct toxicity to include behavioural modification and growth regulation, offering multiple avenues for integration into sustainable programmes. However, field performance is significantly influenced by environmental variables that are now being altered by climate change (Dhillon & Gujar, 2010). The range of documented biological activities and representative botanical sources are summarised in Table 1.

5.1. Climate-Dependent Efficacy

Translation of lab-demonstrated bioactivity to reliable field

performance critically depends on environmental conditions, which are becoming more extreme and unpredictable due to climate change (Chen & McCarl, 2001; Dhillon & Gujar, 2010) (Table 2).

These climate-induced vulnerabilities underscore the critical need for robust predictive models. Future pest management decision-making requires models that integrate regional climate projections, pesticide degradation kinetics, pest phenology, and crop growth stages to optimise the timing, formulation, and application rates of botanical biopesticides in dynamic agricultural landscapes (Delcour et al., 2015).

6. ADVANCED FORMULATION FOR ENHANCED FIELD PERSISTENCE AND CLIMATE RESILIENCE

Despite potent and diverse botanical biological activities, widespread adoption has been significantly hampered by practical field level limitations. Properties making essential oil components effective fumigants, namely high volatility and susceptibility to UV radiation, heat and moisture degradation, result in short residual activity (Koul et al., 2008). These challenges are intensified by more extreme climate change-associated weather conditions (Dhillon & Gujar, 2010 and Acheuk et al., 2022). Bridging the critical lab-to-field gap requires advanced formulation and delivery systems that protect active ingredients, control release and enhance environmental stress resilience (Nuruzzaman et al., 2016 and Kumar et al., 2019).

6.1. The Formulation Challenge

Advanced formulation primary goals are creating

Table 2. Impact of climatic factors on botanical efficacy

Abiotic factor	Observed impact & mechanism	Specific compound/ context	Source
Temperature	Persistence vs Acute Toxicity Trade-off: Elevated temperatures increase component volatility, leading to rapid dissipation and reduced residual field activity. Conversely, it increases the metabolic rate of ectothermic insects, accelerating toxin uptake and potentially increasing acute efficacy.	Essential oil components (General)	Boina et al., 2009, Dhillon & Gujar, 2010
UV Radiation	Photodegradation: Acts as a primary degradation driver, causing significant loss of biological activity (e.g., antifeedant and IGR effects). <i>Azadirachtin</i> half-life is reduced to 48 minutes under direct 254 nm UV, compared to 2.47–3.98 days under natural conditions.	Azadirachtin; Pyrethrins	Sundaram, 1996, Jansen et al., 2010, Kookana et al., 2014
Precipitation & Humidity	Physical Wash-off & Synergism: Heavy rainfall physically washes formulations off plant surfaces, limiting efficacy. Conversely, high humidity promotes the germination and infectivity of entomopathogenic fungi, offering potential for synergistic application with botanicals.	Botanical formulations (General); Entomopathogenic fungi	Koleva & Schneider, 2009, Dhillon & Gujar, 2010

protective barriers around bioactive cores, shielding them from premature degradation while ensuring target-site availability over desired periods. Ideal formulations should enhance physical and chemical stability, improve solubility (especially lipophilic oils in aqueous sprays), increase plant surface adhesion and provide controlled or sustained release profiles (Kumar et al., 2019). Microencapsulation and nanoformulations represent the two most promising technologies (Camara et al., 2019).

6.2. Microencapsulation for Stability and Controlled Release

Microencapsulation involves surrounding tiny core material particles or droplets (for example, essential oils) with continuous polymeric films, forming microcapsules (1-1000 micrometres in diameter). Polymer shells act as physical barriers, protecting cores from volatilisation and environmental degradation while modulating release (Nuruzzaman et al., 2016). Common techniques include interfacial polymerisation, in which oil and water phase polymer precursors react at emulsion interfaces to form solid polymer shells (for example, polyurea) around oil droplets, and spray drying, a scalable industrial process wherein core material-wall material emulsions are atomised into hot air streams, leaving solid microparticles with entrapped cores.

By entrapping active compounds, microencapsulation significantly enhances shelf life and field persistence. Studies show that microencapsulated repellents maintain high efficacy for considerably longer periods than non-encapsulated formulations. Release is triggered by various mechanisms (diffusion, rupture, and biodegradation), enabling the design of predictable release kinetics (Nuruzzaman et al., 2016).

6.3. Nano-formulations: Enhancing Bioavailability and Climate Resilience

Nanotechnology offers sophisticated botanical pesticide delivery solutions, with the global nanopesticides market projected to exceed USD 2 billion by 2032 (Coherent Market Insights, 2025 and Precedence Research, 2025). Nanoformulations (nanoemulsions and nanocapsules) reduce active ingredient particle or droplet sizes to nanometre scales (typically less than 100 nm) (Kumar et al., 2019).

Nanoemulsions are kinetically stable colloidal dispersions of immiscible liquids (for example, essential oils-water) stabilised by surfactants. Extremely small

droplet sizes provide key advantages: dramatically increased surface area-to-volume ratios enhancing bioavailability and biological activity, improved lipophilic oil solubility and water dispersibility and better hydrophobic plant cuticle adhesion and wettability (Anjali et al., 2010 and Kumar et al., 2019). Nanocapsules and polymeric nanoparticles enclose active ingredients within nanoscale polymer shells or matrices. Nanoencapsulation provides superior environmental degradation protection compared to microencapsulation, enables highly targeted and controlled release and enhances photoprotection of sensitive compounds (Camara et al., 2019). Biopolymers like chitosan and alginate increasingly serve as shell materials due to their biodegradability and biocompatibility (Wani & Khan, 2016).

These advanced formulations represent transformative steps. By systematically addressing primary botanical compound weakness of environmental instability, these technologies can enhance field performance, improve cost-effectiveness and make botanicals more reliable and competitive for mainstream, climate-resilient agriculture (Camara et al., 2019). Smart formulations tailored to specific pest-crop combinations and climate zones represent the next frontier in botanical biopesticide development.

7. STRATEGIC INTEGRATION INTO CLIMATE-SMART INTEGRATED PEST MANAGEMENT SYSTEMS

The ultimate value of botanical biopesticides is realised not as simple one-to-one synthetic replacements, but when they are strategically integrated into holistic IPM programmes (Pedigo, 2002). IPM is an ecosystem-based strategy focusing on long-term pest prevention through combined techniques, using pesticides only when monitoring indicates necessity at established thresholds. Within this framework, botanical pesticides serve as powerful, multifunctional tools that enhance sustainability, manage resistance, and improve overall programme efficacy, contributing to climate-smart agricultural system development (Rao et al., 2022).

7.1. A Cornerstone of Insecticide Resistance Management

One of the most critical roles of botanical pesticides in modern agriculture is insecticide resistance management (IRM) (Hawkins et al., 2019 and Matzrafi, 2019). Relentless single-target synthetic insecticide use creates intense

selection pressure driving rapid resistant pest population evolution, potentially accelerated in warming climates through shorter generation times and larger population sizes.

Botanical pesticides counter this threat in two fundamental ways. First, many products, particularly crude extracts and essential oils, are complex bioactive compound mixtures engaging numerous pest molecular targets simultaneously (Koul et al., 2008). For pest resistance evolution, multiple independent resistance mechanisms must develop simultaneously, a far less probable event than single-target chemical resistance (Matzrafi, 2019). Second, even single botanical compounds such as azadirachtin and eugenol often exhibit multiple action modes (antifeedant and growth-regulating, or neurotoxic and metabolic). Consequently, rotating or tank-mixing single-target synthetics with complex multi-modal botanicals represents a highly effective IRM strategy, extending the useful lifespan of all available control products (Georghiou & Taylor, 1977).

7.2. Synergistic Applications

Beyond IRM, botanical pesticides combine with other control agents to produce synergistic effects, in which combined efficacy exceeds the sum of the individual components (Srivastava et al., 2011). This approach builds multi-layered, robust defence systems more resilient to pest adaptation and environmental volatility.

7.2.1. Synergy with synthetic pesticides

Botanicals can enhance the potency of synthetic pesticides, often enabling reduced application rates (Pavela, 2014). This frequently occurs through inhibition of detoxification enzymes. Certain plant oils inhibit the pest

oxidase and glutathione S-transferase (GST) enzymes, which normally metabolise and detoxify synthetic insecticides such as pyrethroids and organophosphates (Srivastava et al., 2011). An azadirachtin-pyrethroid (cypermethrin) mixture showed a significantly lower lethal concentration (LC50) against red flour beetles (*Tribolium castaneum* Herbst [Coleoptera: Silvanidae]) than either compound alone, indicating a potent synergistic interaction.

7.2.2. Synergy with microbial control agents

Botanical pesticides can be compatible with and enhance microbial biopesticide efficacy, such as entomopathogenic fungi (Wraight & Ramos, 2005 and Surekha & Reddy, 2016). Azadirachtin demonstrates synergistic effects when combined with *Beauveria bassiana* (Balsamo) Vuill. (Fungi: Cordycipitaceae). Botanicals may weaken the immune system or disrupt the cuticle, thereby increasing susceptibility to fungal infections. Matrine demonstrates synergistic interactions with *Akanthomyces attenuatus* (Entomopathogenic Fungi) against thrips, with combined applications significantly lowering pest defensive enzyme activity (Wang et al., 2021). However, compatibility is key; some synthetic fungicides routinely used inhibit beneficial fungal growth and the efficacy of *B. bassiana* (Surekha & Reddy, 2016).

7.2.3. Synergy with entomopathogenic nematodes

Recent research explores botanical compatibility with soil-dwelling biological control agents such as entomopathogenic nematodes (EPNs) (Oso et al., 2021). Studies show *Alepidia amatymbica* Eckl. & Zeyh. and *Elephantorrhiza elephantina* (Burch.) Heine extracts are compatible with several strains of *Steinernema* and *Heterorhabditis* nematodes. Surviving nematodes retained

Table 3. Case studies of botanical integration in integrated pest management (IPM)

Crop / context	Target pest	IPM intervention / botanical strategy	Outcome & efficacy	Source
Brinjal (Eggplant)	Brinjal shoot and fruit borer (<i>Leucinodes orbonalis</i>)	Mechanical removal of infested shoots + Neem-based product sprays	Significantly lower pest infestation and higher marketable yields.	Wang et al., 2021
Tomato	Tomato fruit borer (<i>Helicoverpa armigera</i>)	Neem seed kernel extract (NSKE) + Nuclear polyhedrosis virus (NPV)	Demonstrated synergistic effects against the borer.	Verma et al., 2021
Meta-analysis (153 field trials on organic insecticides)	Several key pests	Pyrethrin - azadirachtin combination products	Provided >75% control of the targeted key pests.	Pavela, 2014
Maize & Cabbage	General pests (Large-scale IPM programs)	Biological controls (<i>Bacillus thuringiensis</i> & <i>Trichogramma</i> spp.) + Cultural practices	Led to >10% yield increases and significant reduction in chemical pesticide use.	CABI, 2015

virulence against target pests, suggesting soil-applied botanical-EPN combinations could improve control compared to individual applications, targeting pests in different environmental niches (foliar and soil) simultaneously.

7.3. Evidence from the Field: Case Studies in IPM

Various case studies across different regions of the globe demonstrate the use of botanicals as effective tools for pest management against a wide range of pests (Table 3).

8. GLOBAL COMMERCIALISATION

Despite compelling evidence of efficacy and ecological benefits, botanical biopesticides currently represent only a small fraction of the global pesticide market (Damalas & Koutroubas, 2018). This significant gap between scientific potential and commercial reality results from a complex interplay of economic, logistical, and regulatory barriers that collectively hinder widespread adoption (Villaverde et al., 2014). Bridging this lab-to-field gap requires understanding these challenges and developing strategies to overcome them.

8.1. Economic Viability and Farmer Adoption Barriers

The path from a promising plant extract to a commercially viable product on the farmer's shelf is fraught with practical difficulties. Biopesticide production often involves plant material cultivation, extraction and purification, potentially more complex and costly than

conventional chemical synthesis (Damalas & Koutroubas, 2018). While many botanicals exhibit high pest specificity, an ecological advantage, this translates to smaller niche markets, making recovery of research and development investment difficult. Inherent instability and short shelf-life of many botanical active ingredients also pose significant logistical challenges, requiring specialised formulations and more complex supply chain management than those for robust synthetic chemicals (Pathma et al., 2021).

Farmers operate in high-risk environments, often hesitant to abandon the predictability of synthetic pesticides in favour of newer technologies. However, growing evidence demonstrates botanical economic viability. Field studies show botanicals can be highly cost-effective; neem seed extract (NSE) for Pakistani tomato pest management generated a cost-benefit ratio of 19.26 (significantly higher than 13.23 for synthetic emamectin benzoate) while achieving comparable marketable yields (Akhter et al., 2023). This demonstrates that, despite potentially higher initial costs or slower action, lower input costs of crude extracts can yield higher net profits for smallholder farmers (Mkindi et al., 2020; Verma et al., 2021).

8.2. The Regulatory Maze: A Comparative Global Analysis

Perhaps the most formidable barrier to botanical biopesticide commercialisation is the complex, costly and time-consuming regulatory approval process (Chandler et

Table 4. Comparative overview of regulatory frameworks for botanical biopesticides in major markets

Country/region	Regulatory body	Key legislation/framework	Timeline for biopesticides	Key features for botanicals
United States	EPA (Environmental Protection Agency)	FIFRA; Biopesticide Registration Programme	12 to 18 months	Dedicated biopesticide pathway; reduced data requirements; lower registration fees
European Union	EFSA; Rapporteur Member States	Regulation EC No. 1107/2009	120 days (low-risk) to 12 months	Stringent two-tier system; no separate biopesticide category; high data burden
China	MARA (Ministry of Agriculture and Rural Affairs)	Pesticide Administration Regulations (2022)	18 to 24 months	Streamlined pathway for biopesticides; growing acceptance of botanicals
Brazil	MAPA/ANVISA/IBA MA	Law 14.785/2023 (New Pesticide Law)	Significantly reduced	Most biopesticide-friendly major market; expedited approval for botanicals
India	CIB&RC (Central Insecticides Board and Registration Committee)	Insecticides Act, 1968; Schedule IV provisions; National Biopesticide Policy	24 to 36 months (conventional); reduced for approved botanicals	Schedule IV lists approved botanicals; CIBRC subcommittee for biopesticides; state-level registration for local botanicals; emphasis on neem products (EC 9%); GAP certification under NPOP
Australia	APVMA (Australian Pesticide and Veterinary Medicines Authority)	Agricultural and Veterinary Chemicals Code Act 1994	12 to 24 months	Reduced data requirements for low-risk botanicals; pathway for essential oil products

al., 2011). Most regulatory frameworks were designed to evaluate the risks of single synthetic chemical molecules, creating a fundamental paradigm mismatch when applied to complex, multi-component botanical extracts (Bailey et al., 2010; Villaverde et al., 2014). The very property giving botanicals their key biological advantage, phytochemical complexity, becomes their greatest regulatory disadvantage. A comparative overview of key regulatory frameworks is provided in Table 4.

8.2.1. India: Regulatory framework for botanical biopesticides

India presents a particularly relevant regulatory context for botanical biopesticides given the country's rich biodiversity of medicinal plants and the scale of its agricultural sector. The primary regulatory authority is the Central Insecticides Board and Registration Committee (CIB&RC), which operates under the Insecticides Act, 1968, and its subsequent amendments. Botanical biopesticides, including neem-based formulations, pyrethrum extracts and essential oil products, are listed under Schedule IV of the Insecticides Act, which provides a degree of regulatory facilitation compared to synthetic chemicals.

Neem-based products have received special attention under Indian regulation. The Bio-pesticides Sub-Committee of the CIB&RC reviews and approves botanical products, and neem formulations meeting minimum specifications (Neem based Pesticides containing Azadirachtin at 0.03% EC and above) are registered with relatively streamlined documentation. The National Policy on Biopesticides aims to promote the adoption of biopesticides, and the Ministry of Agriculture and Farmers Welfare has included biopesticides in flagship schemes such as the National Mission for Sustainable Agriculture (NMSA) and the Paramparagat Krishi Vikas Yojana (PKVY). Several Indian states also maintain state-level registration provisions for locally used botanical preparations under farmer field school and organic farming promotion programmes. The National Programme for Organic Production (NPOP) under APEDA recognises botanical pesticides compliant with international organic standards, facilitating access to export markets.

Despite these provisions, challenges remain. The registration process for novel botanical extracts or combination formulations under CIB&RC still demands extensive dossiers similar to synthetic pesticide registration, which is financially prohibitive for small manufacturers. Harmonisation of standards with Codex Alimentarius and alignment with Integrated Pest Management Cell

recommendations would significantly accelerate the commercialisation of Indian botanical biopesticide products.

A fundamental paradigm shift is needed in global regulatory science, requiring the re-evaluation and development of methodologies for assessing the safety and efficacy of complex botanical mixtures as systems rather than as single-component entities (Villaverde et al., 2014). Greater international harmonisation of data requirements and mutual recognition of approvals would significantly reduce market-entry costs and time, stimulating innovation (AgroPages, 2023; World Bioprotection Forum, 2024).

9. CONCLUSION AND FUTURE PERSPECTIVES

Botanical biopesticides derived from medicinal plants represent a compelling, scientifically validated pathway towards more sustainable and ecologically resilient global agriculture (Fenibo et al., 2022). Grounded in sophisticated plant-herbivore chemical ecology, these natural products offer bioactive compounds with diverse, multimodal mechanisms that are fundamentally advantageous for pest resistance management in warming climates. IPM integration has demonstrated a reduction in reliance on synthetic chemicals, enhanced efficacy of other control agents through synergy, and contributed to safer food production systems (Srivastava et al., 2011).

However, the journey from promising laboratory results to widespread adoption is impeded by significant, interconnected challenges. Many botanical limitations, such as instability under heat and UV radiation, are exacerbated by climate change (Sundaram, 1996 and Dhillon & Gujar, 2010). Formidable economic barriers, farmer risk aversion and regulatory frameworks designed for different technological paradigms have stifled commercialisation and limited availability (Isman & Grieneisen, 2014 and Villaverde et al., 2014). Unlocking the full potential requires concerted, interdisciplinary effort.

9.1. Future Success Priorities

The future success of botanical biopesticides depends on the synergistic advancement of a triad of innovations encompassing molecular biology, formulation science, and regulatory science. Progress in only one or two areas is insufficient; coordinated advances across all three represent the only viable pathway elevating botanicals from niche status to mainstream, indispensable global food system components.

9.1.1. Mechanism-driven bioprospecting

Future botanical discovery should shift from random screening to targeted, mechanism-driven approaches (Sparks & Nauen, 2015). By identifying novel vulnerable pest molecular targets (chordotonal organs and specific metabolic enzymes) and screening for plant compound interactions, research becomes efficient and effective. Genomics, proteomics and computational biology advances will be instrumental.

9.1.2. Advanced formulation engineering for climate resilience

Overcoming stability and persistence issues arguably represents the most critical technological hurdle (Camara et al., 2019). Sustained investment in formulation science, particularly in scalable microencapsulation and nanoformulation technologies, is essential (Nuruzzaman et al., 2016; Kumar et al., 2019). The goal should be to develop smart delivery systems protecting active ingredients from environmental stressors (heat and UV) while providing tailored release profile optimised for specific pests and cropping systems.

9.1.3. Optimising synergistic IPM systems

Robust, large-scale field research is urgently needed to validate botanical performance in real-world, climate-smart IPM programmes (Ghosh et al., 2024). Research should prioritise synergistic combination studies and explore optimal botanical rotation and mixing with synthetic pesticides, microbial agents (*Beauveria* and *Bacillus*), and other biological controls (EPNs and parasitoids) to maximise efficacy and build system resilience (Furlong & Zalucki, 2017; Wang et al., 2021).

9.1.4. Regulatory reform and harmonisation

Future frameworks must evolve to accommodate the unique nature of complex botanical mixtures and to develop new safety and efficacy assessment methodologies. Brazil's reforms offer potential models for reducing timelines; greater harmonisation of international data requirements and mutual recognition of approvals would significantly reduce costs and time, thereby stimulating innovation (World Bioprotection Forum, 2024).

9.1.5. Integration with precision agriculture and genomics

The future of pest management involves biopesticide integration with precision agriculture technologies (drones and sensors) for targeted application, improving efficacy and reducing waste (Nuruzzaman et al., 2016 and Skendi et al., 2021). Biotechnology for selecting or genetically improving microbial agents for enhanced climate tolerance

will be crucial in maintaining synergistic IPM strategy efficacy under future climate scenarios.

9.2. Realising the Potential

While challenges are substantial, they are not insurmountable. Through strategic interdisciplinary research investment, supportive policy reforms, and a commitment to closing lab-field-farmer gaps, botanical biopesticides can become foundational components of the global food system, prepared for the challenges of a changing climate. The paradigm shift from chemical-dependent, single-target pest management towards complex, multi-modal botanical integration into climate-smart IPM represents not merely incremental improvement but a fundamental transformation towards sustainable, ecologically resilient agriculture.

9.2.1. Key research hypotheses addressed.

This review demonstrated that botanical chemical complexity provides intrinsic resistance management advantages; advanced formulations can adequately address environmental stability challenges and strategic IPM integration represents the viable pathway for climate-resilient pest management. Future research must validate these hypotheses across diverse agroecologies and scales.

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CRedit authors contribution statement

Anand Jejal: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. Samiksha Pandey: Writing - review and editing, Investigation, Methodology, validation. Anurag Saxena: Supervision, Validation, Writing - review and editing, Methodology, Validation. Magan Singh: Supervision, Validation, Writing - review and editing

Conflict of interest

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative artificial

intelligence (AI) tools or AI-assisted technologies were used in the writing, editing or revision process of this manuscript. All text was generated through original scholarly effort and critical analysis.

Data availability statement

All data generated or analysed during this study are included in this published article.

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Colonization Dynamics of Lichens Along Altitudinal Gradients in the Kumaun Himalaya, India

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ABSTRACT: Despite ecological relevance and susceptibility to environmental change, the Kumaun Himalaya, a biologically rich region of Uttarakhand, has received scant attention on the diversity and dynamics of lichens. In this context, the goal of this study was to examine the distribution and variety of lichens in the Hawalbagh block region of Kumaun Himalaya. For this purpose, lichen samples were collected between 1400 and 2200 m in altitude to document their diversity and distribution patterns using a stratified random sampling method. The study area was categorized into three elevation zones: lower elevation (1000-1400 m) dominated by pure pine forest; middle elevation (1400 - 1800 m) was characterized by pine-oak mixed forest, and the higher elevation (100-2200) forest zone was dominated by oak trees. An extensive field survey was conducted to collect primary data. During the study, a total of 46 species of lichen belonging to 29 genera and 12 families were encountered. Maximum 60.87% species were found as foliose, followed by crustose (19.57%) and fruticose (15.22 %) lichens. Whereas dimorphic, squamulose, and fruticose forms of lichen contributed 2.17 %, respectively. A single species of dimorphic type (*Cladonia verticillata*) was observed on the soil surface in the higher region. During the field survey, it was also observed that lopping of *Quercus* trees for fuel and fodder, increasing tourism activity, construction of motorable roads and resorts, particularly in the high-altitude region at Shitlakhhet-Syahi Devi, frequent occurrence of forest fire, and grazing at middle and lower altitudes in pine forests, provide a threat to the lichen taxa of the region. Besides heavy anthropogenic activities, lichens on soil and rock surfaces, even on lower regions of tree trunks, are also threatened by their survival. This study suggests that habitats should be protected to support the lichen flora along with other vegetation of the region. It would be helpful to prevent the colonisation of several terricolous and saxicolous lichens on ground and rock surfaces, which are declining due to frequent occurrence of forest fires in the lower reaches of the study area.

Keywords: Lichen diversity, Forest types, Altitudinal gradient, Bioindicators, Kumaun Himalaya.

1. INTRODUCTION

The Indian Himalayan region, which spans a large altitudinal gradient and is home to a broad range of flora and animals, is one of the world's most ecologically varied and geologically dynamic mountain ranges (Ellis et al., 2025). In the context of Kumaun Himalaya in the east and the Garhwal

Himalaya in the west, the two main administrative and biogeographical divisions of Uttarakhand's Central Himalaya are the Kumaun Himalaya and the Garhwal Himalaya. The Kumaun Himalaya, which includes districts such as Almora, Nainital, Bageshwar, and Pithoragarh, is distinguished by a variety of forest types ranging from subtropical to alpine, offering ideal habitats for a broad range

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of cryptogamic diversity, including lichens (Tanta et al., 2024). Lichens are special symbiotic relationships that create a self-sustaining composite creature between a mycobiont (fungus) and a photobiont (green alga or cyanobacterium).

Lichens are among the pioneers of terrestrial ecosystems and constitute a unique group of plants consisting of two unrelated organisms, an alga and a fungus, living together in a tight symbiotic relationship (Awasthi, 2000). At the global level, lichens are an important component of terrestrial biodiversity, with thousands of species spread across several biomes, from polar to tropical locations. Their ecological significance is generally acknowledged, especially as bioindicators of environmental quality, biomonitors of air contaminants, and contributors to ecosystem stability. However, anthropogenic factors such as deforestation, urbanisation, habitat degradation, and climate change are jeopardising lichen diversity, leading to localised losses of vulnerable species (Rawat & Kumar, 2025). The Himalayan region is regarded as a lichen biodiversity hotspot because of its altitudinal complexity and diverse microclimatic conditions. Several investigations conducted in the Kumaun region have found a significant variety of lichens. Mishra & Upreti (2016) identified 246 macrolichen species from the Kumaun region, with foliose forms and families such as Parmeliaceae and Physciaceae predominating. Similarly, Maurya & Mishra (2023) also reported 199 lichen taxa in the Almora district across multiple forest locations, demonstrating high species richness and geographic variation. Studies in surrounding areas, such as the Darma Valley and Kumaun's sacred groves, have also revealed diverse lichen assemblages, highlighting the roles of host tree diversity, altitude, and microclimatic variables in lichen dispersion (Joshi et al., 2018; Chandra & Joshi, 2018). Various morphological as well as physiological characteristics of lichens and the presence of their secondary metabolites allow them to bloom in varied substrata at different localities. Muggia et al. (2013) reported approximately 25,000 species of lichens worldwide, and the Indian Himalayan region accounts for 10% of this global diversity. Based on their habitat, lichens grow over the rock surface (saxicolous, on tree bark (corticolous), and on soil (terricolous). Lichens play a significant ecological role in forest succession, species composition, and as bioindicators (Upreti, 1980). Besides, economically, they are very useful as food (spices), fodder, dyes, perfumery, cosmetics, medicines, etc. Thus, the goal of this study was to examine

the distribution and diversity of lichens in the Hawalbagh block of the Kumaun Himalaya. This study aims to minimise the current research gap and to identify ecological factors that reflect lichen diversity, particularly in the Himalayan region.

2. MATERIAL AND METHODS

2.1. Study Area

The current collection was made from three distinct elevation zones of a temperate forest of the Almora district, Kumaun Himalaya (Uttarakhand). These sites are located between 29°30'-30°20'N latitude and 79°20'-80°20'E longitude and range from 1000-2200 m altitude along the Almora-Ranikhet state highway. This region is well known for its diverse vegetation and various ecological conditions. The dominant forest types of the region are pure pine, pine-oak mixed, and oak mixed forests, respectively. The entire forest comes under the reserve forest and is managed by the forest department. More than 200 villages have benefited from this reserve forest. The local inhabitants collect fuelwood, fodder, litter, and some NTFPs for their own use (Figure 1).

2.2. Lichen sampling and strategy for data collection

An extensive field survey was conducted using random and stratified sampling (Upreti et al., 2015) across the three selected forest sites to document the region's lichen diversity of the region. Lichen specimens were systematically collected from a variety of substrata, including tree bark (corticolous), rocks (saxicolous), and soil (terricolous). Sampling was conducted following the standard field methods described by Awasthi (2007). For each specimen, detailed field notes were recorded, including growth form, substratum type, altitude, GPS coordinates, date of collection, and name of the collector (Figure 1). Preliminary field identification and examination of thallus morphology were performed using a 10× hand lens. At the same time, a chisel and hammer were used to carefully remove saxicolous and firmly attached specimens to avoid damage. All collected samples were air-dried and preserved in properly labelled paper packets (17×10 cm) for subsequent laboratory examination and taxonomic analysis (Figure 2).

2.2.1. Lower elevation zone (1000-1400 m)

This forest site is located at Kosi along the Ranikhet-Almora Road, approximately 12 km from Almora town (Figure 3). The area experiences substantial anthropogenic pressure because of its proximity to human areas and traffic

networks. The site is characterised by a predominantly monospecific *Pinus roxburghii* forest stand (Singh et al, 2023).

2.2.2. Middle elevation zone (1400-1800m)

The site represents a middle-elevation transition zone located approximately 18 km from Almora town along the Ranikhet-Almora and Sun Temple roads (Figure 3). The vegetation is characterized by a mixed pine-oak forest dominated by *Pinus roxburghii* and *Quercus leucotrichophora*, with a sub-canopy layer comprising

Rhododendron arboreum and *Pyrus pashia*. Due to its proximity to transit routes and religious landmarks, the site is subject to significant anthropogenic pressure, including habitat disturbance and biomass extraction (Kumar & Khanduri, 2024).

2.2.3. Higher elevation zone (1800-2200m)

The site is situated within the temperate belt of the Kumaun Himalaya, encompassing the Shitlakhet-Syahi Devi forest range, approximately 45 km from the town of Almora. Unlike the mid-elevation zones, this site is

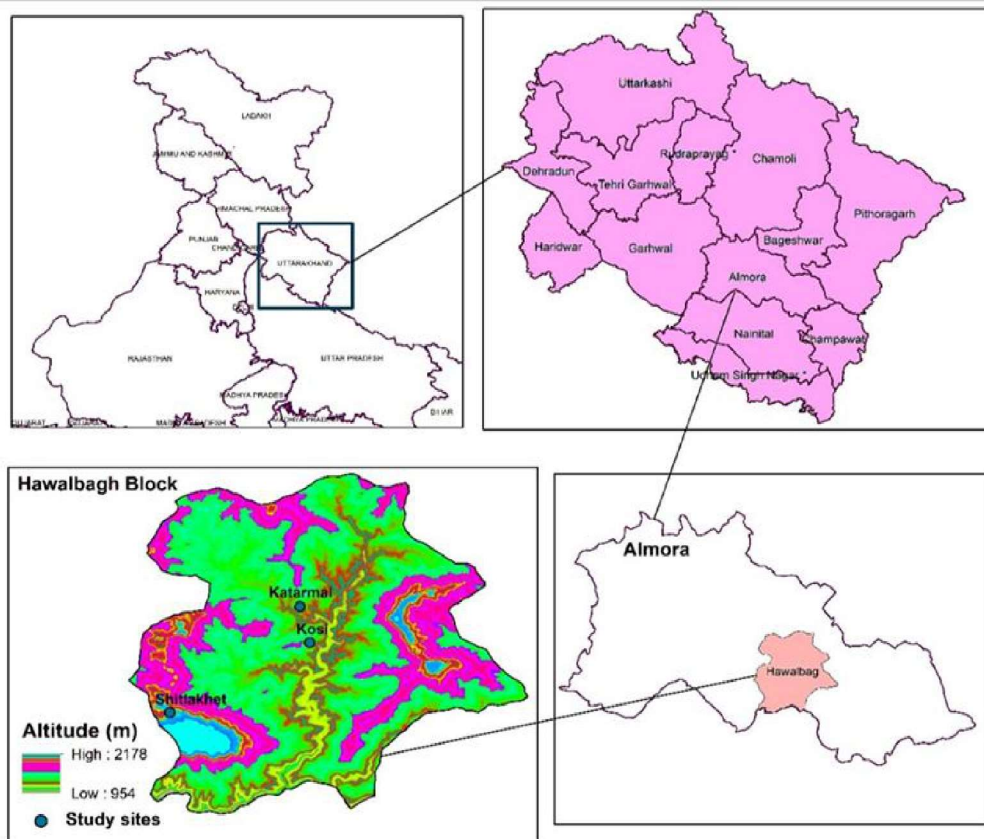


Figure 1. Location map of the study area showing the Hawalbag block of the Almora district

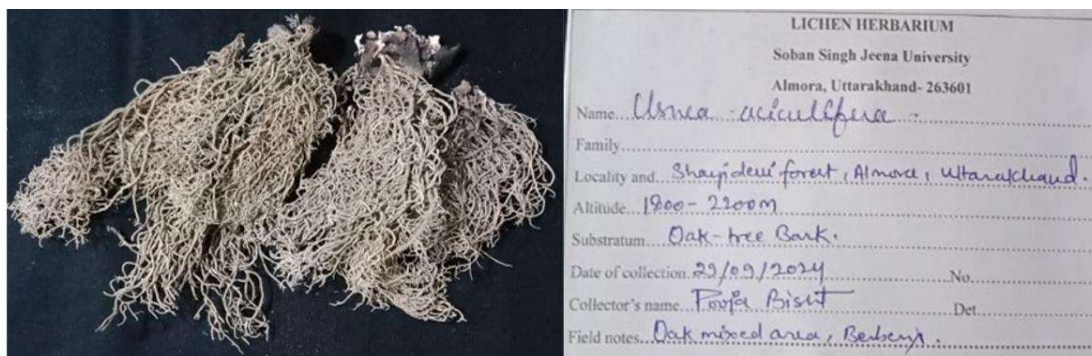


Figure 2. Herbarium sample of *Usnea aciculifera* collected from the study area

characterised by its relatively undisturbed status, largely protected by its religious significance (Syahi Devi temple) and geographical isolation, resulting in a more preserved forest structure (Figure 3).

2.3. Identification of Lichen Taxa

Lichen specimens were processed following standard herbarium protocols. Morphological, anatomical, and chemical characteristics were examined using standard lichenological techniques in the laboratory of the Botany Department at SSJ University Campus, Almora (Uttarakhand) (Figure 2). Taxonomic identification was done using keys and descriptions provided by Awasthi (2007), Divakar & Upreti (2005), and Nayaka (2004). To validate species identity, chemical analyses were performed using thin-layer chromatography (TLC) with solvent systems A and C, following the standardised methods of Orange et al. (2001).

2.4. Data Analysis

Lichen diversity was assessed by calculating species richness and distribution across altitudinal zones and forest types. For the graphical representation of the data, R software version 4.5.0 and Microsoft Excel were used.

Lichen taxa were grouped by family, genus, and growth form (crustose, foliose, fruticose, and dimorphic). Comparative analyses were used to examine variation in diversity patterns across the sites.

3. RESULTS AND DISCUSSIONS

A total of 46 lichen species belonging to 29 genera and 12 families were recorded across the three forest sites of the study area (Table 3). Out of which the predominance of foliose lichens accounted for 28 species (60.87%) of the whole diversity. Besides, nine species (19.57%) of crustose lichens and seven species (15.22%) of fruticose lichens were also recorded from the area (Table 2). Squamulose and dimorphic growth types, on the other hand, were underrepresented, with only a single species, i.e., *Cladonia fruticulosa*, contributing 2.17% to each (Table 1). Similar observations on the distribution of different lichen species were reported by Maurya & Mishra (2023) and Mishra & Upreti (2016) from other parts of the Kumaun Himalaya. The pattern of microclimatic conditions, such as moderate humidity, optimal light availability, and stable substrates, promotes the development of larger, more physiologically complex thalli. In addition, about 19.56% of crustose forms are also contributors to the lichen colonization of the region.

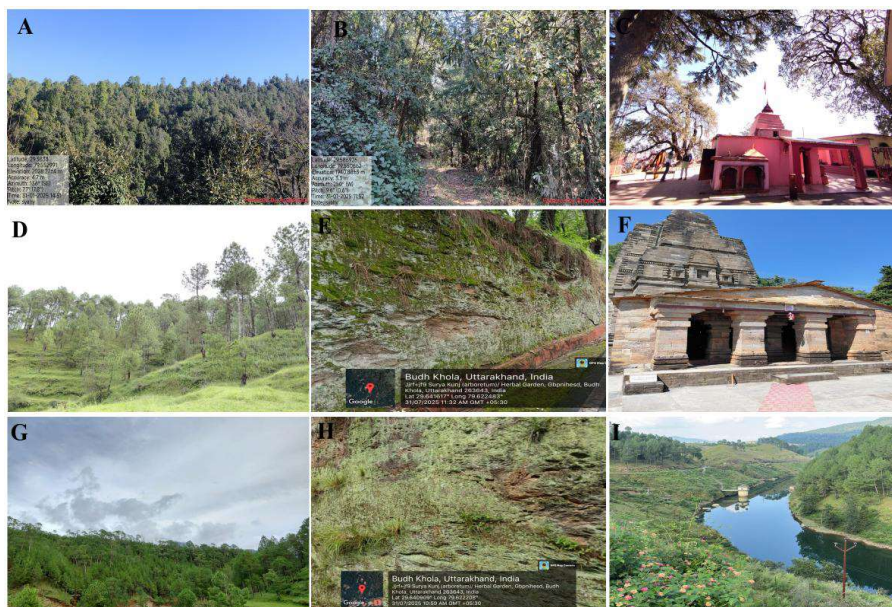


Figure 3. (A). Far view of Syahi Devi forest (B). Under forest view of Syahi Devi (C). Syahi Devi Temple Forest (D). Katarmal forest far view (E). Lichen growth on the wall along the road (F). A sacred site, Suntemple at Katarmal (G). Far view of the Kosi region (H). *Drinaria aegialita* lichen on walls along the Kosi- Ranikhet road (I). Kosi River Dam in the Kosi region

Table 1. Occurrence of various lichen species in different altitudinal zones of the study area

Name of species	Family	Growth forms	Lower altitude (1000-1400m)	Middle altitude (1400-1800m)	Higher altitude (1800-2200m)
<i>Alletraria oakesiana</i> (Tuck) Randl. & thell	Parmeliaceae	Fruticose	-	on bark	-
<i>Aspicilia</i>	Megasporaceae	Crustose	-	on rock	-
<i>Biatora vernalis</i>	Ramalinaceae	Crustose	on rock	-	-
<i>Blastenia ferruginea</i>	Teloschistaceae	Crustose	-	on bark	-
<i>Buellia almorensis</i>	Caliciaceae	Crustose	-	on bark	-
<i>B. betulinoidea</i>	Caliciaceae	Crustose	-	on bark	on bark
<i>Bulbothrix isidiza</i> (Nyl.) Hale	Parmeliaceae	Foliose	-	-	bark & rock
<i>B. setschwanensis</i> (Zahlbr.) Hale	Parmeliaceae	Foliose	-	on bark	Bark
<i>Caloplaca bassiae</i> (Ach.) Zahlbr.	Teloschistaceae	Crustose	on rock	on rock	on rock
<i>Canomaculina subtinctoria</i> (Zahlbr.) Elix	Parmeliaceae	Foliose	-	-	on bark
<i>Canoparmelia pustulescens</i> (Kurok.) Elix	Parmeliaceae	Foliose	on bark	-	-
<i>Catellaria nilgiriensis</i>	Catellariaceae	Crustose	-	on rock	on rock
<i>Cetrelia olivetorum</i> (Nyl.) W.I. Culb. And C.F. Culb.	Parmeliaceae	Foliose	-	-	on bark & twig
<i>Cladonia fruticulosa</i> Krempf.	Cladoniaceae	Dimorphic	-	on soil	-
<i>C. verticillata</i>	Cladoniaceae	Squamulose & fruticose	-	-	on soil
<i>Dermatocarpon vellereum</i>	Verrucariaceae	Crustose	-	on rock	-
<i>Dirinaria aegialita</i> (Afzel. ex Ach.) B.J. Moore	Caliciaceae	Foliose	-	on rock	on rock
<i>Flavoparmelia caperata</i> (L.) Hale	Parmeliaceae	Foliose	-	on bark	on bark
<i>Flavopunctelia borrierioides</i> Kurok	Parmeliaceae	Foliose	-	-	on bark and twig
<i>Hypotrachyna cirrhatum</i> (E.Fries).	Parmeliaceae	Foliose	-	-	on bark
<i>H. nepalense</i> (Taylor) Hale ex Sipman	Parmeliaceae	Foliose	-	-	on bark
<i>Heterodermia diademata</i> (Taylor) D.D. Awasthi	Physciaceae	Foliose	-	-	on soil
<i>H. japonica</i> (M. Sato) Swinscow and Krog	Physciaceae	Foliose	-	-	on soil
<i>H. obscurata</i> (Nyl.) Trevis.	Physciaceae	Foliose	on rock	-	on rock
<i>H. podocarpa</i> (Bel.) D.D. Awasthi	Physciaceae	Foliose	-	-	on twig
<i>Lecanora tropica</i> Zahlbr.	Lecanoraceae	Crustose	on rock	on rock	-
<i>Leucodermia boryi</i>	Physciaceae	Foliose	-	-	on bark
<i>Lobaria retigera</i> var. <i>retigera</i> (Bory) Trevis.	Lobariaceae	Foliose	-	-	on soil
<i>Nephromopsis lei</i>	Parmeliaceae	Foliose	-	-	on bark
<i>Parmotrema austrosinense</i> (Zahlbr.) Hale	Parmeliaceae	Foliose	on rock	on rock	-
<i>P. myelochroa</i>	Parmeliaceae	Foliose	on bark	-	-
<i>P. nilgherrense</i> (Nyl.) Hale	Parmeliaceae	Foliose	-	on bark	-
<i>P. reticulatum</i> (Taylor) M. Choisy	Parmeliaceae	Foliose	on bark	on bark	on bark
<i>P. tinctorum</i> (Despr. Ex Nyl.) Hale	Parmeliaceae	Foliose	on bark	on bark	-
<i>Peltigera canina</i>	Peltigeraceae	Foliose	-	-	on soil
<i>P. rufescense</i>	Peltigeraceae	Foliose	-	-	on soil
<i>Phaeophyscia hispidula</i> (Ach.) Essl.	Parmeliaceae	Foliose	-	-	on rock
<i>Punctelia rudecta</i> (Ach.) Krog	Parmeliaceae	Foliose	-	-	on rock
<i>P. subrudecta</i> (Nyl.) Krog	Parmeliaceae	Foliose	-	-	on bark
<i>Ramalina Conduplicans</i> Vain.	Ramalinaceae	Fruticose	-	-	on twig
<i>R. hossei</i> Vain.	Ramalinaceae	Fruticose	-	-	on twig
<i>R. sinensis</i>	Ramalinaceae	Fruticose	-	on bark	on bark
<i>Usnea aciculifera</i> Vain.	Parmeliaceae	Fruticose	-	-	on bark & twig
<i>U. florida</i>	Parmeliaceae	Fruticose	-	on twig	-
<i>U. orientalis</i> Motyka	Parmeliaceae	Fruticose	-	-	on bark
<i>Xanthoparmelia antleriformis</i> (Elix) Elix and J. Johnst.	Parmeliaceae	Foliose	-	on rock	-

Crustose lichens are responsible for ecological tolerance and surface formation across distant habitats in forest areas (Gupta et al., 2014). As per their habitat basis, corticolous (bark-inhabiting) lichens accounted 41.30% for all the species, and also preferred to grow on various substrata. With a contribution of 26.09%, saxicolous (rock-inhabiting) lichens constitute the second largest group (Figure 4). It was followed by terricolous (soil-inhabiting) lichens about 15.22%. However, 6.52% of the species were ramicolous, meaning they colonize on host twigs (Table 2). In addition, 10.87% of the species were found to be common on tree bark and twigs (Table 2). Upreti et al. (2015) also explored the lichen flora of the Padder valley of the Jammu & Kashmir region. The study revealed the Parmeliaceae as a dominant family, represented by 110 species of lichens, including corticolous forms. Similar observations were made by Sharma & Sheikh (2010) in the J&K region. Several studies have also been carried out on factors such as bark characteristics, texture, moisture retention capacity, and chemical composition responsible for lichen establishment in temperate regions (Löhmus et al., 2023; Borge & Ellis, 2025). Besides, the lichen family Parmeliaceae was also found to be rich in the Nanda Devi Biosphere Reserve of the Kumaun Himalaya (Rawat, 2021). However, the families Physciaceae and Ramalinaceae contribute little to the lichen diversity of the region (Awasthi, 2007; Bajpai & Upreti, 2012). The lichen family Parmeliaceae played a major role in shaping lichen diversity, contributing 50% of the region's total lichen diversity of the region (Table 1). It was followed by Physciaceae and Ramalinaceae, represented by 10.87%

and 8.70%, respectively. Teloschistaceae, Peltigeraceae, and Cladoniaceae each accounted for 4.35%, while Caliciaceae comprised 6.52%. Besides, the families Catillariaceae, Megasporaceae, Lobariaceae, Lecanoraceae, and Verrucariaceae shared very few species (2.17%) of the overall diversity of lichens.

The Syahi Devi region (higher-elevation zone) *Quercus leucotrichophora* forest exhibited the highest number of lichen species, with 29 (63.04%). The assemblage was dominated by foliose and fruticose lichens, including *Bulbothrix isidiza*, *Hypotrachyna cirrhata*, *Punctelia rudecta*, *Lobaria retigera*, *Peltigera canina*, *Ramalina hossei*, and *Usnea orientalis* (Figures 5 and 6). The presence of multiple fruticose (*Usnea* and *Ramalina*) and foliose (*Lobaria* and *Peltigera*) taxa reflects relatively undisturbed nature and mesic microclimatic conditions of the higher region of the study area. The region provides a suitable habitat for the best association of all the growth forms of lichens, particularly for a dimorphic (*C. verticillata*), which was not observed in the middle and lower altitudinal zones. The mid-altitudinal region of Katarmal supported 20 species of 12 genera and 7 families. This community included both

Table 2. Occurrence (%) of different lichen families, genera and species of the study area

Families	Genera (%)	Species (%)
Parmeliaceae	48.28	50
Physciaceae	6.90	10.87
Ramalinaceae	6.89	8.70
Calciaceae	6.89	6.52
Teloschistaceae	6.89	4.35
Peltigeraceae	3.44	4.35
Cladoniaceae	3.44	4.35
Catellariaceae	3.44	2.17
Megasporaceae	3.44	2.17
Lobariaceae	3.44	2.17
Lecanoraceae	3.44	2.17
Verrucariaceae	3.44	2.17

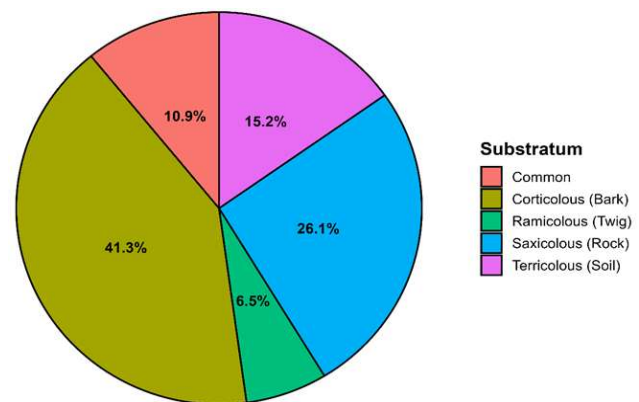


Figure 4. Illustration of species composition as per their habitat preference

Table 3. Contribution (%) of different growth forms of lichens of the study area

Growth forms	Species
Foliose	60.86
Crustose	19.56
Fruticose	15.21
Squamulose	2.17
Dimorphic	2.17



Figure 5. (A). *Dermatocepon vellereum*. (B). *Parmotrema austrosinense*. (C). *Dirinaria aegialita*. (D). *Nephromopsis lei*. (E). *Parmotrema reticulatum*. (F). *Heterodermia diademata*. (G). *Leucodermia boryi* (H). *Xanthoparmelia australasia*. (I). *Parmotrema nilgherrense*. (J). *Peltigera rufescense*. (K). *Usnea aciculifera*. (L). *Parmotrema tinctorum*

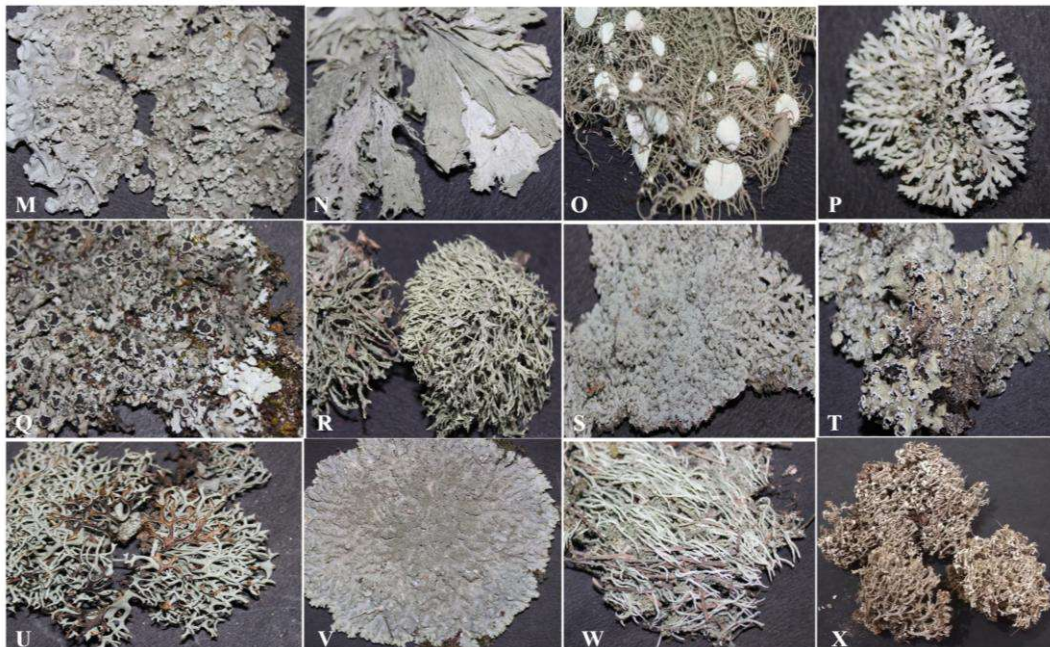


Figure 6. (M). *Flavoparmelia caperata*. (N). *Ramalina sinensis*. (O). *Usnea orientalis*. (P). *Heterodermia obscurata*. (Q). *Phaeophyscia hispidula*. (R). *Ramalina conduplicans*. (S). *Heterodermia japonica*. (T). *Flavopunctelia borrierioides*. (U). *Everniastrum nepalensis*. (V). *Punctelia rudecta*. (W). *Cladonia Fruticulosa*. (X). *Cladonia verticillata*

foliose and crustose lichens, with representatives such as *Dirinaria aegialita*, *Parmelia saxatilis*, *Heterodermia diademata*, and *Physcia tribacia* (Figures 5 and 6). Furthermore, the excellent occurrence of lichens across multiple substrates indicates ecological heterogeneity, particularly in the high-altitude region at Syahi Devi. The coexistence of pine and oak phorophytes created a mosaic of habitats that supported intermediate lichen diversity. The lower altitudinal region contributes the lowest diversity, with only nine species across six genera of four families of lichen. The assemblage was dominated by crustose and a few foliose forms, such as *Lecanora tropica* and *Phaeophyscia hispidula*. However, fruticose forms were absent in the lower-altitude region. Therefore, limited diversity was attributed to higher anthropogenic disturbance, including fuelwood collection, grazing, and tourism pressure. It was also observed that the foliose forms were found abundant, followed by fruticose and crustose forms. Fruticose lichens occurred almost exclusively at higher elevations, whereas crustose taxa were observed in higher numbers at low-altitude regions. The mid-altitude pine-oak mixed forest exhibited a transitional composition, whereas the low-altitude pine forest was depauperated. No fruticose lichens were found in the lower elevation zone. Likewise, the number of species increase with elevation, peaking in the high-altitude oak forest region. During the study, poor colonisation of squamulose and dimorphic forms was also shown. However, ecologically, these growth forms are essential for creating niches that support better colonisation and the region's overall vegetation of the region.

3.1. Higher Elevation Region (1800-2200m at Syahi Devi forest)

This forest was dominated by *Quercus* species, particularly *Q. leucotrichophora*, *Rhododendron arboreum*, and *Myrica esculenta* trees. This region exhibits the highest lichen diversity and is represented by 29 (63.04%) species of lichens. The region provides a suitable habitat for the best association of all the growth forms of lichens, particularly for a dimorphic (*Cladonia verticillata*), which was not observed in the middle and lower altitudinal zones.

3.2. Mid-elevation Region Pine-oak Mixed Forest (1400-1800m, Katarmal)

The transitional mixed forest supported 20 species (43.47%) from 12 genera (41.37%) and 7 families (58.33%).

This community included both foliose and crustose lichens, with their representatives such as *Dirinaria aegialita*, *Parmelia saxatilis*, *Heterodermia diademata*, and *Physcia tribacia*. The coexistence of pine and oak phorophytes created a mosaic of habitats that supported intermediate lichen diversity.

3.3. Low-elevation Region Forest (1000-1400 m, Kosi)

This pine-dominated site recorded the lowest diversity, with only 9 species (19.56 %) across 6 genera (20.68 %) and 4 families (33.33 %) of lichens. The assemblage was dominated by crustose and a few foliose forms, such as *Lecanora tropica* and *Phaeophyscia hispidula*. However, fruticose forms were absent here. Therefore, limited diversity was attributed to higher anthropogenic disturbance, including fuelwood collection, grazing, and tourism pressure.

3.4. Lichen Species Colonization

Parmeliaceae was the most dominant family, contributing 23 species (50% of total diversity), followed by Physciaceae (6 species), Ramalinaceae (4 species), and Caliciaceae (3 species). Lichen families such as Cladoniaceae, Peltigeraceae, and additionally, two species of Teloschistaceae were represented, while five families, such as Verrucariaceae, Lecanoraceae, Lobariaceae, Megasporaceae, and Catillariaceae, were each represented by a single species (Table 1). It was also observed that the foliose growth form for lichens was the most abundant, followed by fruticose and crustose forms. Fruticose lichens occurred almost exclusively at higher elevations, whereas crustose taxa dominated at low-altitude pine forests (Table 2). The mid-altitude pine-oak mixed forest exhibited a transitional composition, whereas the low-altitude pine forest was depauperated. No fruticose lichens were found in the lower elevation zone (Table 3). Likewise, species richness increased with elevation, peaking in the high-altitude oak forest. During the study, poor colonisation of squamulose and dimorphic forms was observed due to various man-made disturbances in the region. However, these growth forms are essential for creating an ecological niche in the region to support better colonisation and forest succession.

3.5. Influences of Altitudinal Variation on Lichen Diversity of the Area

Clear variation in species richness and composition was

observed along the altitudinal gradient. The lichen community assemblages in the Syahi Devi-Katarmal and Kosi corridor exhibit significant turnover driven by the altitudinal gradient (1000-2200m). However, the lower altitude is dominated by crustose forms and moisture-tolerant foliose lichen taxa. The zone acts as a transition point where anthropogenic stress and higher solar radiation limit the development of macrolichens. The mid-altitude ecotonal zone niches support both subtropical and temperate taxa (e.g. *Heterodermia* and *Bulbothrix*). However, the higher altitudinal zone provides a high-moisture, low-temperature refugium. This zone harbours the highest number of fruticose lichens (e.g. *Usnea* and *Ramalina*) and diverse foliose communities (e.g., *Parmotrema*). The presence of dense *Quercus* canopies creates a humid microclimate that fosters complex epiphytic colonization. Table 3 depicts variations in lichen species richness along an altitudinal gradient across three sampling sites. A progressive increase in species number is observed with increasing altitude. Site-I (1200 m) recorded the lowest lichen richness, 9 (19.56% species), followed by Site-II (1600 m) with 20 species (43.47%). The highest richness was observed at Site-III (2200 m), where 29 (63.04%) lichen species were recorded. The positive linear trend shows a strong altitudinal influence on lichen diversity, indicating that higher elevations support greater species richness, likely due to more favourable microclimatic conditions and reduced anthropogenic disturbance.

4. CONCLUSION

It is the first altitudinal-based list of the lichens in the Hawalbagh region of the Kumaun Himalaya. The Sahyadri Devi forest has rich plant diversity, highly significant for medicine, fodder, fibre, timber, and wild edible plants. This study has given far-reaching data on the altitudinal distribution of lichens in Syahi Devi, Katarmal, and Kosi region forests, which will be helpful to the plant researchers, organisers, and particularly to the state forest department for developing strategies and action plans for creating procedures and activity plans for the administration of these biodiversity-rich forests. The present study not only corroborates earlier findings from the Kumaun and Western Himalaya but also fills an important gap by providing localised information on lichen diversity and distribution in the Hawalbagh block. The study underscores the importance of this region as a reservoir of lichen diversity, exhibiting the need for further detailed ecological and biomonitoring studies.

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CRedit authorship contribution statement

Pooja Bisht: Field data collection; laboratory work-lichen herbarium preparation, identification; data analysis; data curation; result compilation; writing original draft of the manuscript. Balwant Kumar: Validation; visualisation; review and editing.

Conflict of interest

The authors have declared no conflicts of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used to write the text, or to create or modify any figure, visuals, graphics, or data.

Data availability statement

Data collected and/or analyzed during the current investigation are available from the corresponding author upon reasonable request.





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Floristic Diversity and Regeneration of Agroforestry Parklands in the Sudano-Sahelian Zone of Cameroon

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ABSTRACT: Agroforestry parklands play a crucial role in biodiversity conservation, soil fertility improvement, and climate resilience in arid and semi-arid regions. This study aimed to assess woody species diversity, regeneration capacity, and agroforestry anthropisation and vulnerability indices and to propose a sustainable management strategy across four sites: Benoue, Mayo-Louti, Mayo-Kani and Diamare in the Sudano-Sahelian zone of Cameroon. Floristic inventories were conducted in 50 m × 50 m plots, where all woody individuals were identified and measured. Species diversity was analysed using Shannon-Wiener, Simpson, and Pielou's equitability indices, with principal component analysis supporting the analysis. Tree density, regeneration rates and anthropogenic pressures were also evaluated. A total of 40 woody species belonging to 36 genera and 22 families were recorded. Dominant species included *Khaya senegalensis*, *Adansonia digitata*, *Balanites aegyptiaca* and *Faidherbia albida*. Tree density ranged from 59 to 179 stems per hectare. Regeneration varied markedly among species, with *B. aegyptiaca* showing a high rate (97.5%) and *A. digitata* a very low rate (0.5%). Deforestation, overgrazing and land clearing were major threats. In conclusion, although agroforestry parklands provide significant ecological and socio-economic benefits, targeted sustainable management measures are essential to ensure their long-term resilience and biodiversity conservation.

Keywords: Agroforestry, Diversity, Regeneration, Vulnerability, Management.

1. INTRODUCTION

Agroforestry parklands are traditional agricultural systems in which trees are intentionally preserved or integrated within croplands. These systems play a crucial role in sustaining rural livelihoods and maintaining

ecosystem services, particularly in arid and semi-arid regions. Agroforestry contributes to biodiversity conservation, soil fertility enhancement, carbon sequestration, and the provision of food, fuelwood, timber, and medicinal products, thereby supporting sustainable rural development (Awazi et al., 2025; Mlambo et al., 2025; Yabi

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et al., 2024). Recent studies published have highlighted the multifunctional role of agroforestry systems across diverse ecological and socio-economic contexts (Sharma et al., 2025). Ulman and Yadav (2024) demonstrated that higher plant diversity in agroforestry systems significantly enhances household income, while Santosh et al. (2024) emphasised the importance of gender roles in the management and sustainability of agroforestry systems. Similarly, Sharma et al. (2025) identified agroforestry as a scientific tool for promoting sustainable livelihoods in rural communities. Studies from different agroecological regions further indicate that agroforestry systems contribute simultaneously to biodiversity conservation, ecosystem service provision and livelihood enhancement (Maurya et al., 2025; Tariyal et al., 2025).

Species-specific and system-based studies also underline the economic and ecological relevance of agroforestry. For example, *Senegalia catechu*-based systems have been shown to generate significant economic returns while maintaining ecological functions (Sharma et al., 2025), whereas sandalwood (*Santalum album*)-based agroforestry systems contribute to income diversification and long-term sustainability (Venkatesh et al., 2023). These findings collectively confirm that agroforestry systems represent resilient land-use strategies capable of balancing production, conservation and socio-economic objectives.

In the Sudano-Sahelian zone of Cameroon, agroforestry parklands play a similarly vital role under conditions of high climate variability, prolonged dry seasons, and increasing land degradation, and are largely owned by individuals and local communities, who also ensure their management. However, despite their importance, comprehensive information on floristic diversity, regeneration potential, anthropisation level and vulnerability of agroforestry parklands in this region remains limited. This knowledge gap constrains effective conservation planning and sustainable management, particularly in landscapes facing increasing anthropogenic pressures. Therefore, the present study aimed to assess the floristic diversity and regeneration potential of agroforestry parklands in the Sudano-Sahelian zone of Cameroon, evaluate anthropogenic impacts and species vulnerability and propose sustainable management strategies to enhance ecosystem resilience and support rural livelihoods.

2. MATERIALS AND METHODS

2.1. Study Area

The research was conducted in the Sudano-Sahelian zone of Cameroon, located at latitude 9°20'43.95"N and longitude

13°34'36.38"E. This agroecological region includes the North and Far North Regions, covering approximately 10.2 million hectares, of which around 0.56 million hectares are currently cultivated (Haiwang, 2001; Seignobos, 2000). Fieldwork was conducted from March to September 2024 across four departments, i.e. (In Cameroon specifically, what used to be called "provinces" are now officially called regions, and departments are the next level below regions), Benoue, Mayo-Louti, Mayo-Kani and Diamare selected for their contrasting agroforestry systems and ecological characteristics (Figure 1).

In the present study, agroforestry parklands were grouped according to the dominant tree species maintained and managed by farmers within cultivated fields. This classification is primarily based on the ecological and structural dominance of tree species, as well as their frequency of occurrence in agroforestry systems. The main types of parklands identified are as follows: *Balanites aegyptiaca*-dominated parklands (PBa), *Adansonia digitata*-dominated parklands (PAd), *Khaya senegalensis*-dominated parklands (PKs) and *Faidherbia albida*-dominated parklands (PFa).

The typology of agroforestry parklands was established based on the following criteria: The presence and dominance of a main tree species, considered as the key structural component of the system; The relative frequency and abundance of this species within agricultural plots; The associated agroecological functions (soil fertility enhancement, shading, ecosystem services, and socio-economic uses); The floristic composition and associated species diversity.

This approach makes it possible to distinguish functionally contrasting agroforestry systems, each reflecting both farmers' management choices and local ecological conditions.

2.2. General Methodological Approach

A mixed-methods approach was employed, integrating both quantitative and qualitative data collection techniques. The methodology included: floristic and structural inventories of woody vegetation; ecological indices (diversity, mortality, regeneration, and anthropogenic pressure); and socioeconomic surveys of local farmers using semi-structured questionnaires. This approach aimed to provide a comprehensive understanding of the structure, composition and management of agroforestry parklands in the study area.

2.3. Sampling Design and Experimental Setup

This diagram illustrates a stratified sampling design in which the study area is first divided into departments (blocks) and then into agroforestry parkland types (treatments). Within each stratum, plots of 50 m × 50 m (Figure 2) are randomly selected and replicated to ensure representativeness and statistical reliability. Data are then collected at the plot level on floristic composition, dendrometric parameters, regeneration and anthropogenic pressures.

The study was conducted using a Randomized Complete Block Design (RCBD), with departments considered as blocks and 50 m × 50 m plots as experimental units. Each type of agroforestry parkland was treated as a separate treatment. Four plots were established per parkland type in each department, resulting in 16 replicates per site (Figure 3). The location of each plot was determined randomly using GPS to avoid selection bias. Individual plots serving as the experimental units. Each treatment was replicated five times per department, yielding a robust dataset for statistical analysis.

2.4. Floristic Inventory

Floristic inventory was conducted following the Lejoly (1993) protocol. Taxonomic identification of species was carried out using Arbonnier (2002), and classification followed the APG IV system (Angiosperm Phylogeny Group, 2016). Sampling was performed in rectangular plots

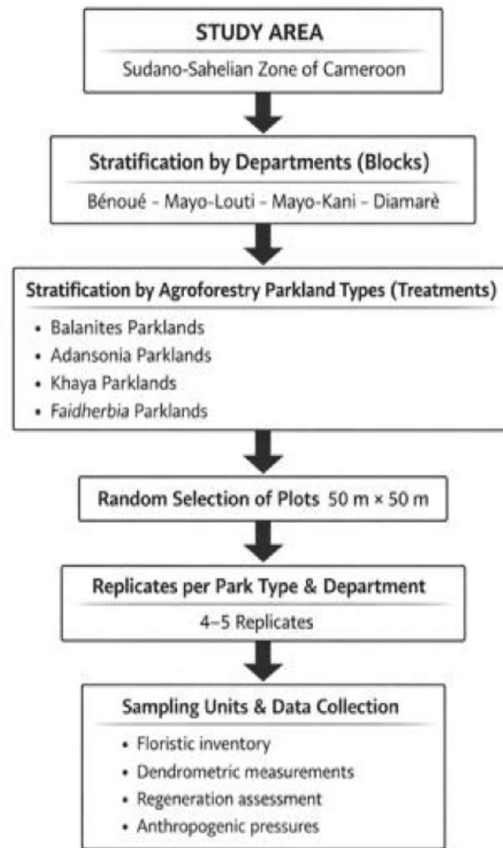


Figure 2. Stratified sampling design of agroforestry parklands in the Sudano-Sahelian zone of Cameroon

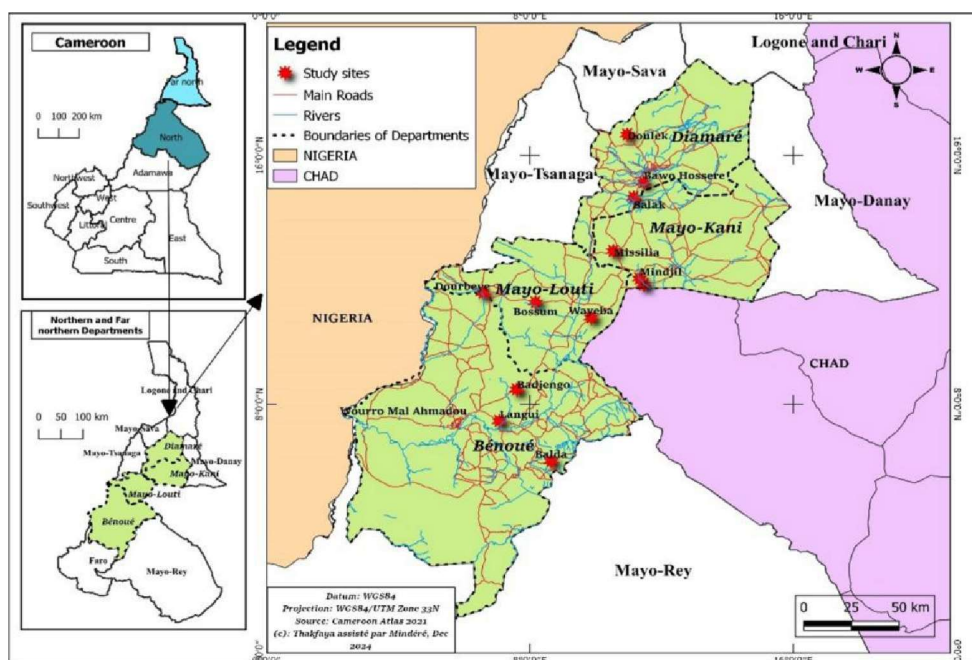


Figure 1. Location of the study area

(Figure 3) measuring 50 m × 50 m (2,500 m²), randomly established in agroforestry parklands. Each plot was considered as a replicate within a stratified sampling design. All woody plant species with a diameter at breast height (DBH) ≥ 10 cm were recorded. The following field equipment was used: a GPS (Garmin Terex 10) to record geospatial coordinates (latitude, longitude, altitude); a decameter and measuring tape for measuring plot boundaries and tree dimensions; a graduated pole for estimating tree height; a machete and string for demarcating plots; a camera (Canon EOS 4000D) for photographic documentation; and a laptop (HP Pavilion) for data entry and processing.

2.5. Structural Characterisation of Agroforestry Parklands

In this study, the structural characterisation of these parklands focused on the analysis of floristic diversity, species composition, and key dendrometric parameters such as tree density, basal area and natural regeneration dynamics. Woody species diversity was quantified using Shannon's diversity index (H'), Pielou's evenness index (EQ) and Simpson's dominance index (S), in order to assess both species richness and distribution patterns within the stands. Particular attention was given to dominant agroforestry species known for their functional roles in parklands systems *K. senegalensis*, *A. digitata*, *B. aegyptiaca* and *Faidherbia albida*. These species, commonly preserved in cultivated plots, interact closely with crops and significantly influence the structural integrity, ecological resilience and productivity of the agroforestry system.

2.6. Dendrometric Measurements

Structural parameters and floristic composition, as well as dendrometric characteristics including tree height (measured using a graduated pole), diameter at breast height (DBH) were assessed to analyse the parkland's structure.

Tree height was measured for both adult and young individuals using a graduated pole, while DBH was calculated from circumference measurements taken at 1.30 m above ground level (Song et al., 2021). Crown diameter was determined using perpendicular measurements in the North–South and East–West directions, providing information on tree canopy cover and spatial distribution.

2.7. Diversity and Floristic Composition

Floristic diversity was quantified by calculating species richness, relative frequency, and relative density. Diversity was further assessed using standard ecological indices, including the Shannon–Wiener diversity index ($H' = -\sum p_i \log_2 p_i$) (Shannon, 1948), Simpson's diversity index ($D = \sum N_i(N_i - 1) / N(N - 1)$) (Simpson, 1949), and Pielou's evenness index ($J' = H' / \log_2 N$) (Pielou, 1966). These indices provide complementary information on species dominance, distribution patterns and overall floristic balance across the studied sites.

2.8. Mortality rate (M)

Dead individuals were identified during the field inventory through direct observation, based on visible signs such as dryness, uprooting, absence of leaves, or lack of regrowth. Each dead individual was recorded within the corresponding plot and compared to the total number of

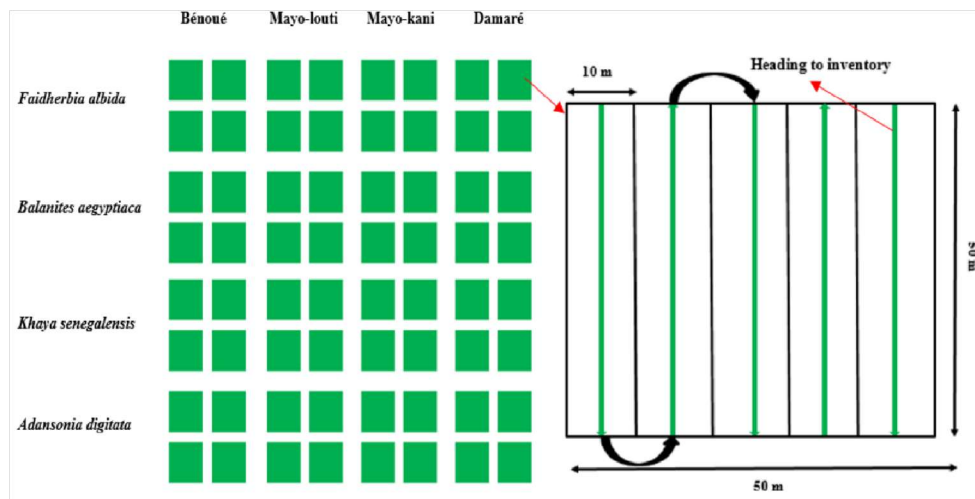


Figure 3. Inventory design (a) and Experimental setup (b)

living individuals. The mortality rate (M) was then calculated as the ratio of dead individuals (n) to the total number of living individuals (N), using the formula: $M = (n / N) \times 100$

2.9. Anthropisation Indices

Anthropogenic factors were recorded through direct field observations. These included the presence of fire traces, tree cutting, bark stripping, pruning and agricultural activities. Such observations provided insights into the frequency of human actions across the different study sites. The frequency of each anthropogenic activity was determined using the following formula (Eq. 1):

Frequency of activity = Number of a given anthropogenic trace / Total number of traces $\times 100$ (1)

2.10. Regeneration

The regeneration potential was assessed by identifying and counting young individuals of tree species (*K. senegalensis*, *A. digitata*, *B. aegyptiaca*, and *F. albida*) in agroforestry parklands. An individual was considered to be regenerating if it had: a stem diameter of less than 10 cm, and a height of less than 4 meters. Their counting allows for estimating the natural renewal capacity of tree populations in these agroforestry systems.

2.11. Assessment of Anthropogenic Pressures and Species Vulnerability

Anthropogenic Pressures and Species Vulnerability such as logging, pruning, fire traces and bark stripping were recorded to assess anthropisation indices. The vulnerability index, adapted from Betti (2001) and Traoré et al. (2011), was used to classify species into low, moderate, or high-risk categories, based on their extraction method, usage frequency and regeneration capacity (Table 1). These

analyses highlight the critical role of conservation strategies in maintaining agroforestry parklands sustainability.

2.12. Farmers' Surveys and Management Perceptions

The management plan was developed based on systematic surveys conducted among farmers in four departments: Benoue, Mayo Louti, Mayo Kani and Diamare. A total of 400 farmers were interviewed, 100 from each site, using semi-structured questionnaires to ensure equitable representation of local contexts. The surveys gathered farmers' perceptions, expectations and suggestions regarding the sustainable management of agroforestry parklands. Key themes included current management practices, priorities such as conservation, reforestation and resource rationalization and ideas for improving the management and productivity of the parklands.

Participants were selected using simple random sampling from lists of cultivators provided by local agricultural services, ensuring equitable representation of local contexts. The surveys focused on farmers' management practices, perceptions and expectations regarding agroforestry parklands. Key themes included current strategies, priorities such as conservation, reforestation and sustainable resource use, as well as farmers' suggestions for improving the management and productivity of the parklands.

Quantitative responses were analysed using descriptive statistics, while qualitative data were examined through content analysis. The insights gained from these surveys served as the basis for developing a context-specific management plan tailored to the needs and realities of each department.

2.13. Data Analysis

All quantitative data were analysed using XLSTAT 2022 and Statgraphic Plus 5.1. Descriptive statistics (mean,

Table 1. Important parameters considered for calculating the vulnerability index

Code	Evaluated parameter	Vulnerability to uncontrolled exposure		
		Low (scale=1)	Medium (scale=2)	High (scale=3)
Q1	Frequency of use (FU)	FU < 20%	Medium 20% ≤ FU <60 %	High FU ≥60%
Q2	Number of uses (NU)	NU < 2	2 ≤ NU ≤ 4	NU ≥ 5
Q3	Plant organ used	Leaf	branch Wood	seed, bark, root, flower
Q4	Collection method	Collection		Picking, cutting
Q5	Stage of development	Old or senescent	Adult	Young
Q6	Relative frequency (RF)	RF ≥ 2/3 Fm	1/3 Fm ≤ RF <2/3 Fm	RF <2/3 Fm

Adapted from (Betti, 2001; Traoré et al., 2011)

standard deviation, percentages) were computed. Analysis of variance (ANOVA) was used to test for differences in floristic parameters across sites, followed by Duncan's Multiple Range Test to separate significant means at $p \leq 0.05$. Graphs were generated in Microsoft Excel (histograms, curves, pie charts). Multivariate analyses were used to examine patterns of diversity and structure among agroforestry parklands. Diversity indices were standardised prior to analysis. Principal component analysis (PCA) and hierarchical clustering were applied to identify overall similarity patterns among parklands. Non-metric Multidimensional Scaling (NMDS) was used to explore compositional dissimilarities, and model fit was assessed using stress values. Multiple factor analysis (MFA) integrated diversity, structural and regeneration variables to identify the main ecological gradients structuring the parklands. Laboratory support for species identification and data validation was provided by the Botany and Ecology Laboratory of the University of Ngaoundéré, in collaboration with the Regional Delegations of Environment and Forestry in the North and Far North Regions.

3. RESULTS AND DISCUSSION

3.1. Floristic Composition per Parkland Types and Department

The floristic composition of agroforestry parklands showed marked variation according to the dominant tree species, whereas no significant differences were observed among departments. However, species richness, as well as genus and family diversity, were significantly influenced by the Department ($p < 0.05$).

Parklands dominated by *Khaya senegalensis* and *Adansonia digitata* exhibited the highest floristic diversity, with up to 18 species, 17 genera, and 13 families. In contrast, parklands dominated by *Faidherbia albida* showed the lowest diversity levels, with only 4 species, 4 genera, and 4 families. The absence of significant differences between Department means suggests that floristic variability in these agroforestry systems is primarily driven by the ecological influence of dominant tree species rather than by geographical location.

Overall, inventory revealed the presence of 40 woody species distributed among 36 genera and 22 families, highlighting the substantial ecological and socio-economic

Table 2. Floristic composition of agroforestry parklands according to department and dominant species

Department	Parameter	Type of parkland				Mean ±SD
		<i>B. aegyptiaca</i>	<i>A. digitata</i>	<i>K. senegalensis</i>	<i>F. albida</i>	
Benoue	Nber of individuals	134 ^b	87 ^b	179 ^a	108 ^b	127.00 ± 40.62
	Nber of species	9 ^c	15 ^b	18 ^a	4 ^d	11.50 ± 6.08
	Nber of genera	9 ^c	15 ^a	17 ^a	4 ^d	11.25 ± 5.68
	Nber of family	6 ^b	11 ^a	11 ^a	4 ^c	8.00 ± 3.16
Mayo Louti	Nber of individuals	84 ^{ab}	106 ^a	59 ^c	78 ^b	81.75 ± 19.42
	Nber of species	11 ^c	18 ^a	15 ^b	10 ^c	13.50 ± 3.70
	Nber of genera	10 ^b	17 ^a	15 ^a	9 ^b	12.75 ± 3.86
	Nber of family	8 ^b	13 ^a	10 ^b	9 ^b	10.00 ± 2.16
Mayo Kani	Nber of individuals	133 ^a	76 ^b	107 ^{ab}	103 ^{ab}	104.75 ± 23.63
	Nber of species	9 ^b	10 ^b	15 ^a	6 ^c	10.00 ± 3.74
	Nber of genera	8 ^b	10 ^b	14 ^a	6 ^c	9.50 ± 3.42
	Nber of family	7 ^b	8 ^{ab}	10 ^a	5 ^c	7.50 ± 2.08
Diamare	Nber of individuals	149 ^a	86 ^c	88 ^c	132 ^b	113.75 ± 31.42
	Nber of species	8 ^c	9 ^c	14 ^a	11 ^b	10.50 ± 2.65
	Nber of genera	6 ^c	14 ^a	13 ^a	9 ^b	10.50 ± 3.87
	Nber of family	6 ^b	10 ^a	10 ^a	7 ^b	8.25 ± 2.06

B. egyptiaca: *Balanites aegyptiaca*, *A. digitata*: *Adansonia digitata*, *F. albida*: *Faidherbia albida*, *K. Senegalensis* : *Khaya senegalensis*, Nber = Number , SD= Standard deviation, Different superscript lowercase letters (a, b, c, ab, d) indicate significant differences between dominant species ($p < 0.05$)

value of agroforestry parklands in the Sudano-Sahelian zone of Cameroon. This level of species richness exceeds that reported in similar systems in Niger and Chad, and is comparable to values documented in Burkina Faso and Mali, indicating that these parklands remain relatively well conserved.

Comparable patterns of high floristic diversity have also been reported in traditional agroforestry systems in India, where long-standing land-use practices and farmer-led tree conservation play a key role in maintaining species richness and ecosystem stability (Ulman and Yadav, 2024; Sharma et al., 2025). These similarities suggest that the observed diversity in northern Cameroon may likewise result from local management practices that favor the conservation of multipurpose woody species.

3.2. Floristic Diversity by Parkland Types and Department

Analysis of diversity indices (Table 3) revealed that Shannon diversity (H'), Pielou's equitability (EQ) and Simpson's dominance (S) were primarily influenced by dominant tree species rather than by the geographical location of Department. Across all Departments, parklands dominated by *Khaya senegalensis* consistently showed the highest Shannon diversity values, indicating a greater complexity of associated woody species. In contrast, *Faidherbia albida* -dominated parklands exhibited the lowest values for all diversity indices, highlighting their

limited contribution to overall floristic diversity and a simpler community structure.

Adansonia digitata -dominated parklands showed the highest equitability values, reflecting a more balanced distribution of associated species and reduced dominance effects. This pattern suggests that *A. digitata* creates ecological conditions favourable to the coexistence of multiple species with relatively similar abundances. Conversely, the lower equitability and diversity values associated with *F. albida* indicate stronger dominance by a few species and reduced structural heterogeneity.

Spatial variation in diversity indices among Department further reflects differences in land-use intensity, management practices and local ecological conditions. Higher Shannon diversity and evenness values observed in Benoue Department suggest more stable and structurally complex agroforestry systems, likely linked to lower anthropogenic pressure and better conservation of woody species. In contrast, lower diversity values recorded in Mayo-Kani indicate a tendency toward dominance by a limited number of species, reflecting higher land-use pressure and reduced ecosystem resilience.

These findings are consistent with observations from dryland agroforestry systems in India, where increasing anthropogenic disturbance has been shown to simplify species composition and reduce functional diversity (Maurya et al., 2025). Additionally, variations in tree density across dominant species and the Department emphasise the

Table 3. Diversity index values of agroforestry parklands according to department and dominant species

Department	Parameter	Type of parkland				Mean ±SD
		<i>B. aegyptiaca</i>	<i>A. digitata</i>	<i>K. senegalensis</i>	<i>F. albida</i>	
Benoue	H' (Shannon)	1.38 ± 0.11 ^b	1.94 ± 0.09 ^a	2.09 ± 0.11 ^a	1.49 ± 0.09 ^b	1.73 ± 0.31
	EQ (Equitability)	0.72 ± 0.03 ^{ab}	0.79 ± 0.04 ^a	0.73 ± 0.03 ^{ab}	0.65 ± 0.09 ^b	0.72 ± 0.06
	S (Simpson)	0.05 ± 0.03 ^b	0.02 ± 0.04 ^a	0.05 ± 0.03 ^b	0.01 ± 0.01 ^a	0.03 ± 0.02
Mayo Louti	H' (Shannon)	1.65 ± 0.11 ^b	1.96 ± 0.09 ^{ab}	2.12 ± 0.08 ^a	1.27 ± 0.08 ^c	1.75 ± 0.37
	EQ (Equitability)	0.74 ± 0.02 ^b	0.83 ± 0.03 ^a	0.71 ± 0.03 ^b	0.46 ± 0.02 ^a	0.69 ± 0.16
	S (Simpson)	0.03 ± 0.01 ^b	0.05 ± 0.03 ^b	0.06 ± 0.05 ^b	0.01 ± 0.01 ^a	0.04 ± 0.02
Mayo Kani	H' (Shannon)	1.85 ± 0.11 ^{ab}	1.51 ± 0.09 ^b	2.04 ± 0.08 ^a	1.92 ± 0.16 ^a	1.83 ± 0.22
	EQ (Equitability)	0.74 ± 0.16 ^a	0.78 ± 0.05 ^a	0.71 ± 0.02 ^{ab}	0.58 ± 0.02 ^b	0.70 ± 0.09
	S (Simpson)	0.05 ± 0.02 ^a	0.04 ± 0.03 ^a	0.05 ± 0.03 ^a	0.03 ± 0.03 ^a	0.04 ± 0.01
Diamare	H' (Shannon)	0.92 ± 0.27 ^c	1.59 ± 0.09 ^{ab}	1.79 ± 0.08 ^a	1.21 ± 0.10 ^b	1.38 ± 0.33
	EQ (Equitability)	0.74 ± 0.03 ^{ab}	0.79 ± 0.03 ^a	0.74 ± 0.03 ^{ab}	0.59 ± 0.02 ^b	0.72 ± 0.08
	S (Simpson)	0.05 ± 0.03 ^a	0.03 ± 0.02 ^a	0.06 ± 0.03 ^a	0.05 ± 0.03 ^a	0.05 ± 0.01

B. egyptica: *Balanites aegyptiaca*, *A. digitata*: *Adansonia digitata*, *F. albida*: *Faidherbia albida*, *K. Senegalensis*: *Khaya senegalensis*, Different superscript lowercase letters (a, b, c, ab) indicate significant differences between dominant species (p<0.05), SD= Standard deviation

combined influence of farmer preferences and site-specific ecological conditions in shaping the structure and diversity of agroforestry parklands.

3.3. Tree Density by Parkland Type and Department

Tree density in agroforestry parklands did not show significant variation between Departments, with mean values ranging from 81.75 ± 19.36 trees ha^{-1} in Mayo-Louti to 127.0 ± 39.64 trees ha^{-1} in Benoue, all belonging to the same statistical group ($p > 0.05$). However, tree density differed significantly according to the dominant species ($p < 0.05$), with *Balanites aegyptiaca* dominated parklands recording the highest mean density (125.0 ± 24.51 trees ha^{-1}), significantly greater than that of *Adansonia digitata* (88.75 ± 10.85 trees ha^{-1}), while densities of *Khaya senegalensis* (108.25 ± 42.38) and *Faidherbia albida* (106.0 ± 13.53) were intermediate and not significantly different from those of *B. aegyptiaca*. These results indicate that tree density is primarily determined by the dominant species rather than the Department location. High densities of *B. aegyptiaca* reflect strong selection and deliberate maintenance farmers due to its multifunctionality and drought tolerance, whereas low densities of *A. digitata* likely result from its large crown size and shading effect, which limit the number of individuals tolerated in cultivated plots. Intermediate densities of *K. senegalensis* and *F. albida* reflect management compromises aimed at balancing agricultural productivity and ecosystem services. The lack of significant differences between Department confirm that geographic or administrative factors play a secondary role in structuring parkland stands, as observed in Sahelian parklands where tree density remains relatively homogeneous at large scales due to comparable agricultural practices and climatic conditions (FAO, 2001). The marked effect of dominant species highlights the importance of farmer decisions in parkland management; high densities of

B. aegyptiaca are consistent with findings by Takimoto et al. (2007), showing that farmers preferentially maintain multifunctional, drought-resistant species, while low densities of *A. digitata* illustrate strategies to reduce competition with crops (Foli et al., 2022). Intermediate densities of *K. senegalensis* and *F. albida* indicate balanced management between agricultural production and ecological functions, with *F. albida* often retained at moderate densities for its positive effect on soil fertility despite its limited contribution to overall stand density (FAO, 2001).

3.4. Regeneration Rate of Species by Agroforestry Parklands

The regeneration rates of woody species varied markedly across different types of agroforestry parklands (Table 5). Several species, including *Agave sisalana*, *Borassus aethiopicum*, *B. dalzielii*, *Daniellia oliveri*, and *Parkia biglobosa*, showed no evidence of natural regeneration in any park type, indicating weak recruitment. In contrast, certain species exhibited relatively high regeneration: *Ziziphus mauritiana* reached up to 67.5% in Adansonia-dominated parklands, *Piliostigma reticulatum* achieved 97.5% in Faidherbia-based parklands, and *Combretum molle* attained 77.5% in Balanites-dominated parklands. Regeneration patterns were clearly influenced by parkland type: Balanites-based parklands favoured the regeneration of *B. aegyptiaca* and *C. molle*, Adansonia-based parklands supported species such as *Acacia seyal* and *Hyphaene thebaica*, Faidherbia-dominated parklands showed strong regeneration of *F. albida* and *P. reticulatum*, whereas Khaya-based parklands generally exhibited low regeneration.

These results reveal pronounced interspecific contrasts in regeneration dynamics. High regeneration rates of *B. aegyptiaca* and *P. reticulatum* stand in sharp contrast to the very low regeneration observed for *Adansonia digitata*,

Table 4. Tree densities of agroforestry parklands according to department and dominant species

Department / Species	<i>B. aegyptiaca</i>	<i>A. digitata</i>	<i>K. senegalensis</i>	<i>F. albida</i>	Mean \pm SD
Benoue	134.0 ± 26.18^{ab}	87.0 ± 9.63^b	179.0 ± 14.86^a	108.0 ± 28.40^{ab}	127.0 ± 39.64^a
Mayo Louti	84.0 ± 13.80^b	106.0 ± 9.06^a	59.0 ± 6.95^c	78.0 ± 18.02^b	81.8 ± 19.36^c
Mayo Kani	133.0 ± 27.72^{ab}	76.0 ± 11.73^b	107.0 ± 14.78^b	103.0 ± 36.22^{ab}	104.8 ± 23.32^b
Diamare	149.0 ± 35.28^a	86.0 ± 13.63^b	88.0 ± 11.68^b	132.0 ± 25.34^a	115.0 ± 23.32^{ab}
Species means \pm SD	125.0 ± 24.51^a	88.8 ± 10.85^b	108.3 ± 4.28^{ab}	106.0 ± 13.53^{ab}	

B. aegyptiaca: *Balanites aegyptiaca*, *A. digitata*: *Adansonia digitata*, *F. albida*: *Faidherbia albida*, *K. Senegalensis*: *Khaya senegalensis*, Letters (a, b, c, ab) indicate significant differences between department and dominant species ($p < 0.05$), SD= Standard deviation

Table 5. Natural regeneration rates of dominant species across different agroforestry parklands types

Species	B. <i>aegyptiaca</i>	A. <i>digitata</i>	F. <i>albida</i>	K. <i>senegalensis</i>
<i>A. ataxacantha</i>	0.0	0.0	0.0	5.0
<i>A. nilotica</i>	17.5	27.5	0.0	2.5
<i>A. seyal</i>	0.0	32.5	5.0	5.0
<i>A. digitata</i>	0.0	0.5	0.5	0.5
<i>A. sisalana</i>	0.0	0.0	0.0	0.0
<i>A. senegalensis</i>	10.0	10.0	17.5	1.5
<i>A. leiocarpus</i>	30.0	0.0	0.0	10.0
<i>A. indica</i>	47.5	47.5	8.5	17.5
<i>B. aegyptiaca</i>	97.5	0.0	0.0	12.5
<i>B. aethiopicum</i>	0.0	0.0	0.0	0.0
<i>B. dalzielii</i>	0.0	0.0	0.0	0.0
<i>C. procera</i>	0.0	0.0	0.0	0.0
<i>C. molle</i>	77.5	0.0	2.5	2.5
<i>D. oliveri</i>	0.0	0.0	0.0	0.0
<i>D. glomerata</i>	0.0	0.0	0.0	0.0
<i>D. mespiliformis</i>	0.0	17.5	0.0	0.0
<i>E. camaldulensis</i>	0.0	0.0	0.0	0.0
<i>F. albida</i>	0.0	10.0	45.0	17.5
<i>F. sycomorus</i>	0.0	7.5	0.0	0.0
<i>F. thonningii</i>	0.0	0.0	0.0	0.0
<i>G. aqualla</i>	0.0	0.0	0.0	0.0
<i>H. monopetalus</i>	2.5	0.0	0.0	0.0
<i>H. thebaica</i>	10.0	50.0	0.0	0.0
<i>I. carnea</i>	0.0	25.0	5.0	0.0
<i>K. senegalensis</i>	0.0	0.0	0.0	0.0
<i>L. schimperii</i>	0.0	0.0	0.0	0.0
<i>M. indica</i>	0.0	0.0	0.0	0.0
<i>M. oleifera</i>	0.0	2.5	0.0	0.0
<i>P. biglobosa</i>	0.0	0.0	0.0	0.0
<i>P. reticulatum</i>	35.0	55.0	97.5	37.5
<i>P. guajava</i>	0.0	0.0	0.0	0.0
<i>S. birrea</i>	20.0	10.0	0.0	0.0
<i>S. singueana</i>	0.0	0.0	0.0	0.0
<i>S. stigeria</i>	0.0	0.0	0.0	0.0
<i>T. indica</i>	20.0	0.0	0.0	0.0
<i>T. glaucescens</i>	20.0	45.0	0.0	0.0
<i>V. paradoxa</i>	0.0	0.0	0.0	0.0
<i>X. americana</i>	0.0	0.0	0.0	0.0
<i>Z. mauritiana</i>	50.0	67.5	60.0	27.5
<i>Z. spina-christi</i>	0.0	2.5	0.0	5.0

highlighting the vulnerability of economically important species to overexploitation, grazing pressure, and poor seedling survival. This pattern of regeneration bottlenecks has been widely reported in both African and Indian agroforestry systems (Bayala et al., 2020; Ulman & Yadav, 2024), and it poses a significant threat to the long-term sustainability and resilience of these parklands. Overall, regeneration dynamics are heterogeneous and largely shaped by the interplay between species traits, parklands types, and ecological conditions.

3.5. Regeneration Rate of Species per Department

The regeneration rates of agroforestry parklands varied among Department; however, these differences were not statistically significant ($p > 0.05$) due to high within-Department variability (Table 6), with the highest mean recorded in Benoue (1.61 ± 1.75), exceeding values in Mayo Kani (0.30 ± 0.28) and Diamare (0.32 ± 0.29), while Mayo-Louti presented an intermediate level (0.67 ± 0.28). In contrast, regeneration differed significantly among dominant tree species ($p < 0.05$), with *Balanites aegyptiaca* showing the highest regeneration (1.82 ± 1.76), largely driven by exceptionally high values in Benoue (4.09). *Khaya senegalensis* exhibited intermediate regeneration (0.82 ± 0.39), whereas *Adansonia digitata* (0.31 ± 0.29) and *Faidherbia albida* (0.45 ± 0.24) displayed significantly lower values. These results indicate that regeneration dynamics are primarily determined by the dominant species rather than by Department location, highlighting the greater regeneration capacity and resilience of *B. aegyptiaca* compared to other species. The observed patterns are consistent with findings in other Sahelian agroforestry systems, where species with multiple economic uses and drought tolerance, such as *B. aegyptiaca*, are preferentially maintained by farmers, resulting in higher natural regeneration rates (Bayala et al., 2020 ; Takimoto et al., 2007). In contrast, species like *A. digitata* often experience regeneration bottlenecks due to overexploitation, grazing, and low seedling survival, which threatens the long-term sustainability of parklands (Ulman & Yadav, 2024). The heterogeneity of regeneration among species underscores the need for targeted management strategies to enhance the recruitment of vulnerable but economically and ecologically important trees, ensuring the resilience and continuity of agroforestry parklands in the Sudano-Sahelian zone.

3.6. Mortality Rate

Table 7 presents the mortality rates of four woody species

(*Balanites aegyptiaca*, *Adansonia digitata*, *Khaya senegalensis* and *Faidherbia albida*) across different Divisions in Northern Cameroon. In the Benoue Department, mortality was 0% for *B. aegyptiaca*, *A. digitata*, and *F. albida*, whereas *K. senegalensis* exhibited a slight mortality rate of 1.67%, indicating a minor sensitivity of this species to local conditions. In the Mayo-Louti, Mayo-Kani and Diamare Department, all four species recorded zero mortality. Overall, plant survival was excellent, with mortality almost negligible, highlighting the strong establishment potential of these species under the studied ecological conditions. The results confirm the hardiness of *B. aegyptiaca*, *A. digitata*, and *F. albida*, while the marginal mortality of *K. senegalensis* suggests that this species may require closer monitoring during early establishment. These findings align with previous studies in Sahelian agroforestry systems, which report high survival rates for drought-tolerant and well-adapted species under appropriate silvicultural management (Bayala et al., 2020; Takimoto et al., 2007). The excellent survival observed also emphasises the feasibility of integrating these species into restoration and agroforestry programs, ensuring sustainable parkland management and long-term ecosystem resilience.

3.7. Anthropisation Index of Agroforestry Parklands by Department

The analysis of anthropogenic pressure in different types

of agroforestry parklands revealed marked variations among practices and Department (Table 8). Cultivation was the most widespread activity, with consistently high values across all Department, peaking in Benoue (35.98) and reaching a minimum in Diamare (22.73). Partial cutting ranked second, particularly high in Benoue (17.05) and Mayo-Louti (14.39), but considerably lower in Diamare (2.27). Peeling and pruning showed generally low intensities, although pruning was relatively more significant in Benoue (4.92) and Mayo-Kani (3.41). Fire trails were almost absent, with minor occurrences only in Mayo-Louti (0.75) and Mayo-Kani (1.14). Among the Departments, Benoue exhibited the highest anthropogenic values across all practices, followed by Mayo Louti, whereas it was lowest in Diamare. Despite these trends, differences among

Table 7. Mortality rate of agroforestry parklands according to department and dominant species

Dominant species	Benoue	Mayo louti	Mayo kani	Diamare
<i>B. aegyptiaca</i>	0	0	0	0
<i>A. digitata</i>	0	0	0	0
<i>F. albida</i>	0	0	0	0
<i>K. senegalensis</i>	3	0	0	0
Mortality	1.67	0	0	0

B.eagyptica: *Balanites aegyptiaca*, *A. digitata*: *Adansonia digitata*, *F. albida*: *Faidherbia albida*, *K. Senegalensis*: *Khaya senegalensis*

Table 6. Regeneration rate of agroforestry parklands according to department and dominant species

Department / Species	<i>B. aegyptiaca</i>	<i>A. digitata</i>	<i>K. senegalensis</i>	<i>F. albida</i>	Mean ± SD
Benoue	4.09 ± 2.00 ^a	0.18 ± 0.02 ^a	1.4 ± 0.00 ^a	0.78 ± 0.01 ^a	1.61 ± 1.75 ^a
Mayo louti	0.89 ± 0.01 ^b	0.33 ± 0.00 ^b	0.91 ± 0.00 ^b	0.53 ± 0.01 ^b	0.66 ± 0.24 ^b
Mayo kani	0.15 ± 0.01 ^c	0.00 ± 0.00 ^c	0.65 ± 0.00 ^c	0.39 ± 0.00 ^c	0.30 ± 0.28 ^b
Diamare	0.15 ± 0.02 ^c	0.71 ± 0.01 ^d	0.30 ± 0.00 ^d	0.11 ± 0.01 ^d	0.32 ± 0.29 ^b
Mean±SD	1.32 ± 1.85 ^a	0.31 ± 0.29 ^c	0.82 ± 0.42 ^b	0.45 ± 0.29 ^c	

B. eagyptica: *Balanites aegyptiaca*, *A. digitata*: *Adansonia digitata*, *F. albida*: *Faidherbia albida*, *K. Senegalensis*: *Khaya senegalensis*, Letters (a, b, c, ab) indicate significant differences between department and dominant species (p < 0.05), SD= Standard deviation

Table 8. Anthropisation index of agroforestry parklands by department

Department/ Practices	Peeling	Pruning	Fire trail	Partial cutting	Cultivation	Mean ± SD
Benoue	5.30 ± 2.01 ^a	4.92 ± 1.87 ^a	0.38 ± 0.14 ^b	17.05 ± 6.46 ^a	35.98 ± 13.63 ^a	12.73 ± 14.39 ^a
Mayo louti	1.51 ± 0.57 ^b	0.75 ± 0.28 ^b	0.75 ± 0.28 ^{ab}	14.39 ± 5.46 ^{ab}	30.30 ± 11.47 ^{ab}	9.55 ± 12.97 ^{ab}
Mayo kani	0.76 ± 0.29 ^b	3.41 ± 1.29 ^{ab}	1.14 ± 0.43 ^a	11.36 ± 4.30 ^b	26.52 ± 10.04 ^b	8.64 ± 10.87 ^{ab}
Diamare	0.65 ± 0.25 ^b	1.62 ± 0.61 ^b	0.00 ± 0.00 ^b	2.27 ± 0.86 ^c	22.73 ± 8.61 ^b	5.45 ± 9.70 ^b
Practice mean	2.06 ± 2.20 ^c	2.68 ± 1.86 ^c	0.57 ± 0.49 ^d	11.27 ± 6.43 ^b	28.88 ± 5.66 ^a	

Different superscript letters (a,b,c,ab) indicate significant differences (p < 0.05); SD= Standard deviation

Departments were not statistically significant ($p > 0.05$). These results indicate that, although the types of anthropogenic activities are relatively homogeneous across regions, their intensity varies considerably, with Benoue experiencing the highest anthropogenic pressure. Anthropisation emerged as a principal driver of ecosystem degradation, as agricultural expansion, partial cutting, and bark harvesting significantly affect floristic composition and natural regeneration. The vulnerability index further identified *Adansonia digitata*, *Khaya senegalensis*, *Balanites aegyptiaca*, and *Sclerocarya birrea* as highly threatened species, corroborating global conservation assessments. These findings align with studies in Sahelian agroforestry and Indian agroecosystems, which report that anthropogenic pressures such as cultivation and selective tree harvesting significantly alter species diversity, regeneration, and ecosystem resilience (Maurya et al., 2025; Sharma et al., 2025).

3.8. Anthropisation Index of Agroforestry Parklands

The analysis of anthropogenic practices across different types of agroforestry parklands revealed notable contrasts. Cultivation (30.59 ± 2.45) and partial cutting (14.11 ± 2.17) were the most prevalent human pressures, while fire trails were the least frequent (0.57 ± 0.49). Among parkland types, *Balanites*-based parklands (PBa) had the highest mean anthropisation index (11.14 ± 14.40), whereas *Faidherbia*-based parklands (PFa) showed the lowest values (9.39 ± 12.06). Large standard deviations indicate substantial variability in the intensity of human activities across parklands types. These results suggest that, although cultivation and partial cutting are consistently practiced across all parklands, their local intensity varies, resulting in heterogeneous levels of anthropogenic pressure. Differences among parkland types were not statistically

significant ($p > 0.05$), implying that parkland type alone does not strongly determine overall anthropisation. This pattern aligns with observations from Sahelian and Indian agroforestry systems, where human activities such as selective tree harvesting and agricultural expansion vary locally but consistently influence species composition, regeneration and ecosystem resilience (Maurya et al., 2025; Sharma et al., 2025).

3.9. Vulnerability Index of Agroforestry Species and Classification According to IUCN

The vulnerability assessment of agroforestry species revealed that most recorded species were classified as Moderately Vulnerable (MV) or Least Concern (LC) according to the IUCN Red List (Table 10). However, certain species were under higher local threat. For example, *Khaya senegalensis*, *Vitellaria paradoxa*, and *Ipomoea carnea* were rated as Very Vulnerable (VU) or Vulnerable (VU), reflecting high anthropogenic pressure and limited natural regeneration. Other species, such as *Boswellia dalzielii* and *Eucalyptus camaldulensis*, were categorized as Near Threatened (NT), while *Agave sisalana* was classified as Endangered (EN). These findings indicate that, although most agroforestry species remain relatively stable, several ecologically and socio-economically important taxa face critical risks. This highlights the need for targeted conservation and management strategies, including controlled harvesting, assisted regeneration, and species-specific protection measures, to maintain biodiversity and ecosystem services. Similar patterns of localized vulnerability due to human pressure and regeneration constraints have been documented in Sahelian and Indian agroforestry systems (Bayala et al., 2020; Ulman & Yadav, 2024), underscoring the importance of integrating vulnerability assessments into sustainable parkland management plans.

Table 9. Anthropisation index of agroforestry Parklands

Anthropisation practice	Peeling	Pruning	Fire trail	Partial cutting	Cultivation	Practice mean \pm SD
PB. <i>aegyptiaca</i>	1.52 ± 0.57^b	2.65 ± 1.00^{ab}	0.76 ± 0.28^a	16.67 ± 6.31^a	34.09 ± 12.91^a	11.14 ± 14.40^a
PA. <i>digitata</i>	3.79 ± 1.43^a	1.89 ± 0.71^b	0.38 ± 0.14^{ab}	14.02 ± 5.30^a	29.55 ± 11.19^{ab}	9.92 ± 12.20^a
PF. <i>albida</i>	2.27 ± 0.86^{ab}	3.79 ± 1.43^a	0.00 ± 0.00^b	11.36 ± 4.30^a	29.55 ± 11.19^{ab}	9.39 ± 12.05^a
PK. <i>senegalensis</i>	0.76 ± 0.28^b	2.65 ± 1.00^{ab}	1.14 ± 0.43^b	14.39 ± 5.45^b	28.79 ± 10.90^b	9.55 ± 12.14^a
Mean \pm SD	2.08 ± 1.29^c	2.75 ± 0.78^c	0.57 ± 0.49^d	14.11 ± 2.17^b	30.49 ± 2.43^a	

P B. *aegyptiaca*: Parkland with *Balanites aegyptiaca*, P A. *digitata*: Parkland with *Adansonia digitata*, P F. *albida* : Parkland with *Faidherbia albida*, P K. *Senegalensis*: Parkland with *Khaya senegalensis*, Different superscript letters (a,b,c,ab,d) indicate significant differences ($p < 0.05$), SD= Standard deviation

Table 10. Vulnerability status of agroforestry species based on IUCN categories

Species	VI	Local status	Status according to IUCN
<i>A. ataxacanta</i>	2.2	MV	LC
<i>A. nilotica</i>	2.2	MV	LC
<i>A. seyal</i>	2.2	MV	LC
<i>A. digitata</i>	2.8	TV	LC
<i>A. sisalana</i>	2.2	MV	EN
<i>A. senegalensis</i>	2.2	MV	LC
<i>A. leiocarpus</i>	2.8	TV	LC
<i>A. indica</i>	2	FV	LC
<i>B. aegyptiaca</i>	2.8	TV	LC
<i>B. aethiopum</i>	2	FV	LC
<i>B. dalzielii</i>	2.2	MV	NT
<i>C. procera</i>	2.2	MV	LC
<i>C. molle</i>	2.2	MV	LC
<i>D. oliveri</i>	2.2	MV	LC
<i>D. glomerata</i>	2.2	MV	LC
<i>D. mespiliformis</i>	2.8	TV	LC
<i>E. camaldulensis</i>	2.2	MV	NT
<i>F. albida</i>	2.2	MV	LC
<i>F. sychomorus</i>	2.2	MV	LC
<i>F. thonningii</i>	2.2	MV	LC
<i>G. aqualla</i>	2.2	MV	LC
<i>H. monopetalus</i>	2.2	MV	LC
<i>H. thebaica</i>	2.2	MV	LC
<i>I. carnea</i>	2.2	MV	VU
<i>K. senegalensis</i>	2.8	TV	VU
<i>L. schimperi</i>	2.2	MV	NT
<i>M. indica</i>	2.2	MV	DD
<i>M. oleifera</i>	2.2	MV	LC
<i>P. biglobosa</i>	2.8	TV	LC
<i>P. reticulatum</i>	2.2	MV	LC
<i>P. guajava</i>	2	MV	LC
<i>S. birrea</i>	2.8	TV	LC
<i>S. singueana</i>	2.2	MV	LC
<i>S. setigera</i>	2	FV	LC
<i>T. indica</i>	2	FV	LC
<i>T. glaucescens</i>	2	FV	LC
<i>V. paradoxa</i>	2.2	MV	VU
<i>X. Americana</i>	2.2	MV	LC
<i>Z. mauritiana</i>	2.2	MV	LC
<i>Z. spina-christi</i>	2.2	MV	LC

MV: Moderately Vulnerable, LC: Least Concern, DD: Data Deficient, TV: Very Vulnerable, FV: Slightly Vulnerable, VU: Vulnerable, NT: Near Threatened, EN: Endangered

3.10. Strategy for an Agroforestry Parklands

Management Plan

The analysis of proposed management strategies for agroforestry parklands revealed notable variations across Departments (Table 11). Overall, the most emphasised strategies were conservation and restoration in agreement with government policies (22.50 ± 2.08) and the rational use of natural resources (16.50 ± 1.29), highlighting the importance of institutional collaboration and sustainable practices. Moderate attention was given to initiatives such as supporting the Green Sahel project (10.75 ± 1.70), raising

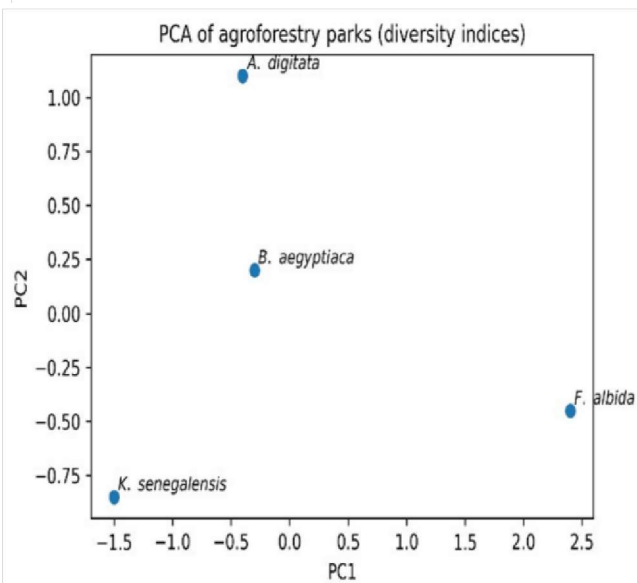
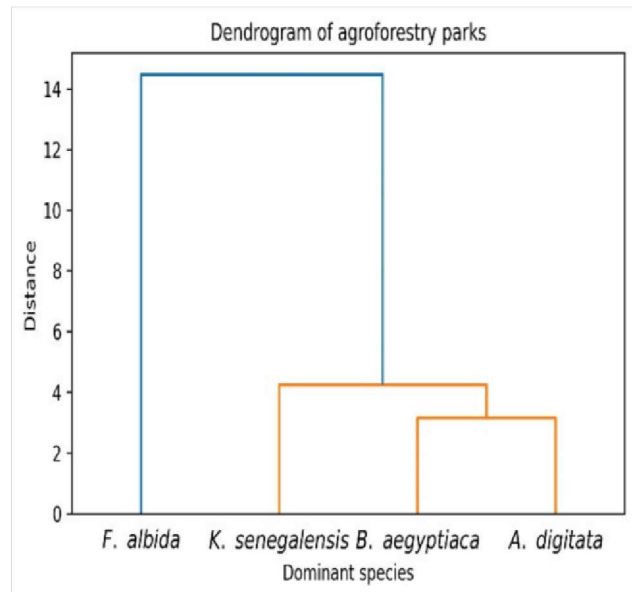


Figure 4. Multivariate structuring of agroforestry parklands based on diversity indices

awareness on the importance of trees (10.00 ± 1.63), and tree planting (10.00 ± 0.82), collectively reflecting a balance between ecological restoration and community engagement. Conversely, less priority was given to protection of young plants (4.50 ± 1.29) and waste protection (4.75 ± 1.26), suggesting that practical, on-the-ground measures for regeneration receive limited emphasis compared to broader policy and awareness-based interventions. Comparisons across Departments showed similar average numbers of strategies implemented (Benoue: 10.00; Mayo-Louti: 10.18; Mayo-Kani: 10.00; Diamare: 9.91), indicating a shared perception of management priorities throughout the study area. These results underscore the predominance of institutional and awareness-driven approaches over strictly technical measures in the management of agroforestry parklands. Similar findings have been reported in Sahelian agroforestry systems, where policy-driven and community

engagement strategies often take precedence over field level technical interventions, emphasising the role of governance and local participation in sustainable management (Foli et al., 2022; Takimoto et al., 2007).

3.11. Multivariate Analysis of Agroforestry Parklands Structure and Diversity

Multivariate analyses consistently revealed a clear structuring of the studied agroforestry parklands according to their dominant tree species and associated ecological characteristics (Figure 4). Hierarchical clustering based on diversity indices highlighted distinct groupings, with *Faidherbia albida*-dominated parklands clearly separated from other parkland types, indicating pronounced differences in species composition and diversity patterns. This separation was further confirmed by principal component analysis (PCA), where *F. albida* parkland was strongly segregated along the first principal axis, reflecting substantial divergence in diversity structure relative to parks dominated by *Khaya senegalensis*, *Balanites aegyptiaca*, and *Adansonia digitata*, which clustered more closely together. These findings suggest that dominant tree species play a key role in shaping community composition and ecological interactions in agroforestry parklands. Similar patterns have been reported in Sahelian and Indian agroforestry systems, where species-specific traits drive species differentiation.

Complementary ordination analyses using Non-metric Multidimensional Scaling (NMDS) and multiple factor analysis (MFA) provided further insights into the ecological gradients shaping agroforestry parklands. The NMDS ordination revealed partial but discernible clustering of

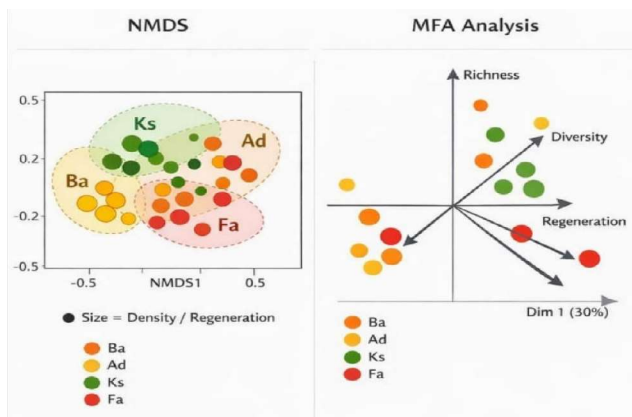


Figure 5. Multivariate analyses of the studied agroforestry parklands using Non-metric multidimensional scaling (NMDS) and multiple factor analysis (MFA)

Table 11. Proposed agroforestry Parklands Management Plan

Strategies	Benoue	Mayo louti	Mayo kani	Diamare	Mean ±SD
Conservation, restoration in agreement with the government	20±3,64 ^a	25±3,86 ^a	23±4,09 ^a	22±4,06 ^a	22.5±2.08 ^c
Tree planting	10±1,82 ^a	08±1,23 ^a	09±1,60 ^a	10±1,85 ^a	9.25±0.95 ^c
Raising awareness of the importance of trees	12±2,18 ^a	09±1,39 ^b	11±1,96 ^{ab}	10±1,85 ^{ab}	10.5±1.29 ^c
Rational use of natural resources	18±3,27 ^a	16±2,47 ^{ab}	15±2,67 ^b	17±3,14 ^a	16.5±1.29 ^b
reducing the pressure to harvest firewood	08±1,46 ^a	07±1,08 ^b	09±1,60 ^a	05±0,92 ^c	7.25±1.70 ^d
Reorganise the green sahel project	10±1,82 ^{ab}	13±2,01 ^a	11±1,96 ^{ab}	09±1,66 ^b	10.75±1.70 ^c
Protecting waste	05±0,91 ^b	03±0,46 ^c	06±1,07 ^{ab}	07±1,29 ^a	5.25±1.70 ^d
Recruitment of forest wardens	09±1,64 ^a	08±1,23 ^a	07±1,25 ^{ab}	06±1,11 ^b	7.5±1.29 ^d
Creation of forest reserves	05±0,91 ^b	06±0,93 ^{ab}	05±0,89 ^b	08±1,48 ^b	6±1.41 ^d
Protection of young plants	03±0,55 ^a	05±0,77 ^{ab}	04±0,71 ^b	06±1,11 ^a	4.5±1.29 ^c
Mean±SD	10±5.49 ^a	10±6.48 ^a	10±5.61 ^a	10±5.41 ^a	10±5.45 ^c

Different superscript letters (a,b,c,ab e,d) indicate significant differences (p < 0.05), SD= Standard deviation

parks according to their dominant species, with some overlap among groups, indicating gradual ecological transitions rather than strictly discrete assemblages. MFA further identified clear relationships between parklands types and key ecological variables: the first dimension was primarily structured by gradients of regeneration versus species richness and diversity. *Faidherbia albida*-dominated parklands were closely associated with higher regeneration levels, whereas *Khaya senegalensis* and *Adansonia digitata* parks were more strongly related to richness and diversity variables. *Balanites aegyptiaca* occupied an intermediate position, reflecting mixed ecological characteristics.

These analyses confirm that dominant tree species strongly influence parkland structure and dynamics. The distinct position of *F. albida* parklands reflects its well-documented ecological role in enhancing soil fertility and promoting understory regeneration, as reported in both African and Indian agroforestry systems (Bayala et al., 2020; Ulman & Yadav, 2024). Overall, the results underscore the importance of assisted natural regeneration, protection of juvenile plants, and community-based management strategies to maintain the long-term resilience, productivity and ecological functionality of agroforestry parklands.

4. CONCLUSION

The present study confirms the ecological and socio-economic importance of agroforestry parklands in the Sudano-Sahelian zone of Cameroon. The recorded woody diversity (40 species belonging to 36 genera and 22 families) and the wide variation in tree density (59–179 stems ha⁻¹) reflect the heterogeneity of parkland structures and management practices. Species-specific differences in regeneration highlight a major sustainability concern, with *Balanites aegyptiaca* showing strong regenerative capacity, whereas *Adansonia digitata*, despite its high basal area and ecological value, exhibits critically low regeneration. Anthropogenic pressures, particularly agricultural expansion, partial cutting and bark harvesting, strongly influence floristic composition and regeneration dynamics, increasing the vulnerability of key multipurpose species. These findings indicate that, although agroforestry parklands continue to provide important ecological functions and livelihood benefits, their long-term resilience is increasingly threatened. Sustainable management should therefore prioritize assisted natural regeneration, protection of juvenile plants and community-based restoration strategies, supported by appropriate policy frameworks.

Such integrated approaches are essential for conserving biodiversity, maintaining ecosystem services, and enhancing the sustainability of agroforestry systems in the Sudano-Sahelian region.

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CRediT authorship contribution statement

Tchakfaya Fanehela Felix: Data curation, Funding acquisition, Investigation, Resources, Supervision, Visualization and Writing – original draft. **Ali Ahmed Davy**: Conceptualization, Data collection, Formal analysis, Funding acquisition, Investigation, Methodology, project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft and Writing-review & editing. **Massai Tchima Jacob**: Visualization. **Tchobsala**: Conceptualization, Data curation, Methodology, project administration, Software, Supervision, Validation and Visualization.

Conflict of interest

The author has no conflicts with any step of the article preparation.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no artificial intelligence tools were used to write this manuscript.

Data availability statement

All data supporting the findings of this study are fully available with the authors.

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New Distributional Records with an Annotated List of Scolopendrid Centipedes (Myriapoda: Chilopoda) from West Bengal, India


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ABSTRACT: The diversity and distribution of scolopendrid centipedes in eastern India, particularly in West Bengal, remain poorly documented despite their ecological importance. Previous studies recorded only thirteen species from the state, indicating a significant knowledge gap. The present study provides a comprehensive and up-to-date account of the family Scolopendridae in West Bengal, based on surveys conducted between 2021 and 2024 across diverse habitats, supplemented by a literature review and examination of specimens. The study also clarifies several earlier taxonomic and distributional ambiguities through the reassessment of doubtful records and updated nomenclatural interpretations. A total of eighteen species are documented, increasing the known diversity from thirteen to eighteen species. Five species are recorded for the first time from the state, namely *Ethmostigmus tristis* (Meinert, 1886), *Rhysida crassispina* Kraepelin, 1903, *Rhysida konda* Joshi, Karanth & Edgecombe, 2020, *Rhysida trispinosa* (Jangi & Dass, 1984), and *Scolopendra subspinipes* Leach, 1815, representing notable range extensions in eastern India. The study provides taxonomic notes, diagnoses, materials examined, and an updated distributional list for all species. These results establish a comprehensive baseline for future taxonomic, ecological, and biogeographical research on scolopendrid centipedes in the region.

Keywords: Centipede, Species, *Rhysida*, *Ethmostigmus*, *Scolopendra*, Scolopendridae, Eastern India.

1. INTRODUCTION

The centipede fauna of India is notably rich and diverse, with 126 species currently documented across the country. Despite the heterogeneous topography and ecological habitats of West Bengal, only 16 species have been reported so far (Kashmeera & Sureshan, 2025). Of these, family Scolopendridae in West Bengal is represented by thirteen

species, viz. *Cormocephalus dentipes* Pocock, 1891, *Cormocephalus pygmaeus* Pocock, 1892, *Ethmostigmus pygomegas* (Kohlrausch, 1881), *Otostigmus nudus* Pocock, 1890, *Otostigmus politus* Karsch, 1881, *Otostigmus rugulosus* Porat, 1876, *Otostigmus spinosus* Porat, 1876, *Otostigmus scaber* Porat, 1876 (recorded from the state as *Otostigmus insularis* Hasse, 1887, which is currently considered a synonym of *Otostigmus scaber*), *Rhysida*

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longipes (Newport, 1845), *Rhysida pazhuthara* Joshi, Karanth & Edgecombe, 2020, *Scolopendra dehaani* Brandt, 1840, *Scolopendra hardwickei* Newport, 1844 and *Scolopendra morsitans* Linnaeus, 1758 (Khanna & Tripathi, 1985; Yadav 1993; Khanna, 1994a, 1997, 2006, 2013, 2020; Balan & Sureshan 2018 and Mukherjee et al., 2026). In light of recent field collections and a critical reassessment of earlier records, the present study aims to update the list of Scolopendridae species known from West Bengal. The present study documents five species of scolopendrid centipedes, thereby increasing the known diversity of Scolopendridae to eighteen species. In addition, the study compiles existing distributional data from the literature, thereby contributing to a broader understanding of scolopendrid centipede distribution patterns across the state and highlighting knowledge gaps in regional biodiversity assessments.

2. MATERIALS AND METHODS

2.1. Collection and Preservation of Scolopendrid Species

Field surveys were conducted across various districts of West Bengal, India, covering a wide range of habitat types, including forested areas, grasslands, agricultural fields, and urban environments (Figure 1). Sampling was undertaken in diverse microhabitats such as beneath logs and stones, within leaf litter, under decomposing wood, and among accumulated organic debris to ensure coverage across diverse ecological conditions. Specimens were collected



Figure 1. Habitat types of the Scolopendridae collection sites across districts of West Bengal: **A.** Purulia, **B.** Paschim Bardhaman, **C.** Jhargram, **D.** Paschim Medinipur

using a combination of methods, including active hand searching under stones, logs, and leaf litter with long forceps, as well as litter sampling, ground collection, and vegetation-based sampling (Balan & Sureshan, 2018). Subsequently, the specimens were preserved in 70% ethanol in separate vials. Relevant field data, including locality, habitat type, date of collection, and collector details, were recorded for each specimen during sampling.

2.2. Identification of Scolopendrid Species

The collected samples were examined for diagnostic morphological characters using a Leica EZ4 educational stereo zoom microscope. High-resolution photographic documentation was carried out using a Leica M205A stereo zoom microscope equipped with a Leica DMC-4500 digital camera. Identification was carried out based on published literature: Jangi & Dass 1984; Lewis, 2010a, 2010b, 2015; Joshi & Edgecombe, 2013, 2018; Siriwut et al., 2016; Balan & Sureshan 2018; Joshi et al., 2020; Schileyko et al., 2020 and Chen et al., 2023.

All examined specimens are deposited in the National Zoological Collections of the Museum and Taxidermy Section (ZSIK-TC) of the Zoological Survey of India, Kolkata, India. Final image processing was completed using Adobe® Photoshop 7.0. The map was prepared using QGIS 3.40 Bratislava (QGIS Development Team, 2026). In addition, an annotated list of Scolopendridae species found in West Bengal is presented in Table 1. Collection details of examined specimens of the remaining species (which are not included here as new records) are provided in the Supplementary material.

3. RESULTS AND DISCUSSION

3.1. Taxonomy

Class - Chilopoda

Order - Scolopendromorpha

Family - Scolopendridae Leach, 1814

Genus - *Ethmostigmus* Pocock, 1898

Type species- *Scolopendra trigonopodus* Leach, 1817

1. *Ethmostigmus tristis* (Meinert, 1886) (Figure 2)

Diagnosis: Antennae consisting of 20 articles, glabrous on the basal four (Figure 2A). The cephalic plate is smooth, with a longitudinal median furrow present on the anterior part of the plate. Tooth plate bearing three teeth (Figure 2B). Tergite with complete paramedian suture from tergite 4 (Figure 2C). Coxopleural process with a dorsal spine, the process is approximately twice as long as the sternite of the ultimate leg-bearing segment (Figure 2D). Ultimate leg

prefemur with one dorsomedial spine on the prefemur.

Material Examined: 1 ex. (ZSIK-TC119), Kangsabati water supply canal, Paschim Medinipur district, 22°24'23" N, 87°18'13" E, 12.viii.2024, Coll. Uttam Kumar Chaudhary.

Distribution in India: Tamil Nadu (Joshi & Edgecombe, 2018), West Bengal (new record).

Remarks: This is the first report of *Ethmostigmus tristis* from West Bengal (Figure 5A). The only characteristic difference found in the examined specimen (ZSIK-TC119) is the presence of two tarsal spurs in the first six pairs of legs (vs in the first three or four pairs of legs, as stated in Joshi and Edgecombe (2018)).

Genus - *Rhysida* Wood, 1862

Type species- *Branchiostoma lithobioides* Newport, 1845

2. *Rhysida crassispina* Kraepelin, 1903 (Figure 3A, B)

Diagnosis: Antennae consisting of 18 articles, glabrous on the first five. The cephalic plate is smooth and narrow, with a longitudinal median furrow present on the anterior part of the plate. Tooth plate bearing four teeth. Sternite with complete paramedian suture (Figure 3A). Coxopleural process approximately twice as long as the sternite of the ultimate leg-bearing segment (Figure 3B). Leg 1 with a tibial and femoral spur.

Material Examined: 1 ex. (ZSIK-TC044), Joytunga, Paschim Medinipur district, 23°15'53" N, 87°15'47" E, 27. ix.2023, Coll. Kaushik Deuti and Party; 1 ex. (ZSIK-TC045), Susunia, Bankura district, 23°23'42" N, 86°59'14" E, 27. ix. 2023, Coll. Kaushik Deuti and Party.

Distribution in India: Kerala, Maharashtra, Meghalaya, Uttarakhand, Uttar Pradesh (Khanna, 1994a, 2010; Joshi et al. 2020 and Kashmeera et al. 2025), West Bengal (new record).

Table 1. Annotated list of scolopendrid centipede species from West Bengal

Species	Distribution in West Bengal	Source
* <i>Cormocephalus dentipes</i> Pocock, 1891	Kolkata, Nadia, Paschim Medinipur, Purulia	Khanna & Tripathi, 1985
<i>Cormocephalus pygmaeus</i> Pocock, 1892	Darjeeling	Khanna, 1994a, 2006
<i>Ethmostigmus pygomegas</i> (Kohlrausch, 1881)	Reported in earlier literature without a precise locality.	Khanna, 1994a
** <i>Ethmostigmus tristis</i> (Meinert, 1886)	Paschim Medinipur	
* <i>Otostigmus nudus</i> Pocock, 1890	Jhargram, Nadia, Paschim Medinipur	Mukherjee et al. 2026
<i>Otostigmus politus</i> Karsch, 1881	Darjeeling	Khanna, 1994a, 1997, 2020
* <i>Otostigmus rugulosus</i> Porat, 1876	Alipurduar, Bankura, Birbhum, Jhargram, Nadia, Paschim Bardhaman, Paschim Medinipur, Purulia, South 24 Parganas	Mukherjee et al. 2026
* <i>Otostigmus scaber</i> Porat, 1876	Alipurduar	Khanna, 1994a, 2008
<i>Otostigmus spinosus</i> Porat, 1876	Reported in earlier literature without a precise locality.	Khanna, 1994a
** <i>Rhysida crassispina</i> Kraepelin, 1903	Bankura, Paschim Medinipur	
** <i>Rhysida konda</i> Joshi, Karanth & Edgecombe, 2020	Jhargram, Paschim Bardhaman, Paschim Medinipur	
* <i>Rhysida longipes</i> (Newport, 1845)	Bankura, Birbhum, Jhargram, Kolkata, Paschim Bardhaman, Purba Bardhaman, Purulia.	Khanna & Tripathi, 1985; Khanna, 1994a.
* <i>Rhysida Pazhuthara</i> Joshi, Karanth & Edgecombe, 2020	Jhargram, Kolkata, Nadia, Paschim Bardhaman, Paschim Medinipur	Mukherjee et al. 2026
** <i>Rhysida trispinosa</i> (Jangi & Dass, 1984)	Paschim Medinipur, Purulia	
* <i>Scolopendra dehaani</i> Brandt, 1840	Darjeeling, Hooghly, Howrah, Kolkata, North 24 Parganas, Purba Bardhaman	Khanna, 1994a
* <i>Scolopendra hardwickei</i> Newport, 1844	Mentioned without a precise locality.	Yadav, 1993; Khanna, 2013; Balan & Sureshan, 2018
<i>Scolopendra morsitans</i> Linnaeus, 1758	Bankura, Purba Bardhaman	Balan & Sureshan, 2018
** <i>Scolopendra subspinipes</i> Leach, 1815	Purba Bardhaman	

* Previously reported species with new locality data added in the present study

**Species recorded for the first time from West Bengal in this study

Remarks: This is the first report of *Rhysida crassispina* from West Bengal (Figure 5B). The examined specimen (ZSIK-TC045) exhibits minor variations compared to the previously redescribed material. These include:

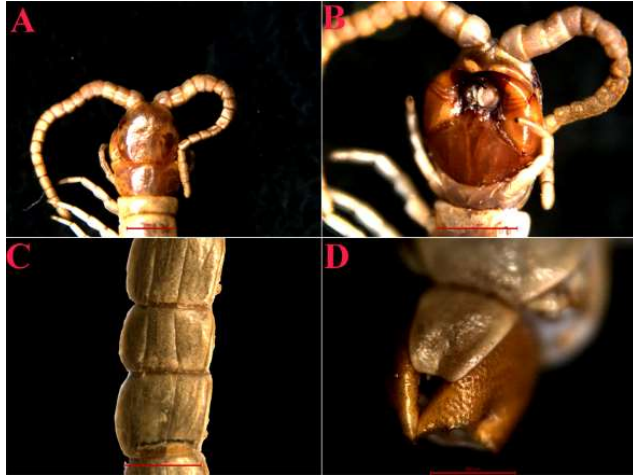


Figure 2. Diagnostic characters of *Ethmostigmus tristis* (Meinert, 1886): **A.** Cephalic plate with antenna, **B.** Tooth plate, **C.** Tergite with paramedian sutures, **D.** Sternite 21 with coxopleural process. Scale bars: A-C: 1mm; D: 0.5 mm

Coxopleural process with two apical spines and one lateral spine (vs two apical spines only), no tarsal spur in leg 2-20 (vs one tarsal spur in leg 2-20) (Joshi et al., 2020).

3. *Rhysida konda* Joshi, Karanth & Edgecombe, 2020 (Figure 3C, F)

Diagnosis: Antennae consisting of 18 articles. The cephalic plate is smooth, with a longitudinal median furrow present on the anterior part of the plate. Tooth plate bearing four teeth into two groups (Figure 3C). Sternite with paramedian suture 5-10% of length. Coxopleural process with two apical, one subapical and one lateral spine. Ultimate leg prefemur with 3 ventrolateral, 2 ventromedial and 3 dorsomedial spines (Figure 3F). Leg 1-4 with tibial spur. Leg 1-7 two tarsal spurs. One tarsal spur in leg 8-20.

Material Examined: 1 ex. (ZSIK-TC125), Kuradi, 14 No. coliary, Paschim Bardhaman district, 23°22'51" N, 87°01'27" E, 3. viii.2024, Coll. Uttam Kumar Chaudhary; 1 ex. (ZSIK-TC162), Saldanga, Paschim Medinipur district, 23°39'14" N, 87°35'33" E, 11.viii.2024, Coll. Uttam Kumar Chaudhary; 1 ex. (ZSIK-TC165), Beachi village, towards Khandarni dam, Jhargram district, 22°40'17" N, 86°42'39" E, 9.viii.2024, Coll. Uttam Kumar Chaudhary; 2 exs. (ZSIK-TC171 and ZSIK-TC172), Astajuri, Jhargram district,

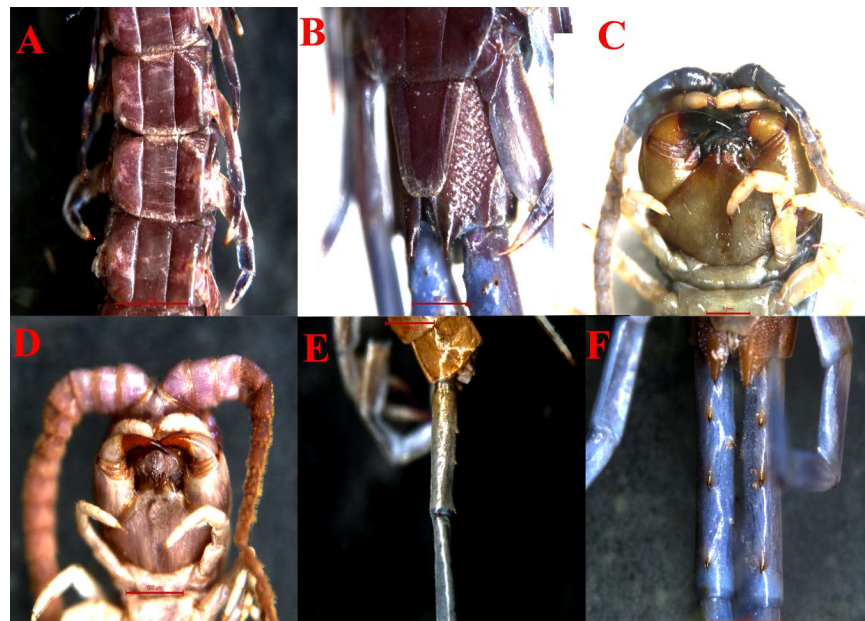


Figure 3. Diagnostic characters of *Rhysida crassispina* Kraepelin, 1903: **A.** Sternite, **B.** Coxopleural process ventrolateral view; diagnostic characters of *Rhysida konda* Joshi, Karanth & Edgecombe, 2020: **C.** Tooth plate, **F.** Ultimate leg prefemur and diagnostic characters of *Rhysida trispinosa* (Jangi & Dass, 1984): **D.** Tooth plate, **E.** Ultimate leg prefemur. Scale bars: A, C & F: 1 mm; B, D & E: 0.5 mm

22°38'59" N, 86°45'15" E, 7.viii.2024, Coll. Uttam Kumar Chaudhary.

Distribution in India: Chhattisgarh, Odisha (Joshi et al. 2020; Mukherjee et al. 2025), West Bengal (new record).

Remarks: This is the first report of *Rhysida konda* from West Bengal (Figure 5B). One of the examined specimens (ZSIK-TC125) shows minor variation compared to the previously redescribed material, such as the dorsomedial spine in the ultimate leg prefemur being absent (vs three dorsomedial spines present, as mentioned in Joshi et al., 2020).

4. *Rhysida trispinosa* (Jangi & Dass, 1984) (Figure 3 D, E)

Diagnosis: Antennae consisting of 20-21 articles. The cephalic plate is smooth, with a longitudinal median furrow present on the anterior part of the plate. Tooth plate bearing four teeth (Figure 3D). Sternite with paramedian suture 5-10% of length. Coxopleural process approximately 1.5 times of the sternite of the ultimate leg-bearing segment. Coxopleuron with two apical and one subapical spine. Ultimate leg prefemur with 2 ventrolateral, 1 median and 1 dorsomedial spine (Figure 3E). Leg 1-18 with two tarsal spurs, 19 and 20 with one spur.

Material Examined: 1 ex. (ZSIK-TC136), Murguma Dam, near Jhalda, Purulia district, 23°18'51" N, 86°02'59" E, 6. viii.2024, Coll. Uttam Kumar Chaudhary; 1 ex. (ZSIK-TC161), Saldanga, Paschim Medinipur district, 23°39'14" N, 87°35'33" E, 11.viii.2024, Coll. Uttam Kumar Chaudhary.

Distribution in India: Andhra Pradesh, Karnataka, Kerala, Maharashtra, Tamil Nadu (Jangi & Dass, 1984; Balan & Sureshan, 2018; Joshi et al., 2020), West Bengal (new record).

Remarks: This is the first report of *Rhysida trispinosa* from West Bengal (Figure 5B).

Genus - *Scolopendra* Linnaeus, 1758

Type species- *Scolopendra morsitans* Linnaeus, 1758

5. *Scolopendra subspinipes* Leach, 1815 (Figure 4A-E)

Diagnosis: Antennae consisting of 18 articles, glabrous on the first six (Figure 4A), broken on one side. The cephalic plate is smooth, with a longitudinal median furrow present on the anterior part of the plate. Tooth plate bearing five teeth (Figure 4B). Tergite 3-20 paramedian suture and margination are complete from tergite 14-21 (Figure 4D). Sternite 3-20 with Complete paramedian suture (Figure 4C). Coxopleural process with two apical spines but lacking lateral and dorsal spines. Ultimate leg prefemur with 2 ventrolateral, 1 median and 3 dorsomedial spines (Figure

4E). Leg 1-20 with one tarsal spur.

Material Examined: 1 ex. (ZSIK-TC090), Bagilla village, Memari, Purba Bardhaman district, 23°09'08" N, 88°07'49" E, 15. ix. 1993, Coll. Chandra Bhan Prasad.

Distribution in India: Andaman and Nicobar Islands, Tamil Nadu (Khanna, 2001), West Bengal (new record).

Remarks: *Scolopendra subspinipes sensu stricto* has never been reported from West Bengal, as all earlier records refer to *Scolopendra subspinipes dehaani*. So, this study presents the first confirmed report of the species from the state (Figure 5C).

3.2. Key Insights

The distributional patterns of the newly recorded scolopendrid species across the districts of West Bengal are illustrated in Figure 5. In addition to these species, several other Scolopendrid species have been reported from West Bengal in earlier literature but are not currently present in the state. Notably, *Cormocephalus macrosestrus* (Attems, 1928) was originally described with two type localities, one of which was Calcutta. However, this was later deemed erroneous, and Bonato et al. (2016) restricted the type locality to the Western Ghats. Khanna (1994b, 2010, 2020) reported the presence of *Rhysida afra* (Peters, 1855) in West Bengal. However, this species has since been restricted to South Africa (Joshi et al., 2020). Similarly, *Rhysida nuda*



Figure 4. Diagnostic characters of *Scolopendra subspinipes* Leach, 1815: **A.** Cephalic plate with antennal articles, **B.** Tooth plate, **C.** Sternite with Paramedian Suture, **D.** Tergite with margination, **E.** Tergite 21 with Ultimate leg Prefemur. Scale bars: A-E: 1 mm

immarginata (Newport, 1845) was recorded from the state by Khanna (1994a, 2006, 2010), but *Rhysida nuda* is now considered endemic to Australia (Joshi et al., 2020). *Rhysida immarginata immarginata* (Porat, 1876), also reported from West Bengal by Balan and Sureshan (2018), has likewise been restricted to Southeast Asia by Joshi et al. (2020).

In the present study, most of the species documented from the state are endemic to India, except *Scolopendra subspinipes* Leach, 1815, which exhibits a broad global distribution, including regions such as Africa, Hong Kong, Irian Jaya, Laos, Peninsular Malaysia, Philippines, Sarawak, Seychelles, Singapore, South America, Sri Lanka, Sulawesi, Sumatra, Surinam, and Vietnam.

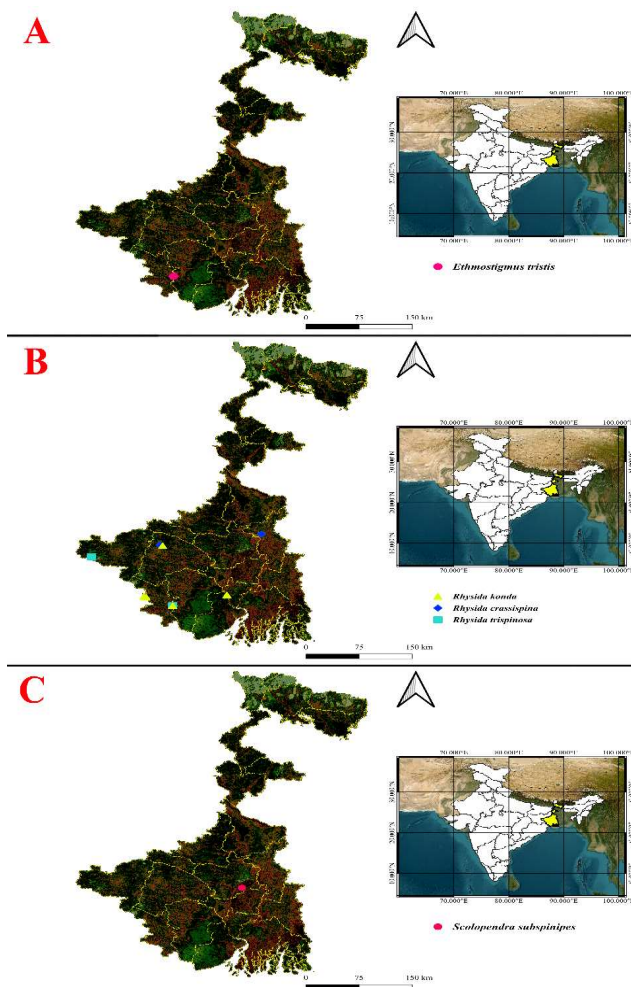


Figure 5. Mapping the distribution of newly recorded Scolopendridae species across West Bengal, India: **A.** *Ethmostigmus tristis* (Meinert, 1886), **B.** *Rhysida* spp., **C.** *Scolopendra subspinipes* Leach, 1815

3.3. List of Members of the Family Scolopendridae in West Bengal

A comprehensive compilation of a total of eighteen species of Scolopendrid centipedes collected and documented to date from various districts of West Bengal is presented in Table 1. Among these, *Ethmostigmus pygomegas* (Kohlrausch, 1881) has previously been reported from the Eastern Himalaya (Schileyko & Stagl, 2004; Schileyko & Stoev, 2016); therefore, its distribution in West Bengal is presumed to be confined to the Himalayan districts. *Otostigmus scaber* Porat, 1876 was previously recorded from West Bengal as *Otostigmus insularis* Hasse, 1887, without a precise locality (Khanna, 1994a), and was subsequently synonymised by Chao and Chen (2003). As we have collected and examined the specimens of this species during the present study, its occurrence in this state is hereby confirmed. *Scolopendra dehaani* Brandt, 1840, was previously recorded from West Bengal as *Scolopendra subspinipes dehaani*. Kronmüller (2012) elevated it to the species level. Overall, this study refines the taxonomy and distributional records of scolopendrid centipedes in West Bengal, providing a more accurate baseline for future research and biodiversity assessments.

4. CONCLUSION

As a result of this study, five species of Scolopendrid centipedes are newly recorded, thereby increasing the total number of species known from West Bengal to eighteen (Table 1). These findings indicate that the scolopendrid fauna of West Bengal remains inadequately explored despite the state possessing a wide range of physiographic and ecological conditions. The diverse topography of West Bengal, extending from the Himalayan foothills and Terai region in the north to the Gangetic plains, lateritic western plateau, coastal tracts, and mangrove ecosystems of the Sundarbans, provides a variety of habitats suitable for centipede diversity and distribution. Further extensive and systematic surveys across the state, particularly in understudied regions with diverse microhabitats such as seasonal rainfall zones, open canopies, leaf-litter accumulation, fluctuating temperature and moisture conditions, are likely to reveal additional new or poorly known species, thereby enhancing our understanding and documentation of the region's biodiversity.

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CRediT authorship contribution statement

Soumi Mukherjee: Conceptualisation, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft. Sumidh Ray: Methodology, Formal analysis, Writing - Original Draft. Uttam Kumar Chaudhary: Data Curation, Investigation, Methodology, Formal analysis, Writing - Original Draft. Priyadarshi Girija Sankar Sethy: Validation, Writing - Review & Editing, Resources, Supervision, and Funding acquisition.

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Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare no use of AI tools in the writing process.

Data availability statement

Data is available and can be made available on demand.

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Supplementary Material Link

https://www.indianj ecol.org/storage/publication_files/supplementary_IJECOL-26-0097_1779797184.pdf





Drought Resilience of *Pinus kesiya* Royle ex Gordon Populations in Manipur, North-East India


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Abstract: This study evaluated water-use efficiency (WUE) and drought-stress-related traits in two *P. kesiya* populations, the Eastern (Andro) and Western (Langol), from the valley region of Manipur. The populations were sampled from sites with bioclimatic and edaphic conditions that were significantly similar but in reproductive isolation. Five trees per population were sampled for needle and wood cores for measuring wood moisture content (WMC), stomatal number per length (SNL), relative water content (RWC), and proline concentration, along with growth traits viz. height, girth at breast height. Significant differences were observed in SNL, WMC, and proline content, but not in RWC. Andro showed higher SNL (67) than Langol (57.11), suggesting greater stomatal conductance and potentially lower WUE under drought conditions. Langol's slightly lower SNL and higher proline (0.19 mM, albeit Andro's 0.14 mM) indicate a water conservation strategy and stronger biochemical defenses against drought stress. Andro's higher WMC (46.18) than Langol's 33.10 supports hydraulic resilience. Both populations maintained high RWC, indicating effective leaf hydration. These findings highlight *P. kesiya*'s physiological plasticity, with Langol adapted to drier conditions and Andro suited to wetter environments, informing afforestation and forest management strategies in North-East India amid escalating climate change impacts.

Keywords: *Pinus kesiya*, Water-use efficiency, Drought tolerance, Climate change, Forest resilience.

1. INTRODUCTION

Climate change profoundly alters global forest ecosystems, with projections indicating increased frequency and intensity of droughts, elevated temperatures, and erratic precipitation patterns (IPCC, 2022), severely affecting the biodiversity of tropical and subtropical regions of India, such as the North-Eastern Region (NER). NER, encompassing eight states and characterized by its unique subtropical wet forests, faces additional pressures of drastic changes from anthropogenic activities such as shifting

cultivation (jhum) and deforestation, which interact synergistically with climate change to degrade forest health (Chaturvedi et al., 2011; Roy et al., 2021; Gogoi & Lahon, 2022). Along with these pressures, the NER has also been experiencing heightened climate variability, including prolonged dry seasons and reduced monsoon reliability, which exacerbates drought stress on vegetation (Singh & Kumar, 2022; Roy et al., 2021). These changes pose severe threats to endemic forests and keystone species like *Pinus kesiya* Royle ex Gordon (Khasi Pine), a dominant conifer that plays critical roles in carbon sequestration, soil

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stabilisation, and local livelihoods (Patricio & Tulod, 2010).

Drought resilience in trees involves a suite of physiological, biochemical, and morphological adaptations that enable survival and productivity under water-limited conditions (McDowell et al., 2008; Allen et al., 2010). Water-use efficiency (WUE), the ratio of carbon assimilated to water transpired, is a pivotal indicator of drought tolerance, enabling plants to optimise growth while minimising water loss (Bramley et al., 2013; Frank et al., 2015). In conifers, WUE is modulated by traits such as stomatal density (or number per length in needle leaves), relative water content (RWC), wood moisture content (WMC), and osmo-protectant accumulation like proline (Szabados & Saviouré, 2010; Corcuera et al., 2012; Duan et al., 2024). Proline, in particular, acts as an osmo-protectant, stabilizing cellular structures and mitigating oxidative damage during water deficits (Delauney & Verma, 1993; Newton et al., 1986; Shabnam et al., 2016). RWC reflects leaf hydration status and is a predictor of drought-induced mortality (Sapes & Sala, 2021; Keyvan, 2010; Blackman et al., 2023), while WMC indicates hydraulic capacitance and resilience to embolism (Ziemińska et al., 2020). Stomatal traits regulate gas exchange and transpiration, with lower densities often conferring higher WUE (Kouwenberg et al., 2003; Hepworth et al., 2015; Pirasteh-Anosheh et al., 2016; Bertolino et al., 2019). In the context of NER, where climate models predict a 1-2°C temperature rise and 10-20% precipitation decline by mid-century (Murthy et al., 2011), understanding intraspecific variation in these traits is essential for predicting species responses.

Pinus kesiya, distributed across altitudes of 350-2900 m, exhibits phenotypic plasticity, but population-level adaptations remain understudied (Fan et al., 2019). Previous research on conifers highlights that populations from drier sites often exhibit conservative water-use strategies, such as reduced stomatal conductance and elevated proline levels, which enhance survival but may limit growth (Olano et al., 2014). Conversely, wetter-site populations may prioritise hydraulic efficiency, as in higher WMC (Holbrook, 1995; Fan et al., 2019).

Therefore, the present study aimed to provide a foundation for drought resilience breeding programs on *P. kesiya* populations by expanding on prior works (1) to quantify variation in SNL, RWC, WMC, and proline content between two populations of Manipur; (2) to correlate these traits with growth parameters and bioclimatic data and soil characters; and (3) to discuss implications for climate change adaptation in NER's forests.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in two *P. kesiya* populations within the Central Forest Division of Manipur, India viz. Langol (Imphal West, 24.82°N, 93.95°E, ~800 m asl) and Andro (Imphal East, 24.74°N, 94.04°E, ~900 m asl). Both sites represent subtropical wet forests with a pronounced dry season from November to March and receive annual rainfall of ~1200-1400 mm (IMD, 2023). Bioclimatic variables, including mean annual temperature (18-25°C), precipitation of the driest quarter (~100-150 mm), and isothermality, were extracted from the WorldClim database (Hijman et al., 2005).

2.2. Field Sampling

Field sampling adhered to the National Working Plan Code (2023) guidelines, ensuring minimal disturbance in these reserved forests. Five trees (with GBH >50 cm) per population were randomly selected, maintaining a minimum distance of 50 m along a North-South transect to capture maximum diversity (Vaishnav et al., 2021). Trees were of similar age (estimated 20-30 years via increment borers) and health. Three replicates needle bunches were collected from mid-canopy branches using pole pruners for biochemical and morphological analyses. Wood cores (5 mm diameter) were extracted at breast height (1.3 m) using a Haglof increment borer for WMC assessment (Ziemińska et al., 2020). Growth traits: height (H), girth at breast height (GBH), bark thickness (Bt), and clear bole height (CBH) were measured with a clinometer, measuring tape, and bark gauge, respectively. GPS coordinates were recorded with the help of a Garmin eTrex-30 device for geospatial integration with climate data. Sampling was conducted in March (the dry-season peak) to capture stress responses (Pumijumnonng & Wanyaphet, 2006; Fan et al., 2019).

2.3. Laboratory Work

Proline was extracted from fresh needles using a modified eco-friendly ninhydrin protocol (Carillo & Gibon, 2011; Shabnam et al., 2016) and quantified by spectrophotometry at 520 nm against L-proline standards. Using epidermal impressions and microscopy, SNL was determined on both needle abaxial and adaxial surfaces (Kouwenberg et al., 2003; Hepworth et al., 2015). RWC was calculated as '(fresh weight - dry weight)/(turgid weight - dry weight) × 100' (Sapes & Sala, 2021; Li et al., 2024). WMC was measured gravimetrically on wood cores after oven-

drying at 105°C (Ziemińska et al., 2020). All measurements were replicated 3 times per tree, whereas wood core samples were replicated only 2 times.

Table 1. Bioclimatic variables of the two sampled populations derived from Worldclim data (Worldclim.org)

Bioclimatic variable	Langol	Andro
Annual Mean Temperature [°C]	22.6	22.6
Mean Monthly Temperature Range [°C]	10.4	10.4
Isothermality (2/7) (* 100)	49.6	49.1
Temperature Seasonality (STD * 100)	346.4	342
Max Temperature of Warmest Month [°C]	30.6	30.9
Min Temperature of Coldest Month [°C]	9.7	9.7
Temperature Annual Range (5–6) [°C]	20.9	21.2
Mean Temperature of Wettest Quarter [°C]	25.6	25.4
Mean Temperature of Driest Quarter [°C]	18.7	18.7
Mean Temperature of Warmest Quarter [°C]	25.7	25.5
Mean Temperature of Coldest Quarter [°C]	17.6	17.6
Annual Precipitation [mm]	1578	1632
Precipitation of Wettest Month [mm]	343	356
Precipitation of Driest Month [mm]	0	0
Precipitation Seasonality (CV)	87.4	91.9
Precipitation of Wettest Quarter [mm]	899	966
Precipitation of Driest Quarter [mm]	52	48
Precipitation of Warmest Quarter [mm]	706	781
Precipitation of Coldest Quarter [mm]	64	59

2.4. Data Analysis

Descriptive statistics and correlations (Pearson's) were performed using Microsoft Excel and PAST v4.0 (Hammer et al., 2001). Significance level was determined at $p < 0.05$. Trait correlations with bioclimatic variables were analysed via regression models ® v4.2).

3. RESULTS AND DISCUSSION

3.1. Bioclimatic Variables, Soil Variables, and Growth Characteristics

The bioclimatic variables from the two sampled

Table 2. Physical and chemical properties of soil at 25cm depth derived from (www.soilgrid.org)

Parameter	Langol	Andro
Organic carbon density (hg/m ³)	183	191
Soil organic carbon (dg/kg)	163	152
Bulk density (cg/cm ³)	127	125
Clay content (g/kg)	348	364
Coarse fragment (cm ³ /dm ³)	209	183
Sand (g/kg)	299	282
Silt (g/kg)	353	353
Volume water content at -10kpa (10 ⁻² cm ³ cm ⁻²)*10	394	407
Volume water content at -33kpa (10 ⁻² cm ³ cm ⁻²)*10	342	356
Volume water content at -1500kpa (10 ⁻² cm ³ cm ⁻²)*10	173	182
Cation exchange capacity (at pH 7)	163	152
Nitrogen (cg/kg)	190	161
pH of water	5.3	5.4

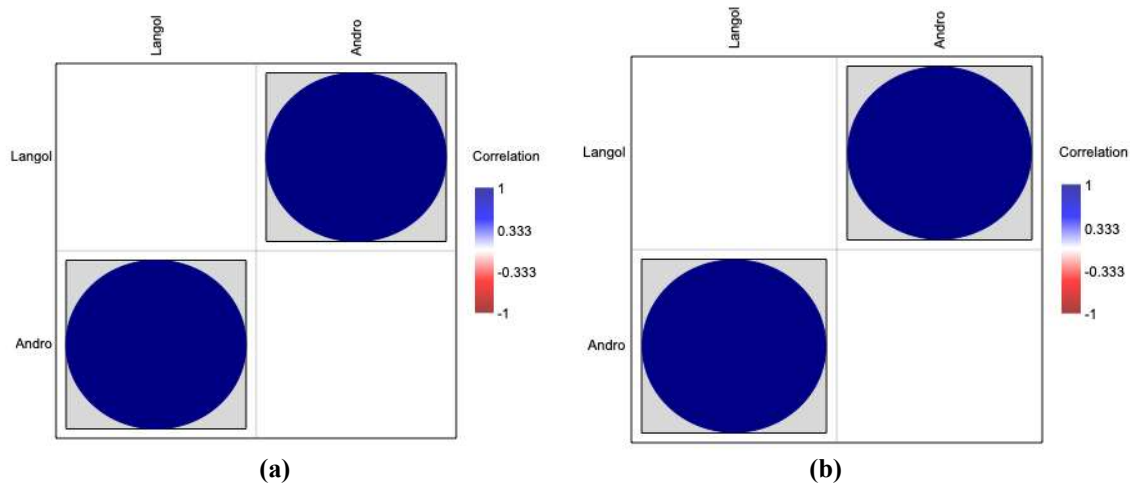


Figure 1. a) Significant ($p < 0.05$) Pearson's correlation between the bioclimatic variables of the two study sites determined the homogeneity of the bioclimatic conditions in the sites. b) significant ($p < 0.05$) Pearson's correlation between the physical and chemical properties of the soil of the two study sites determined the homogeneity of the soil properties of the sites

populations (Langol and Andro) show high similarity, with annual mean temperatures of 22.6°C for both sites, identical minimum temperatures of the coldest month (9.7°C), and comparable precipitation patterns (e.g., annual precipitation of 1578 mm in Langol vs 1632 mm in Andro (Table 1). The soil's physical and chemical properties also show high similarity (Table 2). The significant Pearson's correlation ($p < 0.05$) between the bioclimatic variables and soil properties indicates homogeneous environmental and edaphic conditions across the sites (Figure 1). ANOVA revealed no significant differences among populations in growth traits: GBH, CBH, H, and Bt. The homogeneity of bioclimatic and soil conditions across the two sites supports the hypothesis that trait divergences arise from reproductive isolation and subtle microclimatic influences rather than from environmental heterogeneity.

3.2. Proline Content (Pro)

Proline content was higher in Langol (0.19 mM) than in Andro (0.14 mM) (Table 7), aligning with its role as an osmo-protectant under drought (Szabados & Savouré, 2010; Khoma et al., 2021). It reflects the mirror patterns observed in *Pinus pinaster* (Corcuera et al., 2012) and *Pinus taeda* (Newton et al., 1986), in which drier sites accumulate more proline to enhance cellular stability under water deficits (Table 4). There was no significant variation between populations (or among trees within populations).

Pearson's correlation analysis showed a significant negative correlation between proline content and WMC, but no significant correlations with stomatal number/length (SNL) or RWC (Table 5). The lack of significant between-population differences in proline content, coupled with its negative correlation with WMC, aligns with proline's role during water deficits (Dien et al., 2019; Kijowska et al., 2023; Pory et al., 2023). Similar to the observation of overexpression of proline metabolism exhibiting enhanced drought resistance and proline degradation response sensitively to changing moisture content (Szabados & Savouré, 2010; Khoma et al., 2021). This negative correlation with WMC underscores proline's compensatory role during moisture stress, supporting Langol's water-conserving phenotype and increased drought resilience.

3.3. Stomatal Number/Length (SNL)

The Andro population exhibited a higher mean SNL (67.00) than Langol (57.11) (Table 4), implying lower WUE in Andro due to increased transpiration (Hepworth et al., 2016; Pirasteh-Anosheh et al., 2016). Lower SNL in Langol aligns with drought adaptations in conifers (Kouwenberg et al., 2003; Miyazawa et al., 2006; Moran et al., 2017; Petek et al., 2023). No significant difference in SNL was found between populations, but significant variation was found among trees within populations.

Table 3. Variation based on growth characters of the two populations of the sample trees

Locations	Accessions	GBH (cm)	CBH (m)	H (m)	Bt (cm)
Langol	MNCF_CC2	107	3.43	11.3	1.23
Langol	MNCF_CC3	60	2	5.48	1.13
Langol	MNCF_CC4	101	2	6.85	1.26
Langol	MNCF_CC6	110	5.5	10.9	1.13
Andro	MNAN_HH1	79	2	8.49	1.2
Andro	MNAN_HH2	68	2	8.49	1.2
Andro	MNAN_HH3	147.5	3	12.33	1.83
Andro	MNANLM1	89	2	8.22	0.97
Andro	MNANLM2	83.5	1.5	9.45	1.1

H – height, GBH – girth at the breast height, CBH – clear bole height, Bt – bark thickness

Table 4. Mean values with standard deviation of the functional traits

Population	Pro (mM)	SNL	RWC (%)	MC (%)
Langol	0.19±0.09	57.11±12.14	91.95±13.83	33.10±3.65
Andro	0.14±0.06	67.00±10.03	89.85±1.77	46.18±3.74

Pro- Proline content, SNL- Stomatal number/length, RWC- Relative Water content of leaves, WMC- Wood Moisture Content

Table 5. Correlations between functional traits, based on Pearson's linear correlation coefficients (below the diagonal) and p-values (above the diagonal), confirmed a significant negative correlation between Proline content (Pro) and wood moisture content (WMC)

Population	Pro (mM)	SNL	RWC (%)	MC (%)
Pro (mM)		0.08	0.05	0.01*
SNL	-0.31		0.99	0.06
RWC (%)	-0.35	-0.00		0.44
MC (%)	-0.53*	0.41	-0.18	

*Significant at $p < 0.05$

Pro- Proline content, SNL- Stomatal number/length, RWC- Relative Water content of leaves, WMC- Wood Moisture Content

3.4. Relative Water Content (RWC)

RWC of leaves was slightly higher in Langol (91.95 %) than in Andro (89.85 %) (Table 5). In the RWC, no significant variation between populations was observed, but significant differences among trees within populations were observed. The non-significant differences in RWC between populations indicate that leaf hydration levels are maintained consistently in both Langol and Andro, reflecting effective water-retention mechanisms. Although not significantly correlated with other traits, the trend of negative associations with proline suggests a potential trade-off where higher RWC might reduce the need for osmoprotectants, contributing to overall drought tolerance strategies in these populations. RWC exhibited significant within-population variation, serving as a reliable marker of hydration status and drought response. Declines in RWC under stress are common, but resilient plants recover rapidly post-drought. The stability across populations, despite intraspecific variability, indicates inherent tolerance, as RWC correlates positively with root traits and overall survival. RWC reductions during drought highlight adaptive strategies such as stomatal regulation. This trait's pattern suggests both sites are drought-suitable, with populations capable of maintaining turgor through other biochemical adjustments (Fu et al., 2017; Meena & Kaur, 2019; Haghpanah, 2024).

3.5. Wood Moisture Content (WMC)

WMC was markedly higher in Andro (46.18%) than in Langol (33.10%), reflecting better water-use efficiency of Langol (Table 4). There was significant variation between populations, but no significant differences among trees within populations. A significant negative correlation was found with proline, while correlations with SNL and RWC (Table 5) were non-significant, suggesting that as WMC declines, proline compensates to stabilise cellular functions.

This is consistent with findings that drought intensity alters biomass distribution, favouring roots for improved water uptake (Wang et al., 2024). Therefore, the langol population with lower WMC is more suited to prolonged drought, as it promotes adaptive mechanisms like dehydration tolerance

This study hypothesises that, despite similar bioclimatic conditions, reproductive isolation between the Andro (Eastern) and Langol (Western) populations of *P. kesiya* in Manipur has led to divergent drought adaptation strategies. Specifically, we posit that the Langol population, potentially exposed to microclimatic drier spells due to soil differences (sandy loam vs clay loam), will exhibit traits indicative of higher drought tolerance, including lower SNL, higher proline accumulation, and lower WMC, reflecting a water-conserving phenotype. In contrast, the Andro population is expected to show traits favouring growth under moist conditions, such as higher SNL and WMC, but potentially lower WUE under stress. This hypothesis is grounded in evolutionary ecology principles, where local selection pressures drive trait divergence (Choat et al., 2018; Anderegg et al., 2016). Justifying the aims, this research addresses a critical gap in NER's conifer studies by integrating multiple WUE traits to inform climate-resilient reforestation. Amid projections of intensified droughts (Aragão et al., 2018), identifying tolerant genotypes could enhance forest management, biodiversity conservation, and carbon sinks in vulnerable regions (Patricio & Tulod, 2010; Olano et al., 2014; Duan et al., 2024).

4. CONCLUSION

The analysis of bioclimatic, edaphic, growth, and functional trait data from the Langol and Andro populations of *Pinus kesiya* demonstrates overall similarity in environmental conditions and morphological development, affirming their adaptation to a shared subtropical climate with pronounced seasonal variations of the functional traits (Pro, SNL, RWC,

WMC). Andro appears more suitable for the tree species studied. This is evidenced by lower proline content (indicating reduced osmotic stress), higher stomatal number/length (suggesting improved photosynthetic potential), more stable relative leaf water content (lower variability), and significantly higher wood moisture content (enhancing hydration and structural resilience). In contrast, Langol shows signs of mild stress (elevated Pro and variable RWC) and lower WMC, which may compromise long-term performance despite similar growth outcomes. If selecting for optimal physiological health, Andro is recommended; however, site-specific management could mitigate differences.

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CRediT authorship contribution statement

Z. Philamazan Peace Shimray: Field data collection; laboratory work, data curation; result compilation; writing original draft of the manuscript. Hira Soraisam: Field data collection; laboratory work, data curation; result compilation; writing original draft of the manuscript. Vivek Vaishnav: Conceptualisation, Supervision, Validation, visualisation, review and editing.

Data availability statement

All data generated or analysed during this study are included in this published article. However, any additional data are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflict of interest.

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Adsorption Behaviour of Selected Acid Herbicides on Activated Carbon: Equilibrium, Kinetic and Laboratory–Field Assessment

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ABSTRACT: The present study evaluates the adsorption behaviour of selected acid herbicides (MCPA, Mecoprop-P, 2,4-D, and Triclopyr) on activated carbon under controlled laboratory conditions and assesses the relevance of this behaviour to aquatic environmental protection. Batch adsorption experiments were conducted to investigate the influence of contact time, adsorbent dosage and initial herbicide concentration. Adsorption equilibrium data were analysed using Langmuir and the Freundlich isotherm models, while adsorption kinetics were evaluated using pseudo-first-order and pseudo-second-order models. Freundlich modelling showed better agreement with the experimental data, suggesting heterogeneous surface interactions during adsorption. Langmuir modelling demonstrated high intrinsic adsorption capacities, reaching 116.82 mg g⁻¹ for MCPA. Kinetic analysis revealed that the pseudo-second-order model provided the best fit (R^2 up to 0.996), suggesting that it adequately describes the adsorption kinetics. Regression analysis indicated a statistically significant preliminary relationship ($R^2 = 0.933$, $p \leq 0.05$) between intrinsic adsorption capacity and field removal efficiency. The findings highlight the practical relevance of laboratory-derived adsorption parameters for preliminary assessment of the performance of activated carbon in agricultural water treatment systems and contribute to ecological protection by supporting improved management of herbicide contamination in water resources.

Keywords: Activated carbon, Acid herbicides, Adsorption isotherms, Kinetic modeling, Agricultural runoff.

1. INTRODUCTION

Acid herbicides are widely used in agricultural systems for effective weed control and improvement of crop productivity. However, their persistence and mobility in the environment increase the risk of contamination of surface and groundwater resources. Monitoring studies have reported the presence of herbicide residues in rivers, reservoirs and drinking water sources, raising ecological concerns due to their potential toxicity and long-term environmental impact (de Souza et al., 2020; Silva et al., 2019). Continuous exposure of aquatic ecosystems to pesticide residues may affect non-target organisms and

disturb the ecological balance. Several studies have highlighted the ecological implications of pesticide contamination in aquatic ecosystems and emphasised the need of develop effective treatment strategies to protect water resources (Herrero-Hernández et al., 2020; Silva et al., 2019).

Among various treatment technologies, adsorption using activated carbon has been widely used of remove organic micropollutants from water. Activated carbon possesses a highly developed porous structure, a large surface area, and diverse surface functional groups that facilitate interactions with organic contaminants such as phenoxy acid herbicides (Bansal & Goyal, 2005; Snyder et al., 2007). Several studies have demonstrated the effectiveness of activated carbon in

removing pesticides and other emerging contaminants from water treatment systems (Mojiri et al., 2020; Ighalo et al., 2021).

The adsorption behaviour of organic contaminants is influenced by several physicochemical parameters, including contact time, adsorbent dosage, initial concentration and surface characteristics of the adsorbent. Equilibrium isotherm models, such as the Langmuir and Freundlich models, are widely used to understand adsorption mechanisms and surface interaction behaviour, while kinetic models provide insight into the rate-controlling steps governing the adsorption process (Foo & Hameed, 2010; Ho & McKay, 1999).

Recent studies have reported significant advances in herbicide adsorption using activated carbon and related materials, highlighting improved removal efficiency and enhanced understanding of adsorption mechanisms under varying environmental conditions (McGinley et al., 2022; Taylor et al., 2022; Astuti et al., 2022; Butkovskiy et al., 2021). Therefore, integrating adsorption modelling with environmentally relevant remediation performance remains an important research gap.

Recent investigations have further demonstrated the growing importance of advanced adsorption materials and environmentally relevant remediation strategies for herbicide and pesticide removal from contaminated water systems. These studies have highlighted improved adsorption efficiency, enhanced surface functionality and the role of sustainable adsorbent materials under variable environmental conditions, supporting the continued development of adsorption-based treatment technologies for agricultural water remediation.

Unlike conventional adsorption investigations, the present study integrates laboratory adsorption modelling with field-scale remediation observations to improve understanding of the environmental relevance and practical applicability of activated carbon-based treatment systems for agricultural water remediation.

Despite extensive research on adsorption processes, the relationship between intrinsic adsorption capacity obtained from laboratory experiments and removal efficiency observed under field conditions has not been adequately explored. Establishing such relationships is important for improving the understanding and practical applicability of adsorption-based water treatment technologies in agricultural catchments.

However, most existing studies on phenoxy acid herbicide adsorption using activated carbon are limited to

controlled laboratory conditions, focusing primarily on equilibrium and kinetic modelling. These studies provide valuable mechanistic insights but often fail to demonstrate how laboratory-derived adsorption parameters translate to real-world environmental systems. In particular, there is a lack of studies that integrate laboratory adsorption performance with field-scale removal efficiency under variable environmental conditions.

To address this gap, the present study combines laboratory-based adsorption modelling with field-scale performance data obtained from agricultural water systems. This laboratory–field integration enables evaluation of the practical relevance of intrinsic adsorption parameters (e.g., Q_{max}) for real-world herbicide removal. By establishing a statistical relationship between laboratory adsorption capacity and field removal efficiency, this study provides a more applied and environmentally relevant framework for assessing the effectiveness of activated carbon in agricultural water treatment systems.

Therefore, the present study aims to evaluate the adsorption behaviour of selected acid herbicides on activated carbon using equilibrium and kinetic modelling under controlled laboratory conditions. Furthermore, laboratory-derived adsorption parameters were statistically integrated with field-scale removal performance to provide mechanistic insight into adsorption efficiency and its ecological relevance for protecting agricultural water systems from herbicide contamination.

2. MATERIALS AND METHODS

2.1. Chemicals and Adsorbents

Analytical-grade standards of acid herbicides including MCPA, Mecoprop-P, 2,4-D and Triclopyr were procured and used without further purification. Stock solutions were prepared using deionized water and subsequently diluted to obtain the desired working concentrations for experimental analysis.

Two types of activated carbon were used as adsorbents: granular activated carbon (GAC) and coconut-based activated carbon (CAC). The adsorbent materials were sieved to obtain a uniform particle size fraction of 0.5–1.0 mm. Prior to experimentation, the adsorbents were washed thoroughly with deionized water to remove surface impurities and dried at 105°C for 24 hours.

2.2. Batch Adsorption Experiments

Batch adsorption experiments were conducted at a controlled temperature of $25 \pm 1^\circ\text{C}$ using 250 mL

Erlenmeyer flasks containing 100 mL of herbicide solution. An adsorbent dosage of 0.5 g L⁻¹ was used for all experiments. Initial herbicide concentrations ranged between 10 and 200 mg L⁻¹, and the solution pH was maintained at 6.5 ± 0.2 during the experimental period to simulate environmentally relevant conditions typically observed in agricultural surface water systems.

The experimental pH was selected to represent environmentally relevant agricultural water conditions commonly observed in irrigation runoff and surface water systems. Near-neutral pH conditions were considered environmentally relevant for evaluating adsorption performance of phenoxy acid herbicides under realistic environmental scenarios, as these compounds exhibit pH-dependent ionisation behaviour. Adsorbent dosage, contact time, and concentration ranges were selected based on preliminary optimisation trials, adsorption equilibrium requirements, and previously reported activated carbon adsorption studies involving herbicide contaminants.

Acid herbicides exhibit pH-dependent ionisation behaviour, which significantly influences adsorption behaviour. At near-neutral pH, these compounds predominantly exist in partially dissociated form, allowing effective interaction with activated carbon surfaces.

Although pH variation can influence adsorption performance, the present study focused on evaluating adsorption behaviour under representative environmental conditions to enable meaningful comparison with field-scale observations. The selected pH, therefore, provides a practical basis for assessing adsorption efficiency under realistic water quality conditions.

The flasks were agitated at a constant speed using a mechanical shaker. Samples were collected at predetermined time intervals ranging from 0 to 240 minutes. The collected samples were filtered through 0.45 µm membrane filters prior to analysis. Equilibrium was assumed when the variation in concentration between successive measurements was less than 2%. All adsorption experiments were conducted in triplicate, and the reported values represent mean ± standard deviation (n = 3). Standard deviation-based error bars have been incorporated into Figures 1 and 2 to illustrate the variability among replicate observations. The mean values were used for subsequent adsorption modelling and statistical analyses.

The adsorption capacity at time t (q_t) and at equilibrium (q_e) was calculated using Equation (1) and Equation (2),

respectively:

$$q_t = \frac{(C_o - C_t)V}{m} \dots\dots\dots(1)$$

$$q_e = \frac{(C_o - C_e)V}{m} \dots\dots\dots(2)$$

where C_o , C_t and C_e (mg L⁻¹) represent the initial, time-dependent and equilibrium concentrations, respectively, V (L) represents the volume of solution and m (g) denotes the mass of adsorbent used in the experiment.

2.3. Adsorption Isotherm and Kinetic Modelling

The adsorption equilibrium behaviour was evaluated using the Langmuir and the Freundlich isotherm models. Nonlinear regression fitting was applied for estimation of isotherm model parameters. The Langmuir model assumes monolayer adsorption on a homogeneous surface, whereas the Freundlich model describes multilayer adsorption on heterogeneous surfaces.

Adsorption kinetics were analysed using pseudo-first-order and pseudo-second-order kinetic models in order to determine the rate controlling mechanisms involved in the adsorption process.

The model performance was evaluated using statistical indicators including the coefficient of determination (R^2), chi-square (χ^2) test and the standard error of estimate (SEE). The most suitable model was identified based on higher R^2 values and lower χ^2 and SEE values.

2.4. Surface Characterization

The surface morphology of the activated carbon samples was examined using scanning electron microscopy (SEM), which revealed a well-developed porous structure with irregular cavities and surface heterogeneity favourable for adsorption. Energy-dispersive X-ray spectroscopy (EDX) analysis confirmed the presence of major elements such as carbon and oxygen, indicating the availability of functional groups that can facilitate adsorption interactions.

Although advanced characterization techniques such as Brunauer–Emmett–Teller (BET) surface area analysis, pore size distribution and Fourier transform infrared spectroscopy (FTIR) provide additional quantitative insight into adsorbent properties, the adsorption performance of activated carbon is widely governed by its porous structure and surface functionality, as supported by existing literature.

In the present study, the adsorption performance was primarily evaluated through equilibrium and kinetic modelling. The high adsorption capacity (Q_{max}) and

favourable Freundlich behaviour observed in this study indirectly suggest the presence of heterogeneous surface sites and well-developed porosity, which are consistent with characteristics reported for activated carbon materials.

Although detailed physicochemical characterization such as BET surface area and FTIR analysis would provide additional insights, the results obtained in this study are consistent with previously reported properties of activated carbon materials in similar applications.

Therefore, the combined interpretation of SEM–EDX analysis and adsorption modelling provides a reasonable basis for understanding adsorption behaviour, even in the absence of detailed physicochemical characterization.

2.5. Field Data Description

Field-scale data used in this study were obtained from controlled monitoring investigations conducted in agricultural runoff environments as part of earlier experimental work. These investigations were designed to evaluate the performance of activated carbon-based systems under practical environmental conditions.

The field studies involved the installation of passive treatment units containing activated carbon media within drainage pathways influenced by agricultural activities. Monitoring of herbicide concentrations was conducted at defined sampling points before and after the implementation of these treatment systems to assess removal performance.

Sampling was carried out over multiple time intervals to capture variability in environmental conditions and pesticide transport dynamics. The monitored compounds included commonly used phenoxy acid herbicides such as MCPA, Mecoprop-P, 2,4-D, Triclopyr and Fluroxypyr.

The field observations demonstrated measurable reductions in herbicide concentrations following treatment, confirming the effectiveness of activated carbon under real environmental conditions. These findings are consistent with recent field-scale investigations, which demonstrate the effectiveness of activated carbon-based systems for pesticide removal under real environmental conditions, highlighting the importance of system design and operational optimisation. These field-derived removal efficiencies were subsequently utilised to establish a statistical relationship with laboratory-derived adsorption parameters in the present study.

The field dataset utilised in this study comprised monitoring observations collected under varying environmental conditions, representing a range of flow regimes and contaminant loading scenarios. The dataset was

derived from controlled monitoring investigations conducted as part of the author's doctoral research, ensuring consistency in experimental design and data reliability. While the dataset provides a practical basis for evaluating laboratory–field relationships, variations in field conditions, such as flow dynamics, organic matter content, and competing solutes, may influence adsorption performance.

2.6. Laboratory–Field Statistical Integration

To evaluate the practical significance of laboratory-derived adsorption parameters, previously reported field intervention datasets were analysed to determine the relationship between intrinsic adsorption capacity (Q_{max}) and field removal efficiency.

Linear regression analysis was performed to examine the relationship between laboratory adsorption capacity and field-scale removal performance. Statistical significance was evaluated at $p < 0.05$, and 95% confidence intervals were calculated for the regression parameters.

The laboratory–field statistical comparison performed in the present study was intended as a preliminary exploratory assessment to evaluate the environmental relevance of adsorption behaviour observed under controlled laboratory conditions. The field-scale observations used for comparative interpretation were derived from previously documented remediation interventions conducted under environmentally relevant agricultural water conditions. However, the available field observations were limited in number and may involve variability in water quality characteristics, operational conditions, environmental factors, and remediation practices across different field settings. Therefore, the observed statistical relationship should be interpreted with caution as an exploratory comparative association rather than as definitive mechanistic validation or predictive ecological modelling. Additional large-scale field investigations under diverse environmental and operational conditions are necessary to establish robust predictive relationships and improve understanding of field-scale adsorption performance. Because the adsorption experiments were conducted under controlled batch conditions, the observed relationships may not fully represent adsorption behaviour under dynamic continuous-flow environmental treatment systems.

3. RESULTS AND DISCUSSION

3.1. Adsorption Equilibrium Behaviour

Adsorption equilibrium data obtained from batch experiments were analysed using the Langmuir and the

Freundlich isotherm models to evaluate the adsorption of acid herbicides on granular activated carbon. The Freundlich model exhibited superior fit to the experimental data, with coefficients of determination (R^2) values ranging from 0.995 to 0.999. This suggests that the adsorption process occurs on heterogeneous surfaces with multilayer adsorption characteristics. Figure 1 shows that the Freundlich model exhibited better agreement with the experimental adsorption data than the Langmuir model for the investigated herbicides.

The Freundlich exponent ($n > 1$) further confirmed favourable adsorption conditions for all investigated herbicides. Although the Langmuir model also provided statistically acceptable fits ($R^2 \geq 0.978$), the corresponding χ^2

values were comparatively higher than those obtained for the Freundlich model, suggesting that adsorption occurred on energetically heterogeneous surfaces rather than forming a strictly monolayer coverage.

The maximum monolayer adsorption capacity (Q_{max}) predicted by the Langmuir model reached 116.82 $mg\ g^{-1}$ for MCPA and 116.76 $mg\ g^{-1}$ for Triclopyr, while 2,4-D exhibited a slightly lower adsorption capacity (112.47 $mg\ g^{-1}$). These findings are consistent with previously reported adsorption behaviour of phenoxy acid herbicides on activated carbon materials, indicating strong adsorption affinity associated with heterogeneous porous surfaces.

The suitability of the isotherm models was evaluated using statistical indicators including the coefficient of

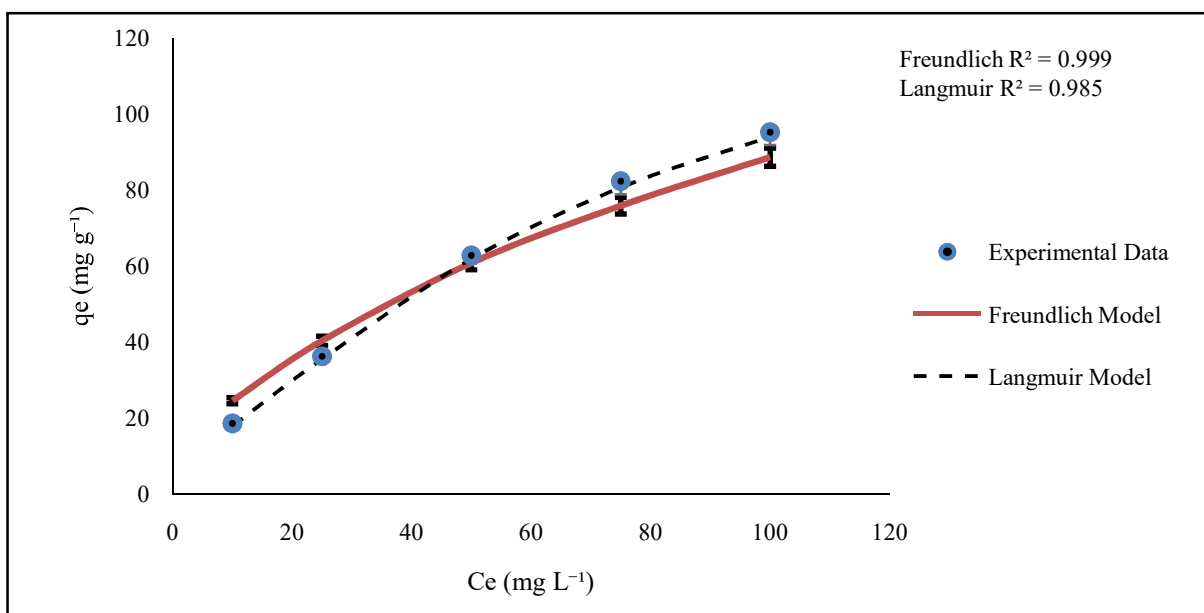


Figure 1. Adsorption isotherms of acid herbicides on granular activated carbon. Data points represent mean \pm standard deviation ($n = 3$) obtained from triplicate experiments. Experimental data (\bullet) along with Freundlich (—) and Langmuir (---) model fits are presented. The figure illustrates equilibrium adsorption behaviour and the comparative performance of the isotherm models

Table 1. Langmuir and the Freundlich isotherm fitting curves for the adsorption of MCPA on granular activated carbon, showing experimental adsorption equilibrium behaviour

Herbicide	Model	Q_{max} ($mg\ g^{-1}$)	KL ($L\ mg^{-1}$)	KF ($(mg\ g^{-1})(L\ mg^{-1})^{1/n}$)	n	R^2	χ^2
MCPA	Langmuir	116.820	0.084	-	-	0.985	0.214
MCPA	Freundlich	-	-	38.742	2.611	0.999	0.041
Triclopyr	Langmuir	116.760	0.079	-	-	0.982	0.268
Triclopyr	Freundlich	-	-	36.115	2.534	0.998	0.053
2,4-D	Langmuir	112.470	0.071	-	-	0.978	0.314
2,4-D	Freundlich	-	-	34.280	2.417	0.995	0.067

determination (R^2), chi-square (χ^2) and the standard error of estimate (SEE). The lower χ^2 values obtained for the Freundlich model indicate better agreement between experimental and predicted values, confirming its superior applicability. The SEE values also supported this observation, although the differences were less pronounced compared to χ^2 .

The observed adsorption trend suggests heterogeneous surface interaction behaviour consistent with previously reported activated carbon systems investigated for phenoxy herbicide remediation under environmentally relevant conditions.

The Freundlich constant (KF) reflects adsorption capacity, while the exponent ($n > 1$) reflects favourable adsorption and surface heterogeneity. This suggests that the adsorption process is governed by multilayer interactions on energetically non-uniform surfaces of activated carbon.

In contrast, the Langmuir model assumes monolayer adsorption on homogeneous surfaces. The relatively higher χ^2 values observed for the Langmuir model suggest deviation from idealised monolayer adsorption behaviour.

Overall, adsorption of acid herbicides on activated carbon is predominantly governed by multiple adsorption-related interactions rather than idealised monolayer adsorption.

3.2. Kinetic Behaviour

The adsorption kinetics of the investigated herbicides were evaluated using pseudo-first-order and pseudo-second-order kinetic models. The pseudo-second-order model showed superior agreement with the experimental data, with R^2 values reaching up to 0.996. The calculated equilibrium adsorption capacities ($q_{e,calc}$) obtained from the pseudo-second-order model closely matched the experimental values, suggesting that the model adequately describes the adsorption kinetics.

Figure 2 illustrates the superior fitting performance of the pseudo-second-order kinetic model compared to the pseudo-first-order model.

The observed kinetic behaviour suggests that adsorption likely involves multiple adsorption interactions and diffusion-related processes rather than a single rate-controlling mechanism.

However, it is important to note that a good fit of the pseudo-second-order model does not necessarily imply chemisorption or specific surface-controlled mechanisms. The kinetic results primarily reflect the overall adsorption rate behaviour and may involve multiple processes,

including surface adsorption and intraparticle diffusion.

In contrast, the pseudo-first-order model produced comparatively lower correlation coefficients ($R^2 = 0.914\text{--}0.921$), demonstrating its limited suitability for describing the adsorption kinetics of the investigated herbicides. Intraparticle diffusion analysis revealed multilinear characteristics, suggesting that pore diffusion contributes to the adsorption process but is not the sole rate-controlling step.

The multilinear behaviour observed in the intraparticle diffusion plots suggests that adsorption proceeded through multiple sequential stages, including external surface adsorption followed by gradual pore diffusion. The deviation of the diffusion plots from the origin further indicates the contribution of additional mass transfer processes during adsorption.

The adsorption performance observed in the present study may be associated with the porous surface morphology and functional surface characteristics of activated carbon materials reported in previous studies. Earlier investigations on granular activated carbon have demonstrated that surface heterogeneity, pore structure, and oxygen-containing functional groups contribute significantly to herbicide adsorption behaviour. FTIR-based interpretations reported in previous studies on activated carbon have suggested the involvement of hydroxyl, carbonyl, and aromatic functional groups in adsorption interactions. At the same time, SEM observations have demonstrated a porous surface morphology favourable for adsorption processes. These physicochemical characteristics likely contributed to the observed Freundlich adsorption behaviour and favourable adsorption kinetics observed in the present investigation. However, detailed surface-specific mechanistic evaluation was beyond the scope of the present study.

The adsorption behaviour observed under near-neutral pH conditions may also be influenced by the ionisation characteristics of phenoxy acid herbicides and the surface charge behaviour of activated carbon materials. Since phenoxy herbicides exist predominantly in dissociated form near neutral pH, electrostatic interactions, surface heterogeneity, and pore diffusion processes may collectively contribute to adsorption performance. However, detailed pH-dependent adsorption investigations were beyond the scope of the present study and should be explored in future research to improve mechanistic understanding under varying environmental conditions.

Similarly, temperature-dependent thermodynamic

investigations involving ΔG° , ΔH° , and ΔS° analysis were not included in the present study and should be explored in future research to provide additional insight into adsorption energetics and spontaneity.

The adsorption capacities observed in the present study are comparable with previously reported activated carbon-based systems for herbicide remediation. McGinley et al. (2022) and Kodali et al. (2021) similarly reported favourable adsorption behaviour of herbicides onto porous carbon materials under environmentally relevant conditions. The strong Freundlich model conformity observed in the present study is also consistent with earlier investigations reporting heterogeneous adsorption interactions on activated carbon surfaces. Similar observations regarding adsorption affinity and kinetic behaviour have also been reported in recent studies investigating pesticide remediation using activated carbon and biochar-derived materials. However, unlike most previous studies focused exclusively on laboratory-scale adsorption evaluation, the present work additionally integrates field-scale removal observations to improve the practical applicability of adsorption modelling for agricultural water treatment systems.

3.3. Laboratory–Field Statistical Integration

To examine the practical relevance of laboratory-derived

adsorption parameters, regression analysis was performed to evaluate the relationship between intrinsic adsorption capacity (Q_{max}) and field removal efficiency. The regression equation obtained was:

$$y = 0.2864x + 62.945$$

The field monitoring dataset comprised multiple observations ($n = 12$) collected under different monitoring events and flow conditions. For laboratory–field comparison, herbicide-specific mean removal efficiencies were calculated from the broader field monitoring dataset and used in the regression analysis presented in Figure 3. Consequently, Figure 3 displays five herbicide-level averaged data points corresponding to MCPA, Mecoprop-P, 2,4-D, Triclopyr and Fluroxypyr rather than all individual monitoring observations. While the dataset provides a useful basis for establishing relationships, the results should be interpreted considering inherent variability in field conditions.

The coefficient of determination ($R^2 = 0.933$, $p < 0.05$) demonstrates a statistically significant positive relationship between intrinsic adsorption capacity and field-scale removal performance. The relatively narrow 95% confidence intervals further support the reliability of the regression relationship. These findings suggest that laboratory adsorption parameters may provide useful

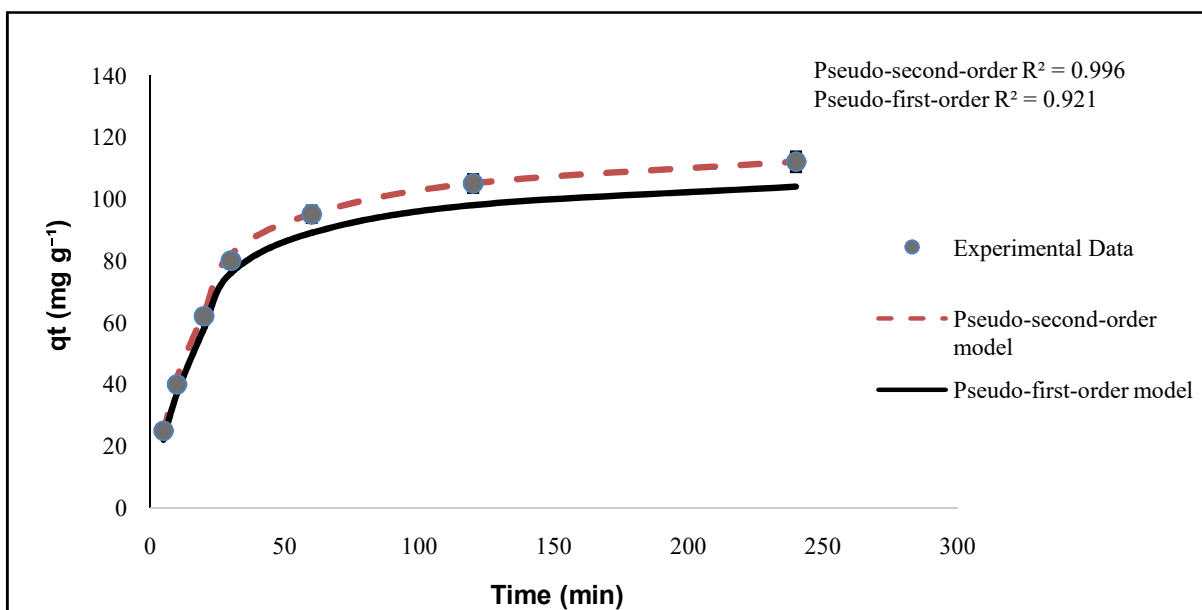


Figure 2. Kinetic model fitting curves for the adsorption of MCPA on granular activated carbon. Data points represent mean \pm standard deviation ($n = 3$) obtained from triplicate experiments. Experimental data (\bullet) along with pseudo-second-order (—) and pseudo-first-order (---) model fits are presented. The pseudo-second-order model shows better agreement with the experimental data. Intraparticle diffusion plots exhibited multilinear characteristics, indicating the contribution of multiple adsorption stages

preliminary indicators for understanding field-scale treatment performance under environmentally relevant conditions.

Figure 3 demonstrates a significant positive relationship between intrinsic adsorption capacity (Q_{max}) and field-scale removal efficiency.

The incorporation of field-derived data enhances the applicability of the study by validating laboratory adsorption behaviour under real environmental conditions and bridging the gap between controlled experiments and practical environmental implementation.

conditions. The plotted values represent herbicide-specific mean removal efficiencies derived from multiple

field monitoring observations ($n = 12$). Consequently, five herbicide-level averaged data points corresponding to MCPA, Mecoprop-P, 2,4-D, Triclopyr and Fluroxypyr are presented in the regression analysis. The figure illustrates a statistically significant positive relationship between intrinsic adsorption capacity (Q_{max}) and field removal efficiency.

3.4. Integrated Mechanistic Interpretation

The adsorption trend observed in this study reflects the combined influence of molecular characteristics of herbicides, surface heterogeneity of activated carbon, equilibrium adsorption behaviour and kinetic behaviour.

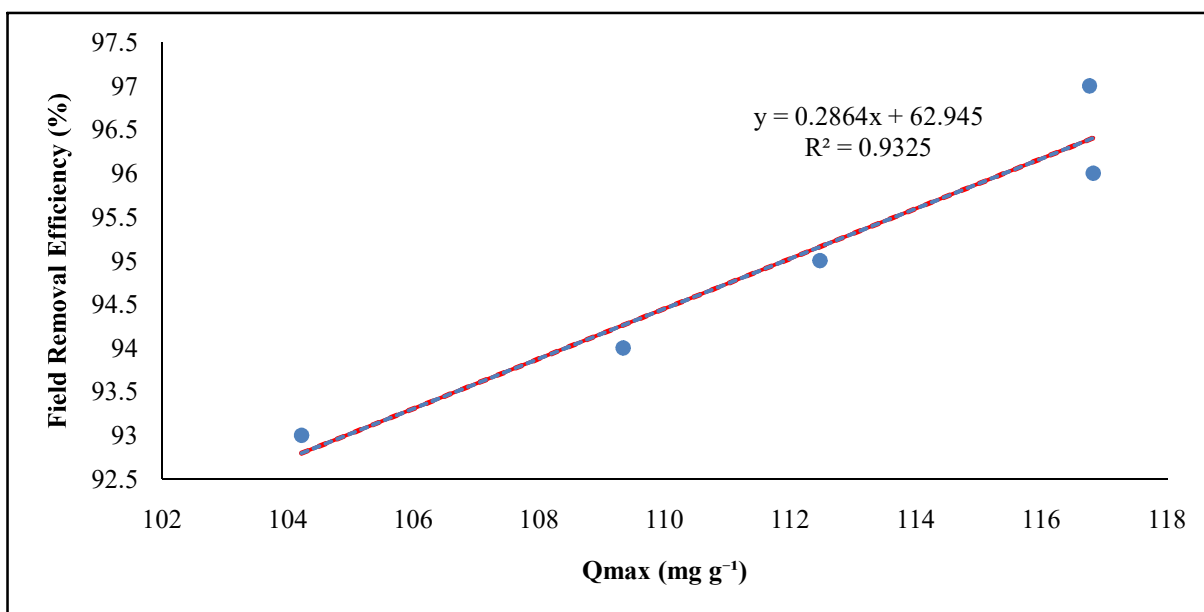


Figure 3. Relationship between intrinsic adsorption capacity (Q_{max}) and field-scale herbicide removal efficiency observed under environmentally relevant agricultural water treatment conditions. The plotted values represent herbicide-specific mean removal efficiencies derived from multiple field monitoring observations ($n = 12$). Consequently, five herbicide-level averaged data points corresponding to MCPA, Mecoprop-P, 2,4-D, Triclopyr and Fluroxypyr are presented in the regression analysis. The figure illustrates a statistically significant positive relationship between intrinsic adsorption capacity (Q_{max}) and field removal efficiency

Table 2. Kinetic model parameters for acid herbicide adsorption on granular activated carbon

Herbicide	Model	k_1 (min ⁻¹)	k_2 (g mg ⁻¹ min ⁻¹)	$q_{s,calc}$ (mg g ⁻¹)	R^2
MCPA	Pseudo-first-order	0.041	-	104.280	0.921
MCPA	Pseudo-second-order	-	0.00082	112.940	0.996
2,4-D	Pseudo-first-order	0.036	-	101.360	0.914
2,4-D	Pseudo-second-order	-	0.00076	109.780	0.994
Triclopyr	Pseudo-first-order	0.039	-	103.420	0.918
Triclopyr	Pseudo-second-order	-	0.00080	111.560	0.995

Although intrinsic adsorption capacity strongly influences contaminant removal potential, field-scale performance is also affected by hydraulic residence time, flow dynamics and operational conditions.

Figure 4 presents the simplified laboratory–field integration framework linking adsorption behaviour with field-scale herbicide removal performance.

From an environmental management perspective, the adsorption efficiencies observed in the present study indicate the potential of activated carbon systems to reduce herbicide concentrations in agricultural runoff and surface water environments. Such reductions may contribute to minimising ecological exposure risks to aquatic organisms

and supporting compliance with water quality protection objectives associated with pesticide contamination control. Although detailed ecological risk quantification and toxicity threshold assessment were beyond the scope of the present investigation, the observed adsorption performance demonstrates the practical environmental relevance of adsorption-based treatment systems for improving agricultural water quality.

The comparatively higher removal efficiency of GAC compared to CAC may be associated with its relatively well-developed pore structure and adsorption affinity, as commonly reported for granular activated carbon systems in previous studies.



Figure 4. Simplified laboratory–field integration framework illustrating the relationship between adsorption equilibrium behaviour, kinetic performance and field-scale herbicide removal efficiency ($R^2 = 0.933$)

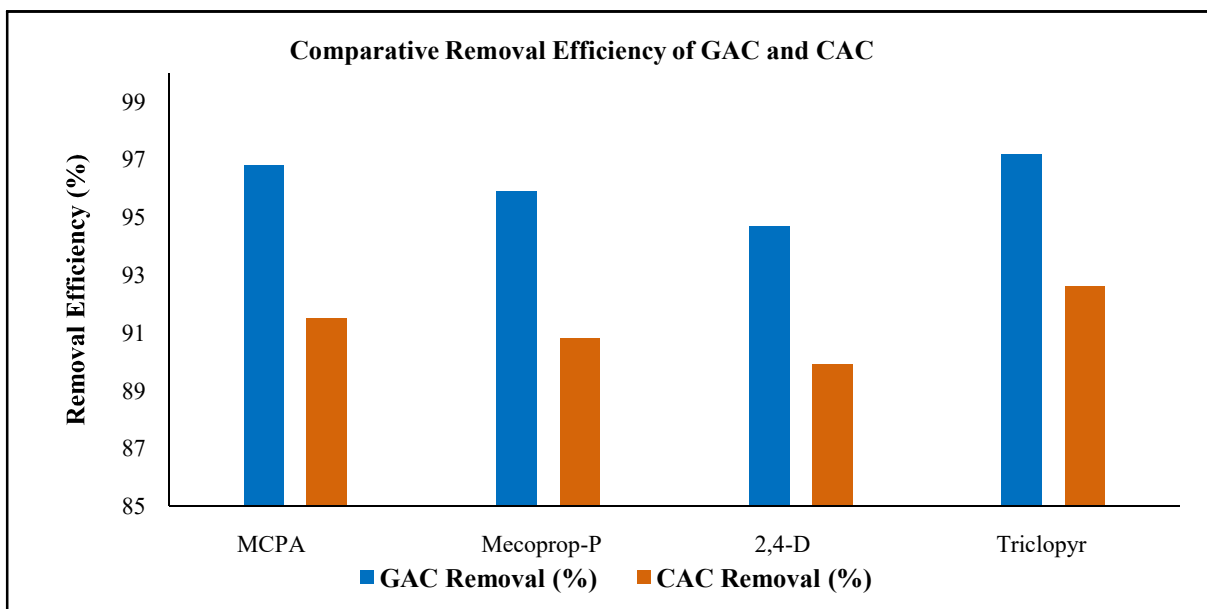


Figure 5. Comparative field-scale removal efficiency of granular activated carbon (GAC) and coconut-based activated carbon (CAC) for selected herbicides. The figure shows the comparative removal efficiencies of GAC and CAC under field-scale conditions, with GAC consistently exhibiting higher performance across all investigated herbicides

Figure 5 shows the comparatively higher removal efficiency of granular activated carbon (GAC) than coconut-based activated carbon (CAC) under field-scale conditions.

Although high intrinsic adsorption capacity enhances the removal potential of herbicides, compliance with drinking water regulatory limits requires appropriate integration of adsorption characteristics with hydraulic design and operational conditions. Laboratory modelling provides valuable insights into adsorption affinity and rate-controlling mechanisms; however, field-scale performance is also influenced by hydrodynamic conditions, system configuration, and operational optimisation.

While a strong laboratory–field correlation was observed in the present study, the experiments were conducted under controlled batch conditions with limited field datasets. Therefore, further validation under continuous-flow operational scenarios is recommended to improve real-world applicability and strengthen the predictive reliability of adsorption-based treatment systems.

The findings of this study highlight the ecological significance of adsorption-based treatment strategies in reducing herbicide contamination in agricultural water systems, thereby contributing to the protection of aquatic ecosystems from pesticide-induced stress.

4. CONCLUSION

The present study evaluated the adsorption behaviour of selected acid herbicides on activated carbon through equilibrium modelling, kinetic analysis and laboratory–field statistical integration. The results demonstrated that the Freundlich isotherm provided a better representation of adsorption behaviour, indicating heterogeneous surface interactions between herbicide molecules and activated carbon. Langmuir modelling revealed high intrinsic adsorption capacities, while kinetic analysis confirmed that the adsorption process predominantly followed pseudo-second-order behaviour.

A statistically significant relationship between intrinsic adsorption capacity and field removal efficiency highlights the practical relevance of laboratory-derived adsorption parameters. These findings suggest that adsorption modelling can provide useful guidance for predicting treatment performance and optimizing the deployment of activated carbon systems for the removal of herbicide contaminants in water treatment applications.

Overall, the integrated laboratory–field approach presented in this study contributes to improved

understanding of adsorption mechanisms and provides scientific evidence for the design and optimization of activated carbon-based treatment systems for mitigating herbicide contamination in agricultural catchments and safeguarding water resources. From an ecological perspective, the integration of adsorption modelling with field-scale performance evaluation provides an important framework for improving the management of pesticide contamination in agricultural landscapes and protecting freshwater ecosystems. The observed removal efficiencies further indicate the potential applicability of activated carbon systems for reducing herbicide concentrations towards environmentally acceptable levels in agricultural water systems, thereby supporting improved ecological protection, water quality management objectives, and compliance with agricultural water quality protection goals.

Further investigation involving thermodynamic parameters (ΔG , ΔH and ΔS), continuous-flow column systems, competitive ion interactions, dissolved organic matter effects, pH-dependent adsorption behaviour, temperature variation, and variable environmental conditions would provide deeper insight into the adsorption mechanism and improve understanding of real-world field-scale applicability of activated carbon-based treatment systems.

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CRediT authorship contribution statement

Pradip Salve: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

The author used limited AI-assisted tools only for language editing and grammatical refinement. All scientific interpretation, data analysis, results, and conclusions were independently developed by the author.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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Ecological Roles of Indian Palm Squirrels (*Funambulus* spp.) as Floral Visitors Across Five Indian States: First Comprehensive Account


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ABSTRACT: Flower visitation by non-flying mammals remains poorly documented in South Asia. The ecological roles of palm squirrels as floral visitors and potential pollinators in India have received limited systematic attention. This study aimed to document flower visitation by all four Indian palm squirrel species (*Funambulus* spp.), assess their roles as nectar robbers, flower predators, or occasional floral visitors across diverse plant species. Floral visitation was recorded opportunistically through direct observation, photographic and video documentation across gardens, agricultural landscapes, forest edges and protected areas in five Indian states, encompassing 40 independent observation events between December 2019 and March 2021. Observations were classified into three functional categories: nectar robbers, flower predators, and occasional visitors. A presence-absence bipartite interaction network was constructed to visualise interaction structure across squirrel species. All four palm squirrel species were recorded visiting flowers of 20 plant species, belonging to 11 families, of which 11 represent novel interaction records. Most visits involved nectar access with floral damage, indicating roles as nectar robbers or flower predators, particularly on small, fragile entomophilous flowers. Visits to large, robust flowers were less destructive and occasionally involved contact with reproductive structures, raising the possibility of opportunistic pollen transfer. The bipartite network indicated unequal interaction occurrence among squirrel species, with *Funambulus tristriatus* recording the maximum number of plant associations, whereas *Funambulus sublineatus* showed the minimum interactions. These findings highlight the underappreciated role of palm squirrels as frequent floral visitors in Indian plant communities.

Keywords: Palm squirrels, *Funambulus*, Nectar robbing, Flower visitation, Mammal-plant interactions, Floral damage, Pollination ecology.

1. INTRODUCTION

Interactions between flowering plants and their visitors constitute one of the most ecologically significant mutualisms in terrestrial ecosystems, sustaining plant reproduction and supporting food webs across diverse

landscapes (Ollerton et al., 2011; Hale et al., 2020; Dicks et al., 2021). Among vertebrate pollinators, bats and birds have received the greatest research attention, while the contribution of non-flying mammals to floral visitation and pollination has remained comparatively neglected, particularly in tropical Asia (Carthew & Goldingay, 1997;

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Ganesh & Devy, 2006; Kobayashi et al., 2019; Kobayashi et al., 2021). Non-flying mammals such as rodents and small marsupials, visit flowers primarily for nectar, and their interactions with plants range from mutualistic pollination to antagonistic behaviours, including nectar robbing and flower predation (Irwin et al., 2010; Kobayashi et al., 2017). Nectar robbing refers to the extraction of nectar without following the normal pollination pathway, typically by piercing floral tissues and avoiding contact with anthers and stigmas, whereas flower predation involves the consumption or destruction of floral structures, including reproductive organs, that can directly reduce plant reproductive success (Richman et al., 2021; Boaventura et al., 2022; Xiao et al., 2025). Consequently, mammalian flower visitors may function as pollinators, nectar robbers or flower predators depending on both floral architecture and their mode of resource exploitation (Kobayashi et al., 2017). Rodents, in particular, have been documented as frequent floral visitors in tropical systems across Asia, Africa and the Americas, where they often exploit nectar rewards opportunistically without consistently delivering pollination services (Deng et al., 2004; Deng et al., 2015). Squirrels (family Sciuridae) are among the more conspicuous rodent floral visitors, having been recorded as pollinators, nectar robbers and incidental flower visitors across a variety of plant species in tropical and subtropical regions (Kobayashi et al., 2015; Kobayashi et al., 2017; Kobayashi et al., 2018a; Kobayashi et al., 2018b). Notably, squirrel pollination has been confirmed for *Mucuna macrocarpa* in Taiwan, where body size and floral architecture are well matched for pollen transfer (Kobayashi et al., 2017), while in the Western Ghats of India, the Indian giant squirrel and the dusky-striped squirrel were recorded as flower predators on *Cullenia exarillata*, consuming floral parts without contributing to pollination (Ganesh & Devy, 2006). Together, these contrasting examples illustrate that the functional role of squirrels at flowers is not fixed, but is contingent on the morphological compatibility between the visiting animal and the flower being exploited. This contingency is best understood through the concept of therophily, the pollination syndrome associated with non-flying mammals, which predicts that flowers tolerant of mammalian visitors typically share a suite of traits: large, robust, bowl-shaped structures capable of withstanding physical disturbance, freely accessible nectar, drab colouration and musty or yeasty scents that appeal to olfactory-oriented foragers (Carthew & Goldingay, 1997;

Wester et al., 2023). Importantly, therophily does not guarantee pollination; where the visitor's body size or foraging posture precludes consistent contact with anthers and stigmas, even structurally accommodating flowers may yield only nectar robbery rather than pollen transfer (Lai et al., 2024)

Palm squirrels of the genus *Funambulus* are among the most widely distributed and ecologically familiar small mammals of the Indian subcontinent, occurring abundantly across natural forests, forest edges, agricultural landscapes, gardens and urban areas (Chakravarthy & Thyagaraj, 2012). Despite their conspicuousness and close association with flowering vegetation, systematic documentation of their interactions with flowers across India remains sparse. Early observations identified palm squirrels as visitors to coconut palm (*Cocos nucifera*) flowers and suggested a possible role in pollination (McCann, 1933). More recently, it was documented that the foraging activities of palm squirrels, *F. palmarum*, on coconut inflorescences in plantation ecosystems highlighted their potential contribution to pollination through therophily (Chakravarthy & Thyagaraj, 2012). Scattered records exist for individual species visiting specific plant species. However, a comprehensive account covering all four Indian *Funambulus* species across a range of plant families and geographic regions has not been attempted. This gap is significant given that palm squirrels, by virtue of their abundance and behavioural flexibility (Perodaskalaki et al., 2023; Abedin et al., 2025), may exert a non-trivial influence on the reproductive ecology of the plants they visit, whether as flower predator and nectar robbers that reduce floral resources available to legitimate pollinators, or as incidental pollen vectors on structurally suitable flowers (Irwin et al., 2010; Chakravarthy & Thyagaraj, 2012).

The present study was therefore undertaken to compile and document opportunistic records of flower visitation by all four Indian palm squirrel species across multiple states, characterise the nature of their interactions with flowering plants and assess their functional roles as nectar robbers, flower predators or occasional visitors. A bipartite interaction network was additionally constructed to examine patterns of interaction structure across squirrel species. By synthesising these observations, the study will contribute to a broader understanding of non-flying mammal floral interactions in South Asia and stimulate further experimental investigations into their ecological consequences.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was carried out across five states of India, namely West Bengal, Odisha, Maharashtra, Tamil Nadu and Kerala, spanning a broad ecological range (Figure 1). These states collectively encompass a diverse array of biogeographic zones, including the Eastern Ghats, Western Ghats, Deccan Plateau and the eastern coastal plains, and support populations of all four Indian palm squirrel species. Observations were made across a variety of land-use types, including home gardens, urban parks, agricultural landscapes, forest edges and protected areas. The wide geographic scope of the study reflects the opportunistic nature of the documentation, which was carried out wherever palm squirrels were encountered visiting flowers during the study period. Direct observations by the first

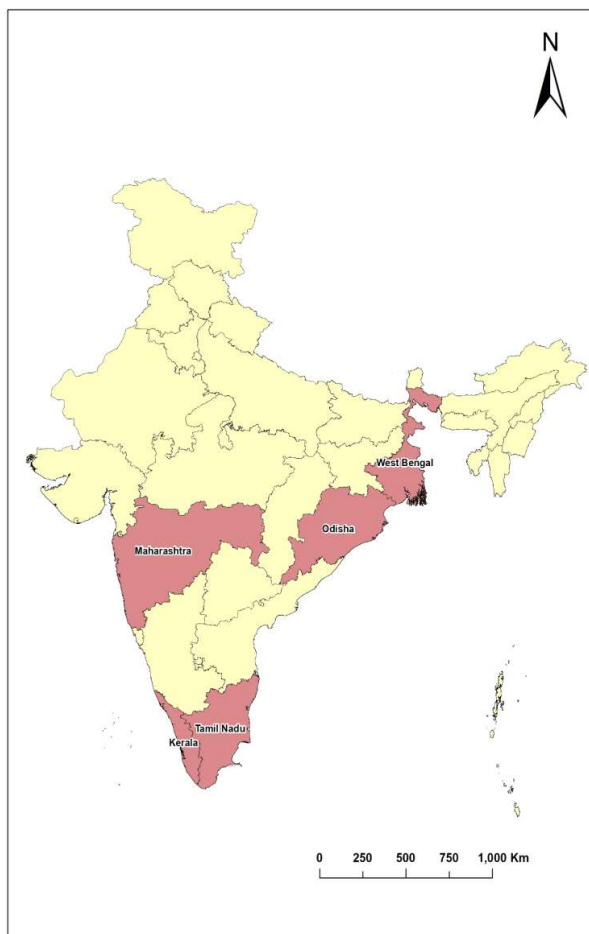


Figure 1. Study area map of India showing the five states, West Bengal, Odisha, Maharashtra, Tamil Nadu and Kerala, from which opportunistic records of flower visitation by palm squirrels (*Funambulus* spp.) were documented

author were carried out at the AJC Bose Indian Botanical Garden, Howrah, West Bengal, India (22°33'31"N, 88°17'28"E; ca. 10 m a.s.l.), where *Funambulus pennantii* was recorded visiting the flowers of *Asystasia gangetica* in an urban botanical garden setting.

2.2. Study Species

The four palm squirrel species native to India, namely *Funambulus palmarum* (Linnaeus, 1766), *Funambulus pennantii* Wroughton, 1905, *Funambulus tristriatus* (Waterhouse, 1837) and *Funambulus sublineatus* (Waterhouse, 1838), were the focal taxa of the present study. All four species are diurnal, arboreal to semi-arboreal rodents belonging to the family Sciuridae that collectively occupy a broad geographic range across India. However, individual species differ in their distributions and habitat associations (Wilson & Reeder, 2005). They are omnivorous, consuming fruits, seeds, insects, bark and plant exudates, and are commonly observed in close association with flowering vegetation across both natural and human-modified landscapes (Chakravarthy & Thyagaraj, 2012).

All four native Indian *Funambulus* species were easily identified from photographs and videos using a standardised matrix of diagnostic phenotypic traits and geographic distribution boundaries (Dissanayake et al. 2012; Nivetha et al. 2023). The Northern Palm Squirrel (*F. pennantii*) was identified by its characteristic five pale dorsal stripes (Yousefi et al., 2013). The remaining three-striped congeners were differentiated based on body size, stripe prominence, pelage characteristics, habitat preference, and geographic occurrence (Talmale et al., 2013). The Three-striped Palm Squirrel (*F. palmarum*) was recognised by its distinct three-striped pattern and its occurrence in southern and central India (Samson et al., 2017). The Jungle Palm Squirrel (*F. tristriatus*) was distinguished by its relatively larger body size, darker pelage, and association with woodland habitats of the Western Ghats (Talmale et al., 2013). The Nilgiri Striped Squirrel (*F. sublineatus*) was identified by its notably small size, faint dorsal striping, and restriction to high-altitude montane forests of the southern Western Ghats (Dissanayake et al., 2012; Rajamani, 2021). The squirrel species was identified by the co-author, Muhamed Jafer Palot (Zoological Survey of India), who is an expert on Indian small mammals.

2.3. Focal Observation

Floral visitation by palm squirrels was documented

opportunistically between December 2019 and March 2021. Observations were made whenever squirrels were encountered interacting with flowers during the course of routine field activities and dedicated wildlife surveys. A few records were contributed by citizen-science observers across India, who submitted photographic documentation of palm squirrel floral visitation via personal communication (see Acknowledgement section). Independent observation events from 5 states of India were recorded. Each interaction was recorded through direct observation, supplemented, wherever possible, by photographic and video documentation to facilitate subsequent verification of both squirrel identity and the nature of the interaction. No animals

were handled, or subjected to any form of experimental manipulation during the course of this study.

Plant species visited were identified to species level using standard floristic references and verified against herbarium records where necessary. In addition to original observations, published records of floral visitation by *Funambulus* species in India were reviewed and incorporated (Ganesh & Devy, 2006; Chakravarthy & Thyagaraj, 2012). Literature records were included only when squirrel and plant species could be reliably identified and floral visitation was clearly documented. These records were incorporated to complement original observations and provide a broader overview of palm squirrel-flower interactions in India. Because the present study compiled opportunistic natural-history observations from multiple observers, only conspicuous, day-active behaviour of palm squirrels in human-frequented areas was documented.

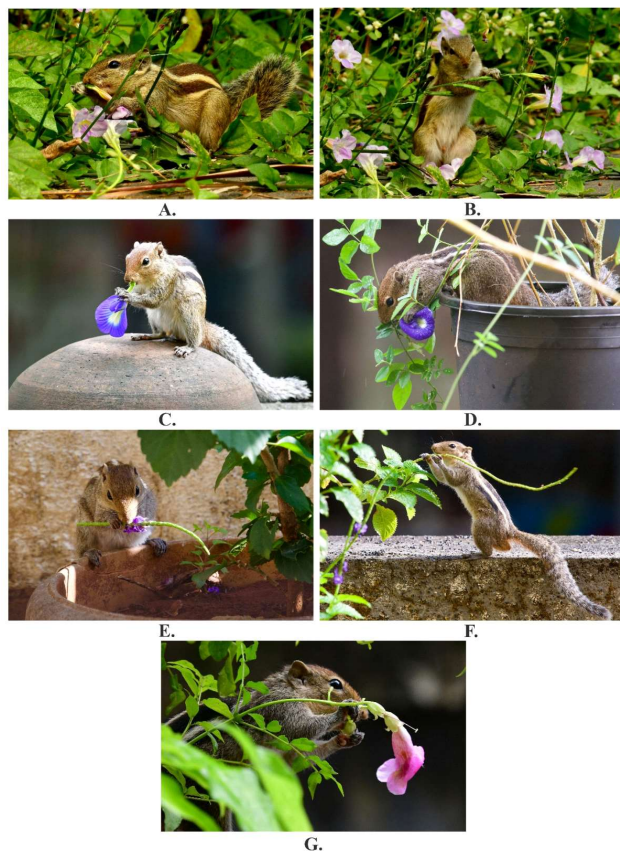


Figure 2. A. *Funambulus pennantii* was found feeding nectar from the corolla of *Asystasiagangetica*, B. *F. pennantii* dislodged several flowers of *A. gangetica*, C. *F. tristriatus* was found feeding on floral parts by plucking the entire flower of *Clitoriaternatea*, D. *F. tristriatus* was biting the corolla tube of *C. ternatea*, E and F. *F. tristriatus* was nectaring from *Stachytarpheta jamaicensis* by taking away the entire flower spike, G. *F. tristriatus* was nectaring on flowers of *Tecoma stans*

2.4. Floral Interactions

Each recorded interaction was assigned to one of three functional categories (Inouye, 1980; Irwin et al., 2010). Interactions were classified as nectar robbing when squirrels obtained nectar by piercing or biting floral tissues such as the corolla tube or calyx, causing structural damage and often facilitating subsequent visits by secondary robbers. Interactions were classified as flower predation when squirrels consumed floral parts, including petals, anthers, ovaries or entire flowers. Interactions were classified as occasional visitation when squirrels accessed floral rewards without exhibiting morphological or behavioural specialisation for pollination, making incidental, and generally non-destructive contact with anthers and stigmas. Pollen adhesion to body fur and nasal bristles was observed during some visits, suggesting the possibility of incidental pollen contact. However, quantitative pollen load analysis was beyond the scope of the present study. These visits were distinguished from nectar robbing by the absence of visible floral damage and from flower predation by the absence of flower or floral-part consumption.

2.5. Bipartite Network Analysis

To visualise the structure of interactions between squirrel species and plant species, a presence-absence bipartite interaction network was constructed based on the recorded visitation data (Robinson et al., 2015; Khorsand et al., 2025). Each squirrel-plant pair with at least one confirmed interaction was treated as a binary link. Binary network metrics, including connectance, nestedness (NODF), and

modularity, were calculated using the 'networklevel' function in the bipartite package to characterise the structural properties of the network. The network was constructed and visualised using the bipartite package v2.18 (Dormann et al., 2008) in R v4.1.2 (R Core Team, 2023) to summarise interaction patterns among the four *Funambulus* species. As the study was conducted in citizen-science mode, interaction frequencies were not recorded, and weighted metrics were therefore not calculated. Only squirrel-plant interactions recorded during the study period were included as binary links, representing the occurrence of interactions (Chacoff et al., 2012).

3. RESULTS AND DISCUSSION

3.1. Floral Visitation Records and Plant Diversity

All four palm squirrel species occurring in India, *Funambulus palmarum*, *F. pennantii*, *F. tristriatus* and *F. sublineatus* were observed visiting flowers. Across all four species, 40 observation events were recorded on 20 plant species belonging to 11 families (Table 1). *Funambulus tristriatus* contributed the highest number of observation events, followed by *F. pennantii*, *F. palmarum*, and *F. sublineatus*. Of the 20 plant species recorded, 8 were associated exclusively with occasional visitation, 5 exclusively with flower predation, and 1 exclusively with nectar robbing, while 6 species exhibited multiple interaction behaviours. During most visits, squirrels accessed nectar by biting or tearing floral tissues. This often resulted in damage to corollas or removal of entire flowers, particularly in small or delicate entomophilous species such as *Asystasia gangetica*, *Stachytarpheta jamaicensis* and *Lantana camara*. In several instances, flowers were plucked and discarded after nectar extraction.

In contrast, visits to large and robust flowers and inflorescences, including *Butea monosperma*, *Callistemon citrinus*, *Musa* spp. and *Cocos nucifera*, involved nectar feeding with comparatively little floral damage. In these cases, contact between the squirrel's head or snout and floral reproductive structures was occasionally observed while accessing nectar (Figure 3F & 3G for the pollen attached to the snout of the squirrel).

3.2. Bipartite Interaction Network

The bipartite interaction network (Figure 4) illustrated the visitation pattern of all four *Funambulus* species across the 20 recorded plant species (Figure 4). Binary network metrics revealed a connectance of 0.325. The network exhibited moderate nestedness (temperature = 34.16; NODF

= 26.53) and moderate modularity ($Q = 0.450$), with four distinct modules identified, each predominantly associated with a single squirrel species. Network-level specialisation, $H2'$ was 0.704. All metrics were computed from presence-absence data, and weighted metrics were not calculated as recording interaction frequencies were not a part of this study.

Funambulus tristriatus, a widely distributed species showed the maximum host visits, with floral interactions recorded across the highest number of plant species, while *F. sublineatus*, being a forest species had the least interaction, with only two observation events (Table 1) both recorded on

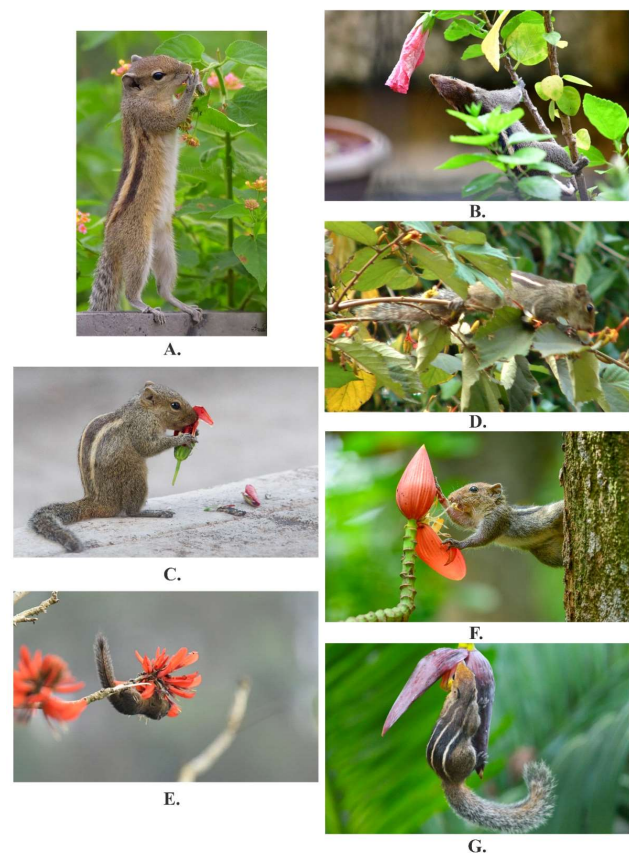


Figure 3. A. *F. palmarum* was taking nectar and damaging the entire inflorescence of *Lantana camara*, B. *F. tristriatus* was nectaring on the flowers of *Hibiscus* sp., C. *F. tristriatus* was plucking the flowers of *Hibiscus* sp., D. *F. tristriatus* was feeding nectar of *Helicteresisora*, without damaging the flower heads, E. *F. sublineatus* found feeding on the flowers of *Erythrina variegata*, F. *F. tristriatus* was feeding nectar from the inflorescence of *Musa ornata* and the entire flowers are taken away G. *F. tristriatus* was visiting inflorescence of *Musa paradisiaca*

Table 1. Flower visitation by Indian palm squirrels (*Funambulus* spp.) recorded across selected states in India

Plant species (Common name; Scientific name; Family)	Pollination syndrome	Observation locality	Date and documentation	Number of times observed	Behaviour category	Remark
* Chinese violet; <i>Asystasia gangetica</i> ; Acanthaceae	Entomophilous (Liow et al. 2001).	Howrah (Kolkata, West Bengal)	13-12-2019	8	Flower predator & Nectar robber	<i>F. pennantii</i> fed on nectar from the corolla (Fig.2A) and dislodged flowers (Fig.2B).
*Asian pigeonwing; <i>Clitoria ternatea</i> ; Fabaceae	Entomophilous (Girish, 2017)	Thane (Mumbai, Maharashtra)	23-09-2020	2	Flower predator	<i>F. tristriatus</i> fed on floral parts by plucking the entire flower (Fig.2C) or biting the corolla tube (Fig. 2D)
*Blue porterweed; <i>Stachytarpheta jamaicensis</i> ; Verbenaceae	Entomophilous; butterfly nectar plant (Manoj et al. 2021)	Thane (Mumbai, Maharashtra)	22-03-2020	2	Flower predator	<i>F. tristriatus</i> removed entire flower spikes while nectaring (Fig. 2E, Fig. 2F)
*Yellow elder; <i>Tecoma stans</i> ; Bignoniaceae	Entomophilous and ornithophilous (Dhnaya et al. 2013)	Thana (Mumbai, Maharashtra)	02-12-2020	1	Occasional visitor	<i>F. tristriatus</i> nectaring on flowers (Fig. 2G)
*Common lantana; <i>Lantana camara</i> ; Verbenaceae	Entomophilous; butterfly nectar plant (Manoj et al. 2021)	Coimbatore (Tamil Nadu)	02-12-2020	2	Nectar robber & Flower predator	<i>F. palmarum</i> nectaring and damaging the inflorescence (Fig.3A)
*Red powder puff; <i>Calliandra haematocephala</i> ; Fabaceae	Entomophilous, chiropterophilous; butterfly nectar plant (Churiet al. 2021)	Vaniyambalam, (Malappuram district Kerala)	11-10-2020	1	Occasional visitor	No photo documentation, but observed <i>F. tristriatus</i> nectaring on flowers (personal Communication by Dasan)
* <i>Hibiscus</i> sp.; Malvaceae	Butterfly nectar plant (Churiet al. 2021)	Thane (Mumbai, Maharashtra)	15-07-2020	2	Nectar robber & Flower predator	<i>F. tristriatus</i> nectaring (Fig.3B) and plucking flowers (Fig.3C)
Silk cotton tree; <i>Bombax ceiba</i> ; Malvaceae	Ornithophilous, chiropterophilous, occasionally entomophilous (Raju et al. 2005)	Payangadi Kannur, (Kerala)	18-02-2020	1	Occasional visitor	Though not photo documented, observed <i>F. tristriatus</i> visiting flowers.
Indian coral tree; <i>Erythrina variegata</i> ; Fabaceae	Entomophilous; ornithophilous; chiropterophilous (Fleming et al. 2009)	Munnar, Idukki, (Kerala)	22-10-2020	2	Flower predator	<i>F. sublineatus</i> (Fig.3E) and <i>F. tristriatus</i> observed feeding on flowers
Drumstick tree; <i>Moringa oleifera</i> ; Moringaceae	Entomophilous; nectar plant & ornithophilous (Jyothi et al. 1990; Sharma, 2019)	Bhuvaneswar, (Odisha) Patnadevi, Gautala-Autramghat WLS, Jalgaon, (Maharashtra)	18-06-2020 14-01-2021	2	Occasional visitor	<i>F. palmarum</i> frequently visited flowers; frequent nectar-feeding visits by <i>F. pennantii</i> were observed.
*Copperpod; <i>Peltophorum pterocarpum</i> ; Fabaceae	Entomophilous (Aluri & Reddi, 1996)	Pune, (Maharashtra)	29-03-2021	2	Flower predator	<i>F. pennantii</i> and <i>F. palmarum</i> skimmed through flower clusters feeding on flowers.

Cont...

Table 1. Flower visitation by Indian palm squirrels (*Funambulus* spp.) recorded across selected states in India

Plant species (Common name; Scientific name; Family)	Pollination syndrome	Observation locality	Date and documentation	Number of times observed	Behaviour category	Remark
*Lindley's aporosa; <i>Aporosa cardiosperma</i> ; Phyllanthaceae	Pollinators unknown (Schot, 2004)	Aralam WLS, Kannur, (Kerala)	January, 2020	2	Occasional visitor & Flower predator	<i>F. tristriatus</i> frequently visited and fed on flowers.
Champak tree; <i>Magnolia champaca</i> ; Magnoliaceae	Entomophilous (WWF 2019)	Bhuvaneswar, (Odisha)	2-07-2020	2	Occasional visitor	Regular visits by <i>F. palmarum</i> on flowers.
*Indian laburnum; <i>Cassia fistula</i> ; Fabaceae	Entomophilous (Murali, 1993)	Pune, (Maharashtra)	27-03-2021	2	Flower predator	<i>F. pennantii</i> attended flowers while skimming through flower clusters.
Bottle brush tree; <i>Callistemon citrinus</i> ; Myrtaceae	Entomophilous & ornithophilous (Sharanya et al. 2014)	Raichak, (West Bengal) and Uttar Pradesh	14-02-2020	1	Occasional visitor, Nectar robber	<i>F. pennantii</i> nectaring with little flower damage.
Indian screw tree; <i>Helicteresisora</i> ; Malvaceae	Entomophilous & ornithophilous (Atluri, 2000)	Calicut University Campus, Malappuram, (Kerala)	01-04-2020	1	Occasional visitor	<i>F. tristriatus</i> fed on nectar without damaging flower heads (Fig.3D),
Flame of the forest; <i>Butea monosperma</i> ; Fabaceae	Ornithophilous, therophilous, chiropterophilous (Tandon et al. 2003; Fleming et al. 2009)	Akurdi, Pune, (Maharashtra)Mukkali, Silent Valley National Park, Palakkad, (Kerala)	28.03. 2020	3	Occasional visitor	<i>F. palmarum</i> , <i>F. pennantii</i> and <i>F. tristriatus</i> observed nectar feeding.
Ornamental banana; <i>Musa ornata</i> ; Musaceae	Ornithophilous, therophilous, chiropterophilous (Nur, 1976; Subbaraya & Baudoin, 2006)	Kozhikode (Kerala) Several places in Maharashtra	Feb, 2020 Throughout the year	2	Flower predator / Occasional visitor	<i>F. tristriatus</i> removed flowers while feeding (Fig. 3F); <i>F. palmarum</i> nectaring observed.
Common banana; <i>Musa paradisiaca</i> ; Musaceae	Chiropterophilous (Fleming et al. 2009)	Malappuram, (Kerala)	10-01-2020	1	Occasional visitor	<i>F. tristriatus</i> visiting inflorescences (Fig. 3G).
Coconut palm; <i>Cocos nucifera</i> ; Areaceae	Entomophilous, ornithophilous, therophilous (Thomas & Kumar, 2013; Chakravarthy & Thyagaraj, 2012)	Karnataka and northern part of Kerala.	16-03- 2020	1	Occasional visitor	<i>F. tristriatus</i> observed on coconut palms.

*Indicates first report of flower visitation by palm squirrels

a single plant species (Figure 4), *Erythrina variegata*. *Funambulus palmarum* and *F. pennantii* showed intermediate host breadths. Nonetheless, the pattern is consistent with the known habitat associations of these species: *F. tristriatus* is a generalist of disturbed and edge habitats where floral diversity is high, whereas *F. sublineatus* is a forest interior specialist with a more limited geographic and ecological range (Chandrasekar & Sunquist, 1996; Molur & Nameer, 2016; Middleton & Ferguson, 2020;

Rajamani, 2021). The network further revealed that several plant species, including *Musa* spp. and *Moringa oleifera*, were associated with more than one *Funambulus* species, suggesting overlap in floral resource use at the species level.

3.3. Nectar Feeding and Floral Damage

Nectar feeding by palm squirrels appears to be opportunistic and primarily related to energy acquisition. Frequent damage or removal of flowers indicates that

squirrels often function as nectar robbers or flower predators, especially on flowers not structurally adapted to mammalian visitors. Similar patterns of nectar robbing and floral damage by rodents and other mammals have been documented in tropical systems (Deng et al., 2004; Irwin et al., 2010). Such damage is likely to reduce floral longevity and may limit access to nectar for legitimate pollinators.

3.4. Context-dependent Pollination Potential

In contrast to damaging visits on small, fragile entomophilous flowers, palm squirrels visiting large, structurally robust flowers occasionally came into contact with anthers and stigmas. Nine plant species recorded in this study, *Tecoma stans*, *Calliandra haematocephala*, *Bombax ceiba*, *Moringa oleifera*, *Magnolia champaca*, *Helicteres isora*, *Butea monosperma*, *Musa paradisiaca*, and *Cocos nucifera*, were categorised exclusively as occasional visitors, with no flower damage recorded. This behavioural distinction suggests that floral morphology plays a decisive role in determining visit outcome.

Among these, several species possess floral traits particularly conducive to incidental pollen transfer. *Bombax ceiba* produces large, open, cup-shaped flowers with a prominent exerted stamen column, with no morphological restriction for access to nectar, making anther contact with the squirrel's muzzle or facial fur highly probable during nectar feeding (Raju et al., 2005). Similarly, *Magnolia champaca* bears large, robust tepals with anthers and stigmas in close spatial proximity along a central receptacle axis, a floral architecture associated with generalist vertebrate visitation (Thien et al., 2000). *Callistemon citrinus* displays a bottlebrush inflorescence architecture in which stamens are exerted several centimetres beyond the corolla, virtually ensuring contact with a visiting mammal (Sharanya et al., 2014). *Butea monosperma*, visited by all three *Funambulus* species in the present study, has previously been reported to be squirrel-pollinated. *Funambulus tristriatus* was observed to contact both the anthers and the stigma while foraging for nectar, thereby facilitating pollen deposition and fruit set (Tandon et al., 2003). *Helicteres isora* is particularly noteworthy: the observed individual fed on nectar without damaging flower heads, suggesting a non-destructive foraging mode compatible with pollinator function; its flowers are large, zygomorphic, with a prominent androgynophore that positions stamens accessibly for vertebrate visitors (Atluri et al. 2000).

This raises the possibility that they may function as potential incidental pollen vectors when floral morphology is compatible with their body size and handling behaviour. However, pollen deposition and transfer were not directly measured in this study. Similar interactions have been reported where squirrels act as opportunistic rather than specialised pollinators, and pollen transfer has been inferred in flowers with sturdy construction, accessible nectar, and exposed reproductive organs (Ganesh & Devy, 2006;

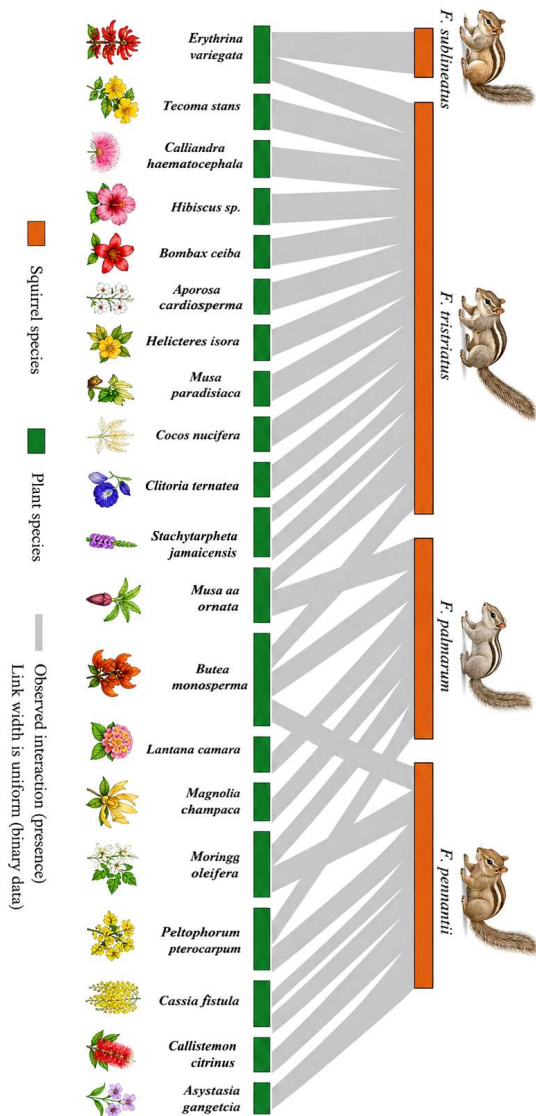


Figure 4. Bipartite network illustrating the flower visitation interactions between four *Funambulus* species (orange nodes, top) and 20 plant species from 11 families (green nodes, bottom) documented across five Indian states. Lines connect each squirrel species to the plant species it was recorded visiting

Kobayashi et al., 2017). Such trait matching between floral architecture and mammalian foragers is a recurring feature of non-flying mammal pollination systems (Carthew & Goldingay, 1997), and future studies incorporating pollen load analysis would be needed to confirm whether palm squirrels contribute meaningfully to pollination in Indian plant communities.

3.5. Ecological Implications

The wide taxonomic range of flowers visited and the occurrence of floral visitation across diverse landscapes indicate substantial behavioural flexibility in palm squirrels. As common and abundant mammals in many Indian ecosystems, their frequent nectar feeding may influence plant-pollinator interactions by competing with legitimate pollinators and altering floral resource availability (Irwin et al., 2010; Chakravarthy & Thyagaraj, 2012).

The bipartite interaction network provides a preliminary structural overview of the squirrel-plant interaction system documented in this study. The asymmetry in host breadth across species with *F. tristriatus* being notably more generalist than *F. sublineatus* likely reflects a combination of unequal observation effort and biological differences in distribution range, habitat use and abundance rather than dietary specialization per se, as all four species appear to exploit floral resources opportunistically. The occurrence of shared plant species across multiple squirrel species raises the possibility of interspecific competition for floral resources, though this cannot be evaluated without quantitative visitation data. Such interaction networks, even when based on presence-absence records, can serve as a useful baseline for future studies that incorporate visit frequency, pollen load analysis and experimental studies to rigorously assess the ecological roles of non-flying mammals in Indian plant communities (Dormann et al., 2017; Blüthgen & Staab, 2021)

4. CONCLUSION

The study highlights floral visits of palm squirrels in India, spanning a taxonomically diverse range of plant species and providing a comprehensive synthesis of squirrel-flower interactions from the country to date. The interactions involved nectar robbing or florivory on delicate entomophilous flowers, particularly species lacking morphological traits associated with conspicuous therophilic syndromes. However, they may serve as potential pollen vectors for structurally robust therophilous flowers such as *Butea monosperma* and *Musa* spp. Floral

visitation by palm squirrels is not always ecologically neutral; nectar robbing and florivory can deplete floral rewards and damage reproductive structures, reducing resource availability for legitimate pollinators such as bees, butterflies, birds or mammals and potentially compromising plant reproductive fitness. Non-flying mammals, often overlooked in pollination ecology research, can meaningfully influence plant-animal interaction networks, and future studies should move beyond natural history observations toward experimental evaluation of the ecological consequences of floral visitation by Indian palm squirrels.

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CRediT authorship contribution statement

Prodipta Biswas: Data collection, Methodology, Software, Writing original draft, Formal analysis, Tabulation, Data curation. **K. Rajmohana:** Conceptualization, Supervision, Reviewing and editing, Visualization. **Muhamed Jafer Palot:** Methodology, Reviewing and editing, Visualization.

Conflict of interest

The authors declare that they have no conflicts of interest

Data availability statement

All data generated or analysed during this study are included in this published article. The interaction records are presented in their entirety in Table 1. The corresponding author retains raw photographic and video evidence supporting the documented observations and will be made available upon reasonable request.

Declaration of generative AI AND AI-assisted technologies in the writing process

The authors declare that no artificial intelligence tools were used to write this manuscript.

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Checklist of Hemipteran Insects Collected on Cucurbitaceous Crops of Punjab, India

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ABSTRACT: Cucurbits are warm-season crops that constitute the most widely cultivated category of summer vegetables. The family Cucurbitaceae comprises 120 genera and 800 species, including 31 genera and 94 species found in India. India is the second-largest global producer of vegetables, accounting for approximately 15% of the world's overall output. The current survey-cum-collection study illustrates the current status and taxonomic characteristics of hemipteran species visiting cucurbitaceous vegetables in the state of Punjab, India, and provides a checklist of these insects. In total, 14 insect species in the order Hemiptera were observed in eight families and 11 genera, including Aleyrodidae and Coreidae (1 genus, 1 species), Geocoridae and Lygaeidae (3 genera and 3 species), Pentatomidae, Pseudococcidae (1 genus, 1 species), Pyrrhocoridae, and Reduviidae (2 genera and 2 species). This checklist provides basic information about the current status of regional biodiversity in Punjab's agricultural systems, which in turn will help formulate innovative integrated pest management (IPM) approaches. Species-wise morphometric observations, key diagnostic features, distribution, and host records in India are provided based on literature searches and field observations.

Keywords: Cucurbits, Diversity, Hemiptera, Punjab, India.

1. INTRODUCTION

Punjab, also known as the "Granary of India," is a state located in north-western India that ranks third in total foodgrain production after the Uttar Pradesh and Madhya Pradesh states in the country (Ministry of Finance, 2026), and is known to be a major contributor to the production of food grains in India (Singh, 2020). Some economically valuable cucurbitaceous crops cultivated in all regions of the state are cucumber (*Cucumis sativus* L.), bottle gourds (*Lagenaria siceraria* (Molina) Standl.), bitter gourd (*Momordica charantia* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and muskmelon (*Cucumis melo* L.) (Dhaliwal, 2017; Mercy & Nmom, 2017 and Ingle

& Shyamrao, 2020). Various insect pests infest cucurbitaceous crops, and hemipteran insects constitute an important part of the total pest complex (Atwal & Dhaliwal, 2018).

All hemipteran insect species associated with cucurbitaceous crops are either pest species that directly damage the crop through feeding or beneficial predatory species that contribute to natural pest control (Omkar & Kumar, 2013 and Cuthbertson, 2020). Basic knowledge of species composition and distribution is critical for developing effective integrated pest management (IPM) and sustainable agricultural management practices (Gyawali et al., 2024; Tartanus & Malus, 2024).

Earlier studies on the hemipteran fauna of Punjab have

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been scattered and mostly focused on specific pest species (Distant, 1902, 1904). Comprehensive checklists documenting the diversity of hemipteran insects in cucurbitaceous crops in the Punjab region are scarce (Kaur et al., 2012). The purpose of this study was to revise the list of hemipteran species collected from various cucurbitaceous crops in five agro-climatic regions of Punjab, to provide a checklist of their diagnostic characteristics, and to document their distribution in India.

2. MATERIALS AND METHODS

2.1. Study Area

Hemiptera bug collections were conducted from April 2022 to October 2022 in five agro-climatic regions in Punjab, India (sub-mountain, undulating region, undulating plain region, central plain region, western plain region, and western region) (Table 1, Figure 1).

The state of Punjab in India has five distinct agro-climatic regions, with the cooler, rain-fed Shivalik foothills in the northeast and the arid southwest plains. Differences determine various environmental and social realities. First, the Sub-Mountain Undulating Region (Zone I or Kandi region) is a transitional, semi-arid tract along the northeastern border of Punjab, situated at the base of the Shiwalik hills and, to the north, bordering Himachal Pradesh state. Second, the Undulating Plain Region (Zone II) directly borders and lies south of the Shiwalik foothills of Himachal Pradesh. These regions are mainly undulating with hilly topography and is prone to serious soil erosion due to higher rainfall. These regions mainly support the rural population,

which depends on mixed farming and rain-fed crops. As one move inwards the regions undulating plains region is a transitional region of a moderately irrigated region with some undulating topography that quickly flattens out to the hyper-fertile Central Plain Region (Zone III), which is the rich agricultural region regarded as the breadbasket of India, following an intensive wheat rice rotation producing a rich farming community (Kaur et al., 2024; Singh, 2013). However, extensive and sophisticated network of tubewells have resulted in severe groundwater depletion. Finally, Western Plain Region (Zone IV) and Western Region (Zone V) bordering north-western Rajasthan state has sandy soils, low rainfall and high summer temperature. The local

Table 1. Survey locations in five agro-climatic regions of Punjab during the study

Agro-climatic region	Location of collection	GPS coordinates
Sub-Mountain Undulating region (Z1)	Khanpur, Mukerian	31°54'42.1"N 75°37'31.8"E
Undulating Plain region (Z2)	Kolar, SBS Nagar	31°04'28.0"N 76°23'20.5"E
Central Plain region (Z3)	Malerkotla	30°32'20.7"N 75°51'21.8"E
Western Plain region (Z4)	Lehra Bega, Bathinda	30°14'13.8"N 75°06'44.3"E
Western region (Z5)	PAU RRS, Abohar	30°10'15.0"N 74°12'22.7"E
	Nihalkhera, Abohar	30°13'42.7"N 74°08'02.1"E

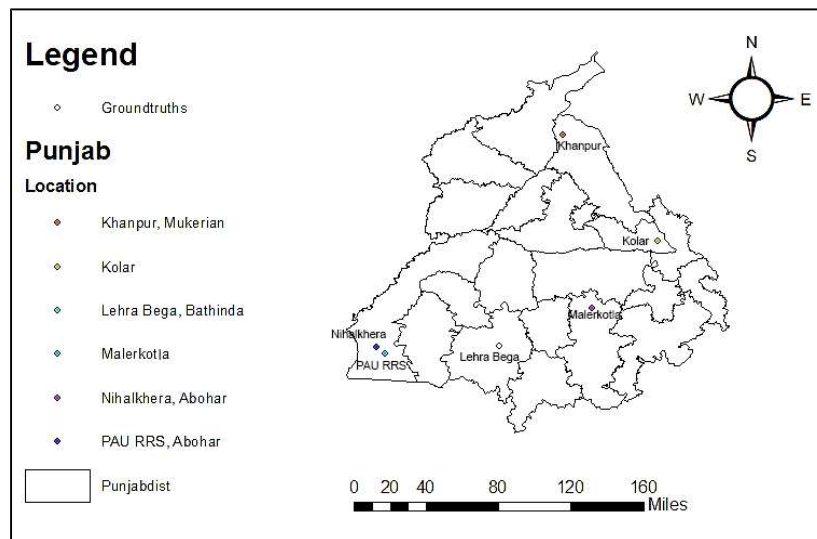


Figure 1. Collection sites of hemipteran bugs in Punjab

population depends on canal irrigation for growing cotton and citrus crop. Soil salinity and waterlogging are important environmental challenges in these regions (Singh, 2013 and Bandumula et al., 2018).

2.2. Collection, Identification, and Morphometry

2.2.1. Field surveys and sampling

Multiple field surveys were conducted in cucurbit-growing areas in Punjab to collect data on cucumbers, bottle gourds, bitter gourds, watermelons, and muskmelons. Insect samplings were performed in farmers' fields and on research plots at PAU Ludhiana campus, Ludhiana, Regional Research Station (RRS), Bathinda, and Dr. J C Bakshi RRS, Abohar during the 2021-22 cropping season (Table 1).

Various standard entomological techniques were employed for specimen collection, including visual searches, light traps, sweep nets, and direct host collection. The collected specimens were curated and identified using standard taxonomic keys and morphological characteristics (Triplehorn & Johnson, 2005). Taxonomic identification was performed based on taxonomic articles and relevant taxonomic data (Distant, 1902, 1904; Chandra et al., 2018). Experts were consulted to reconfirm the species or genus.

2.2.2. Morphometry

The adult insect specimens were identified using Nikon SMZ25 Motorized Stereo Zoom Microscope (Nikon Corp., Japan) housed in the Insect Taxonomy Lab of the Department of Entomology, PAU, Ludhiana. The dorsal habitus of each pinned specimen was photographed with the

mounted Nikon D5300 camera body on the aforementioned microscope. The morphometric observations of the head, thorax, and abdomen for each specimen were measured using the ocular micrometer (Nikon Corp., Japan) available within the eyepiece of the same microscope. The measurements were expressed in millimetres (Table 2). The existing literature has been utilised to compile Indian distributional data for the fauna of the region, taxonomic records, and habitat surveys (Distant, 1902, 1904; Gupta & Singh, 2013; Chandra & Boaz, 2018; and Chandra et al., 2018).

3. RESULTS AND DISCUSSION

3.1. Systematic Account

The dorsal habitus of all 14 hemipteran species or genera recorded during the survey is shown in Figure 2, with each specimen labelled by its scientific name, its authority, and scale. Figure 2 provides a visual reference for easy diagnosis of hemipteran insects found on various cucurbits cultivated in Punjab, in which each family, genus, and species is described along with its key morphological characters, host records, and their extended distribution within India.

3.2. Order HEMIPTERA Linnaeus, 1758

3.2.1. Family ALEYRODIDAE Westwood, 1840

Genus *Bemisia* Quaintance & Baker, 1914

Bemisia tabaci (Gennadius, 1889)

Common name: Silverleaf whitefly, Cotton whitefly

Diagnostic characters: Adult insects were smaller than 2

Table 2. Morphometric observations of genera and species of hemipteran fauna collected from Punjab, India

Genus/Species	Family	Head (mm)	Thorax (mm)	Abdomen (mm)
<i>Bemisia tabaci</i> (Gennadius, 1889)	Aleyrodidae	0.09	0.11	0.23
<i>Cletus signatus</i> Walker, 1871	Coreidae	1.20	3.87	4.09
<i>Geocoris</i> spp.	Geocoridae	0.49	1.01	2.8
<i>Graptostethus servus</i> (Fabricius, 1787)	Lygaeidae	1.16	1.95	5.34
<i>Spilostethus pandurus</i> (Scopoli, 1763)	Lygaeidae	0.73	2.45	7.95
<i>Oxycarenus</i> spp.	Lygaeidae	0.82	1.47	2.37
<i>Bagrada hilaris</i> (Burmeister, 1835)	Pentatomidae	0.99	1.24	3.13
<i>Dolycoris indicus</i> Stål, 1876	Pentatomidae	2.07	6.17	3.49
<i>Eysarcoris</i> spp.	Pentatomidae	0.56	4.55	1.2
<i>Nezara viridula</i> (Linnaeus, 1758)	Pentatomidae	1.87	7.72	3.82
<i>Planococcus</i> spp.	Pseudococcidae	0.25	0.54	1.78
<i>Dysdercus evanescens</i> Distant, 1902	Pyrrhocoridae	1.87	5.21	10.33
<i>Physopelta gutta</i> (Burmeister, 1834)	Pyrrhocoridae	1.42	5.23	8.03
<i>Zelus</i> spp.	Reduviidae	2.47	3.18	4.79

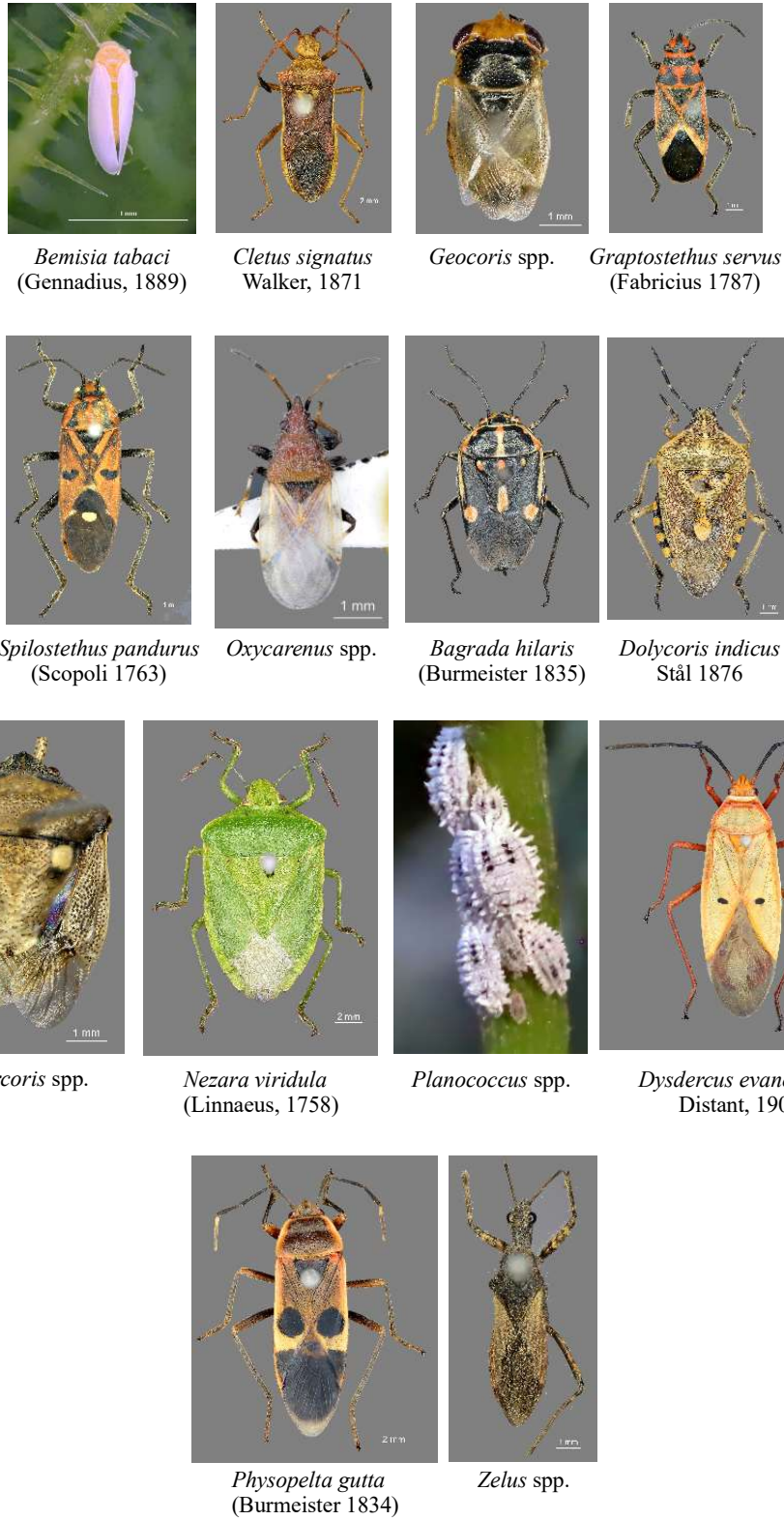


Figure 2. Dorsal habitus of various hemipteran insects collected from cucurbitaceous crops grown in Punjab, India, during 2022. Each specimen is labelled with its scientific name and authority; scale bars are shown on the individual photographs

mm, and their wings were covered with a whitish waxy powder coating. The wings were opaque, whitish, and powdery. The fore and hind wings were somewhat equal in size to the hind wings. The pupal case was colourless to brown. Caudal setae were invariably strong and usually as long as the vasiform orifice. The orifice, situated slightly within the edge of the pupal case, was shorter than that of the caudal furrow. The sides of the orifices were nearly straight. These observations coincided with those reported by Martin (1987), Hodges and Evans (2005) and Chaubey et al. (2015).

Host plants: Polyphagous in many plant families, such as Cucurbitaceae, Solanaceae, Malvaceae, and Leguminosae (Martin, 1987 and Hodges & Evans, 2005). This insect was found feeding on pumpkins in Punjab.

Distribution in India: Throughout India - Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal (Martin, 1987 and EPPO, 2025).

Remarks: The major pests of cucurbitaceous crops cause direct feeding damage and are known to transmit plant viruses.

3.2.2. Family COREIDAE Leach, 1815

Genus *Cletus* Stål, 1859

Cletus signatus Walker, 1871

Common name: Squash bug, Leaf-footed bug

Diagnostic characters: Medium-sized bugs with body lengths ranging between 12 and 15 mm. The adult body was moderately elongated and dark in colour. A leaf-like expansion was observed in the hind tibia. The humeral spine was not acute. The outer angle of the 7th abdominal sternite was acute. The spermathecal bulb was of uniform length, and the outer angle of the abdominal sternite 7 was sharp. Well-developed scent glands were observed between the middle and hind coxae. These characteristics are consistent with those reported by Triplehorn and Johnson (2005) and Gupta and Singh (2013).

Host plants: Several cucurbitaceous crops, such as squash, pumpkin, gourds, etc. (Schaefer & Panizzi, 2000 and Gupta & Singh, 2013). This insect feeds on pumpkin and bottle gourds in Punjab.

Distribution in India: Widely distributed in several states, including Assam, Bihar, Gujarat, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal (Gupta & Singh, 2013).

Remarks: Inflict damage due to sucking plant juices, especially in developing fruits.

3.2.3. Family GEOCORIDAE Baerensprung, 1860

Genus *Geocoris* Fallén, 1814

Geocoris spp.

Common name: Big-eyed bugs

Diagnostic characters: Small-bodied bugs, measuring 3.5-4.0 mm in length. Adults are characterized by the presence of large reniform compound eyes protruding from the head. The body was dark, whereas the head and rostrum were yellow-brown. The compound eyes were reddish-brown in colour. The antennae were black, and the first and apical joints were light brown and yellowish, respectively. A yellowish-brown coloration was present on the lateral margins of the thoracic pronotum and the corium of the forewing, whereas the scutellum and pronotum were black. The 3rd and the 4th abdominal segments had spiracles on their dorsal side. Similar characteristics have been reported by Henry (2009); Kóbor (2018, 2020) and Triplehorn and Johnson (2005).

Host(s): These are generalist predators that prey on several small insects on cucurbitaceous crops (Sweet, 2000; Mead, 2011 and Varshney, 2023). An adult of this insect was collected from a half-opened bitter melon flower in Khanpur, Punjab.

Distribution in India: Throughout India, including all major states, such as the North Indian foothills, Punjab, Haryana, Uttar Pradesh, Bihar, Odisha, West Bengal, Assam, Tamil Nadu, Karnataka, Maharashtra, Gujarat, Arunachal Pradesh, and Rajasthan (Distant, 1910, 1918; Kóbor, 2018 and Varshney, 2023).

Remarks: Beneficial predatory bugs that feed on sedentary pests such as aphids, whiteflies, thrips, scales, and other small insect pests.

3.2.4. Family LYGAEIDAE Schilling, 1829

Genus *Graptostethus* Stål, 1868

Graptostethus servus (Fabricius, 1787)

Common name: Seed-eating bug

Diagnostic characters: Medium-sized bugs, approximately 8-9 mm long. The body was reddish-brown with fine hair. The triangular head and its width were nearly equal to its length. The antennae were 4-segmented, and the 2nd segment was the longest. The compound eyes were large, round, and black. The rostrum was elongated and extended into the hind coxae. The thorax had a distinct shape, with a quadrangular pronotum that was wide at the base and gradually tapered towards the front. The scutellum was black, whereas the clavus and corium displayed a combination of red and black, all adorned with subtle greyish pubescence.

In contrast, the abdominal sternum was pale gray. All

abdominal spiracles were positioned dorsally. the wing membrane had deep brown, featuring straight longitudinal veins. Our findings corroborate those by Distant (1904), Triplehorn and Johnson (2005) and Chandra et al. (2018), and are similar to these studies.

Host plants: Adults and nymphs feed on the seeds of several plant species, including cucurbits (Distant, 1904; Schuh & Slater, 1995 and Chandra et al., 2018). This insect was collected from bitter gourds, pumpkins, and bottle gourds in Punjab.

Distribution in India: Extensively distributed in Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal (Distant, 1904 and Chandra et al., 2018).

Remarks: Feeds mainly on seeds but can damage the growing fruits

Genus *Spilostethus* Stål, 1868

***Spilostethus pandurus* (Scopoli, 1763)**

Common name: Seed bugs

Diagnostic characters: The bugs were large, ranging in size from 13 to 16 mm. The body was red, with black markings on the head apex, inner edges of the compound eyes, and two longitudinal patches on the pronotum, antennae, rostrum, scutellum, and legs. The wing membrane was reddish-brown in colour, with a prominent white spot. The femora had spines underneath them. The rostrum extended to the mid-coxae. Our findings extend those reported by Distant (1904) and Chandra et al. (2018).

Host plants: Adult and nymphal stages of this insect feed on the seeds of various cucurbitaceous vegetable crops (Schuh & Slater, 1995 and Chandra et al., 2018). This insect was collected from pumpkin, bitter gourd, and bottle gourd in Punjab.

Distribution in India: Across India, from Kashmir to Tamil Nadu, including Punjab, Haryana, Maharashtra, North Bihar, West Bengal, and Odisha, Andhra Pradesh, Karnataka, Gujarat, and Rajasthan (Chandra et al., 2018).

Remarks: It is mainly a seed feeder; however, it can damage developing fruits.

Genus *Oxycarenus* Fieber, 1837

***Oxycarenus* spp.**

Common name: Dusky cotton bug

Diagnostic characters: Oblong-bodied small-to-medium-sized bugs. The head was triangular and projected in front of the antenniferous tubercles. The rostrum was thin and long. The pronotum was trapezoidal with a midline constriction. The scutellum was triangular and barely wider than it was

long. The hemelytra with straight lateral margins completely covered the abdomen. The moderately thickened femora were finely spined. These observations are consistent with those of Distant (1902) and Schuh and Slater (1995).

Host plants: Cotton, cucurbits, etc. (Distant, 1902 and Ahmad & Kamaluddin, 1985). This insect was collected from bottle gourds and sponge gourds in Punjab.

Distribution in India: Found throughout India, especially in cotton-growing tracts such as Punjab, Haryana, Rajasthan, Gujarat, Maharashtra, Karnataka, Andhra Pradesh, Karnataka, and Tamil Nadu (Distant, 1902).

Remarks: Primarily a pest of cotton, but may also occur on cucurbitaceous crops.

3.2.5. Family PENTATOMIDAE Leach, 1815

Genus *Bagrada* Stål, 1862

***Bagrada hilaris* (Burmeister, 1835)**

Common name: Painted bug, Bagrada bug

Diagnostic characters: Medium-sized bugs with subovate, shield-shaped bodies. It had a black and shiny head. The lateral sides of the head, edges of the eyes, lateral and anterior margins of the pronotum, and pronotum had a median longitudinal stripe that extended to the scutellum, and the sides of the corium were pale off-white in colour (Rider, 2017 and Chandra et al., 2018). The antennae and tarsi were five- and three-segmented, respectively, which is consistent with those reported by Triplehorn and Johnson (2005), Rider (2017), and Chandra et al. (2018).

Host plants: Cruciferous crops and cucurbits (Palumbo et al., 2016; Rider, 2017 and Chandra et al., 2018). This insect was collected from pumpkin, bitter gourds, and bottle gourds in Punjab.

Distribution in India: North India - Punjab, Haryana, Rajasthan, Gujarat, Himachal Pradesh, Jammu & Kashmir, and some parts of Uttar Pradesh (Chandra et al., 2018).

Remarks: Mainly a cruciferous crop pest, but sometimes a pest of cucurbitaceous crops.

Genus *Dolycoris* Mulsant & Rey, 1866

***Dolycoris indicus* Stål, 1876**

Common name: Indian shield bug

Diagnostic characters: The medium-to-large-bodied and oval-shaped adult bugs were brownish-ochraceous in colour, with thick punctures covering the body. The five-segmented antennae were black, with the basal joint and bases of the other joints being luteous (orange-yellow or greenish-yellow) coloured. Small black spots were observed near the coxae. Tarsi three-segmented. The thoracic sternum did not have a longitudinal median keel. The scutellum was large and covered a large portion of the abdomen. The lateral

areas of the prosternum and abdomen had sparse black punctures, and the connexivum was spotted black at the incisures. The membrane was greyish, with slightly darker veins; similar observations were recorded by Distant (1902), Triplehorn and Johnson (2005), and Rider (2017).

Host plants: Many crops, including cucurbits, legumes, and vegetables (Distant 1902, Rider 2017). This insect was collected from bitter gourds and bottle gourds in Punjab.

Distribution in India: This insect was collected from sponge gourds, bitter gourds, pumpkin, and bottle gourds in Punjab. Throughout India, from most Indian states, including Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Maharashtra, Gujarat, and Rajasthan (Distant, 1902).

Remarks: Polyphagous pest found damaging through sucking plant sap.

Genus *Eysarcoris* Hahn, 1834

***Eysarcoris* spp.**

Common name: Shield bugs

Diagnostic characters: Small- to medium-sized pentatomid bugs with a typical shield-shaped body. The body was obovate in shape, moderately broad, and somewhat strongly convex beneath it. The head was deflected, with a rounded apex, and the central lobe was as long as or slightly longer than the lateral lobes. The five-segmented antennae had a basal joint that did not reach or nearly reached the apex of the head. The tarsi were three-segmented. Body shape varied from rounded to oval. The pronotum was deflected forward, while the scutellum was approximately the same length as its width at the base or slightly longer. The scutellum was triangular and did not cross the apex of the abdomen. These observations are consistent with the findings of Distant (1902), Triplehorn and Johnson (2005), Salini (2006, 2015) and Rider (2017).

Host plants: Different types of crops, such as cucurbits and vegetables (Distant, 1902, 1904 and Rider, 2017). This insect species was collected from bottle gourds and bitter gourds in Punjab.

Distribution in India: Widely distributed pan-India in Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Gujarat, Rajasthan, and Himachal Pradesh (Distant, 1902, 1904).

Remarks: Minor pest of various crops, including cucurbitaceous plants.

Genus *Nezara* Amyot & Serville, 1843

***Nezara viridula* (Linnaeus, 1758)**

Common name: Southern green stink bug

Diagnostic characters: Large pentatomid bugs, 12-17 mm in length. Colourful insects with a bright green colour may vary depending on the season. Five-segmented antenna. Three-segmented tarsi. Shield-shaped body characteristics of the family. Although the scutellum was large, it did not reach the apex of the abdomen. These diagnostic characteristics are in accordance with those reported by Triplehorn and Johnson (2005) and Rider (2017).

Host plants: Highly polyphagous and feed on many crops, including cucurbits, legumes, cereals, and vegetables (Ahmad & Kamaluddin, 1985; Jones, 1988 and Rider, 2017). This insect pest was collected from pumpkin, bottle gourds, and bitter gourds in Punjab.

Distribution in India: Throughout India - this is one of the most widespread pentatomids and is distributed in all the major states, as far as Punjab and Haryana southwards to Uttar Pradesh and Bihar, westwards to West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, and Maharashtra, and eastwards to Gujarat, and Rajasthan (Distant, 1902, 1904 and Ahmad & Kamaluddin, 1985).

Remarks: This pentatomid bug is an important polyphagous insect pest that is known to cause economic losses in many field crops.

3.2.6. Family PSEUDOCOCCIDAE Cockerell, 1905

Genus *Planococcus* Ferris, 1950

***Planococcus* spp.**

Common name: Mealybugs

Diagnostic characters: These mealy bugs are soft-bodied and produce a powdery white wax that covers their bodies. Adult females are wingless. Their bodies are broadly oval-shaped and appear convex dorsally and relatively flat ventrally. Dorsally, adult females show yellowish, brownish, or greyish-yellow colouration while yellowish ventrally. In female mealybugs, the colouration on the dorsal surface is mostly hidden due to the presence of a dense layer of white powdery substance. Similar observations have also been reported by Williams and Granara de Willink (1992), Miller et al. (2000) and Joshi et al. (2021).

Host plants: Polyphagous on many crops, including cucurbits, citrus, and ornamentals (Williams & Granara de Willink, 1992 and Miller et al., 2000). Adult female insect were collected from bottle gourds in Punjab.

Distribution in India: All over India, collected from all major agricultural states, namely, Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Maharashtra, Gujarat, and Rajasthan (Williams & Granara de Willink, 1992).

Remarks: It is an agriculturally important insect pest that is

known to cause direct damage to crops and transmit plant viruses.

3.2.7. Family PYRRHOCORIDAE Amyot & Serville, 1843

Genus *Dysdercus* Guérin-Ménéville, 1831

Dysdercus evanescens Distant, 1902

Common name: Red cotton bug

Diagnostic characters: Adult bugs were medium to large in size, measuring 10- 14 mm. The body was brilliantly red and black. All abdominal spiracles were located dorsally. In general, the head was narrower than the pronotum. The antennae were four-segmented. The pronotal collar was either white or the same colour as the rest of the body. The adults were concolourous, with the pronotal disc, anterior angles, and posterior margin ranging from a light grayish-ochraceous colour to a deep red shade. The corium exhibited a small transverse spot that was centrally located and relatively smaller than the size of the insect. These observations are consistent with those of Distant (1902), Kapur and Vazirani (1956) and Ahmad and Kamaluddin (1985).

Host plants: Cotton, cucurbits, and other crops (Distant, 1902 and Ahmad & Kamaluddin, 1985).

Distribution in India: Found throughout India - most prominently in the cotton-growing areas of Punjab, Haryana, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh, Karnataka, and Tamil Nadu (Distant, 1902 and Ahmad & Kamaluddin, 1985). This insect pest was collected from pumpkin, bottle gourds, and bitter gourds in Punjab.

Remarks: Primarily, a cotton pest. However, it is occasionally found in cucurbitaceous crops.

Genus *Physopelta* Amyot & Serville, 1843

Physopelta gutta (Burmeister, 1834)

Common name: Red bug

Diagnostic characters: Medium-sized bugs with distinctive red and black colourations. The elongated body was dull reddish-yellow in colour with a pilose texture. Four-segmented antennae. The base of the apical joint of the antennae was yellow. The apical angles of the corium and membrane were black, and the center of the corium was characterized by a black round mark on each side. The rostrum, legs, sternum, abdominal incisures, and three sublateral spots exhibited a dark brownish-gray coloration. The lateral margins of the prosternum, base of the first joint of the antennae, basal angle of the membrane, coxae, trochanters, and femora beneath exhibited a dull reddish-yellow coloration. All abdominal spiracles were dorsally present, which is typical of familial characteristics. These

observations are congruous with those of Distant (1902), Schuh and Slater (1995), Stehlik (2013) and Chandra and Boaz (2018).

Host plants: Several plants, such as cucurbits and other crops (Distant, 1902, 1904 and Schuh & Slater, 1995). Adults were collected from bottle gourds and bitter gourds in Punjab.

Distribution in India: Widely distributed in Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Gujarat, Rajasthan, and the northeastern states (Distant, 1902, 1904).

Remarks: Attacks a wide range of plants, including cucurbitaceous crops.

3.2.8. Family REDUVIIDAE Latreille, 1807

Genus *Zelus* Fabricius, 1803

Zelus spp.

Common name: Assassin bugs

Diagnostic characters: Medium-to large-bodied, elongated predatory bugs. Typically, they were slender. The colour ranged from yellowish-brown to black, with a generally consistent hue. The head was narrowed behind the compound eyes, forming a distinct neck. The ocellus was raised. The three-segmented rostrum was short and curved. The antennae were four-segmented. The front legs were often developed to catch prey. The scape and basiflagellomere were long and subequal, whereas the pedicel and distiflagellomeres were short. The scape was at its maximum thickness. The legs were elongated and slender, with nearly equal femoral diameters. The lengths of the pro- and metafemora were greater than that of those mesofemur. The hemelytron extended beyond the end of the abdomen. The abdomen typically had subparallel lateral margins, with a ventral outline that was usually straight or concave. Wings were well-developed when present. Similar observations have been reported by Ambrose (2003), Triplehorn and Johnson (2005) and Zhang et al. (2016).

Hosts: Prey on several insects that feed on cucurbitaceous and other crops (Ambrose, 2003 and Weirauch & Munro, 2009). Only one adult was collected from a bitter gourd field in Punjab.

Distribution in India: Common throughout India, including all major states, namely Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Maharashtra, Gujarat, and Rajasthan (Distant, 1902, 1904 and Ambrose, 2003).

Remarks: Beneficial predatory species that consume various pest insects in cucurbits. The morphometric

observations (in mm) of all the species or genera described in this study are provided in Table 2.

In the current study, eight families, five genera, and nine species were collected from cucurbitaceous crops in Punjab, India, during the April 2022 to October 2022 growing season and taxonomically identified using available keys. Among the eight Hemipteran families, Pentatomidae was the most predominant, represented by three species and one genus. In the family Lygaeidae, two species were successfully identified, while in the family Pyrrhocoridae, one genus and two species were successfully identified. However, in five families (eg. Aleyrodidae, Coreidae, Geocoridae, Pseudococcidae, and Reduviidae) one species each was identified. Morphometric measurements of the head, thorax and abdomen were recorded from the dorsal side of all 14 adult insects and expressed in mm. Thus, multiple insect pest species and a few beneficial predators inhabiting cucurbitaceous crops form intricate ecological associations. This biodiversity is necessary in sustainable agricultural environments.

3.3. Insect Pest Species

The nine insect species described and depicted in this checklist are key cucurbitaceous crop pests and predators of insects on cucurbits. For instance, *Bemisia tabaci* is one of the most damaging insect pests, known for its direct feeding injuries to crops. Additionally, *B. tabaci* is a known insect vector of a few plant viruses (Martin, 1987). *N. viridula* is a highly polyphagous insect that causes economic damage to various agricultural crops (Ahmad & Kamaluddin, 1985 and Jones, 1988). Vegetable crops, such as brinjal and okra, are attacked by *N. viridula*. They cause damage through sap-sucking, thereby negatively affecting plant growth and reducing fruit development (Bhosale & Kamble, 2022). Recent studies by Prabhakar et al. (2023) have identified *N. viridula* as an emerging insect pest of maize crops in India. The peak activity of *N. viridula* during the critical growth period of the crop results in significant crop yield losses. *C. signatus* appears to be injurious because it sucks the sap of developing fruits (Gupta & Singh, 2013). Owing to the damage to developing seeds and fruits, several seed bugs (e.g., *G. servus*, *S. pandurus*) cause both direct and indirect damage to the seeds and fruits (Chandra et al., 2018).

3.4. Beneficial Species

The two families represented in this checklist contain beneficial predatory species. Common predators of aphids, thrips, and other small insect pests are *Geocoris* spp.

(Family: Geocoridae) (Sweet, 2000; Mead, 2011 and Varshney, 2023). *Zelus* spp. (Family: Reduviidae) have been reported as generalist predators that feed on various insects (Ambrose, 2003; Weirauch & Munro, 2009).

3.5. Biogeographical Significance

The species presented have broad distributions in India, as the majority of the reported species are found across many states (Distant, 1902, 1904; Chandra et al., 2018). This indicates the agricultural significance of cucurbitaceous crops in India, as well as the adaptability of cucurbitaceous crops in different climates in which hemipteran species can survive.

4. CONCLUSION

In this study, nine species of hemipteran insects, belonging to eight families and 11 genera, were collected and identified from various cucurbitaceous crops cultivated in Punjab, India. Among all families, Pentatomidae was the most abundant family, representing four species. In this study, it was observed that the insect community represented both pest species (e.g., silver leaf whitefly (*B. tabaci*), southern green stink bug (*N. viridula*), and beneficial species (e.g., *Geocoris* spp. or *Zelus* spp.). The coexistence of insect pests and beneficial predators suggests the need for an integrated pest management (IPM) program that includes the conservation of biocontrol agents and the management of harmful insect species. Many of the species presented in this study had high distribution values throughout India; therefore, the results of this study have implications for agricultural systems throughout India.

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CRedit authorship contribution statement

Abu Bakar Farooq Babu: Insect Collection and Insect Curation, Writing - Draft Preparation. **Prasad S. Burange:** Conceptualization, Methodology, Insect Collection, Supervision, Writing-Reviewing and Editing.

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Conflict of interest

The authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no AI tool was used in the preparation of this manuscript.

Data availability

The data and insect specimens are available from Prasad S. Burange.

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Population Fluctuation of Bihar Hairy Caterpillar, *Spilarctia obliqua* (Walker) and its Natural Enemies on Castor Under Semi-Arid Conditions of Gujarat, India


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ABSTRACT: Bihar hairy caterpillar, *Spilarctia obliqua* (Walker), is a polyphagous lepidopteran pest, known to cause substantial damage to a wide range of agricultural and horticultural crops. In recent years, its occurrences have become increasingly severe in castor (*Ricinus communis* L.) cultivation in Western India. Therefore, to study the seasonal incidence and damage potential, a study was undertaken on castor (GCH-8) during *Kharif*, 2024-25, at the Centre for Oilseeds Research, S. D. Agricultural University, Sardarkrushinagar. The results revealed that peak larval incidence (43.52 larvae/plant) was observed during the 45th SMW, coinciding with maximum foliar damage (58.44%) during the 44th SMW. The larval parasitoid, *Cotesia ruidus* (Wilkinson), was recorded from natural field infestations in castor. The highest population (1.60 spiders/plant) of spider (adult and spiderling) was recorded at 49th SMW, and the maximum (1.00 coccinellids/plant) coccinellids (adults) were recorded during 44th SMW, and the maximum population of *Chrysoperla* (1.40 *Chrysoperla*/plant) was recorded during 44th SMW. Multiple regression analysis revealed that abiotic factors had a strong influence on pest incidence, particularly larval population ($R^2 = 0.66$) and leaf damage ($R^2 = 0.61$) while, natural enemies showed weak to moderate relationships ($R^2 = 0.18-0.45$), indicating a greater role of abiotic factors.

Keywords: *Spilarctia obliqua*, Castor, Population dynamics, Parasitoid, Natural enemies, Regression

1. INTRODUCTION

An important non-edible oilseed crop of the spurge (Euphorbiaceae) family, castor (*Ricinus communis* L.), is thought to have originated in Ethiopia. Because it does not compete with food crops or food-grade oils, requires only moderate rainfall, and places low demands on soil fertility, it is widely distributed throughout the tropics and subtropics. It also adapts well to the world's temperate climates. Long used as a lubricant, castor oil and its derivatives are widely used in a variety of industries, including the pharmaceutical,

paper, textile, chemical, plastics, grease, hydraulic, brake fluid, paint, varnish, linoleum, plasticisers, soap, cosmetics, and even the electronics and telecommunications sectors. Additionally, it is employed in the production of synthetic leather. Cosmetics and allied items employ it because of its ability to unclog (Sumit, 2013). Due to high nitrogen content (4.5%), it is utilised as organic manure in agriculture. In addition to being used as fuel or thatching material, the stalks of the castor plant are also used to make paper pulp for writing, printing, and wrapping. Castor cake is used as manure once the oil from the seeds has been extracted.

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Along with a few minerals, it contains potassium (1%), phosphoric acid (2.5%), and nitrogen (6.4%) (Tomar et al., 2017). On castor, about 100 insect pests are known to occur, including sucking insects and leaf feeders (Basappa & Lingappa, 2001). Defoliators such as semilooper, *Achaea janata* L.; tobacco caterpillar, *Spodoptera litura* Fab.; capsule borer, *Conogethes punctiferalis* Guen; and Bihar hairy caterpillar, *Spilarctia obliqua* (Walker) are the main pests affecting castor. Additionally, sucking pests such as leaf hoppers, *Empoasca flavescens* Fab., whiteflies, *Trialeurodes ricini* Misra thrips, *Retithrips syriacus* Mayet (Lakshminarayana & Raoof, 2005) and *Scirtothrips dorsalis* Hood (Chaudhary et al., 2023b) attack at various stages of growth. Among them, the Bihar hairy caterpillar, *S. obliqua*, a polyphagous pest, is seriously harming several important crops. In India, it is reported to attack 126 plant species across more than 24 plant families, including several commercially significant plants such as pulses, grains, cereals, and oilseeds (Singh et al., 2004). In recent years, ecological-based pest management strategies have gained increasing importance due to their sustainability and environmental safety. In this context, regular field monitoring forms the backbone of any effective pest management programme, as it helps understand pest incidence, distribution, and population dynamics in the crop. Seasonal incidence studies are particularly useful for planning need-based plant protection strategies, as they indicate the exact peak activity periods and pest-free intervals of major insect pests. The population of insect pests in castor is influenced by various abiotic factors, such as temperature, relative humidity, and rainfall, as well as biotic factors, including natural enemies and other biological control agents. Therefore, the present study was undertaken to assess the effect of abiotic factors on the incidence and population dynamics of insect pests on castor.

2. MATERIALS AND METHODS

2.1. Experimental Site and Season

A dynamics study was carried out during *Kharif*, 2024-25 at Centre for Oilseeds Research, S. D. Agricultural University, Sardarkrushinagar, located at Jorapura Farm in Gujarat, India. The experimental site lies at an elevation of 116.56 meters above sea level, positioned at 24°32' North latitude and 72°28' East longitude. The region experiences a sub-tropical, semi-arid climate, characterised by extremely hot and humid summers. The average annual temperature ranges from 19.2°C to 33.8°C, with an average annual

rainfall of 940 mm (Anonymous, 2025).

2.2. Experiment Details

Castor hybrid GCH-8 was sown during the 2nd week of July, which was raised as per the local package of practices without any insecticidal sprays. The castor hybrid GCH 8 was grown in an area of 180 m² at a spacing of 150 cm × 120 cm.

2.3. Observations Procedure

Observations were recorded on five plants selected at each location in the plot, and from five locations, 25 plants were selected. The meteorological data during the crop period were used to determine the correlation of insect pest population with weather parameters.

2.4 Statistical Analysis

Starting from the larvae's first appearance until the crop was harvested, weekly observations of the *Spilarctia obliqua* larval population and leaf damage percentage were recorded every week. Analysis was carried out using the number of larvae per plant and associated leaf damage. Microsoft Excel (version 2019) was used to organise all data collected during the study period, and IBM SPSS Statistics (version 32) was used for analysis. Pest population (larvae per plant) and leaf damage percentage were the dependent variables, and weather parameters like maximum and minimum temperatures, morning and evening relative humidity, evaporation, rainfall, wind speed, and hours of bright sunshine were the independent variables. The degree and direction of the relationship between the pest population and specific weather variables were ascertained using the correlation coefficient (r). The significance of the correlation coefficient was examined at the 1% and 5% significance levels (Pearson, 1895). The coefficient of determination (R^2) was used to assess the regression model's quality of fit, and the significance of the regression coefficients was examined at the 5% probability level.

3. RESULTS AND DISCUSSION

3.1. Bihar Hairy Caterpillar

The adult female deposited eggs in clusters on the bottom and upper surface of the leaf epidermis. While the gregarious juvenile caterpillars feed on soft, green tissues, giving the appearance of a net or web, the older larvae skeletonise the leaves (Figures 1a and 1b). The data from Figure 2 revealed that the larval population of the Bihar hairy caterpillar

appeared during the third week of September (37th SMW) and persisted until the third week of January (3rd SMW). Initially, its population was low (13.22 larvae/plant), which

gradually increased in its numbers and attained a peak level (43.52 larvae/plant) during the 2nd week of November (45th SMW). Thereafter, larval population steadily decreased



Figure 1. (a) Gregarious larvae feeding on the leaves



Figure 1. (b) Skeletonization of leaf lamina

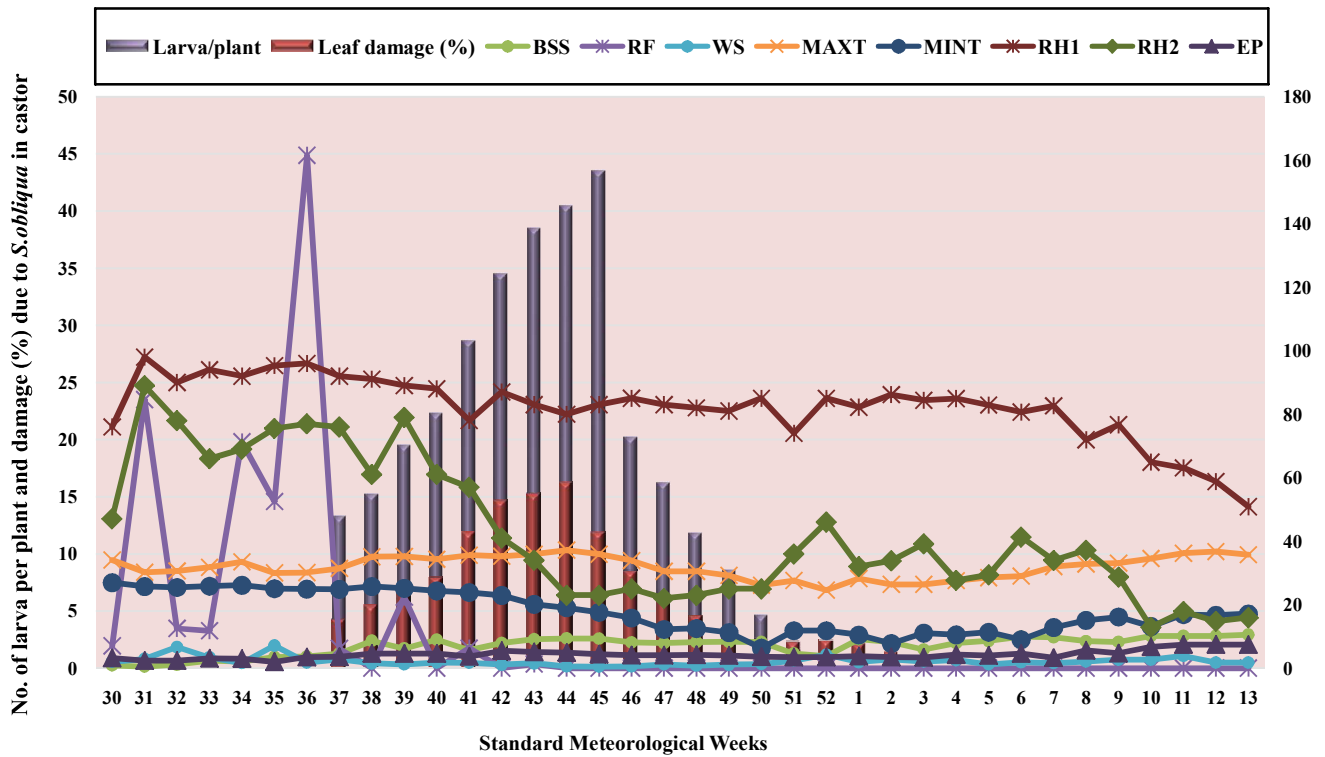


Figure 2. The incidence of *S. obliqua* larvae and corresponding leaf damage in association with prevailing abiotic factors during Kharif 2024

from the 46th SMW (20.14 larvae/plant) to the 3rd SMW (0.24 larvae/plant). During the 39th to 44th SMW, *S. obliqua* larvae were found most active with their maximum larval population. The data on leaf damage (%) were directly associated with the larval population. Damage was observed from the 37th standard meteorological week (3rd week of September) to the 1st standard meteorological week (1st week of January). As the larval population increased, the damage also increased in castor. The leaf damage was 15.22% at the initial appearance, with peak damage (58.44%) recorded during the 44th standard meteorological week (1st week of November), which also coincided with the maximum larval population. The correlation coefficient analysis revealed that the larval population and leaf damage (%) had highly significant positive correlations with maximum temperature ($r=0.553^{**}$, $r=0.475^{**}$, respectively). In contrast, the minimum temperature had a less significant positive correlation ($r=0.285^*$, $r=0.195$). Wind speed ($r=-0.422^*$, $r=-0.455^{**}$) showed a significant negative correlation, while rainfall ($r=-0.182$, $r=-0.226$) showed a negative but non-significant correlation. Other abiotic factors, such as morning relative humidity ($r=0.125$, $r=0.121$), evaporation ($r=0.136$, $r=0.120$), and bright sunshine hours ($r=0.244$, $r=0.255$), showed positive but non-significant correlations with larval population and leaf damage (%). The larval population showed a moderate level of association with weather parameters, with a coefficient of determination (R^2) of 0.66. Among the variables, positive effects were observed for X_1 , X_4 , X_5 , and X_6 , indicating that these factors favoured larval buildup, whereas X_2 , X_3 , X_7 , and X_8 had negative effects. This suggests that specific climatic conditions play a crucial role in regulating larval dynamics. Leaf damage also showed a similar trend, with an R^2 of 0.61, indicating that the selected abiotic factors explained 61% of the variation. Positive contributions from X_4 , X_5 , and X_6 indicated their role in enhancing crop damage, while other variables showed a suppressive effect (Table 3). Similarly, Gaur (2014) observed that the hairy caterpillar appeared on castor during the 35th SMW. Patel and Patel (2015) recorded the abundance of *S. obliqua* on castor. They revealed that the pest was active on the castor crop from the 4th week of September to the 3rd week of November, with the highest activity occurring from the 2nd week of October to the 3rd week of November. Patel et al. (2016) found that the gregarious larval population first appeared on the crop (42.7 larvae/plant) during the 33rd SMW (3rd week of August) and was recorded on the crop till the 42nd SMW (3rd week of October). Further, Chaudhary (2023a) indicated that the

Bihar hairy caterpillar appeared on castor crop during the 40th SMW and remained active up to the 49th SMW. Patel et al. (2024) also observed that the Bihar hairy caterpillar was first recorded on mung beans during the 32nd standard week (1.92 larvae/plant), which reached its maximum during the 41st standard week (11.93 larvae/plant). Present findings on the correlation of weather parameters are closely related to Patel and Patel (2015), who studied the seasonal abundance of *S. obliqua* on castor and showed that there is a highly significant negative correlation with wind speed, minimum temperature, morning and evening relative humidity and morning as well as evening vapour pressure, while a significant positive correlation with maximum temperature. Further, studies by Patel et al. (2016) on the seasonal abundance of *S. obliqua* across various oilseed crops and correlation analysis revealed that minimum temperature had a significant positive association with the number of larvae ($r=0.612^*$). Also, Shivakumara et al. (2024) reported that the maximum temperatures (-0.52 and -0.49) were negatively correlated with per cent pest incidence and larval population. Meena et al. (2024) found that the infestation of *S. obliqua* on black gram was negatively correlated with rainfall ($r=-0.55$) and positively correlated with minimum temperature ($r=0.33$) and morning relative humidity ($r=0.33$).

3.2. Natural Enemies

3.2.1. Spiders

The spider population began in the 2nd week of August (32nd SMW) at 0.20 spiders per plant (Table 1). The data is shown in the Figure 3 revealed that the population steadily increased to a maximum level (1.60 spiders/plant) during the 2nd week of December (49th SMW). Thereafter, the spider population declined (0.52 spiders/plant) in the 5th week of December (52nd SMW). The data on spiders depicted in Table 2 indicated that bright sunshine hours ($r=0.159$) and morning relative humidity ($r=0.124$) showed positive and non-significant correlations. Whereas rainfall, wind speed, maximum temperature, minimum temperature, evening relative humidity, and evaporation ($r=-0.117$, $r=-0.166$, $r=-0.125$, $r=-0.243$, $r=-0.203$, $r=-0.066$) show negative and non-significant correlations with the spider population. In contrast, the spider population exhibited a very low coefficient of determination ($R^2=0.18$), indicating a weak dependence on the weather parameters considered. This suggests that spider abundance may be influenced more by prey availability and ecological interactions rather than abiotic factors alone (Table 3). These studies are in line with Reddy (2002), who reported that the spider was found

predominant in November-sown castor crop among all three sowings at Guntur (Andhra Pradesh). Shambhavi et al. (2023) also recorded the population of spiders on castor crop during the 27th SMW (vegetative stage of the crop) with a

population of 0.20 spiders per plant and maximum activity during the 35th SMW (maturity stage of the crop) with a population of 0.29 spiders per plant. Further, Chaudhary (2023a) noted that the spider population on castor during the

Table 1. Population dynamics of Bihar hairy caterpillar infesting castor during *Kharif* 2024-25

Months	Weeks	SMW	Number of larva(e)/ plant	leaf damage (%) / plant	No. of Spiders /plant	No. of Coccinellids /plant	No. of <i>Chrysoperla</i> /Plant
July, 2024	4	30	0.00	0.00	0.00	0.00	0.00
August, 2024	1	31	0.00	0.00	0.00	0.00	0.00
	2	32	0.00	0.00	0.20	0.00	0.00
	3	33	0.00	0.00	0.20	0.00	0.00
	4	34	0.00	0.00	0.80	0.42	0.20
September, 2024	1	35	0.00	0.00	0.60	0.80	0.40
	2	36	0.00	0.00	0.40	0.40	0.20
	3	37	13.22	15.22	0.20	0.60	0.62
	4	38	15.16	19.62	0.80	0.40	0.80
	5	39	19.54	23.84	0.80	0.82	0.40
October, 2024	1	40	22.32	28.44	0.40	0.80	0.40
	2	41	28.62	42.82	0.80	0.40	0.82
	3	42	34.54	52.64	1.20	0.88	0.86
	4	43	38.42	54.66	1.40	0.80	1.20
November, 2024	1	44	40.42	58.44	1.20	1.00	1.40
	2	45	43.52	52.60	0.64	0.82	1.22
	3	46	20.14	30.42	0.40	0.84	0.82
	4	47	16.20	22.48	0.82	0.20	0.20
December, 2024	1	48	11.82	16.24	1.22	0.40	0.44
	2	49	8.60	10.28	1.60	0.20	0.28
	3	50	4.56	8.24	1.20	0.20	0.24
	4	51	1.58	8.66	1.40	0.42	0.22
	5	52	0.52	7.56	0.52	0.60	0.62
January, 2025	1	1	0.68	4.84	0.32	0.20	0.80
	2	2	0.42	0.00	0.36	0.20	0.20
	3	3	0.24	0.00	0.20	0.00	0.20
	4	4	0.00	0.00	0.40	0.00	0.20
February, 2025	1	5	0.00	0.00	0.20	0.00	0.00
	2	6	0.00	0.00	0.20	0.00	0.00
	3	7	0.00	0.00	0.40	0.00	0.00
	4	8	0.00	0.00	0.80	0.00	0.00
March, 2025	1	9	0.00	0.00	0.60	0.00	0.00
	2	10	0.00	0.00	0.60	0.00	0.00
	3	11	0.00	0.00	0.00	0.00	0.00
	4	12	0.00	0.00	0.00	0.00	0.00
	5	13	0.00	0.00	0.00	0.00	0.00

SMW: Standard Meteorological Week

38th SMW remained active up to the 12th SMW. Its population ranged from 0.05 to 1.80 adults per plant, with the highest observed during the 4th SMW (1.80 adults/plant), indicating its activity from September to March. There was a slight variation in the population occurrence of spiders due to environmental conditions, as well as variation in the pest population.

3.2.2. Coccinellids

The data presented on the incidence of coccinellids in Table 1 and graphically depicted in Figure 3 revealed that the adult population of coccinellids ranged between 0.20 and 1.00 per plant. Coccinellids were found active on castor from the 4th week of August to the 3rd week of January (34th to 3rd SMW), with 0.42 coccinellids per plant. The population increased gradually and reached a maximum level of 1.00 coccinellids per plant during the 1st week of November (44th SMW) and thereafter declined and reached a lowest level (0.20 coccinellids/plant) during the 2nd week of January (2nd SMW). The data on coccinellids presented in Table 2 revealed that morning relative humidity (r = 0.368*) showed a positive and significant correlation. Maximum temperature (r = 0.237), minimum temperature (r = 0.293),

bright sunshine hours (r = 0.004), rainfall (r = 0.052), and evening relative humidity (r = 0.193) showed positive and non-significant correlations with coccinellids. Wind speed (r = -0.183) and evaporation (r = -0.129) showed negative but non-significant correlations with the coccinellid population. Coccinellids showed a moderate relationship (R² = 0.37), with a slight positive influence from X₁ and X₆, while most other variables had negligible or negative effects. This indicated limited but notable climatic influence on their population (Table 3). The study conducted by Chaudhary (2023a) revealed that ladybird beetles on castor appeared during the 41st SMW and remained active up to the 11th SMW. Its population ranged from 0.05 to 1.40 adults per plant, with the highest observed during the 10th SMW (1.40 adults/plant).

3.2.3. Green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen)

The green lacewing population began in the 4th week of August (34th SMW), with 0.20 *Chrysoperla* per plant (Table 1 and Figure 3). The population steadily increased and reached a maximum level (1.40 *Chrysoperla* per plant during the 1st week of November (44th SMW). Thereafter, a

Table 2. Correlation between larval population and natural enemies with weather parameters during *Kharif* 2024-25

Weather parameters	No. of <i>S. obliqua</i> larvae /plant	Leaf damage (%)	No. of spiders/plant	No. of Coccinellids/plant	No. of <i>Chrysoperla</i> /plant
Maximum temperature (Max. T) °C	0.553**	0.475**	-0.125	0.237	0.256
Minimum temperature (Min. T) °C	0.285*	0.195	-0.243	0.293	0.107
Morning relative humidity (M. RH) %	0.125	0.121	0.124	0.368*	0.256
Evening relative humidity (E. RH) %	-0.029	-0.097	-0.203	0.193	-0.049
Evaporation (EP) mm	0.136	0.120	-0.066	-0.129	-0.039
Rainfall (RF) mm	-0.182	-0.226	-0.117	0.052	-0.154
Wind speed (WS) kmhr ⁻¹	-0.422*	-0.455**	-0.166	-0.183	-0.315
Bright sunshine (hours/days)	0.244	0.255	0.159	0.004	0.200

* Significance at 5 per cent level of significance (‘r’= 0.330); ** Significance at 1 % level of significance (‘r’=0.424) n=36 week

Table 3. Multiple regression equation of *S. obliqua* with leaf damage and natural enemies of castor with various environmental factors infesting castor

Pest and natural enemies	Multiple regression equation	Coefficient of determination R ²
Larvae	Y = - 139.36+0.28X ₁ -0.112X ₂ -2.11X ₃ +2.58X ₄ +0.09X ₅ +0.95X ₆ -0.19X ₇ -0.52X ₈	0.66
Leaf damage	Y = - 114.74-0.38X ₁ -0.19X ₂ -3.92X ₃ +2.11X ₄ +1.10X ₅ +0.97X ₆ -0.43X ₇ -1.60X ₈	0.61
Spiders	Y = - 0.497-0.03X ₁ -0.00X ₂ -0.01X ₃ +0.00X ₄ +0.00X ₅ +0.02X ₆ -0.01X ₇ -0.01X ₈	0.18
Coccinellids	Y = - 1.251+0.02X ₁ -0.00X ₂ -0.03X ₃ -0.00X ₄ +0.00X ₅ +0.01X ₆ -0.00X ₇ -0.03X ₈	0.37
<i>Chrysoperla</i>	Y = - 2.246+0.03X ₁ -0.00X ₂ -0.03X ₃ +0.01X ₄ +0.03X ₅ +0.02X ₆ -0.01X ₇ -0.06X ₈	0.45

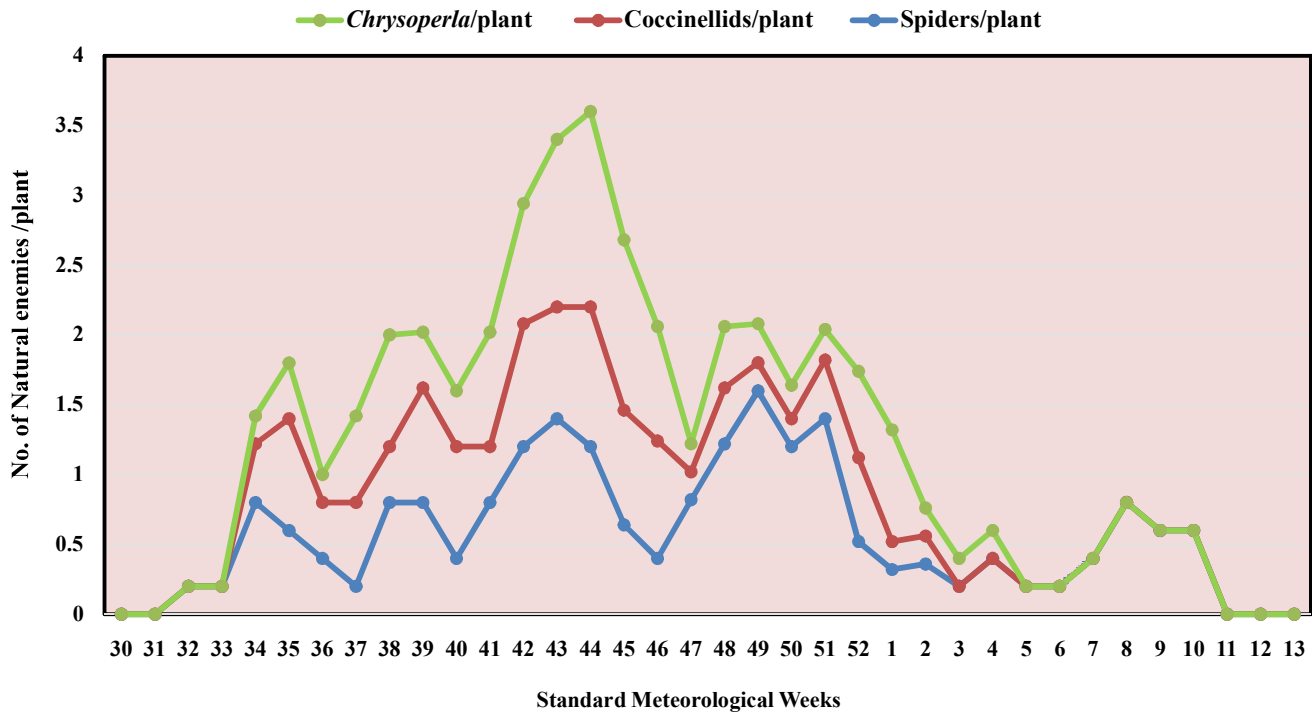


Figure 3. The abundance of natural enemies (spiders, coccinellids, and *Chrysoperla*) during various SMW on castor during kharif 2024

low population of *Chrysoperla* was noticed and declined (0.20 *Chrysoperla* /plant) by the 4th week of January (4th SMW). The data on *Chrysoperla* (Table 2) indicated that maximum temperature ($r = 0.256$), minimum temperature ($r = 0.107$), morning relative humidity ($r = 0.256$), and bright sunshine hours ($r = 0.200$) showed positive but non-significant correlations. Rainfall ($r = -0.154$), wind speed ($r = -0.315$), evening relative humidity ($r = -0.049$), and evaporation ($r = -0.039$) had negative and non-significant correlations with the *Chrysoperla* population (Table 3). Similarly, the *Chrysoperla* population showed a moderate association ($R^2 = 0.45$), indicating that abiotic factors accounted for nearly 45% of the population's variation. Positive contributions from X_1 , X_4 , X_5 , and X_6 indicate favourable conditions for their activity, while negative coefficients for X_3 , X_7 , and X_8 suggest adverse effects. Similarly, Reddy (2002) reported that *Chrysoperla* sp. was predominant in the November-sown castor crop among the three sowings at Guntur (Andhra Pradesh). Further, Chaudhary (2023a) observed that *Chrysoperla* appeared on the castor crop during the 39th SMW and remained active up to the 12th SMW. Its population ranged from 0.43 to 1.90 adults per plant, with the highest observed during the 44th SMW (1.90 adults/plant), and its activity occurs from

September to March.

Overall, the results indicated that abiotic factors had a stronger influence on pest populations (larvae and leaf damage) compared to natural enemies. Natural enemy populations were less dependent on weather variables and may be more regulated by biotic factors such as prey density and habitat conditions. These findings are consistent with earlier studies that reported that pest incidence is more directly influenced by climatic factors, whereas natural enemies respond to both climatic and ecological cues.

4. CONCLUSION

The study confirms that *Spilarctia obliqua* is a major defoliator of castor, with its incidence closely regulated by seasonal climatic conditions. Temperature emerged as a key driver of pest buildup, while rainfall and wind exerted a suppressive effect, underscoring the dominant role of abiotic factors in shaping pest dynamics. The occurrence of natural enemies, including spiders, coccinellids, and *Chrysoperla zastrowi sillemi*, highlights their ecological significance, with their abundance largely influenced by prey availability and, to a lesser extent, by weather variables. Overall, the findings emphasise the importance of integrating weather-based pest monitoring with biological control components

for developing reliable forecasting systems. This approach can support timely, eco-friendly integrated pest management strategies for the sustainable management of *S. obliqua* in castor. Further studies over multiple seasons are required to confirm the consistency of the observed seasonal incidence patterns of the Bihar hairy caterpillar on castor.

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Parmar Dhruv N. Performed the experiments; drafted the manuscript. **Chandaragi MK:** Designed the research, revised the manuscript. **Koosi Sai Thilak:** Reviewing and editing

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The authors have no conflicts of interest to declare.

Data availability statement

The data supporting the findings of this study are available within the article.

Statement on generative AI and AI-assisted technologies in the writing process

The authors declare that no artificial intelligence tools were used to write this manuscript.

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