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Temporal Patterns of Leopard Predation on Livestock in Hamirpur District, Himachal Pradesh, India

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ABSTRACT: Livestock depredation by leopards is widely reported across the Indian Himalayan region. This study investigated long-term trends in leopard-induced livestock depredation over 23 years (2001–2023) in Hamirpur District, Himachal Pradesh, a representative Shivalik Himalayan landscape. To our knowledge, this is one of the longest continuous assessments of leopard–livestock conflict in the Shivalik Himalayas. Data were obtained from official Forest Department compensation records and therefore represent reported depredation incidents rather than absolute predation rates. Mann–Kendall trend analysis revealed a significant decline in annual livestock mortality ($Z = -2.01$, $p = 0.045$), although occasional peaks occurred. A total of 1,420 livestock mortalities were recorded, comprising 1,195 (84.15%) small livestock and 225 (15.85%) large livestock. Among 1,079 reported depredation incidents, 744 (68.95%) occurred in animal sheds, 272 (25.21%) in private land/pasture areas, and 63 (5.84%) in forests. Seasonal mortalities were highest during summer (32.46%), followed by monsoon (26.41%), autumn (20.77%), and winter (20.35%). Hamirpur Range accounted for 73.73% of recorded livestock mortalities, followed by Aghar (12.96%), Barsar (8.31%), Nadaun (3.10%), and Bijhari (1.90%). Livestock predation was significantly associated with prey size, season, attack location, and forest range. The predominance of attacks in animal sheds highlights the importance of livestock husbandry practices in shaping human–leopard conflict. Predator-resistant enclosures, improved nighttime confinement, community-based mitigation measures, and continued long-term monitoring are recommended to reduce livestock losses and support coexistence between rural communities and leopards in the Shivalik Himalayan region.

Keywords: Hamirpur, Leopard, Conflict, Depredation, Livestock, Himalayan, Sustainability.

1. INTRODUCTION

Predation by leopards (*Panthera pardus*) on livestock is a complicated convergence of social, economic, and ecological issues, especially in areas where human settlements and leopard habitats are closely located (Dhakal et al., 2023). As an adaptable apex predator, the leopard frequently encounters human conflict because of the

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perceived or actual harm it poses to livestock. This results in retaliatory killings and further jeopardizes leopard populations (Carter et al., 2014; Cleave et al., 2018; Kumar et al., 2017; Kichloo et al., 2024). Human activities such as grazing cattle and the collection of non-timber forest produce in forests and protected areas result in overlapping use of space with leopards, increasing the likelihood of leopard attacks (Gurung et al., 2008; Shivakumar et al., 2023). The proximity of humans to protected areas is also one of the factors contributing to the increase in wildlife attacks on livestock in recent years (Sijapati et al., 2021).

The leopard's diet is highly diversified, and it is extremely adapted to various ecological conditions. Due to their adaptability to rapidly changing habitats, leopards can readily coexist with human settlements by preying on livestock and stray animals (Daniel, 1996; Hayward et al., 2006; Kumar et al., 2017., Raza et al., 2024). Decline of the prey base in forest areas compels leopards to search for prey near livestock sheds and other human habitations, where domesticated animals such as cows, sheep, goats, and other domestic species become easy targets (Kala et al., 2013). Common leopards' predation of livestock fluctuates with the seasons (Bhardwaj et al., 2024).

The leopard population in Himachal Pradesh is reported to be 510, according to the 2024 estimate by ZSI. In recent years, there has been an increase in human–wildlife conflict in Himachal Pradesh due to several factors, mainly urbanization, expansion of roads, industrialization, and housing development to meet the needs of a growing population (Kumar et al., 2017; Thakur et al., 2024). Increasing interactions between people and wildlife have created management challenges and have multiple implications, ranging from fear of wildlife to severe attacks on humans. Livestock depredation by different carnivores in their habitat and in unprotected forests has increased in India, causing economic loss to farmers, which may contribute to the rise in human–carnivore conflict (Woodroffe et al., 2007; Qamar et al., 2010; Ramesh et al., 2020; Treves & Karanth, 2003). The thriving and sustainability of leopard populations have also been associated with the availability of domestic livestock (Athreya & Belsare, 2007).

Human–wildlife interactions are increasingly common in landscapes where people and wildlife share space. While coexistence can provide ecological and social benefits, negative interactions such as livestock depredation may occur when the needs of humans and wildlife overlap, creating challenges for both conservation and local livelihoods (Treves & Karanth, 2003; Carter et al., 2014; Ripple et al., 2014).

Livestock depredation by leopards can impose substantial economic costs on rural households and may contribute to negative perceptions towards wildlife conservation. Such losses are particularly important in mountain farming systems where livestock constitute a major component of household livelihoods. Understanding the factors influencing leopard predation on livestock is important for developing effective conflict mitigation strategies and promoting human–leopard coexistence. According to Greyling et al. (2023), the main causes of leopard predation on livestock include habitat degradation and fragmentation, prey depletion, and poor livestock management practices. Habitat degradation forces leopards to hunt outside their usual habitats, and they frequently enter livestock enclosures (McManus et al., 2022). Decline in prey numbers in protected areas forces leopards to use livestock as a substitute food source (Davis et al., 2021). Inefficient livestock management practices, such as leaving livestock to roam unsupervised or using poorly constructed enclosures, further increase vulnerability to predation (Puls et al., 2021). Moreover, a gap between scientific research on sustainable practices and animal management methods exacerbates the problem (Pozo et al., 2021).

Although human–leopard conflict has been documented in Himachal Pradesh, systematic long-term assessments of livestock depredation patterns spanning more than two decades remain scarce in the Shivalik Himalayan region. The objectives of the present study were to (i) quantify long-term temporal patterns of leopard depredation on livestock during 2001–2023, (ii) assess variation in depredation among seasons, livestock categories, attack locations, and forest ranges, and (iii) identify management implications for reducing human–leopard conflict in Hamirpur District of Himachal Pradesh.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in the Hamirpur District of Himachal Pradesh, located in the lower west-central outer Himalayas of the Shivalik Hills, at elevations ranging from 400 m to 1,232 m above sea level. The western Hamirpur area covers the entire Barsar region, including. In contrast, parts of southern Nadaun tehsil, while the eastern Hamirpur area includes the entire Bhoranj tehsil area of the Shivalik Hills. The geographical area of the study site is 1,118 km².

The boundary of Hamirpur District coincides with Hamirpur Forest Division, which is further divided into five forest ranges (Hamirpur, Aghar, Barsar, Bijhari, and Nadaun). The geographical location of Hamirpur District and the forest ranges included in the study are shown in Figure 1.

The study was based on secondary data obtained from official livestock depredation compensation records maintained by the Forest Department, Hamirpur Forest Division, Himachal Pradesh, for the period 2001–2023. These records document verified livestock depredation incidents for which compensation claims were processed. The dataset was derived from official compensation records submitted by livestock owners and maintained by the Forest Department. Such records may underestimate actual livestock depredation because some incidents may remain unreported, although the magnitude of this reporting bias could not be quantified from the available data. As with all compensation-based datasets, the possibility of reporting bias cannot be excluded. Some depredation events may remain unreported if livestock owners do not submit compensation claims, whereas reporting rates may vary over time due to differences in awareness, accessibility of compensation mechanisms, or administrative procedures. Consequently, the dataset represents reported depredation incidents rather than absolute predation rates. However, because the same administrative reporting system was used throughout the study period, the records provide a valuable basis for examining long-term temporal patterns in reported livestock depredation.

2.2. Study Period

The study covered a period of 23 years (2001–2023). A depredation incident was defined as a reported leopard attack resulting in the loss of one or more livestock animals. For seasonal analysis, livestock depredation records were classified into four seasons based on the regional climatic pattern of Himachal Pradesh: winter (December–February), summer (March–June), monsoon (July–September), and autumn (October–November). Because these seasonal categories differ in duration, seasonal comparisons based on

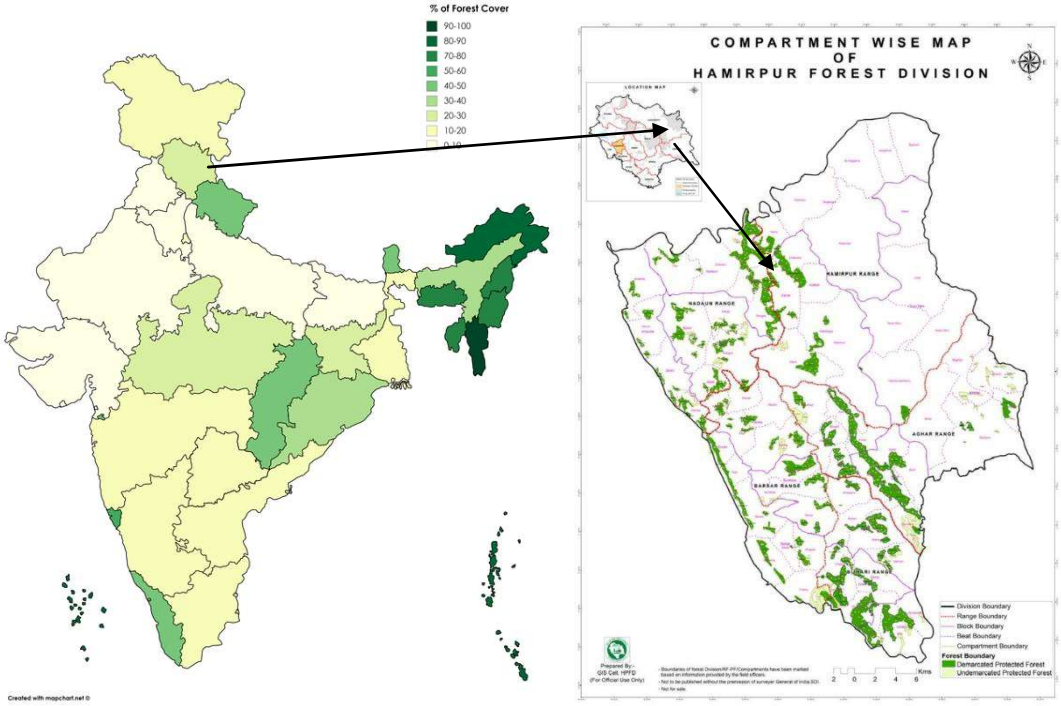


Figure 1. Map showing the location of Hamirpur District and the five forest ranges included in the study in Himachal Pradesh, India. The study area comprises the Hamirpur, Aghar, Barsar, Nadaun, and Bijhari forest ranges, which together cover the entire forest administrative jurisdiction of Hamirpur District. These ranges provided the spatial framework for analysing 23 years (2001–2023) of officially reported leopard-induced livestock depredation incidents based on Forest Department compensation records

total counts should be interpreted with caution. For analytical purposes, livestock were classified into two categories. Small livestock included goats and sheep, whereas large livestock included cattle, buffaloes, oxen, buffalo calves, horses, and mules. The classification was used to examine differences in depredation patterns between relatively small-bodied and large-bodied domestic animals commonly reared in the study area.

Forest range-wise distribution of incidents was also recorded. Descriptive statistics, including annual frequency counts, total livestock mortalities, proportional distributions (%) across livestock categories, attack locations, seasons, and forest ranges, and kill-per-incident ratios, were calculated to summarize livestock depredation patterns during the study period.

2.3. Statistical Analysis

Descriptive statistics were used to summarize livestock depredation patterns across seasons, livestock categories, attack locations, and forest ranges. Chi-square (χ^2) tests of independence were used to examine associations between depredation frequency and season, livestock category, attack location, and forest range. Statistical significance was assessed at $\alpha = 0.05$. All expected cell frequencies exceeded five, satisfying assumptions for chi-square analysis.

To evaluate long-term temporal trends in livestock mortality, a non-parametric Mann–Kendall trend test was performed using annual livestock mortality data from 2001 to 2023. The Mann–Kendall test statistic (τ) and associated p-value were used to assess statistical significance, while Sen's slope estimator quantified the magnitude and direction of temporal change (Mann, 1945; Kendall, 1975; Sen, 1968). The Mann–Kendall test was selected because it is widely used for ecological time-series data and does not require assumptions of normality.

As the dataset was derived from official compensation records, analyses reflect reported depredation incidents rather than absolute predation rates. All statistical analyses were performed using R software (version 4.5.1).

3. RESULTS AND DISCUSSION

3.1. Temporal Trends in Livestock Depredation

A total of 1,079 reported depredation incidents resulted in 1,420 livestock mortalities during the study period (2001–2023), corresponding to an average kill-per-incident ratio of 1.32 livestock per depredation event. Annual livestock mortality varied considerably among years, ranging from 10 animals in 2004 to 158 animals in 2001 (Figure 2).

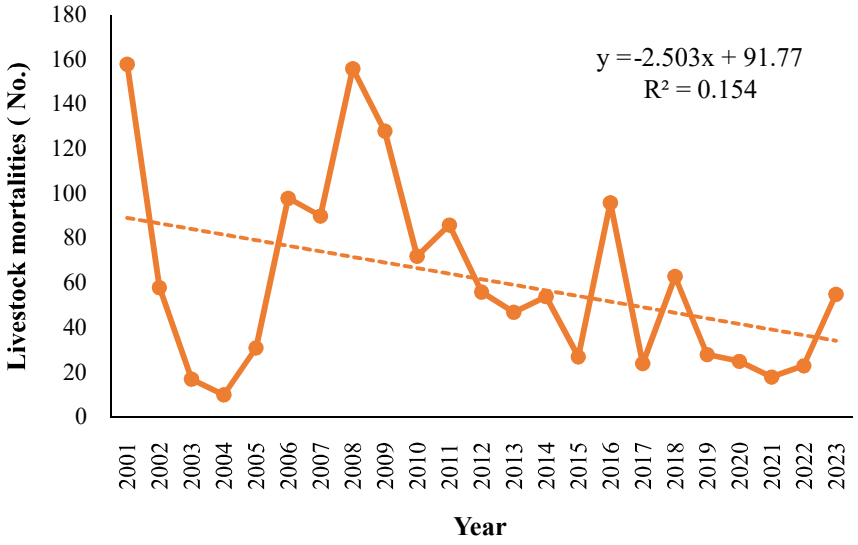


Figure 2. Annual livestock mortalities resulting from leopard depredation in Hamirpur District, Himachal Pradesh, during 2001–2023. The figure illustrates year-to-year variation in reported livestock mortalities based on official Forest Department compensation records. Mann–Kendall trend analysis indicated a significant declining trend in annual livestock mortality over the study period

Mann–Kendall trend analysis revealed a significant declining trend in annual livestock mortality during the study period ($\tau = -0.40$, $p = 0.045$). Sen's slope estimator indicated an average decline of approximately 2.92 livestock mortalities per year. Although periodic increases were observed in certain years, particularly during 2008, 2009, and 2016, the overall long-term trend was significantly negative.

3.2. Seasonal Variation in Livestock Depredation

Seasonal variation in livestock mortality due to leopard depredation was significant ($\chi^2 = 164.63$, $df = 3$, $p < 0.01$). Of the 1,420 livestock mortalities recorded during the study period, 461 (32.46%) occurred during summer, followed by 375 (26.41%) during the monsoon season, 295 (20.77%) during autumn, and 289 (20.35%) during winter (Figure 3). The higher number of reported livestock mortalities during summer and monsoon may reflect increased exposure of livestock to leopard predation during these periods.

Seasonal comparisons should be interpreted cautiously because seasonal categories differed in duration (summer = 4 months, monsoon = 3 months, autumn = 2 months, and winter = 3 months). Nevertheless, significant seasonal variation in reported livestock mortality was evident across the study period.

Livestock depredation differed significantly between livestock categories ($\chi^2 = 373.51$, $df = 1$, $p < 0.01$). Of the total 1,420 livestock mortalities recorded during the study period, 1,195 (84.15%) involved small livestock, whereas 225 (15.85%) involved large livestock (Figure 4).

3.4. Site-wise Distribution of Depredation Incidents

A total of 1,079 reported depredation incidents were analyzed according to attack location. Most incidents occurred in livestock sheds (744; 68.95%), followed by grassland/grazing areas (272; 25.21%), whereas forest areas accounted for the lowest proportion of incidents (63; 5.84%) (Figure 5). Depredation frequency differed significantly among attack locations ($\chi^2 = 351.47$, $df = 2$, $p < 0.01$).

3.5. Range-wise Distribution of Livestock Depredation

Range-wise analysis revealed significant variation in livestock mortality among the forest ranges of Hamirpur Division ($\chi^2 = 915.29$, $df = 4$, $p < 0.01$). Of the 1,420 livestock mortalities recorded during 2001–2023, the highest proportion occurred in Hamirpur Range (1,047;

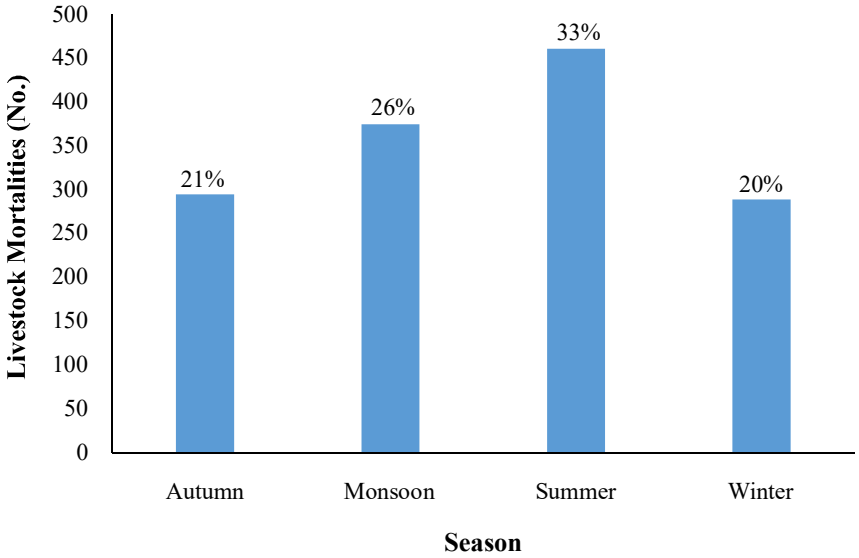


Figure 3. Seasonal distribution of livestock mortalities caused by leopard depredation in Hamirpur District, Himachal Pradesh, during 2001–2023. Bars represent the total number of livestock mortalities recorded in each season from official Forest Department compensation records, with percentages indicating each season's contribution to the overall total. The figure shows that reported livestock mortalities were highest in summer and lowest in winter

73.73%), followed by Aghar Range (184; 12.96%), Barsar Range (118; 8.31%), Nadaun Range (44; 3.10%), and Bijhari Range (27; 1.90%) (Figure 6).

3.6. Temporal Trends in Livestock Depredation

The present study revealed a significant declining trend in livestock mortality caused by leopard depredation during 2001–2023. Mann–Kendall trend analysis confirmed a statistically significant decrease in annual livestock mortality ($Z = -2.01$, $p = 0.045$), although occasional fluctuations were observed in certain years. Notably, elevated livestock mortality was recorded in 2016 and 2018; however, the available compensation records did not contain ecological, climatic, or management-related information that could explain these short-term increases. Consequently, the causes of these fluctuations could not be formally evaluated and no causal inferences can be drawn from the present dataset. Such temporal variability may be influenced by a combination of ecological and socio-economic factors, including changes in livestock management practices, reporting behaviour, local prey availability, and leopard movement patterns. However, these factors were not directly measured in the present study, and therefore their relative contribution to the observed decline in livestock mortality could not be formally assessed.

Despite the significant declining trend, livestock depredation remained a recurring source of human–leopard conflict throughout the study period. Moreover, substantial spatial variation was observed among forest ranges, with Hamirpur Range accounting for the majority of reported livestock mortalities. The present dataset does not permit identification of the specific drivers responsible for the observed decline, and it therefore remains unclear whether the trend reflects improved livestock management, mitigation efforts, changes in reporting behaviour, ecological factors, or a combination of these influences. Continued monitoring and research are therefore necessary to better understand the drivers of depredation and to support evidence-based conflict mitigation in the region.

3.7. Seasonal Patterns of Depredation

Seasonal variation in livestock depredation was significant, with the highest proportion of livestock mortality recorded during the summer season. Comparable seasonal patterns have been reported from other Himalayan and South Asian landscapes where livestock grazing practices influence predator–livestock encounters (Lamichhane et al., 2023; Kshettry et al., 2017). The increased incidence during summer may be associated with seasonal livestock management practices. Owing to reduced fodder availability

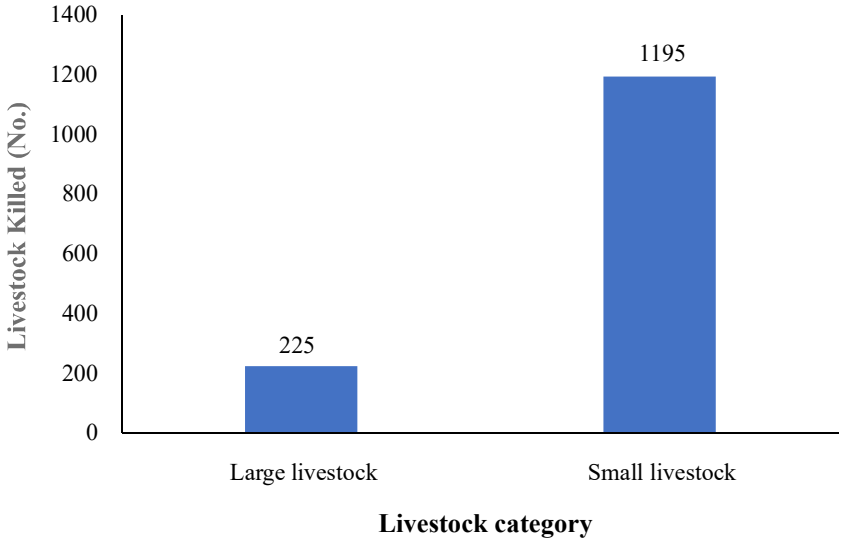


Figure 4. Distribution of livestock mortalities by livestock category in Hamirpur District, Himachal Pradesh, during 2001–2023. Bars show the total number of reported livestock mortalities in each livestock category, and percentages indicate their contribution to the overall total. Small livestock accounted for a substantially higher proportion of reported mortalities than large livestock

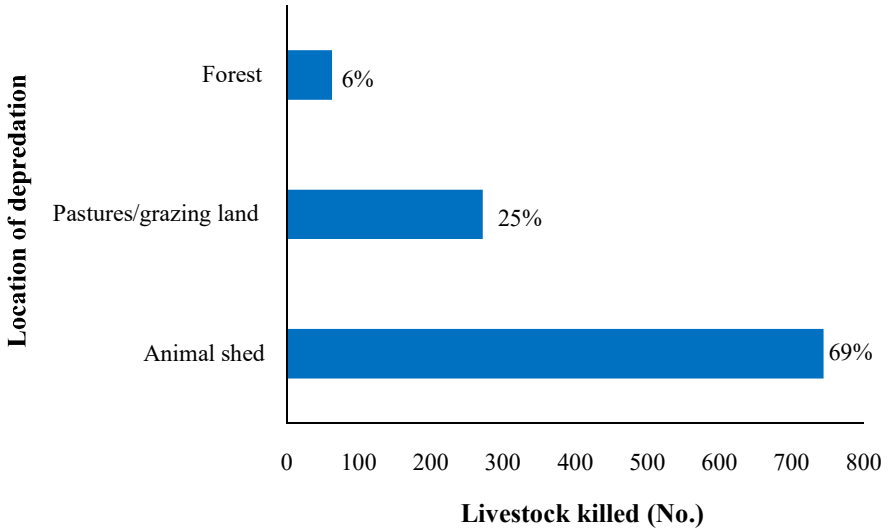


Figure 5. Distribution of reported leopard depredation incidents across different attack locations in Hamirpur District, Himachal Pradesh, during 2001–2023. Bars represent the total number of reported depredation incidents recorded in animal sheds, pastures/grazing land, and forest areas based on official Forest Department compensation records, with percentages indicating each location's contribution to the overall total. The figure shows that most reported depredation incidents occurred in animal sheds, whereas relatively few were reported from forest areas

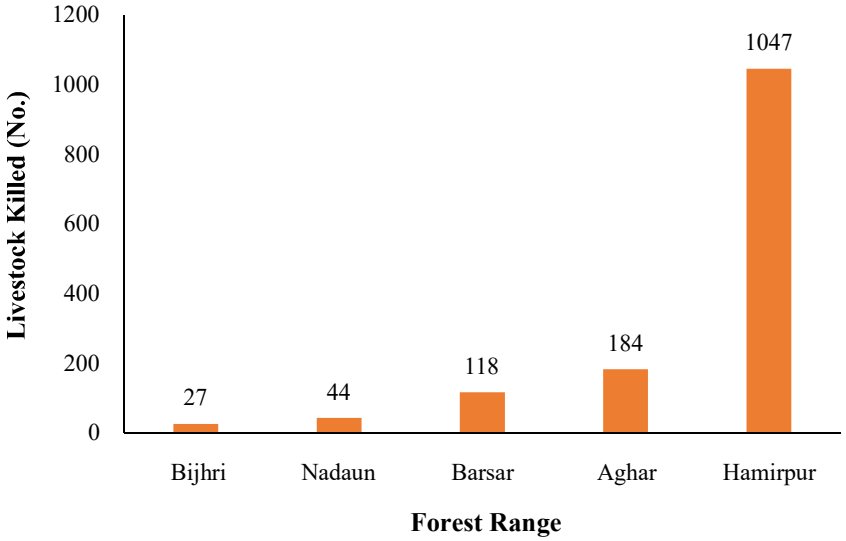


Figure 6. Forest range-wise distribution of livestock mortalities attributed to leopard depredation in Hamirpur Forest Division, Himachal Pradesh, during 2001–2023. Bars show the total number of reported livestock mortalities in each forest range, and percentages indicate their contribution to the overall total. Reported livestock mortalities were concentrated in Hamirpur Forest Range, with considerably lower numbers in the remaining four forest ranges

around settlements, livestock are often grazed in nearby grasslands and forest-adjacent areas, thereby increasing the likelihood of encounters with leopards. In some instances, leopards may also follow livestock movements and attack animals near livestock enclosures.

However, grazing intensity, fodder availability, and leopard movement patterns were not quantified in the present study. Therefore, these explanations should be considered as plausible interpretations rather than confirmed causal mechanisms.

3.8. Livestock Category Preferences

A substantial majority of livestock mortalities recorded during the study period involved small livestock, particularly goats and sheep. This pattern is consistent with the general predatory behaviour of leopards, which often prefer smaller prey because they are easier to subdue, transport, and consume while minimizing the risk of injury. Similar observations have been reported from several Himalayan and South Asian landscapes, where goats and sheep frequently constitute the most vulnerable livestock categories owing to their smaller body size and ease of capture (Odden et al., 2002; Athreya et al., 2013).

The predominance of small livestock depredation may also reflect local livestock husbandry practices, as goats and sheep are frequently grazed in open areas and may be housed in comparatively less secure enclosures. However, the present dataset did not include information on livestock abundance or management practices; therefore, these explanations should be interpreted cautiously.

3.9. Attack Location Patterns

One of the most notable findings of the present study was that nearly 69% of reported depredation incidents occurred in animal sheds. This suggests that livestock enclosures represent an important interface for human–leopard interactions in the study area. Livestock confined within poorly protected sheds may be particularly vulnerable to nocturnal leopard attacks, especially where enclosures are located close to forest fragments, agricultural fields, or natural vegetation cover. Poorly constructed livestock sheds, inadequate night-time security, and the proximity of enclosures to forest fragments or dense vegetation may further increase vulnerability to leopard attacks. In rural Himalayan landscapes, livestock are often housed in traditional structures that may not effectively prevent predator entry. Similar observations have been reported from other human-dominated landscapes, where poorly protected night-time enclosures increase livestock vulnerability to carnivore attacks (Inskip and Zimmermann, 2009; Athreya et al., 2013). The concentration of attacks in animal sheds suggests that livestock protection measures implemented at the household level could substantially reduce depredation risk. This finding highlights the importance of prioritizing predator-resistant housing as a key component of conflict mitigation programmes.

Table 1. Chi-square analysis of livestock depredation according to season, livestock category, attack location, and forest range

Variable	Category	Number (n)	χ^2	d.f	p-value
Season	Winter	289			
	Summer	461			
	Monsoon	375	164.63	3	<0.001
	Autumn	295			
Livestock category	Small livestock	1195			
	Large livestock	225	373.51	1	<0.001
Attack location	Animal shed	744			
	Pasture/Grazing land	272	351.47	2	<0.001
	Forest	63			
Forest range	Hamirpur	1047			
	Aghar	184			
	Barsar	118			
	Nadaun	44	915.29	4	<0.001
	Bijhari	27			

In contrast, depredation incidents in grazing areas were comparatively low, possibly because livestock are often accompanied by shepherds, guard dogs, or farmers during grazing. Similar observations have been reported from other leopard-inhabited landscapes, where livestock protected by human supervision and guarding animals experience lower predation risk than those housed in poorly secured enclosures. The predominance of shed-based attacks highlights the importance of improving livestock housing through predator-resistant enclosures, secure doors, reinforced fencing, and enhanced night-time protection measures.

3.10. Range-wise Variation

Range-wise analysis revealed that the majority of livestock mortalities occurred in Hamirpur Range. Anthropogenic landscape modification may play an important role in shaping human–leopard interactions in the study area. Fragmented forest patches interspersed with agricultural fields and expanding rural settlements could increase spatial overlap between livestock shelters and leopard movement areas. Such habitat configurations may facilitate opportunistic predation, particularly where livestock enclosures are located near forest edges. Human–carnivore interactions are often intensified in fragmented landscapes where wildlife habitats, agricultural land, and human settlements occur in close proximity, thereby increasing opportunities for livestock depredation (Carter et al., 2014).

Leopards are known for their behavioural plasticity and adaptability in human-dominated landscapes (Cleave et al., 2018; Shivakumar et al., 2023), which may enable them to utilize livestock as an alternative prey source under certain conditions. However, habitat characteristics, prey abundance, and leopard movement patterns were not directly measured in the present study; therefore, these explanations should be considered as plausible interpretations rather than confirmed causal mechanisms.

From an economic perspective, even small-scale livestock losses can have disproportionate impacts on rural household income, thereby intensifying negative perceptions towards wildlife conservation. Addressing human–leopard conflict, therefore, requires integrated management strategies that consider ecological, landscape, and socioeconomic dimensions.

3.11. Management Implications

The findings of the present study have important implications for conflict mitigation and livestock protection in the region. Since nearly 69% of reported depredation incidents occurred in animal sheds, improving the design and security of livestock enclosures should be a priority management intervention. Predator-resistant sheds, reinforced doors, secure fencing, and improved night-time livestock confinement may substantially reduce livestock vulnerability to leopard attacks.

Additional measures such as community awareness programmes, improved livestock-guarding practices, timely compensation mechanisms, and targeted monitoring in conflict-prone areas may further help reduce human–leopard conflict. Capacity-building programmes for livestock owners focusing on predator-safe livestock management, enclosure reinforcement, recognition of leopard presence, and improved livestock guarding practices may further reduce livestock vulnerability. Successful implementation of conflict mitigation measures will require active participation of local communities, sustained institutional support, and adoption of practical livestock protection measures at the household level. Adoption of site-specific and evidence-based mitigation strategies will be essential for promoting coexistence between local communities and leopards in the Himalayan landscape

3.12. Study Limitations and Future Research

The present study was based on official compensation records maintained by the Forest Department. Although these records provide a standardized and long-term source of information on livestock depredation, they may be subject to reporting bias. Under-reporting may occur when livestock owners do not file compensation claims, whereas minor depredation events may remain undocumented. Administrative inconsistencies or misclassification of incidents also cannot be entirely ruled out. Consequently, the findings reflect patterns in reported depredation incidents rather than absolute predation rates.

The dataset did not include independent estimates of leopard population density, wild prey abundance, habitat change, climatic variables, land-use change, or other ecological covariates. Therefore, causal relationships between ecological factors and depredation patterns could not be formally evaluated. Similarly, temporal variation in reported incidents may partly reflect differences in reporting efficiency, awareness, and accessibility of compensation mechanisms.

Future studies integrating ecological monitoring, prey-base assessments, spatial habitat analyses, and advanced

statistical modelling would improve understanding of the drivers of livestock depredation and support more effective conflict mitigation strategies.

4. CONCLUSION

The present study provides a comprehensive assessment of leopard-induced livestock depredation in Hamirpur District, Himachal Pradesh, based on 23 years of official compensation records (2001–2023). A total of 1,079 reported depredation incidents resulted in 1,420 livestock mortalities, with small livestock accounting for the majority of losses. Most depredation incidents occurred in animal sheds, while Hamirpur Range experienced the highest proportion of livestock mortality.

Mann–Kendall trend analysis revealed a significant declining trend in annual livestock mortality during the study period, although temporal fluctuations were observed in certain years. Significant variation in depredation patterns was also observed across seasons, livestock categories, attack locations, and forest ranges. These findings highlight the importance of livestock husbandry practices and local landscape characteristics in influencing human–leopard interactions.

Given that nearly 69% of reported depredation incidents occurred in animal sheds, strengthening livestock protection measures through predator-resistant enclosures, reinforced fencing, improved night-time confinement, and community-based conflict mitigation strategies should be prioritized. Capacity-building programmes for livestock owners focusing on predator-safe livestock management, enclosure reinforcement, recognition of leopard presence, and improved livestock guarding practices may further reduce livestock vulnerability. While compensation mechanisms currently provide financial relief to affected livestock owners in the study area, additional preventive measures are required to reduce depredation risk and improve long-term coexistence. Successful implementation of conflict mitigation measures will require active participation of local communities, sustained institutional support, and adoption of practical livestock protection measures at the household level. Continued monitoring of depredation patterns, combined with ecological studies on leopard populations, prey availability, and habitat dynamics, will be essential for developing evidence-based management strategies that promote coexistence between rural communities and leopards in the Himalayan region.

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Conflict of interest

There are no conflicts of interest related to the content of this manuscript.

CRediT author contribution statement

Sangeeta Chandel: Conceptualization, Resources, Investigation, Data acquisition, Project administration, Writing – review & editing; **Bhagwati Prashad Sharma**: Methodology, Supervision, Validation, Writing – review & editing; **Vijay Kumar:** Investigation, Validation, Data interpretation, Writing – review & editing; **Varun Sankhyan**: Formal analysis, Methodology, Data curation, Visualization, Statistical analysis, Writing – original draft, Writing – review & editing. All authors have read and approved the published version of the manuscript**.**

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

The authors used generative AI tools to assist with language editing and the improvement of readability. All content was carefully reviewed and revised by the authors, who take full responsibility for the content of the manuscript.

Data availability statement

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

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Forest Type Mapping of Mandi District of Himachal Pradesh, India: Using Sentinel-2B Multispectral Satellite Data with Supervised Classification Techniques

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ABSTRACT: Accurate mapping of forest types is essential for their sustainable management, ecological studies, and climate change mitigation. Remote sensing techniques using high-resolution satellite imagery have emerged as a cost-effective and reliable tool for vegetation classification and biomass estimation studies. This study focuses on forest type mapping of Mandi district of Himachal Pradesh using Sentinel-2B multispectral satellite data having 10m resolution. In this supervised classification method with Maximum Likelihood Classifier (MLC) was adopted, which was further supported by ground truth data and ancillary resources like SOI toposheets. A total forest area of 1603.19 km^2(40.50% of the geographical area of the district) was classified into 13 major forest types. The most dominant forest types included Moist Deodar forest (24.17%) followed by Chir Pine forest (21.10%), Western Mixed Coniferous Forest (18.04%), and Ban Oak Forest (11.19%), while the remaining forest types occupied comparatively smaller proportions. The overall classification accuracy was 84.67% with a Kappa coefficient of 0.83 which reflects the strong reliability of the results. These findings highlight the dominance of temperate coniferous and broad leaved forests in the district due to its climatic and altitudinal variations. The study demonstrates the potential of Sentinel-2 data to produce detailed, accurate, and up-to-date forest type maps that can serve as critical inputs for biomass estimation, resource management, and climate change studies in Himalayan ecosystems.

Keywords: Forest type mapping, Sentinel-2B, Remote sensing, Supervised classification, Mandi district, Himachal Pradesh.

1. INTRODUCTION

Forests are one of the most important ecosystems, which provide many ecological, economic, and social services to the world. In the Himalayan region, they play a vital role in biodiversity conservation, carbon sequestration, soil and water conservation, climate regulation, and livelihood security. Accurate mapping of forest types is therefore fundamental for sustainable forest management, biomass estimation, and climate change studies. In India, customary methods of mapping forest types were carried out using forest surveys and visual interpretation of aerial photographs (Champion & Seth, 1968), which were labour-intensive, time-consuming, and had limited spatially coverage.

With remote sensing and the emergence of geospatial data, forest monitoring has become synoptic, affordable, and repeatable over large areas. Satellite data, combined with Geographic Information Systems (GIS), have been extensively applied to vegetation classification, forest inventory, and biomass estimation worldwide (Lu et al., 2005; Franklin & Wulder, 2002). In India, agencies like the Forest Survey of India (FSI) have used IRS and Landsat imagery for vegetation mapping at national and regional scales (FSI, 2019). Studies such as Roy et al. (2006) and

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Reddy et al. (2015) demonstrated the potential of multispectral satellite data for mapping diverse forest formations, while Bijalwan et al. (2010) and Bisht (2015) highlighted applications in Himalayan states, particularly for assessing species composition and above-ground biomass.

Recent advances in sensor technology have further improved classification accuracy. Multispectral data at 10-20 m (30 m globally) spatial resolution with a five-day repeat frequency are provided by the European Space Agency Sentinel-2 mission. This represents a major advance over previous satellite sensors. Research has shown its effectiveness in forest type discrimination, vegetation phenology studies, and biomass estimation (Immitzer et al., 2016; Waser et al., 2019). Sentinel-2 images have been used to classify vegetation in the Himalaya (Jain et al., 2021) and assess carbon stock (Pandey et al., 2022) .The Himalayas present a complex ecological mosaic where phenological cycles strongly influence forest type distribution. Recent studies, such as Singh et al. (2023), have demonstrated the utility of Sentinel-2-based NDVI (Normalised Difference Vegetation Index) time series in capturing seasonal forest dynamics across the Western Himalayas. In this study, we extend these approaches by applying Sentinel-2B imagery for forest type mapping in Mandi district. Such findings highlight the suitability of Sentinel-2 data for forest type mapping in heterogeneous and rugged terrains like Himachal Pradesh.

Mandi district, centrally located in Himachal Pradesh, represents a unique mosaic of forest ecosystems shaped by altitudinal and climatic gradients. The district ranges from subtropical low hills to alpine pastures, resulting in coniferous, broad-leafed, scrub, and alpine vegetation. Although forests occupy almost 28% of the area of the State and provide numerous ecosystem services along with important biodiversity, there is no thorough and up-to-date district scale forest type map available and generally, very little fine-scale heterogeneity information available. This creates a barrier for forest resource planning, biodiversity conservation and climate change mitigation.

The present study aimed to classify and delineate the major forest types of Mandi district using high-resolution Sentinel-2B multispectral imagery supported by ground truth data collected during field surveys. The study further evaluated the accuracy and reliability of the classification results through error matrix assessment and Kappa coefficient analysis. In addition, the spatial distribution of forest types was examined in relation to topographic and climatic variations across the district in order to understand the ecological patterns of Himalayan vegetation. By integrating remote sensing data with field validation techniques, the study sought to develop a reliable and updated database on forest types in Mandi district that could support sustainable forest management, biomass estimation, biodiversity conservation, and ecological research in Himalayan landscapes.

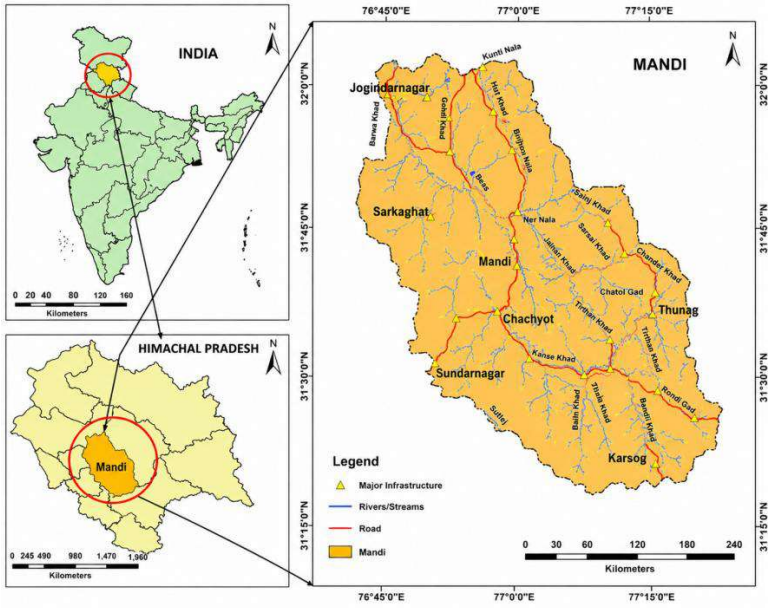


Figure 1. Location map of Mandi District, Himachal Pradesh

The study was based on the hypothesis that high-resolution Sentinel-2B multispectral data, combined with supervised classification techniques and field validation, can effectively discriminate and map the major forest types of Mandi district with high classification accuracy. It was further hypothesised that variations strongly influence the spatial distribution of forest types in the district in altitude, topography, and climatic conditions across the Himalayan terrain.

2. MATERIALS AND METHODS

2.1. Study Area

The study area, Mandi district, was located in central Himachal Pradesh (31°13′-32°05′ N; 76°37′-77°23′ E) in India and covers about 3,950 km² (Figure 1). The elevation ranges from 467 meters (1,532 ft) to 3,350 m (10,991 ft) and the climate ranges from subtropical in the lower region to temperate and alpine in the higher altitudes. Most of the region is drained by the Beas and its tributaries. The district supports diverse forest ecosystems, including chir pine, deodar, ban oak, blue pine, mixed-conifer, birch-rhododendron scrub and alpine pasture. The diversity of forest types makes Mandi ideal study area for satellite based forest mapping

2.2. Data Sources

Sentinel-2B level 1C multispectral data (10 m resolution) were acquired from the Copernicus Open Access Hub. Four bands—Blue (B2: 490 nm), Green (B3: 560 nm), Red (B4: 665 nm), and Near Infrared (NIR) (B8: 842 nm)-were used for vegetation classification (Table 1).

2.3. Image Pre-processing

Cloud-free Sentinel-2B images were corrected radiometrically and geometrically in ERDAS Imagine.

Table 1. Details of spectral bands of Sentinel-2B used in the study

Bands	Resolution	Central wavelength	Description
B2	10 m	490 nm	Blue
B3	10 m	560 nm	Green
B4	10 m	665 nm	Red
B8	10 m	842 nm	Near Infrared (NIR)

Ancillary datasets included conventional Survey of India (SOI) 1:50,000 topographic sheets (52D/12, 52D/16, 53A/9, 53A/13, 53E/1, 53A/10, 53A/14, 53E/2, 53E/6, 53A/15, 53E/3, 53E/7) for geo-referencing and ground validation. Individual spectral bands were stacked to generate False Colour Composites (FCC) (Figure 2). Images were geo-referenced using SOI topographic sheets with RMS error < 0.3, and subsets were extracted based on district boundaries (Figure 3). Composite images and RGB band combinations are shown in Figure 4.

2.4. Forest Type Classification

Forest types were classified following the system of Champion and Seth (1968) using Supervised Classification with the Maximum Likelihood Classifier (MLC). Representative training samples for each forest type were selected based on field observations, visual image interpretation and their spectral reflectance characteristics. The Maximum Likelihood Classifier (MLC) assumed a normal distribution of class statistics. The probability that a pixel with feature vector x belonged to class i was estimated using Eq. 1.

$$p(x|i) = \frac{(2\pi)n}{2}|\Sigma i| \frac{1}{21 \exp\left[-21(x-\mu i)T\Sigma i-1(x-\mu i)\right]} \ldots (1)$$

Where, μi is the mean vector and Σi is the covariance matrix of class i. Each pixel was assigned to the class with

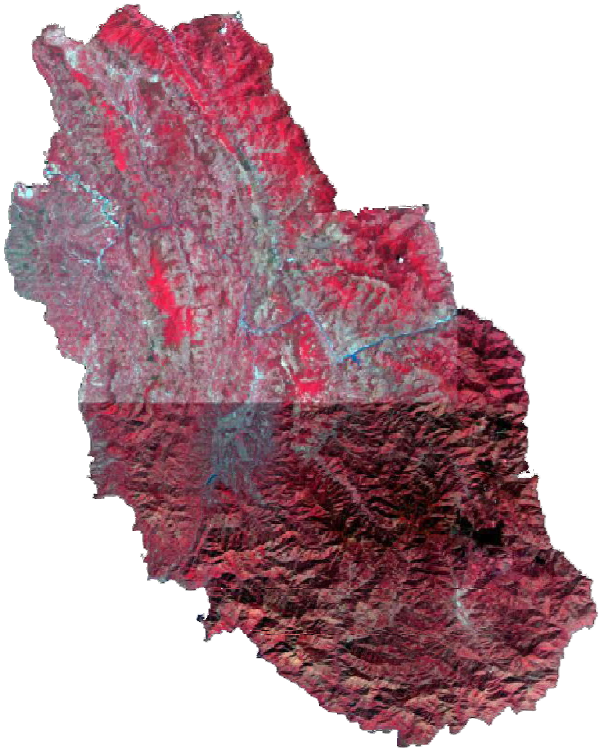


Figure 2. False colour composite (FCC) of the study area

the highest probability. Non-vegetated areas were masked using NDVI derived from Red (B4) and NIR (B8) bands.

2.5. Accuracy Assessment

Accuracy of classification was assessed using stratified random sampling with 150 ground truth points (Eq.2). An error/confusion matrix was generated by comparing reference data with classified outputs (Table 3). Overall classification accuracy and Kappa coefficient (κ) were computed following Congalton (1991). The Kappa coefficient was calculated using Eq. 3.

$$\text{Accuracy assessment percentage} = \left(\frac{\text{No.of ground truthing points classfied correctly}}{\text{Total number of ground truthing points checked}}\right) \times 100 \quad (2)$$

$$K_{hat} = \frac{N\sum_{i=1}^{r} Xii - \sum_{i=1}^{r}(X_{i+} * X_{+i})}{N^2 - \sum_{i=1}^{r}(X_{i+} * X_{+i})} \quad \ldots\ldots(3)$$

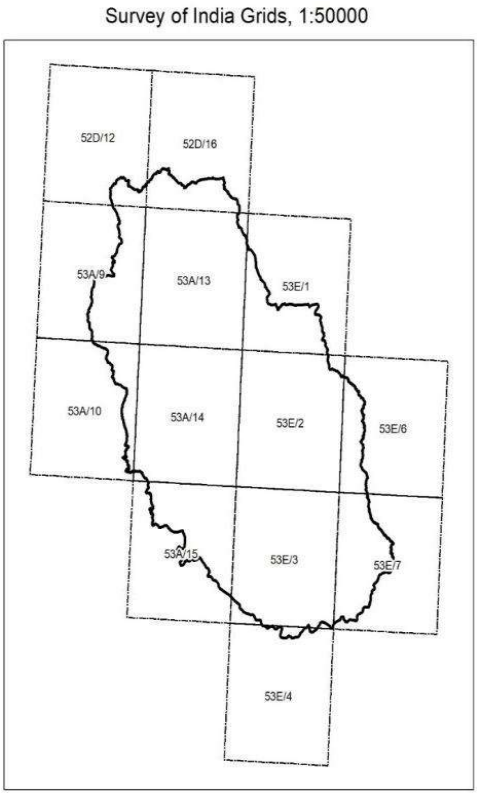


Figure 3. Survey of India (SOI) 1:50,000 scale toposheets covering Mandi district, Himachal Pradesh. The figure illustrates the SOI grid sheets overlapping the study area, which were used as base reference maps for image georeferencing, preparation of the study area boundary, and validation of the forest type map

where

- r = number of rows in the matrix,
- Xii = observations in row i and column i,
- $Xi+$ and $X+i$ = marginal totals of row i and column i,
- N = total number of observations.

2.6. Methodological Framework

The methodology for forest type classification is illustrated in Figure 5 and consists of five main components: data collection, data pre-processing, classification, ground validation, and accuracy assessment.

3. RESULTS AND DISCUSSION

Forest type mapping provides critical baseline information for biodiversity assessment, habitat evaluation, carbon stock estimation, and sustainable forest management

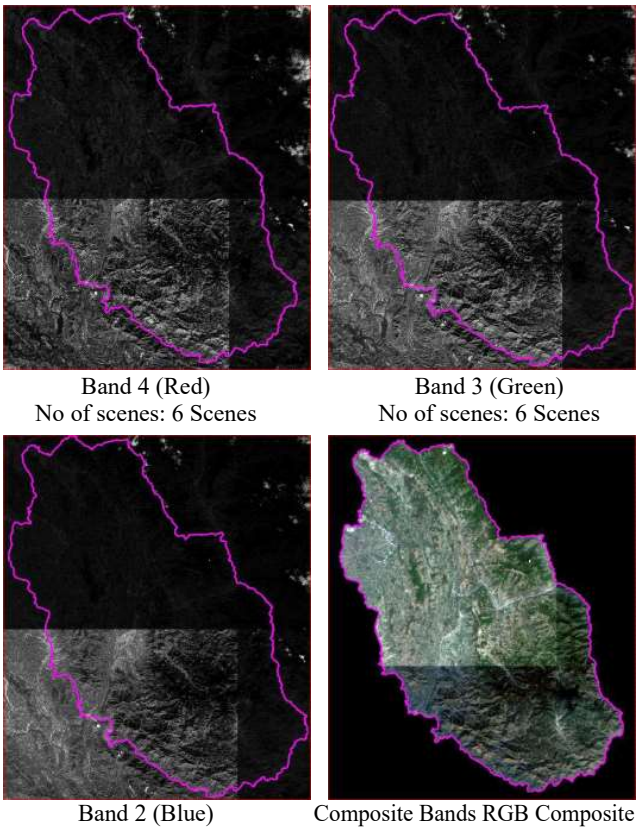


Figure 4. Sentinel-2B multispectral imagery used for image processing, showing the individual spectral bands—Band 4 (Red), Band 3 (Green), and Band 2 (Blue)—along with the RGB composite image generated by layer stacking

in mountainous ecosystems. In the present study, Sentinel-2B multispectral imagery, combined with supervised classification, successfully delineated 13 major forest types in Mandi district, Himachal Pradesh (Figure 6). The spatial distribution and area statistics of these forest types are presented in Table 2. These results show the applicability of Sentinel-2 data for classifying heterogeneous Himalayan vegetation across complex topographic gradients with high accuracy.

3.1. Forest Types of Mandi District

The classified map revealed that Moist Deodar Forest occupied the largest area (38,752.50 ha; 24.17%), followed by Chir Pine Forest (33,834.00 ha; 21.10%) and Western Mixed Coniferous Forest (28,925.90 ha; 18.04%). In contrast, Birch-Rhododendron Scrub occupied the smallest extent (57.37 ha; 0.04%). The dominance of coniferous forests in mid and higher elevations reflects the prevailing temperate climatic conditions and altitudinal zonation of vegetation in the Western Himalayas. Similar vegetation distribution patterns have been reported by Roy (1987), Bisht (2015), and Jain et al. (2021), who observed the predominance of chir pine and deodar forests in Himachal Pradesh under varying elevation regimes.

The observed forest distribution strongly influenced by topographic and climatic variability. Lower elevations were characterised by Northern Dry Mixed Deciduous Forests and Himalayan Subtropical Scrub, while Chir Pine Forests dominated warm subtropical slopes between 800 and 1800 m elevation. Mid-altitude zones supported Moist Deodar Forests, Ban Oak Forests, and Temperate Secondary Scrub, whereas Western Mixed Coniferous Forests, Kharsu Oak Forests, and alpine vegetation characterised higher elevations (>2500 m). Such ecological zonation is consistent with the findings of Singh et al. (2023), who demonstrated that Himalayan forest composition and spectral behaviour in Sentinel-2 imagery is strongly influenced by seasonal vegetation dynamics and altitudinal gradients.

The high spatial resolution and multispectral capability of Sentinel-2B proved highly effective in separating major forest classes with distinct canopy structures and phenological characteristics. Similar observations were

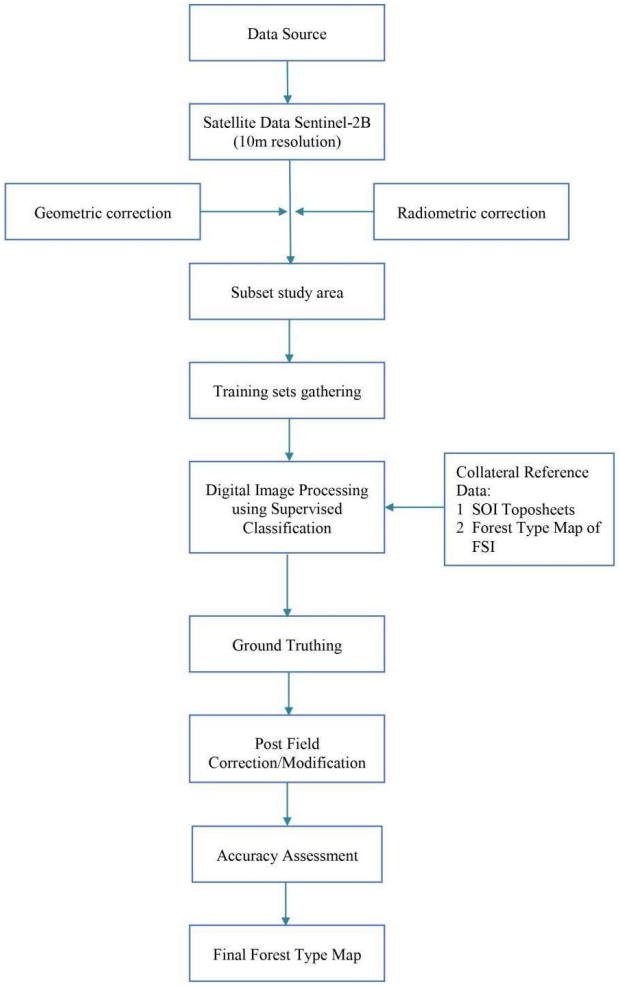


Figure 5. Methodological flow chart illustrating the sequence of steps followed for forest type mapping in Mandi district, Himachal Pradesh

Table 2. Area under different forest types of Mandi district

Forest type classes	Area (ha)	Area (%)
12/C1c Moist Deodar Forest	38752.50	24.17
9/C a&bChir Pine Forest	33834.00	21.10
12/C1d Western Mixed Coniferous Forest	28925.90	18.04
12/C1f Blue Pine Forest	8483.39	5.29
12/C1a Ban Oak Forest	17931.80	11.19
12/C1b Mohru Oak Forest	1062.81	0.66
5B/C2 Northern Dry Mixed Deciduous Forest	5984.50	3.73
12/C2a Kharsu Oak Forest	842.22	0.53
12/Ci/Ds2 Himalayan Temperate Secondary Scrub	6303.38	3.93
9Ci/DS1 Himalayan Subtropical Scrub	4482.73	2.80
12/C1/DS1 Oak Scrub	12316.96	7.68
15/C1 Birch-Rhododendron Scrub	57.37	0.04
12/DS3 Alpine Pastures	1341.55	0.84
Total	**160319.11**	**100**

reported by Immitzer et al. (2016) and Waser et al. (2019), who highlighted the superiority of Sentinel-2 data for tree species discrimination and forest diversity mapping. Furthermore, Vanguri, Laneve, and Hościło (2024) demonstrated that combining Sentinel-2 temporal features with the advanced spectral datasets could improve the discrimination of tree species and biodiversity in heterogeneous forests. Since this study was primarily conducted using Sentinel-2B multispectral bands and not hyperspectral datasets, the classification performance achieved indicates substantial potential for district-level ecological mapping in Himalayan terrain.

3.2. Accuracy Assessment

The classification accuracy assessment as given in Tables 3 and 4 yielded an overall accuracy of 84.67% with a Kappa coefficient (κ) of 0.83, indicating strong agreement between classified outputs and ground reference data. Producer's accuracy ranged from 71.43% for Birch-Rhododendron Scrub to 100% for Alpine Pastures and Non-Forest classes, while user's accuracy ranged from 72.73% for Blue Pine Forest to 100% for Mohru Oak Forest and Birch-Rhododendron Scrub. High classification accuracies obtained for Moist Deodar Forests, Chir Pine Forests, and Western Mixed Coniferous Forests is mainly due to their

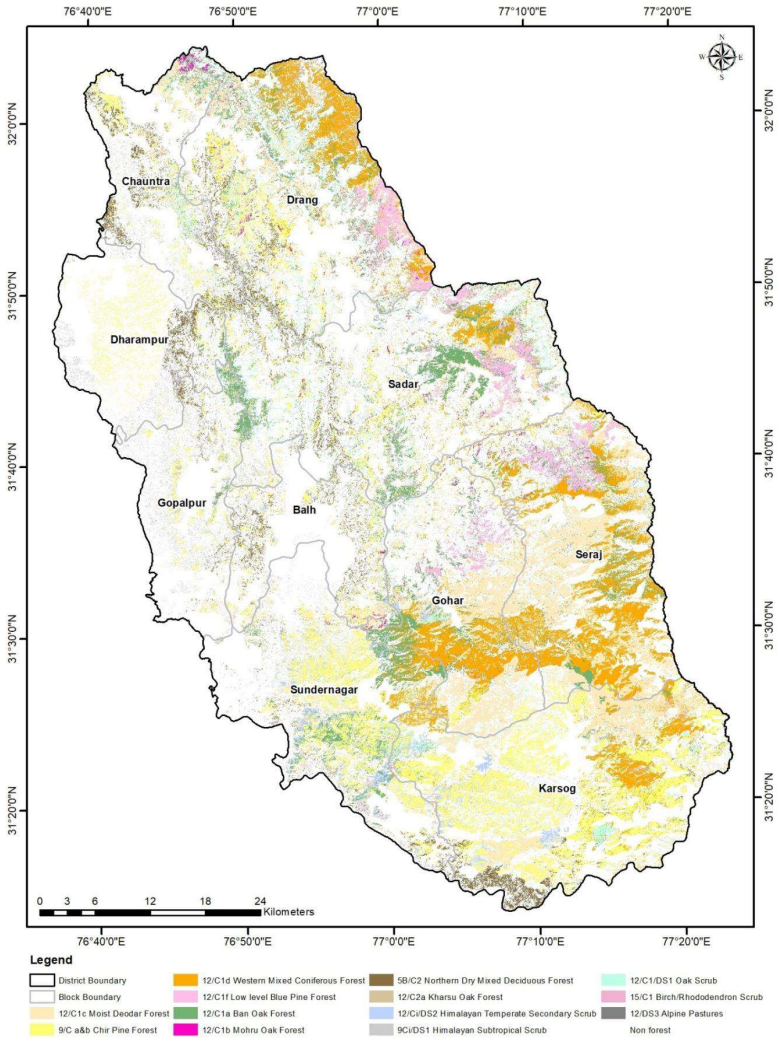


Figure 6. Forest Type map of Mandi District, Himachal Pradesh prepared using Sentinel-2B multispectral imagery and supervised classification. The figure depicts the spatial distribution of major forest types across the district

Table 3. Error matrix for the forest type classification

Classified data	Reference data														
	MDF	CPF	BPF	WMCF	HTSS	NDDF	BOF	HSS	KOF	AP	B/R-S	MOF	OS	NF	Classified total
MDF	16	1	0	0	0	0	0	0	0	0	0	0	0	0	17
CPF	1	17	1	0	0	0	0	0	0	0	0	0	0	0	19
BPF	0	1	8	1	1	0	0	0	0	0	0	0	0	0	11
WMCF	1	0	1	12	0	0	0	0	0	0	0	0	0	0	14
HTSS	0	0	0	0	8	0	0	1	0	0	0	0	1	0	10
NDDF	0	1	0	0	0	9	1	0	0	0	0	0	0	0	11
BOF	0	0	0	0	0	1	9	0	1	0	0	0	1	0	12
HSS	0	0	0	0	1	0	1	6	0	0	0	0	0	0	8
KOF	0	0	0	1	0	0	0	0	5	0	0	0	0	0	6
AP	0	1	0	0	0	0	0	0	0	6	1	0	0	0	8
B/R-S	0	0	0	0	0	0	0	0	0	0	5	0	0	0	5
MOF	0	0	0	0	0	0	0	0	0	0	0	6	0	0	6
OS	0	0	0	0	0	0	0	0	0	0	1	1	9	0	11
NF	0	1	0	0	0	0	0	0	0	0	0	0	0	11	12
Reference total	18	22	10	14	10	10	11	7	6	6	7	7	11	11	150

Overall Classification Accuracy= 84.67 %, Khat= 0.833
Abbreviations: MDF = Moist Deodar Forest; CPF = Chir Pine Forest; BPF = Blue Pine Forest; WMCF = Western Mixed Coniferous Forest; HTSS = Himalayan Temperate Secondary Scrub; NDDF = Northern Dry Mixed Deciduous Forest; BOF = Ban Oak Forest; HSS = Himalayan Subtropical Scrub Forest; KOF = Kharsu Oak Forest; AP = Alpine Pastures; B/R-S = Birch/Rhododendron Scrub; MOF = Mohru Oak Forest; OS = Oak Scrub; NF = Non-Forest

Table 4. Detailed accuracy statistics of image classification

Class name	Reference totals	Classified totals	Number correct	Producers accuracy %	Users accuracy %
Moist Deodar Forest	18	17	16	88.89	94.12
Chir Pine Forest	22	19	17	77.27	89.47
Blue Pine Forest	10	11	8	80.00	72.73
Western Mixed Coniferous Forest	14	14	12	85.71	85.71
Himalayan Temperate Secondary Scrub	10	10	8	80.00	80.00
Northern Dry Mixed Deciduous Forest	10	11	9	90.00	81.82
Ban Oak Forest	11	12	9	81.82	75.00
Himalayan Subtropical Scrub	7	8	6	85.71	75.00
Kharsu Oak Forest	6	6	5	83.33	83.33
Alpine Pastures	6	8	6	100.00	75.00
Birch-Rhododendron Scrub	8	5	5	71.43	100.00
Mohru Oak Forest	7	6	6	85.71	100.00
Oak Scrub Forest	10	11	9	81.82	81.82
Non Forest	11	12	11	100.00	91.67
Total	150	150	127		

relatively distinct spectral signatures and homogeneous canopy structures. In contrast, comparatively lower accuracies for Blue Pine and scrub categories may be attributed to spectral mixing, fragmented patches, shadow effects, and overlapping phenological characteristics in rugged terrain.

The overall classification accuracy obtained in this study is comparable with earlier Himalayan forest mapping studies. Jain et al. (2021) achieved similar accuracies using Sentinel-2 imagery for vegetation classification in the Indian Himalayas, while Srivastava et al. (2022) reported that in complex mountainous environments there is strong performance of machine learning approaches for Sentinel-based land use and vegetation mapping. Likewise, Vanguri et al. (2024) reported improved forest biodiversity mapping accuracy by using Sentinel-2 temporal datasets supported with hyperspectral information. These comparisons signifies that Sentinel-2 imagery remains a highly reliable and cost-effective dataset for ecological and forest type mapping in data-scarce Himalayan regions. Although minor differences were observed when compared with the Forest Survey of India (FSI) vegetation maps, these variations primarily resulted from differences in spatial resolution, image acquisition period, mapping scale, and classification methodology. The use of 10 m Sentinel-2 imagery enabled improved delineation of fragmented forest patches and finer discrimination of forest types compared with conventional vegetation maps. Furthermore, temporal differences between the datasets may reflect natural vegetation succession, anthropogenic disturbances, and ongoing forest management activities. Despite these limitations, the spatial distribution and composition of the major forest types were in good agreement with the regional vegetation characteristics reported by the Forest Survey of India, supporting the reliability of the forest-type map produced in this study.Ecologically, the predominance of deodar, oak, and mixed conifer forests highlights their significance in maintaining biodiversity, slope stability, watershed protection, and carbon sequestration in the Western Himalayas. Oak forests, despite occupying comparatively smaller areas, are particularly important for water conservation and ecosystem resilience. The occurrence of degraded scrub vegetation near human settlements suggests the influence of grazing, fuelwood extraction, forest fires, and land-use change on forest condition. These observations highlight the importance of sustainable forest management and ecological restoration in vulnerable Himalayan landscapes.

4. CONCLUSION

In this study, the forest types of the Mandi district of Himachal Pradesh were mapped and classified using the Sentinel-2B multispectral satellite imagery using a supervised classification method. This resulted in the identification of thirteen major forest types with an overall classification accuracy of 84.67% and Kappa coefficient of agreement 0.83. The major forest types were Moist Deodar Forest, Chir Pine Forest and Western Mixed Coniferous Forest, while minor vegetation types included alpine and scrub vegetation. These results indicate that the Sentinel-2B image can be satisfactorily used for forest-type mapping in the complex and diverse mountainous region of the north-western Himalaya.

The distribution of these different types of forest in the district was largely a function of altitude and climate. The majority of subtropical and deciduous forests occurred at lower altitudes, with temperate coniferous and oak forests occupying higher altitudes in the district. This distribution is consistent with the elements of ecosystem ecology in the Himalayas where precipitation and topography are the major driving forces for the distribution of forest types. The forest-type map may be used in the updating of forest inventories and ecological monitoring, as well as biomass and carbon stock estimation, and biodiversity and watershed management. In addition, satellite imagery, ground observation and GIS have been used to map forest resources in mountainous regions where it is harder to undertake large scale field surveys.

The predominance of coniferous and oak forests highlights their crucial role in carbon storage, water regulation, and biodiversity conservation in the region. In contrast, the presence of degraded scrub vegetation near human settlements shows the effects of grazing, lopping, and other human-induced disturbances on forest ecosystems. These observations emphasise the need of regular ecological monitoring and appropriate management practices for the long-term conservation and sustainable management of forest ecosystems in the region.

The accuracy of forest type mapping can be improved by utilizing multi-season Sentinel-2 imagery, along with hyperspectral and LiDAR datasets, by employing machine learning classifiers such as Random Forest and Support Vector Machines classifiers (SVM). This would further improve mapping accuracy and provide better understanding of the composition and distribution of Himalayan forests.

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CRediT authorship contribution statement

Manoj Thakur: Conceptualization, methodology design, data acquisition, analysis, interpretation of results, and manuscript preparation; **Krishan Thakur:** Assisted in data processing, GIS analysis, data validation, and critical manuscript revision.

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Conflict of interests

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

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

During the preparation of this manuscript, the authors used generative AI and AI-assisted technologies for language refinement, grammar correction, formatting support, and improvement of academic presentation. The authors carefully reviewed and edited the content and take full responsibility for the accuracy, originality, and integrity of the manuscript. No AI tool was used for data generation, analysis, interpretation of results, or drawing scientific conclusions.

Data availability statement

The data used in this study were derived from Sentinel-2B satellite imagery, field observations, and secondary sources obtained from publicly accessible databases and government records. Processed datasets generated during the current study are available from the corresponding author upon reasonable request.

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Effect of Rooting Hormone on Macro-Proliferation of Different Bamboo Species

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ABSTRACT: Bamboo is an ecologically and economically important resource with significant potential to support livelihoods, enhance carbon sequestration, and promote sustainable land management. However, large-scale cultivation is constrained by irregular flowering, limited seed production because most of the species do not produce seed every year, and short seed viability, which limit natural regeneration. Therefore, the development of efficient vegetative propagation techniques is essential to ensure a continuous supply of quality planting material. Therefore, the present study was conducted from February to June 2024 at the Bamboo Resource Centre, College of Forestry, Navsari Agricultural University, Navsari, Gujarat, to evaluate the effect of different auxin concentrations on macro-proliferation of different bamboo species. The experiment was laid out in a factorial completely randomized design using one-year-old seedlings of *Bambusa nutans*, *Bambusa tulda* and *Bambusa polymorpha*, subjected to seven auxin treatments including control, IBA (indole-3-butyric acid) (100 and 200 ppm), NAA (naphthalene acetic acid) (100 and 200 ppm) and their combinations. Observations on sprouting, growth, rooting, survival and multiplication rate were recorded at 120 days after treatment. Results revealed that *Bambusa nutans* exhibited superior growth performance and multiplication rate, whereas *Bambusa polymorpha* performed better in root development and survival. The results revealed that across treatments, G_1 (control) consistently recorded significantly higher values for all parameters. Among the auxin-treated propagules, G_2 (IBA 100 ppm) showed comparatively better performance and remained closer to the control. However, higher concentrations of auxins and their combinations, particularly G_4 (IBA 100 ppm + NAA 200 ppm), G_5 (IBA 200 ppm + NAA 100 ppm), G_3 (IBA 200 ppm), and G_7 (NAA 200 ppm), resulted in a significant reduction in all parameters. Overall, almost all parameters responded positively to the control, while the application of auxins adversely affected the performance of macro-proliferated propagules. The findings demonstrate that exogenous auxin application is not required for macro-proliferation of bamboo under nursery conditions, providing a cost-effective and ecologically sustainable approach for large-scale bamboo propagation and ecological restoration.

Keywords: *Bambusa* spp., Growth and root parameters, Macro-proliferation, Rooting hormones (Auxins).

1. INTRODUCTION

Bamboo, a perennial woody grass belonging to the subfamily *Bambusoideae* under the family *Poaceae,* is one of the most versatile and economically important plant groups in tropical and subtropical regions. Globally, bamboo comprises about 1662 species distributed across 121 genera (Canavan et al., 2017). Owing to its wide range of uses and importance in the rural economy, it is often referred to as "Green Gold", "Poor Man's Timber" and "Cradle to Coffin

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Plant". Bamboo serves as a valuable source of raw material for construction, handicrafts, furniture, paper, and bioenergy, while also playing an important ecological role in soil conservation and carbon sequestration.

The natural distribution of bamboo extends across Asia, Africa and the Americas while Europe has no native bamboo species. Bamboo occupies 1.50 lakh sq. km, representing 17.71% of India's total forest area (ISFR, 2021). India is home to 148 bamboo species, including indigenous and exotic species of bamboo from 29 genera. Bamboos are found in almost all the states of the country, from the tropical to the temperate regions, and the alluvial plains to the high mountains; the only exception where they do not occur naturally is Kashmir. Its occurrence is largely influenced by climatic and edaphic factors such as rainfall, temperature, soil type and altitude. Bamboo is distributed from 47° S to 50° 30′ N latitude and from sea level to about 4300 m altitude (Ahmad et al., 2021). The deciduous and semi-evergreen areas of northeastern India, as well as the tropical moist deciduous forests of northern and southern India, have the highest number of species. Nearly 90 bamboo species are found in India, out of which 41 are indigenous to the hilly areas of northeastern India (Sharma & Nirmala, 2015).

Bamboo is a multifunctional, fast-growing plant on Earth. Bamboo has played a crucial role in the daily lives of millions of people in tropical countries, providing environmental, social, and economic benefits (Ahmad et al., 2021). Bamboo is an essential part of existence, not only in Indian culture but also across Southeast Asian cultures. This group of species is most closely connected to the cornerstone of rural life and culture in our nation due to the variety of functions they serve. Due to its lignified culms, which can serve as a substitute for lumber in many ways, as well as its rapid growth, complex rhizome system, and sustainability, it has evolved into a plant with conservation value that can help to lessen the effects of climate change. Not just pandas, but many other creatures depend on bamboo as a food resource. Like rice, maize, wheat, and sugar cane, bamboo is a vital grass closely tied to human livelihoods. It satisfies needs for shelter, food, paper, and more; bamboo is known as "the plant of a thousand uses" for a reason.

In recent years, the increasing demand for bamboo planting material for plantation forestry, agroforestry, and commercial cultivation has underscored the need for efficient mass-multiplication techniques. Bamboo can be propagated either by seed or by vegetative methods. However, seed propagation is unreliable because most bamboo species flower gregariously only once in their lifetime after long intervals of 12 to 120 years and produce seeds with short viability. As a result, an assured seed supply is rarely available for nursery production. In addition, the monocarpic nature of bamboo and the difficulty of improvement through hybridization make large-scale seed propagation highly uncertain.

The habit of unpredictable gregarious flowering and seeding, followed by death, makes seed propagation of bamboo uncertain, as seeds have short viability. Improving through hybridization is very difficult. To achieve these objectives, large-scale plantation and farming, along with a vegetative propagation technique using PGRs, will offer great potential for the production of high-quality planting material (Ilorkar et al., 2021).

Vegetative propagation of bamboo can be achieved through the branch-cutting method, single-node (bud) cutting method, culm-cutting (flute) method, layering method, and rhizome (offshoot) planting method, all of which are commonly employed (Hamilton et al., 2022). However, these methods often require a large number of explants and usually produce a limited number of propagules. Further, when propagules are obtained from vegetative parts of unknown age, there is a possibility that such planting stock may flower earlier in the field, which can adversely affect plantation performance. Therefore, the production of planting material of known physiological age is highly desirable.

Further, the age of bamboo plants is not known in vegetative saplings until the explants are taken from the mother plant of known age. Using bamboo planting material of unknown age is a big risk, as such a plantation may flower and dry up in the field if it reaches its physiological age. Therefore, nowadays farmers are demanding known-age plants, which is only possible through seedling propagation. However, the bamboos are monocarpic in nature and produce seeds only once in their lifetime; therefore, there is no assured seed supply for the nursery.

Macro-proliferation of bamboo seedlings offers an effective means of producing large numbers of plants from seedling-origin stock of known physiological age. Bamboo seedlings have the capacity to proliferate, and this potential of bamboo can be used for mass propagation through the macro-proliferation method. In this technique, seedlings raised for about six months to one year produce multiple shoots from rhizomes, which are subsequently separated and replanted as independent propagules (Kumar, 1991). The process can be repeated over successive cycles to obtain a large number of planting stocks suitable for field planting.

Since rooting and survival of separated propagules are important for the success of this method, the use of plant growth regulators has often been considered beneficial. Previous studies have reported the positive role of auxins in vegetative propagation of bamboo under nursery conditions (Ilorkar et al., 2021; Venkatesh & Kariappa, 2021). Despite the reported usefulness of plant growth regulators in vegetative propagation, their role in macro-proliferation of bamboo seedlings remains inconsistent and species-specific. Therefore, the present study aims to evaluate whether auxin application is essential for improving macro-proliferation efficiency in bamboo species.

However, the response to exogenous auxins may differ among bamboo species depending upon their genetic constitution and endogenous hormonal balance. Therefore, it is necessary to evaluate the role of rooting hormones in macro-proliferation of important bamboo species under nursery conditions. In this context, the present study was undertaken to assess the effects of different auxin concentrations on the growth, rooting, survival, and multiplication of Bambusa nutans, Bambusa tulda, and Bambusa polymorpha, with a view to developing a suitable protocol for large-scale propagation.

2. MATERIALS AND METHODS

2.1. Experiment Site

The present study was carried out from February to June 2024 at the Bamboo Resource Centre, College of Forestry, Navsari Agricultural University (NAU), Navsari, Gujarat, India. Navsari is situated in the coastal region of South Gujarat at 20°57′ N latitude and 72°54′ E longitude, at an altitude of 9 m above mean sea level. The region falls under the South Gujarat Heavy Rainfall Zone (AES-III). It is characterised by a tropical, humid climate with hot summers, moderately cool winters, and a warm, humid monsoon. The experiment was carried out under nursery conditions.

2.2. Experimental Methods, Treatments and Procedures

2.2.1. Experimental design and treatments

The experiment was conducted under nursery conditions using a factorial completely randomized design (FCRD) in which one-year-old seedlings of three bamboo species, viz., *Bambusa nutans* (B_1), *Bambusa tulda* (B_2) and *Bambusa polymorpha* (B_3) were transplanted into polybags of size 6 × 8 inches and treated with seven different levels of auxin concentrations, namely G_1: control, G_2: IBA 100 ppm, G_3: IBA 200 ppm, G_4: IBA 100 ppm + NAA 200 ppm, G_5: IBA 200 ppm + NAA 100 ppm, G_6: NAA 100 ppm and G_7: NAA 200 ppm. The combination of three bamboo species and seven auxin treatments resulted in twenty-one treatment combinations. Each treatment combination was replicated three times, with fifteen propagules maintained in each replication. Thus, each treatment combination consisted of a total of forty-five propagules (fifteen propagules × three replications). Overall, 945 propagules (21 treatment combinations × 45 propagules) were used in the experiment. The treatment combinations were designated as B_1G_1 to B_3G_7, where B_1, B_2, and B_3 represented the three bamboo species, and G_1 to G_7 represented the seven auxin treatments.

2.2.2. Plant material and preparation of growing media

One-year-old, well-managed seedlings of *Bambusa nutans*, *Bambusa tulda*, and *Bambusa polymorpha*, with multiple culms (three or more) and well-developed roots and rhizomes, were selected for macro-proliferation. Prior to initiation, nursery beds were cleaned, and weeds were removed. The growing medium was prepared using clay soil (54.3% clay) and sand in a 2:1 (v/v) ratio and filled into polybags of size 6 × 8 inches. The polybags were arranged in nursery beds and watered regularly to maintain adequate moisture.

2.2.3. Preparation and application of auxins (PGR)

Auxin solutions of IBA and NAA at concentrations of 100 ppm and 200 ppm were prepared by dissolving 100 mg and 200 mg of IBA and NAA, respectively, in a small quantity of NaOH solution, then diluting with distilled water to a final volume of 1000 ml. Separate stock solutions of IBA and NAA were prepared and mixed in appropriate proportions to obtain the desired treatment combinations. Seven treatment levels were used in the study: G_1 (Control, without auxin treatment), G_2 (IBA 100 ppm), G_3 (IBA 200 ppm), G_4 (IBA 100 ppm + NAA 200 ppm), G_5 (IBA 200 ppm + NAA 100 ppm), G_6 (NAA 100 ppm), and G_7 (NAA 200 ppm). The propagules of each bamboo species were treated with the corresponding auxin solutions according to the assigned treatment combinations, then planted in polybags under nursery conditions.

2.2.4. Macro-proliferation procedure

Macro-proliferation of bamboo seedlings was carried out following the standard procedure described by Kumar (1991), with slight modifications, including the application of rooting hormones to enhance the growth, rooting, and survival of propagules. Seedlings were carefully removed from polybags, and soil adhering to roots and rhizomes was

washed off. Each plant was dissected at the rhizome neck using sharp secateurs to obtain propagules containing the rhizome, roots, and shoot. Approximately 75 per cent of the shoot portion was trimmed to reduce transpiration loss. To minimise mortality, division was preferably performed in the evening or under relatively cool conditions.

The separated propagules were dipped in a fungicide solution of carbendazim at the rate of 1 g L^{-1} for 15–20 minutes to prevent fungal infection. Subsequently, 50 per cent of the length of the basal portion of each propagule was treated with the respective auxin solution for 15 minutes.

2.2.5. Transplanting and maintenance

After treatment, propagules were transplanted into polybags containing prepared growing medium. The planted propagules were maintained under partial shade (50%) in a low-cost polytunnel covered with a 50% green shade net. The shade net reduced incident sunlight by approximately 50% of the incident sunlight, thereby maintaining favourable humidity and temperature conditions for sprouting and establishment of the propagules. Irrigation was applied as required during the initial stage and subsequently at an interval of two days. Routine nursery operations, such as weeding and cleaning, were carried out regularly. After one month, plants were gradually exposed to full sunlight.

2.3. Observations Recorded

Observations were recorded at 120 days after initiation of the experiment on days to first sprouting, number of sprouts, basal diameter, number of leaves per plant, shoot height, root length, number of rootlets, number of rhizomes, survival percentage and multiplication rate.

2.4. Statistical Analysis

The data were analysed using analysis of variance appropriate for a factorial completely randomised design. The standard error of the mean (SEm±) and critical difference (CD) at a 5% level of significance were calculated to compare treatment means following the method described by Panse & Sukhatme (1985).

3. RESULTS AND DISCUSSION

The results are presented under different categories to facilitate a clear understanding of the effects of auxin treatments and their interactions on macro-proliferation performance of three bamboo species. The results of the present investigation of auxin concentrations and their interactions revealed significant variation among bamboo species, providing clear insights into the physiological and propagation responses under macro-proliferation.

3.1. Growth Parameters

Days to the first sprout varied significantly among species, treatments, and their interactions. Among the species, earlier initiation of sprouting was recorded in B_1: *Bambusa nutans* (9.21 days), whereas sprouting was delayed in B_3: *Bambusa polymorpha* (22.73 days). Among auxin treatments, the earliest sprouting was recorded in G_1: control (10.37 days), followed by G_2: IBA 100 ppm, whereas higher concentrations, particularly G_7: NAA 200 ppm, resulted in delayed sprouting (20.41 days). This indicates that exogenous auxin application beyond optimum levels suppressed bud activation. The interaction effect was also significant, and the earliest sprouting was recorded in B_1G_1 (4.29 days; Table 1), indicating a better response of B_1 (*Bambusa nutans*) under untreated conditions, while higher auxin concentrations delayed sprout initiation.

The number of sprouts per propagule differed significantly among species. B_1: *Bambusa nutans* recorded the highest number of sprouts (1.79), whereas B_2: *Bambusa tulda* recorded the lowest (0.85). Among treatments, G_1: control (2.08) showed a significantly higher number of sprouts, while the minimum was recorded in G_7: NAA 200 ppm (0.76). The interaction effect indicated that B_1G_1 recorded the highest number of sprouts (2.56), further indicating that untreated propagules of B_1: *Bambusa nutans* performed best during macro-proliferation (Table 1).

The reduction in sprout production at higher auxin concentrations and in auxin combinations may be attributed to the inhibitory effects of supra-optimal hormone levels on bud differentiation and shoot initiation. Similar findings were also reported by Singh et al.(2002) in bamboo propagation studies.

Basal diameter also varied significantly among species. B_2: *Bambusa tulda* recorded the maximum basal diameter (2.86 mm), whereas B_3: *Bambusa polymorpha* recorded the minimum (1.89 mm). Among treatments, G_1: control recorded the highest basal diameter (3.12 mm), whereas the lowest was observed under G_7: NAA 200 ppm (1.66 mm). The interaction effect showed maximum basal diameter in B_2G_1 (4.09 mm), indicating better growth performance of B_2: *Bambusa tulda* under untreated conditions (Table 1).

The number of leaves per propagule was significantly higher in B_1: *Bambusa nutans* (9.67), whereas B_2: *Bambusa*

Table 1. Effect of different concentration of auxins on macro-proliferation of different *Bambusa* species for growth parameters

Treatment	Days taken for the first				Number of sprouts				Basal diameter (cm)				Number of leaves per rooted				Shoot height (cm)			
	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean
G1 - Control	4.29	9.71	17.11	10.37	2.56	1.67	2.02	2.08	2.82	4.09	2.44	3.12	14.82	6.80	10.96	10.86	48.62	46.11	35.93	43.55
G2 – IBA 100 ppm	6.20	10.80	19.26	12.09	2.22	0.91	1.93	1.69	2.42	3.99	1.87	2.76	13.22	4.11	10.87	9.40	37.55	41.17	34.72	37.81
G3 – IBA – 200 ppm	12.00	18.40	25.98	18.79	1.13	0.56	1.20	0.96	1.70	1.83	1.70	1.75	6.18	2.93	6.44	5.18	15.95	17.83	27.97	20.58
G4 – IBA 100 ppm + NAA 200 ppm	8.27	14.65	20.55	14.49	2.11	0.80	1.44	1.45	2.31	2.85	1.86	2.34	10.44	3.18	8.71	7.44	32.52	33.67	33.19	33.13
G5 - IBA 200 ppm + NAA 100 ppm	9.89	15.98	23.69	16.52	2.04	0.78	1.44	1.42	1.64	2.77	1.83	2.08	9.64	3.16	7.22	6.67	25.63	33.23	32.81	30.56
G6 – NAA 100 ppm	11.78	17.80	24.14	17.91	1.69	0.69	1.40	1.26	1.89	2.76	1.82	2.15	9.16	3.00	7.00	6.39	24.55	25.28	28.09	25.97
G7 – NAA 200 ppm	12.02	20.80	28.40	20.41	0.76	0.51	1.02	0.76	1.54	1.75	1.68	1.66	4.20	2.18	5.09	3.82	15.54	17.31	26.64	19.83
Mean	9.21	15.45	22.73		1.79	0.85	1.50		2.05	2.86	1.89		9.67	3.62	8.04		28.62	30.66	31.34	
	B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G	
S. Em. ±	0.23	0.34	0.60		0.03	0.05	0.09		0.03	0.04	0.07		0.17	0.25	0.44		0.39	0.59	1.03	
C.D. at 5 %	0.64	0.98	1.70		0.09	0.14	0.25		0.08	0.12	0.21		0.47	0.72	1.25		1.11	1.69	2.93	
C. V. %	6.53				11.05				5.56				10.71				5.89			

B1 - Bambusa nutans, B2 - Bambusa tulda and B3 – Bambusa polymorpha

tulda recorded the lowest (3.62). Higher leaf production in Bambusa nutans is directly associated with its higher sprouting ability and faster establishment. Among treatments, G_1: control (10.86) recorded the highest number of leaves, while the lowest was observed in G_7: NAA 200 ppm (3.82). The interaction effect indicated that B_1G_1 recorded the maximum number of leaves (14.82), suggesting that untreated propagules of B_1: *Bambusa nutans* exhibited superior vegetative growth under nursery conditions (Table 1).

Shoot height varied significantly among species and treatments. The maximum shoot height was recorded in B_3: *Bambusa polymorpha* (31.34 cm), whereas the minimum was recorded in B_1: *Bambusa nutans* (28.62 cm). Among treatments, G_1: control recorded the maximum shoot height (43.55 cm), whereas the minimum was observed under G_7: NAA 200 ppm (19.83 cm). The interaction effect showed maximum shoot height in B_1G_1 (48.62 cm), indicating that untreated propagules exhibited better establishment and subsequent shoot growth compared with auxin-treated propagules (Table 1).

These results are in close proximity to the earlier reports by Saharia and Sen (1990) and Agnihotri and Ansari (2000), who observed that the exogenous application of various growth regulators, mostly auxins, had been reported to positively influence growth induction in culm cuttings of bamboos. However, in the present study, untreated propagules performed better indicating that endogenous hormonal balance in seedling-derived propagules is sufficient for sprouting and growth. Similar observations were also reported by Singh et al.(2002) in bamboo cuttings.

The superior performance observed under control conditions may be attributed to adequate endogenous auxin levels present in seedling-derived propagules, which regulate cell division and elongation without external supplementation. The application of higher concentrations of exogenous auxins might have disrupted the internal hormonal balance, leading to inhibitory effects on sprouting and growth. Similar inhibitory effects of excessive auxin concentrations have been reported in vegetative propagation of woody species, where supra-optimal hormone levels adversely affect physiological processes.

3.2. Root Parameters

Root characteristics differed significantly among species and treatments. B_3: *Bambusa polymorpha* recorded the highest root length (18.51 cm), whereas the lowest was observed in B_1: *Bambusa nutans* (16.21 cm). Among treatments, G_1: control recorded significantly higher root length (22.52 cm), while the lowest was recorded under G_7: NAA 200 ppm (12.73 cm). The interaction effect showed maximum root length in B_2G_1 (22.99 cm) (Table 2).

The number of rootlets was also significantly influenced by species and treatments. B_3: *Bambusa polymorpha* recorded the highest number of rootlets (21.42), whereas the lowest was observed in B_1: *Bambusa nutans* (11.90). Among treatments, G_1: control recorded the maximum number of rootlets (19.22), whereas the minimum was recorded under G_7: NAA 200 ppm (12.27). The interaction effect showed the maximum number of rootlets in B_3G_1 (25.00) and the minimum in B_1G_7 (7.00) (Table 2).

The number of rhizomes was significantly higher in B_3: *Bambusa polymorpha* (2.34), whereas the lowest was recorded in B_2: *Bambusa tulda* (1.44). Among treatments, G_1: control recorded the maximum number of rhizomes (2.69), while the minimum was observed under G_7: NAA 200 ppm (1.22). The interaction effect indicated the maximum number of rhizomes in B_1G_1 (3.27) and the minimum in B_2G_7 (0.80) (Table 2). These results are in close conformity with earlier findings of Seethalakshmi et al. (1983) and Nain et al. (2007), who reported variation in rooting behaviour among bamboo species. Similar observations were also reported by Razvi et al. (2015) in Dendrocalamus giganteus and by Rana et al. (1987) and Nautiyal et al. (1991) in other tree species.

The variation in rooting response among species and treatments may be linked to differences in endogenous hormone levels, carbohydrate reserves and genetic constitution. The superior rooting under control conditions suggests that seedling-origin propagules possess sufficient physiological maturity and hormonal balance to initiate root formation. Excessive auxin concentrations may have led to callus formation or inhibited root elongation, thereby reducing overall rooting efficiency.

3.3. Survival Percentage

Species and auxin treatments significantly influenced survival percentage. Among species, B_3: *Bambusa polymorpha* recorded the highest survival (45.71%), whereas B_1: *Bambusa nutans* recorded the lowest (31.43%). Among treatments, G_1: control recorded the maximum survival (59.26%), while the lowest was observed under G_7: NAA 200 ppm (23.70%). The interaction effect indicated that B_2G_1 had the highest survival (73.33%), whereas the

Table 2. Effect of different concentrations of auxins on macroproliferation of different *Bambusa* species for root parameters, survival percentage and multiplication rate

Treatment	Days taken for the first				Number of sprouts				Basal diameter (cm)				Number of leaves per rooted				Shoot height (cm)			
	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean	B1	B2	B3	Mean
G1 - Control	22.89	22.99	21.69	22.52	14.47	18.20	25.00	19.22	3.27	2.00	2.80	2.69	48.89	73.33	55.56	59.26	2.34	1.72	2.22	2.09
G2 – IBA 100 ppm	21.60	22.47	19.95	21.34	11.00	16.80	22.60	16.80	2.80	1.70	2.47	2.32	42.22	55.55	53.33	50.37	1.89	1.68	1.96	1.84
G3 – IBA – 200 ppm	13.00	14.07	17.12	14.73	12.13	12.67	19.40	14.73	1.20	1.13	2.20	1.51	15.56	24.44	40.00	26.67	1.39	1.36	1.36	1.37
G4 – IBA 100 ppm + NAA 200 ppm	19.38	18.86	19.21	19.15	13.53	15.07	22.27	16.96	2.80	1.60	2.40	2.27	40.00	51.11	44.44	45.19	1.88	1.60	1.60	1.69
G5 - IBA 200 ppm + NAA 100 ppm	13.63	18.86	18.80	17.10	12.93	13.40	21.73	16.02	2.20	1.53	2.33	2.02	31.11	42.22	44.44	39.26	1.68	1.57	1.43	1.56
G6 – NAA 100 ppm	13.14	16.53	18.34	16.00	12.27	13.20	19.60	15.02	1.53	1.33	2.27	1.71	26.66	39.90	42.22	36.26	1.63	1.39	1.43	1.48
G7 – NAA 200 ppm	9.86	13.86	14.47	12.73	7.00	10.47	19.33	12.27	0.93	0.80	1.93	1.22	15.56	15.56	40.00	23.70	1.31	1.30	1.22	1.28
Mean	16.21	18.23	18.51		11.90	14.26	21.42		2.10	1.44	2.34		31.43	43.16	45.71		1.73	1.52	1.60	
	B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G		B	G	Interaction B × G	
S. Em. ±	0.20	0.31	0.54		0.24	0.37	0.64		0.04	0.07	0.12		1.04	1.59	2.75		0.02	0.04	0.06	
C.D. at 5 %	0.58	0.89	1.54		0.69	1.05	1.81		0.13	0.19	0.33		2.96	4.52	7.84		0.07	0.10	0.18	
C. V. %	5.30				6.94				10.31				11.86				6.63			

B1 - Bambusa nutans, B2 - Bambusa tulda and B3 – Bambusa polymorpha

lowest survival (15.56%) was observed in B_1G_3, B_1G_7, and B_2G_7. The higher survival under G_1: control may be attributed to better root development and absence of hormonal stress (Table 2).

Reduced survival at higher auxin concentrations may be due to inhibitory effects of excessive hormone application. Similar observations were reported by Tomar et al. (2011), who found that untreated propagules showed better survival under nursery conditions.

3.4. Multiplication Rate

The multiplication rate differed significantly among species and treatments. B_1: *Bambusa nutans* recorded the highest multiplication rate (1.73), whereas B_2: *Bambusa tulda* recorded the lowest (1.52). Among treatments, G_1: control recorded the highest multiplication rate (2.09), while the lowest was observed under G_7: NAA 200 ppm (1.28). The interaction effect showed the maximum multiplication rate in B_1G_1 (2.34) and the minimum in B_3G_7 (1.22) (Table 2).

The higher multiplication rate in B_1: *Bambusa nutans* may be attributed to better sprouting ability and rhizome development. The superior performance under G_1: control indicates that macro-proliferation efficiency is largely governed by the inherent regenerative capacity of the species rather than exogenous hormone application. These findings are consistent with Banik (1995), who reported that bamboo seedlings can be effectively multiplied through rhizome division without the need of growth regulators.

Unlike several earlier reports that advocate the use of auxins for vegetative propagation, the present study clearly demonstrates that exogenous auxin application is not only unnecessary but may even be detrimental to macro-proliferation of seedling-derived bamboo propagules under nursery conditions.

4. CONCLUSION

The present investigation demonstrated that macro-proliferation is an efficient technique for large-scale multiplication of bamboo using seedling-derived planting material of known physiological age. Significant variation was observed among the three bamboo species evaluated. *Bambusa nutans* exhibited superior sprouting, vegetative growth and multiplication rate. Whereas *Bambusa polymorpha* showed better rooting performance and survival percentage. *Bambusa tulda* displayed intermediate performance for most of the parameters. Among the auxin treatments, the control (without auxin application) consistently recorded the highest values for sprouting, growth, rooting, survival and multiplication rate. Although IBA at 100 ppm performed relatively better than the other auxin treatments, none of the auxin-treated propagules surpassed the control. In contrast, higher concentrations of IBA and NAA, particularly NAA at 200 ppm, adversely affected most growth and rooting parameters, resulting in reduced survival and multiplication rates. The superior performance of untreated propagules may be attributed to the adequate regenerative potential and physiological vigour of seedling-derived rhizome segments. The findings indicate that exogenous auxin application is not necessary for successful macro-proliferation of *Bambusa nutans*, *Bambusa tulda* and *Bambusa polymorpha* under nursery conditions. Therefore, macro-proliferation without auxin treatment can be recommended as a simple, cost-effective, and environmentally sustainable approach for the large-scale production of high-quality bamboo planting material for plantation development, agroforestry systems, and ecological restoration programmes.

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CRediT authorship contribution statement

Agravat Shivaniben: Field experimentation, Data collection, Writing-original draft; **Jayesh Pathak:** Conceptualisation, Experiment supervision, writing, reviewing and editing; **S. A. Huse:** Reviewing and editing; **M. B. Tandel:** Reviewing and editing; **Jayendra Chavda:** Field assistance and data collection.

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

No AI tools have been used in the writing process. Basic checks of grammar, spelling and punctuation need no declaration.

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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Functional Diversity of Bacterial and Fungal Endophytes Associated with Mulberry (*Morus* spp.) Cultivated in Southern Karnataka, India

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ABSTRACT: Mulberry (*Morus* spp.) cultivation is frequently constrained by both abiotic and biotic stresses, including insect pests and diseases, which adversely affect silkworm health and silk productivity. Endophytes, defined as microorganisms that colonize internal plant tissues without causing visible harm, represent a promising but underexplored biological resource for enhancing plant vigour, stress tolerance and nutritional quality. The present study evaluated the diversity of bacterial and fungal endophytes associated with eight mulberry varieties grown under field conditions in southern Karnataka, India, and assessed their potential implications for mulberry growth and silkworm biology. Endophytic microorganisms were isolated from surface-sterilized leaf and shoot tissues using standard culture-dependent techniques. A total of four dominant endophytic genera were consistently recovered across samples, including *Bacillus* spp. among bacteria and *Aspergillus* spp., *Trichoderma* spp. and *Fusarium* spp. among fungi. The frequency and distribution of these endophytes varied among mulberry varieties and between plant tissues, indicating clear host genotype- and tissue-specific colonization patterns. In general, shoots harboured comparatively higher fungal diversity, while bacterial endophytes, particularly *Bacillus* spp., were more consistently distributed across both tissues. Although quantitative data on isolation frequency and abundance are presented in the results section, the observed patterns indicate differential colonization efficiency among mulberry genotypes. The dominance of *Bacillus* and *Trichoderma* suggests potential plant growth-promoting functions, including improved nutrient availability, enhanced physiological activity and increased stress tolerance of host plants. These microbial interactions are likely to influence mulberry leaf quality, a critical determinant of silkworm growth performance and cocoon traits.

Keywords: Sericulture, Microbial diversity, Plant growth promotion, Mulberry.

1. INTRODUCTION

Mulberry (*Morus* spp.) forms the backbone of sericulture, as its leaves constitute the sole food source for the silkworm, *Bombyx mori* L. The productivity and profitability of sericulture are therefore intrinsically linked to both the quantity and quality of mulberry leaf yield. Despite its agronomic importance, mulberry cultivation faces serious challenges from abiotic stresses such as drought, soil nutrient depletion and temperature fluctuations, as well as a wide spectrum of biotic agents. More than 300 species of insect and non-insect pests have been reported globally on mulberry, of which over 50 cause significant economic losses by reducing leaf yield, palatability and nutritional value. Furthermore, diseases caused by fungal, bacterial and viral pathogens adversely affect mulberry growth and longevity, thereby indirectly impairing silkworm health, survivability and silk production

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efficiency (Hosamani et al., 2020; Vanitha, 2018). In the context of climate variability and increasing restrictions on chemical inputs, sustainable strategies to enhance mulberry resilience while maintaining leaf nutritional quality have become imperative.

Endophytes are microorganisms that inhabit internal plant tissues for all or part of their life cycle without inducing visible disease symptoms. The term "endophyte" was first introduced by de Bary in 1866 to distinguish internal colonizers from epiphytic microorganisms (Stone et al., 2000). Endophytic communities encompass bacteria, fungi and archaea, many of which establish mutualistic or commensal relationships with their host plants. These microorganisms play critical roles in plant growth promotion, stress mitigation and defense regulation through diverse mechanisms, including the production of phytohormones such as indole-3-acetic acid and gibberellins, solubilization of mineral nutrients, biological nitrogen fixation, synthesis of antimicrobial secondary metabolites and modulation of host defence signalling pathways (Miliute et al., 2015; Malfanova et al., 2013).

In perennial crops such as mulberry, endophytic microorganisms assume greater ecological significance due to long-term host–microbe associations and stable internal niches. Studies have shown that endophytes can enhance host tolerance to drought and nutrient stress by improving water-use efficiency, osmolyte accumulation and antioxidant enzyme activity (Rodriguez et al., 2009; Hardoim et al., 2015). Such stress-alleviating traits are particularly relevant to mulberry cultivation in semi-arid regions, where environmental limitations oftern constrain leaf yield and quality. In addition, endophytes contribute to the biological suppression of plant pathogens through competitive exclusion, antibiosis and the induction of systemic resistance, thereby reducing dependence on chemical pesticides (Harman et al., 2004; Singh et al., 2013).

From a sericultural perspective, the functional role of endophytes extends beyond plant health directly influencing silkworm nutrition and performance. Mulberry leaf biochemical composition particularly protein, carbohydrate, moisture and micronutrient content-is a major determinant of silkworm growth rate, larval duration, survivability and cocoon traits such as cocoon weight, shell weight and filament length (Gunasekhar & Somayaji, 2019; Paul et al., 2005). Endophytes that enhance nutrient assimilation and leaf quality therefore exert an indirect yet profound effect on silkworm biology. Previous studies have reported that mulberry leaves colonized by beneficial endophytes such as *Bacillus* and *Trichoderma* improve larval feeding efficiency, reduce disease incidence and enhance silk yield parameters (Ravikumar et al., 2015; Murugesh et al., 2012).

Despite these potential benefits, systematic information on the diversity, tissue specificity and varietal distribution of endophytic microorganisms in mulberry remains limited, particularly under Indian agro-climatic conditions. Most available studies have focused either on plant growth promotion or disease suppression, with relatively few investigations addressing the integrated mulberry–endophyte–silkworm continuum. Moreover, host genotype plays a crucial role in shaping endophytic community structure, as different mulberry varieties vary in leaf chemistry, phenolic content and defense metabolites, which in turn influence microbial colonization and persistence (Peng et al., 2023; Schulz & Boyle, 2005).

In line with the study titled *"Functional diversity of bacterial and fungal endophytes associated with mulberry (Morus spp.) cultivated in southern Karnataka, India"*, the present investigation was undertaken to isolate and characterize bacterial and fungal endophytic microorganisms from different mulberry varieties grown under rain-fed conditions. The study further aims to elucidate varietal- and tissue-specific patterns of endophytic colonization and to interpret their potential role in influencing mulberry growth performance and the biological responses of silkworms.

2. MATERIALS AND METHODS

2.1. Study Site and Experimental Material

The present investigation was carried out during 2024–2025 at the "A" Block of the College of Agriculture, Vishweshwaraiah Canal Farm, Mandya, Karnataka, India (12°32′ N latitude, 76°53′ E longitude; 690 m above mean sea level). The experimental location lies within the Southern Dry Zone (Agro-climatic Zone-6) of Karnataka, characterized by semi-arid climatic conditions with distinct monsoonal influence. The region receives an average annual rainfall of approximately 751 mm, of which nearly 70–75% is concentrated during the southwest monsoon period from June to November. Mean annual temperatures range between 21 and 34 °C, with moderate relative humidity during the cropping season, creating favourable conditions for mulberry cultivation and microbial colonization.

Eight mulberry (*Morus* spp.) varieties representing traditional, improved and elite varieties namely Mysore Local, Sahana, S36, DD, AR12, V1, G2 and G4 were selected for the study. These varieties were maintained under

uniform agronomic conditions following the recommended package of practices for rain-fed mulberry cultivation, including spacing, pruning schedule, nutrient management and plant protection measures, as prescribed by Dandin and Giridhar (2014). No chemical fungicides or bactericides were applied during the experimental period to avoid interference with native endophytic microbial populations. Plants selected for sampling were healthy, disease-free and of uniform age, ensuring consistency in endophyte isolation and minimizing environmental and physiological variability.

2.2. Isolation of Endophytic Microorganisms

Endophytic microorganisms were isolated from healthy leaf and shoot tissues of each mulberry variety following standard surface sterilization and isolation procedures, as described by Sturz et al. (1999) and Gyaneshwar et al. (2001). The study was designed with a systematic sampling strategy, wherein each mulberry variety was represented by three biological replicates (n = 3) for each tissue type (leaf and shoot). Thus, endophytic populations were independently assessed for leaves and shoots of every variety, ensuring robust comparison across varieties and tissue-specific colonization patterns. All samples were collected from symptom-free, healthy plants and immediately transported to the laboratory in sterile polyethylene bags and processed within 24 h of collection.

Leaf and shoot segments were first washed thoroughly under running tap water to remove adhering soil particles and debris, then rinsed with sterile distilled water. Surface sterilization was carried out in a sequential manner: samples were immersed in 70% ethanol for 1 minute, followed by treatment with 1% sodium hypochlorite (NaOCl) solution for 2–3 minutes and finally rinsed three to four times with sterile distilled water to ensure complete removal of surface sterilants. The effectiveness of surface sterilization was confirmed by plating the final rinse water and performing tissue imprinting on sterile agar plates; the absence of microbial growth after incubation confirmed successful elimination of epiphytic microorganisms and validated the endophytic origin of isolates.

After sterilization, tissues were aseptically blotted dry using sterile filter paper and cut into small segments under laminar airflow conditions. For isolation, leaf and shoot tissues from each replicate were processed separately. Furthermore, bacterial and fungal endophytes were maintained and handled as independent culture sets throughout the study to avoid cross-contamination and ensure accurate characterization of microbial groups. The tissue segments were macerated using a sterile mortar and pestle in sterile phosphate buffer (pH 7.0) to release internal microbial communities.

Appropriate aliquots of the homogenate were inoculated onto specific culture media for isolation. Bacterial endophytes were cultured on Nutrient Agar (NA), while fungal endophytes were isolated on Potato Dextrose Agar (PDA) supplemented with streptomycin (50 mg L^{-1}) to suppress bacterial growth. The inoculated plates were incubated at 28 ± 2 °C under controlled laboratory conditions. Bacterial cultures were incubated for 24–48 hours, whereas fungal cultures were incubated for 5–7 days to allow sufficient colony development.

Distinct colonies emerging from internal tissues were periodically observed, and morphologically different bacterial and fungal colonies were sub-cultured separately to obtain pure cultures. These purified isolates were maintained independently: bacterial isolates on Nutrient Agar slants and fungal isolates on PDA slants, both stored at 4 °C for further morphological, biochemical and molecular characterization.

2.3. Morphological and Biochemical Characterization of Endophytic Isolates

Bacterial endophytes were characterized based on colony morphology, including size, shape, colour, margin and elevation, as observed on Nutrient Agar plates. Microscopic examination was carried out to determine cell shape and arrangement using light microscopy by Gram staining. Motility was assessed using the hanging drop method, while catalase activity was determined by the evolution of oxygen bubbles upon the addition of 3% hydrogen peroxide, following standard microbiological protocols (Cappuccino & Sherman, 2014). These morphological and biochemical traits were used for preliminary identification of bacterial isolates up to the genus level.

Fungal endophytes were identified based on macroscopic and microscopic characteristics. Colony morphology, including growth pattern, pigmentation, texture and sporulation, was recorded on PDA medium. Microscopic features such as hyphal structure, septation, conidiophore arrangement and spore morphology were examined by mounting fungal structures in lactophenol cotton blue stain and observing them under a compound microscope. Identification was carried out using standard mycological keys and descriptions provided by Barnett and Hunter (1998) and Domsch et al. (2007).

Molecular identification (16S rRNA/ITS sequencing) was not conducted due to scope and resource limitations. All endophytic isolates were tentatively identified to the genus level based on morphological and biochemical characteristics, which is an accepted preliminary approach in endophyte ecology studies where the focus is on community composition and functional relevance rather than molecular taxonomy (Schulz & Boyle, 2005; Hyde & Soytong, 2008).

3. RESULTS AND DISCUSSION

3.1. Diversity, Abundance and Tissue-Specific Distribution of Endophytes in Mulberry Varieties

The present investigation demonstrated that mulberry plants harbor a stable and functionally significant endophytic microbial community, comprising both bacterial and fungal taxa colonizing internal leaf and shoot tissues without inducing visible disease symptoms. Across the eight mulberry varieties evaluated, four dominant endophytic genera—*Bacillus*, *Aspergillus*, *Trichoderma* and *Fusarium* were consistently isolated (Figure 1). Similar dominance of these genera has been reported in perennial crop systems, where long-lived tissues favour selective microbial associations rather than random colonization (Stone et al., 2000; Malfanova et al., 2013). The persistence of these genera across varieties indicates strong host compatibility and ecological fitness within mulberry tissues.

A pronounced varietal and tissue-specific colonization pattern was evident. Traditional mulberry varieties such as Mysore Local and DD were consistently colonized by *Aspergillus* spp. in both leaf and shoot tissues, suggesting systemic colonization and long-term host adaptation. In contrast, improved mulberry varieties such as Sahana, S36 and V1 predominantly harbored *Bacillus* spp., reflecting the influence of host genotype on microbial recruitment. Host-driven selection of endophytes is well documented and is often mediated through differences in leaf nutrient composition, phenolics and secondary metabolites shaped by breeding practices (Miliute et al., 2015; Peng et al., 2023).

Leaves supported a higher prevalence of growth-promoting fungi such as *Trichoderma*, while shoot tissues favoured stress-tolerant fungi such as *Fusarium*. Such tissue specificity reflects differences in carbon availability, oxygen concentration and defense signalling within plant organs (Schulz & Boyle, 2005). Similar compartmentalization of endophytes has been reported in mulberry and other woody plants, where leaf tissues serve as hotspots for beneficial symbionts due to their metabolic activity (Nataraja et al., 2011; Rekha et al., 2014).

Table 1. Occurrence of endophytes in plant tissues of mulberry varieties

Variety	Endophytes	
	Leaf	**Shoot**
Mysore local	*Aspergillus* spp.	*Aspergillus* spp.
Sahana	*Bacillus* spp.	*Bacillus* spp.
S36	*Bacillus* spp.	*Bacillus* spp.
DD	*Aspergillus* spp.	*Aspergillus* spp.
AR12	*Trichoderma* spp.	*Fusarium* spp.
V1	*Bacillus* spp.	*Bacillus* spp.
G2	*Trichoderma* spp.	*Trichoderma* spp.
G4	*Trichoderma* spp.	*Fusarium* spp.

3.2. Functional Relevance of *Bacillus* spp. as Dominant Bacterial Endophytes

Bacterial endophytes isolated from Sahana, S36 and V1 exhibited rod-shaped, motile, Gram-positive and catalase-positive characteristics, confirming their tentative identification as *Bacillus* spp. The systemic presence of *Bacillus* in both leaf and shoot tissues indicates its ability to colonize vascular and intercellular spaces efficiently, a trait that has been widely reported for *Bacillus* endophytes in crop plants (Gyaneshwar et al., 2001; Singh et al., 2013).

The predominance of *Bacillus* spp. in high-yielding mulberry varieties is agronomically significant, as members of this genus are known to produce phytohormones such as indole-3-acetic acid, gibberellins and cytokinins, which enhance leaf expansion, chlorophyll synthesis and photosynthetic efficiency (Rao and Sivaprasad, 2007; Rekha et al., 2014). Additionally, *Bacillus* spp. solubilize inorganic phosphates and secrete siderophores, thereby improving nitrogen and phosphorus availability within mulberry leaves (Nagaraju & Giridhar, 2017).

Improved mulberry leaf nutritional status directly influences silkworm biology. Gunasekhar and Somayaji (2019) emphasized that silkworm growth, larval duration and cocoon traits are strongly dependent on leaf protein and carbohydrate content. Silkworms reared on *Bacillus*-associated mulberry leaves have been reported to show higher larval weight, improved efficiency of conversion of ingested food (ECI), increased cocoon weight and shell ratio (Ravikumar et al., 2015). The present findings corroborate

these observations, underscoring the pivotal role of *Bacillus* endophytes in mulberry–silkworm interactions.

Moreover, *Bacillus* spp. produce antimicrobial lipopeptides and endospores that suppress foliar pathogens and enhance plant resilience against biotic stress (Ravikumar *et al.*, 2015). Their natural abundance in elite mulberry varieties such as V1 highlights their potential as biological inputs for sustainable sericulture.

3.3. Ecological and Physiological Role of Fungal Endophytes in Mulberry

Fungal endophytes isolated in the present study belonged predominantly to *Aspergillus*, *Trichoderma* and *Fusarium*, each exhibiting distinct ecological functions. *Aspergillus* spp., isolated consistently from Mysore Local and DD varieties, exhibited septate hyphae and conidial spores typical of endophytic *Aspergillus*. Several studies have demonstrated that *Aspergillus* endophytes enhance phosphorus solubilization, nitrogen metabolism and secondary metabolite synthesis in host plants (Strobel & Daisy, 2003; Kavitha et al., 2011).

In mulberry, *Aspergillus* endophytes have been associated with increased leaf protein and carbohydrate accumulation, thereby improving the nutritional profile for silkworm feeding (Gowda et al., 2009; Zhang et al., 2010). Silkworms reared on such leaves exhibit faster larval growth, improved silk gland activity and superior cocoon parameters, including shell weight and filament denier (Zhang et al., 2010). These findings indicate that *Aspergillus* functions as a beneficial symbiont in the mulberry–silkworm system under non-pathogenic conditions.

3.4. Multifunctional Role of *Trichoderma* spp. in Mulberry–silkworm Systems

Trichoderma spp. were predominantly isolated from AR12, G2 and G4 varieties, with consistent colonisation in G2. *Trichoderma* is widely recognized as a multifunctional endophyte with strong biocontrol and growth-promoting capabilities (Harman et al., 2004; Bisset, 1991). Its ability to produce antifungal metabolites, hydrolytic enzymes and elicitors of induced systemic resistance makes it an effective symbiont in mulberry cultivation (Krishnaveni et al., 2014).

Colonization by *Trichoderma* enhances mulberry shoot biomass, leaf area and chlorophyll content, thereby increasing photosynthetic efficiency and leaf yield (Singh et al., 2011). These improvements translate into enhanced silkworm performance, as leaves with higher protein content and reduced anti-nutritional compounds improve digestibility and assimilation (Murugesh et al., 2012). Silkworms fed on Trichoderma-associated leaves show higher larval survival, reduced disease incidence and increased cocoon shell weight and filament length (Murugesh et al., 2012).

The natural presence of *Trichoderma* in elite mulberry varieties underscores its potential as a biological agent to

Table 2. Morphological and biochemical characterization of bacterial endophytes

Variety	Morphological character		Biochemical character		Tentative genus
	Cell shape	Motility	Gram staining	Catalase test	
Sahana (Leaf and Shoot)	Rod	Motile	Gram-positive	Positive	*Bacillus* spp.
S36 (Leaf and Shoot)	Rod	Motile	Gram-positive	Positive	*Bacillus* spp.
V1 (Leaf and Shoot)	Rod	Motile	Gram-positive	Positive	*Bacillus* spp.

Table 3. Characterization of fungal endophytes

Variety	Hyphal type	Spore type	Tentative genus
Mysore local (Leaf and Shoot)	Septate	Conidial spores	*Aspergillus* spp.
DD (Leaf and Shoot)	Septate	Conidial spores	*Aspergillus* spp.
AR12 (Leaf)	Septate	Conidia (round)	*Trichoderma* spp.
AR12 (Shoot)	Septate	Sickle-shaped	*Fusarium* spp.
G2 (Leaf and Shoot)	Septate	Conidia (round)	*Trichoderma* spp.
G4 (Leaf)	Septate	Conidia (round)	*Trichoderma* spp.
G4 (Shoot)	Septate	Sickle-shaped	*Fusarium* spp.

improve mulberry productivity and silk yield while reducing reliance on chemical pesticides.

3.5. Dualistic Behaviour of *Fusarium* spp. as Asymptomatic Endophytes

Fusarium spp. were isolated from shoot tissues of AR12 and G4 varieties without any visible disease symptoms. Although *Fusarium* is commonly regarded as a plant pathogen, several studies have demonstrated that many *Fusarium* strains behaviour under favourable conditions (Schulz & Boyle, 2005). Such strains may contribute to nutrient cycling and to the mild induction of host defence mechanisms.

In mulberry, endophytic *Fusarium* has been reported to enhance carbohydrate metabolism and nitrogen assimilation, though it may also induce slight increases in phenolic compounds (Murugesh et al., 2012). This dual effect can influence silkworm biology, a marginally prolonged larval duration due to increased phenolic digestion. Nevertheless, cocoon traits generally remain stable or improved, particularly shell weight and filament length (Murugesh et al., 2012).

The restricted distribution of *Fusarium* observed in the present study suggests tight host regulation, preventing

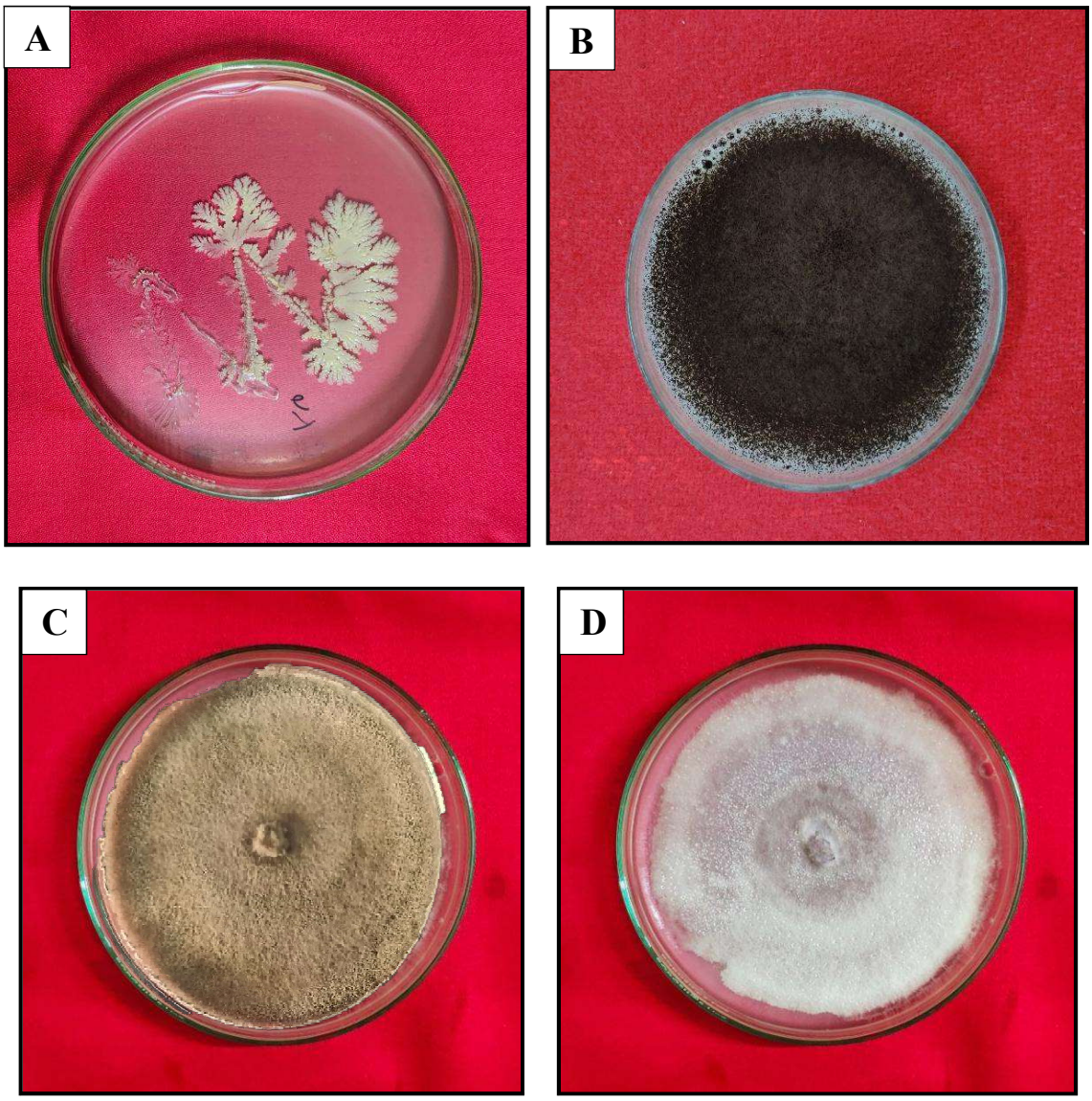


Figure 1. Morphology of endophytes:A. *Bacillus* spp., B. *Aspergillus* spp., C. *Trichoderma* spp., D. *Fusarium* spp.

pathogenic expression while allowing potential functional benefits.

Overall, the identification of bacterial and fungal endophytic isolates in the present study was carried out primarily based on morphological and cultural characteristics, including colony morphology, pigmentation, growth pattern and microscopic features using standard taxonomic keys. Molecular identification techniques such as 16S rRNA gene sequencing for bacterial isolates and ITS region sequencing for fungal isolates were not performed in the present investigation due to resource and scope limitations. This represents a methodological limitation of the study and the isolates were therefore characterised only up to the morphological level. This limitation has been duly acknowledged and should be considered when interpreting the taxonomic resolution of the reported endophytic diversity.

3.6. Implications for Sustainable Sericulture and Applied Microbiology

The present study clearly demonstrates that endophytic microorganisms play a crucial role in regulating mulberry growth, leaf quality and silkworm biology. Beneficial endophytes such as *Bacillus* and *Trichoderma* enhance mulberry physiology, improve resistance to biotic stress and indirectly promote silkworm growth and silk production. Even fungal endophytes with dual roles, such as *Aspergillus* and *Fusarium*, contribute positively when maintained in a balanced symbiotic state. Harnessing these naturally occurring endophytes represents a promising, eco-friendly strategy for sustainable sericulture. Integration of endophyte-based approaches can reduce dependence on chemical fertilizers and pesticides, enhance leaf quality, and improve economic returns to farmers, aligning with the principles of sustainable agriculture and applied microbiology (Vanitha, 2018; Hosamani et al., 2020).

In addition to endophytic interactions, there is also significant scope to explore the role of phyllosphere-associated microbial communities in shaping mulberry–silkworm performance. The phyllosphere microbiome, residing on the leaf surface, may influence leaf surface chemistry, nutrient dynamics and microbial-mediated modulation of plant metabolites, which directly affect silkworm feeding behaviour and physiological performance. These surface-associated microbial populations may also help maintain foliar health by suppressing opportunistic pathogens, thereby indirectly stabilising leaf quality and the nutritional consistency required for optimal silkworm development. However, the present investigation was limited to endophytic microorganisms, and phyllosphere microbial communities were not included in the analysis. Future integrated studies involving both endophytic and phyllosphere microbiomes, along with silkworm bioassays, would provide a more comprehensive understanding of plant–microbe–insect interactions in mulberry-based sericulture systems.

4. CONCLUSION

The present study highlights the ecological and functional significance of endophytic microorganisms in mulberry (*Morus* spp.) and their potential strengthening the mulberry–silkworm production system. The isolation of dominant bacterial and fungal endophytes from the genera Bacillus, Aspergillus, Trichoderma, and Fusarium from multiple mulberry varieties demonstrates that mulberry plants harbour a stable and diverse endophytic community with strong host- and tissue-specificity. The observed variation in endophytic colonization among traditional and improved mulberry varieties underscores the influence of host genotype and internal plant microenvironments in shaping endophyte diversity. The predominance of beneficial endophytes such as *Bacillus* and *Trichoderma*, in high-yielding mulberry varieties suggests their important role in enhancing plant growth, nutrient assimilation and resistance to biotic and abiotic stresses. These improvements in mulberry physiological status and leaf nutritional quality are likely to translate into better silkworm growth, survivability and cocoon traits, thereby reinforcing the close interdependence between plant-associated microbiota and silkworm biology. Even endophytes with dual ecological behaviour, such as *Aspergillus* and *Fusarium*, appear to contribute positively when maintained in a balanced, asymptomatic association within host tissues. Overall, the findings emphasize the potential of exploiting naturally occurring endophytic microorganisms as eco-friendly biological inputs for sustainable mulberry cultivation and sericulture. Integration of endophyte-based strategies can reduce reliance on chemical fertilizers and pesticides, improve leaf quality and enhance silk productivity while maintaining environmental safety. Future studies employing molecular identification, functional assays and silkworm bioassays will further strengthen the understanding of endophyte-mediated mechanisms and facilitate their practical application in sustainable sericulture systems.

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CRediT authorship contribution statement

Nitish Kumar, L.S.: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft preparation; **G. Sugeetha**: Conceptualization, Supervision, Methodology, Validation, Resources, Writing – review and editing; **Asha, N.N.**: Investigation, Data curation, Validation, Formal analysis, Writing – review and editing. All authors have read and approved the final version of the manuscript and agree to its submission for publication.

Conflict of interest

The authors declare no conflicts of interest, either financial or personal, that could have influenced the results or interpretation of this study.

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

During the preparation of this manuscript, AI-assisted writing tools were used solely for language editing, grammatical refinement, and improvement of sentence structure. The scientific content, data interpretation and conclusions were developed, verified and approved entirely by the authors. The use of AI tools did not involve the generation of scientific data, results or references. The authors take full responsibility for the accuracy and integrity of the content presented in this manuscript.

Data availability statement

The data generated and analysed during the current study are included within this manuscript. All datasets supporting the conclusions of this research, including isolation records and observational data on endophytic microbial diversity are available from the corresponding author upon reasonable request. The raw culture isolates are preserved in the laboratory collection and can be made available for further research purposes, subject to institutional guidelines and material transfer conditions.

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Enhancing Germination and Early Seedling Growth of Groundnut through Chemical, Growth Regulator and Hydrothermal Pod Treatments

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ABSTRACT: Groundnut (*Arachis hypogaea* L.) is an important oilseed and food legume crop widely grown in tropical and subtropical regions, where rapid and uniform crop establishment is essential for maximizing productivity under rainfed conditions. However, direct pod sowing often results in delayed germination and poor seedling establishment due to pod shell barriers and uneven water imbibition. This study evaluated the effects of chemical, hormonal and hydrothermal pre-sowing treatments on germination, seedling growth and vigour of groundnut under direct pod sowing conditions. The experiment was conducted in grow bags at the School of Agriculture, VISTAS, Pallavaram, Chennai, India, using a completely randomized design with eight treatments and three replications. The growing medium consisted of red loamy soil, river sand and well-decomposed farmyard manure (2:1:1 v/v/v). Treatments included sulphuric acid (10%), nitric acid (10%), potassium nitrate (1%), gibberellic acid (1%), hydrogen peroxide (1%), hot water (60°C), cold-water soaking and an untreated control. Although all treatments achieved 100% germination, significant differences were observed in germination time, seedling growth, biomass and vigour indices. Hydrogen peroxide (1%) soaking for 12 hours was the most effective treatment, reducing germination time and significantly improving seedling growth, biomass accumulation and seedling vigour. Hot- and cold-water soaking also enhanced early growth compared with the control, whereas acid treatments showed moderate effects and sulphuric acid was the least effective. The findings indicate that hydrogen peroxide-induced reactive oxygen species signaling promotes rapid germination and vigorous seedling establishment, making it a simple and effective pre-sowing treatment for improving direct pod sowing of groundnut under semi-arid conditions.

Keywords: Groundnut, Pod-sowing treatment, Hydrogen peroxide, Hydrothermal treatment, Germination, Seedling vigour.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed and food legume crop cultivated widely in tropical and subtropical regions. It is a major source of edible oil, protein, vitamins and minerals and contributes significantly to food security and livelihoods in semi-arid and rainfed ecosystems. India is one of the leading groundnut-producing countries, where successful crop establishment is essential for achieving higher productivity (2.13 t ha^{-1}). However, direct pod sowing often results in delayed germination and poor seedling establishment due to the hard pod shell, dormancy and slow water imbibition. Therefore, pre-sowing treatments such as chemical scarification, growth regulator soaking and hydrothermal treatments are widely used to improve germination and seedling growth (Copeland &

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McDonald, 2001; Finch-Savage & Bassel, 2016).

Seed germination is a complex physiological process involving imbibition, enzyme activation, mobilization of stored food reserves and cell elongation. Reactive oxygen species (ROS) act as important signalling molecules that regulate seed dormancy, metabolic activation and seedling establishment while also contributing to defence against pathogens (Bailly, 2004; Mittler et al., 2011; Wojtyla et al., 2022). Sulphuric acid and nitric acid enhance germination through scarification, whereas potassium nitrate acts as a nitrate signal to stimulate enzyme activity. Gibberellic acid (GA_3) promotes dormancy breaking by inducing hydrolytic enzymes such as α-amylase (Bewley et al., 2013). Hydrogen peroxide (H_2O_2) regulates antioxidant activity and hormonal balance, thereby improving germination, seedling growth and stress tolerance (Bailly, 2004; Mittler et al., 2011; Nadarajah, 2021). Hydrothermal treatments, including hot- and cold-water soaking, improve water uptake and activate metabolic processes, providing a simple and economical method for enhancing seedling vigour (Baidalina et al., 2025).

Although several studies have investigated seed priming in cereals and pulses, information on the comparative effects of acid scarification, growth regulator and hydrothermal treatments under direct pod sowing conditions in groundnut remains limited. Therefore, the present study was undertaken to evaluate the effectiveness of these pre-sowing treatments on germination, seedling growth, biomass accumulation and seedling vigour of groundnut under direct pod sowing conditions.

2. MATERIALS AND METHODS

2.1. Experiment Site, Crop Management and Measurement of Observations

The experiment was conducted during Kharif 2025 at the School of Agriculture, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. A tropical semi-arid climate with warm temperatures and moderate rainfall characterizes the experimental site. The soil used for the grow-bag experiment was red loamy soil: river sand: Well-decomposed farmyard yard manure (FYM) = 2: 1 : 1 (v/v/v).

Groundnut pods of variety TMV 14 were used for the study. The variety TMV 14 was selected because of its wider adaptability, uniform pod size and suitability for direct pod sowing under Tamil Nadu conditions. Healthy, uniform and mature pods were selected and used for direct pod sowing of intact pods.

Three pods were sown per bag at a depth of 3–4 cm. The experiment was maintained under regular watering and uniform environmental conditions to ensure proper germination and seedling growth. Observations on germination and seedling growth parameters were recorded up to 15 days after sowing.

2.2. Methodology and Pre-sowing Treatments

The experiment was laid out in a completely randomized design (CRD) comprising eight treatments with three replications. The groundnut pods were subjected to various chemical and physical pre-sowing treatments prior to sowing. The treatments included sulphuric acid (H_2SO_4) at 10% concentration for 5 min, nitric acid (HNO_3) at 10% concentration for 5 min, potassium nitrate (KNO_3) at 1% concentration for 12 hours of soaking, gibberellic acid (GA_3) at 1% concentration for 12 hours of soaking and hydrogen peroxide (H_2O_2) at 1% concentration for 12 hours of soaking.

Hydrothermal treatments included hot water treatment at 60°C for 5 min and cold-water soaking for 5 min. Untreated pods served as the control treatment. Following treatment application, acid-treated pods were thoroughly washed with distilled water and shade-dried before sowing.

2.3. Observations Recorded

Observations on germination and seedling growth parameters were recorded systematically. Germination percentage and the number of days to germination were recorded to assess the speed and uniformity of emergence. Seedling growth parameters such as root length, shoot length and total seedling length were measured in centimetres. The number of leaves per seedling was also recorded. Biomass parameters, including the fresh and dry weights of the shoot and root, were measured separately, and the total fresh and dry weights were computed.

Seedling vigour was evaluated using Vigour Index I and Vigour Index II as suggested by Abdul-Baki and Anderson (1973). Vigour Index I was calculated by multiplying germination percentage by total seedling length, and Vigour Index II was calculated by multiplying germination percentage by seedling dry weight.

The magnitude of inhibition or stimulation was evaluated using response index (RI) as described by Williamson and Richardson (1988) using the following formula:

If $T > C$, $RI = 1 - (C/T)$

If $T = C$, $RI = 0$

If $T < C$, $RI = (T/C) - 1$

where T is the treatment mean, and C is the control mean.

2.4. Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with three replications. The experimental data were subjected to analysis of variance (ANOVA) using the AGRES Statistical Software Package (Version 7.01) following the procedure described by Gomez and Gomez (1984). Treatment means were compared at the 5% level of significance (P = 0.05), and the standard error of difference (SEd) and critical difference (CD) were calculated to determine the significance of treatment effects.

3. RESULTS AND DISCUSSION

The present study revealed that chemical and physical pre-sowing treatments significantly influenced germination behaviour, seedling growth, biomass accumulation and vigour of groundnut under direct pod-sowing conditions. Although all treatments recorded 100% germination, substantial differences were observed in the speed of germination, seedling development and vigour indices, indicating that the treatments primarily enhanced physiological performance and seedling establishment rather than final germination percentage. Similar findings were reported in seed priming studies where pre-sowing treatments accelerated metabolic activity and improved seedling vigour without altering germination percentage (Ashraf & Foolad, 2005; Farooq et al., 2019). The observed improvement in seedling establishment has ecological significance under semi-arid and rainfed agro-ecosystems where rapid germination and vigorous early growth are essential for crop survival and resource-use efficiency (Ndlovu et al., 2023).

3.1. Germination and Days to Germination

Significant variation was observed in the number of days required for germination among the treatments (Table 1). Hydrogen peroxide (H_2O_2) 1% soaking (T_5) and nitric acid treatment (T_2) resulted in the earliest germination (7 days), whereas untreated control pods required 12 days for germination. Faster germination in chemically treated pods may be attributed to enhanced water imbibition, activation of hydrolytic enzymes and stimulation of embryo growth. Hydrogen peroxide functions as a reactive oxygen species (ROS) signalling molecule regulating seed dormancy release, cellular metabolism and mitochondrial respiration

Table 1. Effect of pre-sowing treatments on germination and seedling growth of groundnut under direct pod sowing

Treatments	Germination (%)	Number of days to germination	Root length (cm)	Shoot length (cm)	Total length (cm)	No. of leaves	Vigour index-I
T1 - Sulphuric Acid (H_2SO_4) (10%) treatment of Groundnut Pod (5 minutes)	100	8.00	1.03	1.60	2.63	1.06	263
T2 - Nitric Acid (HNO_3) (10%) treatment of Groundnut Pod (5 minutes)	100	7.00	4.60	6.30	15.00	5.16	1500
T3 - Potassium Nitrate (KNO_3) (1%) treatment of Groundnut Pod (12 hours soaking)	100	8.00	1.37	3.00	4.37	1.08	437
T4 - Gibberellic Acid (GA_3) (1%) treatment of Groundnut Pod (12 hours soaking)	100	10.00	2.40	4.60	7.00	2.01	700
T5 - Hydrogen Peroxide (H_2O_2) (1%) treatment of Groundnut Pod (12 hours soaking)	100	7.00	5.90	17.30	23.00	6.51	2300
T6 - Hot Water (60°C) treatment of Groundnut Pod (5 minutes)	100	9.00	2.60	12.58	20.03	5.46	2000
T7 - Cold Water treatment of Groundnut Pod (5 minutes)	100	9.00	2.70	16.60	21.06	6.08	2100
T8 - Control (intact pod)	100	12.00	0.98	1.52	2.50	0.95	250
SEd (±)	NS / NA	0.21	0.07	0.19	0.25	0.08	25.63
CD (P=0.05)	NS / NA	0.46	0.16	0.37	0.57	0.18	58.90

NS – Non-significant due to absence of variation among treatments.
NA – Not applicable.

during germination (Bailly, 2004). Improved germination speed under H_2O_2 treatment may therefore be associated with enhanced energy metabolism and cellular activation leading to rapid radicle emergence. Hydrogen peroxide at optimum concentration functions as a signalling molecule that activates respiratory metabolism, ATP synthesis and antioxidant defence systems during the early phase of germination. It also promotes the activity of hydrolytic enzymes such as α-amylase and proteases, which facilitate rapid mobilization of stored food reserves for embryo growth. Furthermore, H_2O_2-mediated loosening of cell walls and regulation of hormonal balance between abscisic acid and gibberellins enhance cell elongation and radicle protrusion, thereby accelerating seedling emergence and establishment. Similar promotory effects of H_2O_2 on germination metabolism, ROS signalling and early seedling growth were reported by Barba-Espín et al. (2011); Hasanuzzaman et al. (2020); Nadarajah (2021); Wojtyla et al. (2022).

Hydrothermal treatments also reduced germination duration compared to the control. Softening of the pod shell through hot and cold water soaking possibly improved moisture penetration and gas exchange, thereby accelerating metabolic activity during germination. Early germination under direct pod sowing conditions is particularly important in rainfed ecosystems because rapid establishment enables seedlings to escape early-season moisture stress and enhances crop competitiveness under marginal environments (Figure 1).

3.2. Seedling Growth Parameters

Pre-sowing treatments significantly improved root length, shoot length and total seedling length compared to untreated pods (Table 1). Among the treatments, hydrogen peroxide soaking (T_5) recorded the highest root length (5.90 cm), shoot length (17.30 cm) and total seedling length (23.00 cm), followed by cold water treatment (T_7) and hot water treatment (T_6). The control treatment recorded the lowest seedling growth. Enhanced seedling growth under H_2O_2 treatment may be due to increased cell division, cell elongation and efficient mobilization of seed reserves during germination. Hydrogen peroxide is known to interact with



Figure 1. Influence of chemical and physical pre-sowing treatments on seedling growth and root development of groundnut (*Arachis hypogaea* L.) under direct intact pod sowing. Representative seedlings were photographed after germination to illustrate the effects of different treatments on shoot and root growth. Treatments were: T_1 - Sulphuric acid (H_2SO_4, 10%) for 5 min, T_2 - Nitric acid (HNO_3, 10%) for 5 min, T_3 - Potassium nitrate (KNO_3, 1%) for 12 h soaking, T_4 - Gibberellic acid (GA_3, 1%) for 12 h soaking, T_5 - Hydrogen peroxide (H_2O_2, 1%) for 12 h soaking, T_6 - Hot water (60°C) for 5 min, T_7 - Cold water for 5 min, and T_8 - Untreated intact pod (control). The photograph provides a visual comparison of treatment effects on early seedling establishment, demonstrating enhanced shoot and root development in H_2O_2-treated pods compared with the other treatments

phytohormonal pathways involving gibberellins and auxins, thereby promoting seedling growth and tissue differentiation (Barba-Espín et al., 2011).

Improved seedling growth under hydrothermal treatments indicates that physical softening of the pod shell enhanced hydration and activated metabolic enzymes involved in reserve mobilization. Improved root proliferation under hydrothermal and priming treatments may be attributed to enhanced water imbibition, activation of metabolic enzymes and stimulation of cellular division and elongation during early seedling development. Enhanced root growth enables seedlings to explore a greater soil volume for water and nutrient acquisition, thereby improving drought adaptation and establishment under semi-arid conditions (Hasanuzzaman et al., 2020). Hydrothermal treatments may also soften the pod shell and facilitate rapid activation of physiological processes associated with germination and root differentiation. In addition, seed priming treatments are known to enhance membrane repair, antioxidant activity and osmotic adjustment, which collectively contribute to improved seedling vigour and stress tolerance. Similar improvements in seedling growth, root development and early vigour due to hydrothermal and priming treatments were reported by Ndlovu et al. (2023) and Sabongari & Aliero (2024).

3.3. Number of Leaves and Biomass Production

The number of leaves and biomass production were significantly influenced by pre-sowing treatments (Table 2). Hydrogen peroxide treatment recorded the highest number of leaves, fresh biomass and dry biomass accumulation, followed by cold water and hot water treatments. Increased biomass accumulation indicates improved photosynthetic activity, efficient nutrient mobilization and enhanced physiological performance of seedlings. Enhanced dry matter production under H_2O_2 treatment may be associated with improved antioxidant enzyme activity and reduced oxidative damage during early seedling development (Gill & Tuteja, 2010).

Biomass accumulation during the early establishment phase is an important ecological indicator because vigorous seedlings possess greater adaptive capacity under fluctuating environmental conditions. Treatments that improved biomass production may therefore contribute to

Table 2. Effect of pre-sowing treatments on biomass and vigour index-ii of groundnut under direct pod sowing

Treatments	Fresh weight of shoot (g)	Dry weight shoot (g)	Fresh weight of root (g)	Dry weight root (g)	Total fresh weight (g)	Total dry weight (g)	Vigour index-II
T1 - Sulphuric Acid (H_2SO_4) (10%) treatment of Groundnut Pod (5 minutes)	0.10	0.07	0.30	0.23	0.40	0.30	30
T2 - Nitric Acid (HNO_3) (10%) treatment of Groundnut Pod (5 minutes)	0.36	0.21	0.53	0.30	0.80	0.52	52
T3 - Potassium Nitrate (KNO_3) (1%) treatment of Groundnut Pod (12 hours soaking)	0.50	0.13	0.36	0.29	0.94	0.42	42
T4 - Gibberellic Acid (GA_3) (1%) treatment of Groundnut Pod (12 hours soaking)	0.61	0.20	0.66	0.52	1.34	0.72	72
T5 - Hydrogen Peroxide (H_2O_2) (1%) treatment of Groundnut Pod (12 hours soaking)	1.58	0.61	1.18	0.90	2.65	1.90	190
T6 - Hot Water (60°C) treatment of Groundnut Pod (5 minutes)	1.46	0.58	1.16	0.55	1.38	1.14	114
T7 - Cold Water treatment of Groundnut Pod (5 minutes)	1.50	1.02	1.20	0.82	2.52	1.84	184
T8 - Control (intact pod)	0.10	0.07	0.29	0.22	0.38	0.29	29
SEd	0.02	0.01	0.02	0.01	0.03	0.02	1.88
CD (P=0.05)	0.04	0.02	0.03	0.02	0.06	0.04	4.22

improved field establishment and sustainable crop productivity under semi-arid agro-ecosystems.

3.4. Vigour Index I and II

Seedling vigour indices were significantly enhanced by pre-sowing treatments (Table 1 and Table 2). Hydrogen peroxide treatment recorded the highest Vigour Index I (2300) and Vigour Index II (190), followed by cold water and hot water treatments. The untreated control recorded the lowest vigour indices. Higher vigour indices indicate improved seedling establishment potential, faster growth and greater biomass accumulation. Vigour indices are considered reliable indicators of seed quality and field establishment performance under stress-prone environments (Abdul-Baki & Anderson, 1973). The enhanced vigour indices observed under hydrogen peroxide treatment may be attributed to improved metabolic activation, rapid mobilization of seed reserves and enhanced antioxidant defence during germination and early seedling growth. Increased seedling length and biomass accumulation contributed directly to higher vigour index values, indicating superior physiological performance and establishment potential. Hydrogen peroxide-mediated ROS signalling may have stimulated cellular respiration, enzyme activity and hormonal regulation, thereby promoting Vigourous seedling development. Similarly, hydrothermal treatments such as hot water and cold-water soaking improved vigour by facilitating rapid water imbibition and activation of metabolic processes associated with germination. Enhanced seedling vigour is particularly important under rainfed and semi-arid agro-ecosystems, where rapid establishment and early biomass accumulation improve stress tolerance and resource-use efficiency. Similar improvements in seedling vigour due to seed priming and hydrothermal treatments were reported by Ndlovu et al. (2023) and Wojtyla et al. (2022).

Enhanced vigour under H_2O_2 treatment may be associated with ROS-mediated signalling mechanisms that regulate antioxidant defence systems and cellular growth processes. Improved vigour under hydrothermal treatments further demonstrates the potential of low-cost and eco-friendly pre-sowing techniques for enhancing crop establishment in resource-limited farming systems.

3.5. Effect of Acid and Growth Regulator Treatments

Nitric acid treatment significantly improved seedling growth and vigour compared to the control. Acid scarification may have facilitated improved water absorption and oxygen diffusion through partial weakening of the pod shell, resulting in enhanced germination and seedling growth. Potassium nitrate treatment also improved seedling performance due to nitrate-mediated signalling involved in dormancy release and enzyme activation. Gibberellic acid treatment promoted seedling growth through enhanced synthesis of hydrolytic enzymes responsible for mobilization of stored food reserves (Finch-Savage & Leubner-Metzger, 2006).

However, sulphuric acid treatment showed comparatively lower growth and vigour than other treatments. Reduced performance under sulphuric acid treatment may be due to excessive scarification causing slight injury to embryonic tissues. Similar observations regarding reduced seedling growth under strong acid treatments were reported by Copeland and McDonald (2001).

3.6. Response Index of Pre-sowing Treatment of Pod Sowing of Groundnut

The response index (RI) analysis clearly demonstrated the stimulatory effects of pre-sowing treatments on growth and vigour parameters of groundnut (Table 3). Negative RI values for days to germination indicated beneficial reduction in germination duration compared to the control. Hydrogen peroxide and nitric acid treatments recorded the highest reduction in germination duration, confirming their effectiveness in promoting rapid seedling emergence.

Positive RI values observed for root length, shoot length, biomass production and vigour indices indicated strong stimulatory effects of the treatments on seedling development. Hydrogen peroxide treatment recorded the highest positive RI values for most growth parameters, followed by cold water and hot water treatments (Wojtyla et al., 2022). These findings demonstrate that both chemical signalling mechanisms and hydrothermal conditioning effectively enhanced physiological activity and seedling vigour. Hydrogen peroxide-mediated ROS signalling may have stimulated antioxidant defence, respiratory metabolism and hormonal regulation, thereby enhancing seedling establishment and biomass accumulation. Similarly, hydrothermal conditioning likely improved water imbibition, membrane repair and metabolic activation during germination, resulting in improved physiological performance and vigour. Enhanced response index values observed in the present study therefore indicate strong stimulatory effects of the treatments on early seedling

Table 3. Response index (RI) of pre-sowing treatments on growth and vigour parameters of groundnut

Treatments	Number of days to germination	Root length (cm)	Shoot length (cm)	Total length (cm)	No of leaves	Fresh weight of shoot (g)	Dry weight shoot(g)	Fresh weight of root (g)	Dry weight root (g)	Total fresh weight (g)	Total dry weight (g)	Vigour index-I	Vigour index-II
T1 - Sulphuric Acid (H_2SO_4) (10 %) treatment of Groundnut Pod (5 minutes)	-0.33	0.05	0.05	0.05	0.12	0.00	0.00	0.03	0.05	0.05	0.03	0.05	0.03
T2 - Nitric Acid (HNO_3) (10 %) treatment of Groundnut Pod (5 minutes)	-0.42	0.79	0.76	0.83	0.82	0.72	0.67	0.45	0.27	0.53	0.44	0.83	0.44
T3 - Potassium Nitrate (KNO_3) (1 %) treatment of Groundnut Pod (12 hours soaking)	-0.33	0.28	0.49	0.43	0.12	0.80	0.46	0.19	0.24	0.60	0.31	0.43	0.31
T4 - Gibberellic Acid (GA_3) (1 %) treatment of Groundnut Pod (12 hours soaking)	-0.17	0.59	0.67	0.64	0.53	0.84	0.65	0.56	0.58	0.72	0.60	0.64	0.60
T5 - Hydrogen Peroxide (H_2O_2) (1 %) treatment of Groundnut Pod (12 hours soaking)	-0.42	0.83	0.91	0.89	0.85	0.94	0.89	0.75	0.76	0.86	0.85	0.89	0.85
T6 - Hot Water (60°C) treatment of Groundnut Pod (5 minutes)	-0.25	0.62	0.88	0.88	0.83	0.93	0.88	0.75	0.60	0.72	0.75	0.88	0.75
T7 - Cold Water treatment of Groundnut Pod (5 minutes)	-0.25	0.64	0.91	0.88	0.84	0.93	0.93	0.76	0.73	0.85	0.84	0.88	0.84
T8 - Control (intact pod)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

development and stress adaptation under direct pod sowing conditions. Similar response index interpretations and improvements in seedling vigour due to priming and hydrothermal treatments were reported in seed physiology and stress adaptation studies by Ndlovu et al. (2023), in addition to the classical interpretation proposed by Williamson & Richardson (1988).

Among the treatments, hydrogen peroxide (T_5) recorded the highest positive response index values for root length, shoot length, total seedling length, number of leaves, biomass and vigour indices, indicating its strong promotory effect on seedling growth and vigour. Cold water (T_7) and hot water (T_6) treatments also recorded high RI values, showing that hydrothermal treatments significantly improved seedling growth by enhancing water imbibition and metabolic activity (Figure 2). Nitric acid and gibberellic acid treatments showed moderate stimulatory effects, while potassium nitrate showed comparatively lower response. Sulphuric acid treatment recorded very low RI values, indicating minimal improvement over control (Bailly, 2004). Overall, the response index analysis confirmed that hydrogen peroxide treatment was the most effective, followed by cold water and hot water treatments for improving groundnut seedling vigour under direct pod sowing conditions. These findings agree with earlier studies on seed priming and ROS-mediated enhancement of seedling growth and vigour (Farooq et al., 2019; Mittler et al., 2011).

3.7. Ecological Relevance and Practical Implications

The study demonstrated that pre-sowing treatments significantly improved seedling establishment and early vigour of groundnut under direct pod sowing conditions. Rapid germination, improved root growth and higher biomass accumulation are particularly important under semi-arid agro-ecosystems where crops frequently encounter soil moisture deficit during establishment. Among the treatments evaluated, hydrogen peroxide soaking (1% for 12 hours) proved most effective, followed by cold water and hot water treatments.

The improvement in seedling growth and vigour observed under hydrogen peroxide and hydrothermal treatments aligns with the findings of Baidalina et al. (2025), who reported enhanced crop establishment and productivity following pre-sowing seed treatment and growth stimulant application.

Hydrothermal treatments are low-cost, eco-friendly and easily adoptable by farmers without requiring sophisticated

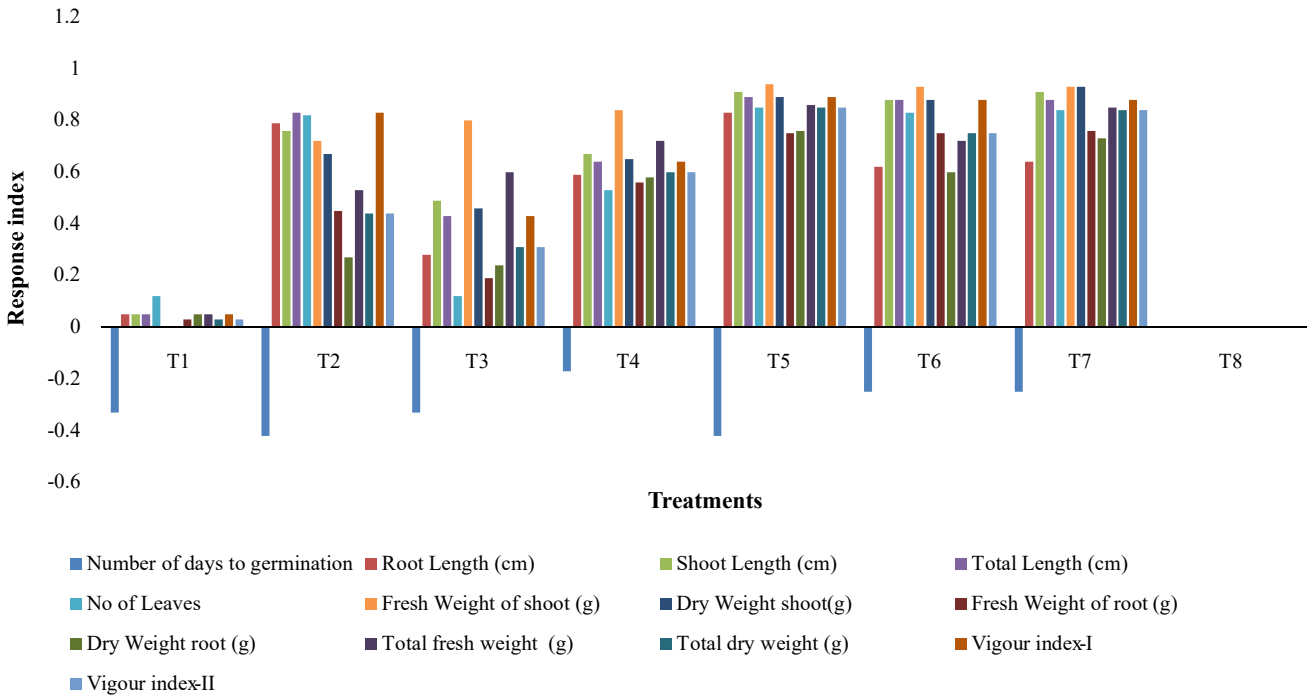


Figure 2. Response Index (RI) showing the influence of different pre-sowing treatments on germination rate, seedling growth, and seedling vigour of direct-sown intact pod groundnut.T_1 - Sulphuric acid (H_2SO_4, 10%) for 5 min, T_2 - Nitric acid (HNO_3, 10%) for 5 min, T_3 - Potassium nitrate (KNO_3, 1%) for 12 h soaking, T_4 - Gibberellic acid (GA_3, 1%) for 12 h soaking, T_5 - Hydrogen peroxide (H_2O_2, 1%) for 12 h soaking, T_6 - Hot water (60°C) for 5 min, T_7 - Cold water for 5 min, and T_8 - Untreated intact pod (control). The Response Index (RI) was calculated relative to the untreated control (T_8), where positive RI values indicate enhancement, negative values indicate inhibition, and zero indicates no change. Higher positive RI values represent greater improvement in germination rate, seedling growth, and seedling vigour resulting from the respective pre-sowing treatment

inputs, thereby making them suitable for sustainable ecological crop management. The findings of the present study therefore align with the scope of the Indian Journal of Ecology by emphasizing sustainable agricultural practices, ecological crop establishment and resource-efficient technologies suitable for rainfed farming systems.

4. CONCLUSION

The present study demonstrated that pre-sowing treatments significantly improved seedling establishment, growth and vigour of groundnut under grow bag conditions of direct intact pod sowing. Among the treatments, 1% hydrogen peroxide (H_2O_2) soaking for 12 hours was the most effective, recording the earliest germination and the highest seedling growth, biomass accumulation, and vigour indices. Cold water and hot water soaking also enhanced seedling establishment, while nitric acid showed moderate improvement and sulphuric acid was comparatively less effective. Based on the overall performance, 1% H_2O_2 soaking for 12 hours is recommended as an effective pre-sowing treatment for direct pod sowing of groundnut, with hydrothermal treatments serving as simple, economical and eco-friendly alternatives for improving crop establishment under rainfed conditions.

Credit authorship contribution statement

Anbarasu Mariyappillai: Conceptualization, Methodology, Supervision, Writing – review & editing; **Aaron Aarputharaj Arputham:** Investigation, Data curation, Formal analysis, Writing – original draft; **Deepa Raju:** Investigation, Data curation, Writing – original draft; **Mageshwarman Keerthi**: Formal analysis, Validation, Visualization; **Srikantha Jayapal:** Resources, Data curation, Review & editing; **Sriram Padmanaban:** Methodology, Software, Formal analysis; **Yamini Priya Boobalan:** Writing – review & editing, Validation.

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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 that AI tools were used only for language editing during manuscript preparation. All scientific content and conclusions are solely the responsibility of the authors.

Data availability statement

The data generated and analysed during the current study are available from the corresponding author upon reasonable request.

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Functional Group Dynamics and Community Composition of Forests in the Northwestern Himalayan Foothills in Haryana, India

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ABSTRACT: Forest ecosystems in the north-western Himalayan Shivalik foothills exhibit high environmental heterogeneity, resulting in complex patterns of species composition, vegetation structure, and ecological functioning. The present study investigated floristic composition, vegetation structure, and functional group dynamics across six forest types in the Yamunanagar (YFD) & Panchkula (PFD) Forest Divisions of Haryana, and assessed species diversity, community organisation, and population structure. A total of 137 plant species belonging to 61 families were recorded. Poaceae was the most species-rich family, followed by Asteraceae and Fabaceae. Dominance–diversity analysis identified *Shorea robusta* C.F. Gaertn. and *Mallotus philippensis* (Lam.) Müll. Arg., *Senegalia catechu* (L.f.) P.J.H. Hurter &Mabb., and *Pinus roxburghii* Sarg. as the dominant tree species across different forest types, while *Lantana camara* L. consistently dominated as the shrub layer. *Heteropogon contortus* (L.) P. Beauv. and *Achyranthes aspera* L. were the most abundant herb species, whereas *Ichnocarpus frutescens* (L.) W.T. Aiton and *Pueraria tuberosa* (Roxb. ex Willd.) DC. predominated among climbers. The Shannon–Wiener diversity index (H′) for trees ranged from 1.237 in subtropical pine forests to 2.510 in mixed deciduous forests of PFD, while Margalef's species richness index varied from 1.744 to 7.440. The concentration of dominance (Cd) ranged from 0.103 to 0.455, with lower values in mixed deciduous forests indicating greater species evenness. Dominance–diversity curves exhibited a log-normal distribution, reflecting balanced niche partitioning. The study demonstrates moderate to high species diversity and considerable compositional heterogeneity among forest types. These findings provide a valuable ecological baseline for long-term biodiversity monitoring, in the north-western Himalayan foothills.

Keywords: Phytodiversity, Community composition, Abundance, Species richness, Diversity indices.

1. INTRODUCTION

Forests are among the richest and most complex terrestrial ecosystems, supporting a variety of life forms and possessing a tremendous intrinsic ability for self-maintenance. Tropical dry deciduous forests occupy approximately 38–41% of total forest area throughout India (Dixit, 1997; ISFR, 2021). Tropical forests are often referred to the most species-diverse terrestrial ecosystems and provide a variety of natural resources to help sustain the livelihoods of local communities (Kumar et al., 2006). These forests are disappearing at alarming rates due to deforestation for the extraction of timber and other forest products (Murphy & Lugo, 1986; Raghubanshi & Tripathi,

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2009; Kushwaha et al., 2018; Bhuyan et al., 2025; Mesa-Sierra et al., 2025). However, many of these forests are losing their resilience due to excessive biotic interference, such as anthropogenic disturbances and uncontrolled grazing. India is a large developing country known for its diverse forest ecosystems and biodiversity. According to the Global Forest Resource Assessment (GFRA, 2020) published by the FAO, India is ranked among the top 10 countries in the world in terms of forest area and holds the 3rd position for the highest annual net gain in forest cover between 2010 and 2020 (ISFR, 2023). The floristic composition of vegetation and species diversity reflect the gene pool and adaptation potential of a community (Odum, 1971). Many tropical forests are under great anthropogenic pressure and require management interventions to maintain overall biodiversity, productivity, and sustainability. Understanding species diversity and distribution patterns is important to help managers evaluate the complexity and resources of these forests. Quantitative floristic inventories have been used in recent years to characterize forest vegetation throughout the tropics (Johnston & Gillman, 1995; Kumar et al., 2011; Condit, 1996; Pascal & Pelissier, 1996; Ayyappan & Parthasarathy, 1999; Parthasarathy, 1999, 2001; Phillips et al., 2003; Kushwaha & Nandy, 2012; Mansingh et al., 2025; Chovatiya et al., 2025). Without data, it is impossible to test these competing claims, and it is imperative that tropical forests, which are rich in biological diversity, be studied intensively to investigate the complex interactions between biodiversity and ecosystem functioning.

With nearly 205250 villages classified as forest-adjoining, there is obviously a huge dependence of communities on forest resources (ISFR, 2021). Forest degradation is usually accompanied by species extinction, loss of biodiversity and decreasing primary productivity. Consequently, there is growing interest in quantifying habitat characteristics, i.e. forest structure, floristic composition, and species richness in Indian forests (Kumar et al., 1999, 2001, 2002; Gupta & Kumar, 2014; Singh et al., 2016; Yadav & Yadav, 2005; Chovatiya et al., 2025). In addition to sustaining biodiversity, tropical dry deciduous forests play a crucial role in climate regulation through carbon sequestration. However, anthropogenic disturbances may not only alter species composition but also influence carbon storage and greenhouse gas emissions, thereby linking biodiversity dynamics with climate change processes to develop and implement adaptation strategies both for biodiversity conservation and protection and for safe guarding the livelihoods of forest dependent people (Bonan, 2008; Pan, et al., 2011; Chaturvedi et al., 2011; Fisher et al., 2014; Gupta & Kumar, 2014; IPCC, 2022; Mansingh et al., 2025).

This study presents a detailed analysis of plant species richness, abundance, and diversity across different physiognomic units in the Shivalik landscape of the north-western Himalaya in Haryana, India, based on qualitative and quantitative analyses. The present study focuses specifically on the tropical dry deciduous forests of Haryana, which are a relatively underexplored region in terms of detailed ecological and floristic assessments. It is an effort to go beyond traditional species inventories by linking floristic diversity with functional attributes**,** thereby contributing to a deeper understanding of how biodiversity influences ecosystem processes and sustainability. In light of increasing anthropogenic pressures and the growing need for sustainable resource management, it is imperative to conduct comprehensive, data-driven studies on tropical forest ecosystems. By integrating floristic diversity, functional attributes, and disturbance dynamics, the present study aims to unravel the complex interactions that govern ecosystem structure and functioning in tropical dry deciduous forests of Haryana. Such insights are essential for developing effective conservation strategies and ensuring the long-term sustainability of these ecologically and economically vital ecosystems.

2. MATERIALS AND METHODS

2.1. Study Area

The study was performed in Shivalik ranges, the foot hills of North-western Himalaya, India located at 30° 00′ 00″ to 30° 30′ 00″N latitude and 70° 00′ 00″ to 77° 00′ 45″E longitudes in forests of Kalesar National Park in Yamunanagar Forest Division (YFD) and between longitude 76° 46′ 40″ to 77° 0′ 33″E and latitude 30° 40′50″ to 30° 54′ 40″N in reserved and protected forests of Panchkula Forest Divisions (PFD) (Figure 1). The altitude varies from 300 to 650 msl at Kalesar forest and 370 to 1100 m at Morni hills. The climate of the area is subtropical monsoonal. Average annual rainfall is1437 mm in Panchkula and 973mm in Yamunanagar district (HARSAC/CRID, Govt. of Haryana 2021a, 2021b). The Shivaliks are composed of alluvial detritus of the solidified and upheaved detritus of the great Himalayan range (Wadia, 1961) in northern India.

2.2. Sampling Design

A vegetation study was conducted in a stratified random manner in six forest types (Champion & Seth, 1968) covering Northern tropical dry deciduous *Shorea robusta* C.F. Gaertn. plain forests, Northern tropical dry deciduous Shivalik *Shorea robusta* C.F. Gaertn. forests, Mixed dry deciduous forests in YFD, Dry deciduous forests, Mixed dry deciduous forests, and Sub-tropical pine forests in PFD. Sampling was done in all the strata, i.e., trees, shrubs, & herbs. Twelve plots of 0.1ha for trees in each of the forest types, twenty-four plots of 5×5m for shrubs, and sixty plots of 1×1m for herbs were sampled within each forest type ecosystem using a stratified random sampling approach (Figure 2). Within each plot, saplings and shrubs were identified to species, and the number of individuals of each species was counted.

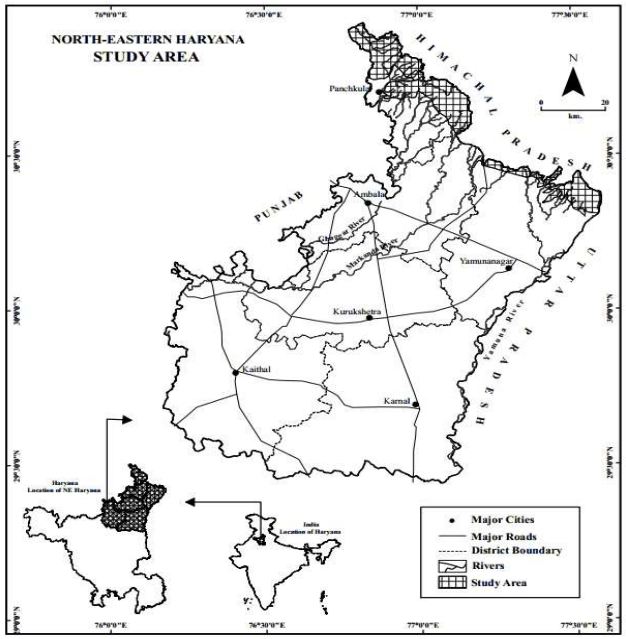


Figure 1. Location of the study site in Haryana. **(**The map has been prepared using ArcGIS software with appropriate Latitude/longitude demarcation of the studied sites)

2.3. Vegetation Functional Group Sampling

The sampling design was to collect field data based on vegetation functional group type strata, i.e., trees, shrubs, herbs, and climbers, to collect macro-habitat-specific relative species abundance information along with other ecologically important variables. Hence, sampling was aimed at addressing the basic issues related to composition, structure, and function through appropriate distribution and intensity across spatial and temporal gradients at the landscape level. For season-specific vegetation, based on the field phenology, sampling was undertaken during rainy, winter, and summer seasons in order to cover the maximum number of occurrences.

2.4. Data Analysis

The collected data were quantitatively analyzed for density, frequency, and abundance according to (Curtis & McIntosh, 1951). Relative frequency, relative density, and relative dominance were calculated following (Phillips, 1959), and the sum of these numbers yielded the importance value index (IVI) of tree species following Curtis (1959). The abundance-to-frequency ratio (A/F) of different species

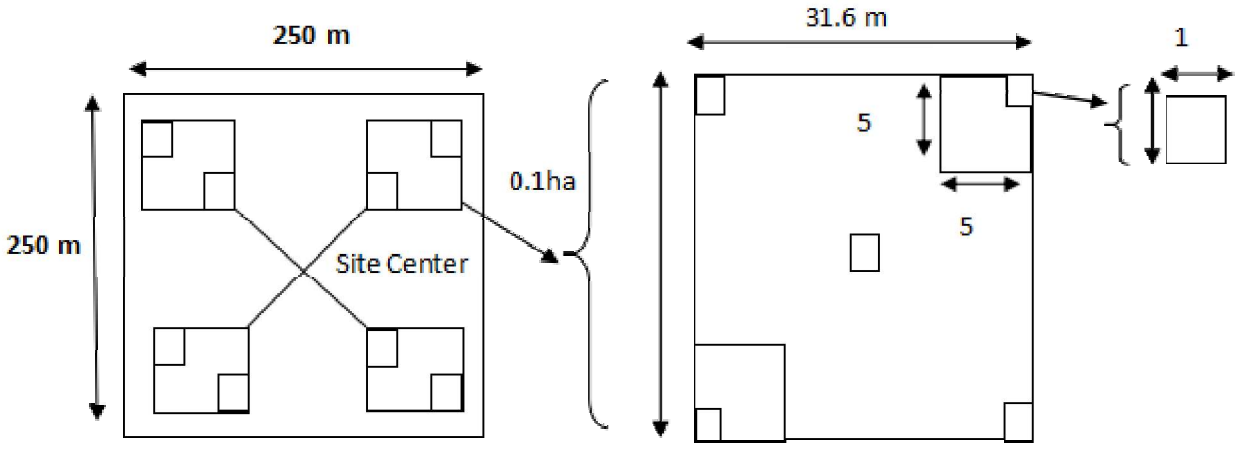


Figure 2. Stratified clustered sampling design. (The figure shows the stratified clustered sampling framework used to ensure representative sampling across different functional groups of the study area)

was computed to assess their distribution pattern of following Whitford (1949). The dominance – diversity curves of species of each site were plotted using log values of IVI and species sequence using Magurran (1988). Shannon-Wiener index of species diversity was computed for various plant species using equation 1. Concentration of dominance (C) was measured using equation 2. Equitability (e) was calculated following equation 3 and Margalef's index of Species richness (d) was calculated using equation 4.

Equation 1: Shannon–Wiener Diversity Index (Shannon & Weaver, 1949)

$$H' = -\sum_{\{i=1\}}^{\{S\}(\backslash frac\ \{N_i\}\{N\})\backslash ln(\backslash frac\ \{N_i\}\{N\})}$$

Equation 2: Simpson's Concentration of Dominance (Simpson, 1949)

$$C = \backslash sum_{\{i=1\}}^{\{S\}\backslash left(\backslash frac\ \{N_i\}\{N\}\backslash right)^2}$$

Equation 3: Pielou's Equitability (Evenness) Index (Pielou, 1966)

$$E = \backslash frac\{H'\}\{\backslash ln\ S\}$$

Equation 4: Margalef's Species Richness Index (Margalef, 1958)

$$D = \backslash frac\{S-1\}\{\backslash ln\ N\}$$

3. RESULTS AND DISCUSSION

3.1. Floristic Composition and Vegetation Structure

The floristic composition and vegetation structure of the studied forest ecosystems, northern tropical dry deciduous plain *Shorea robusta* C.F. Gaertn. forests, northern tropical dry deciduous Shivalik *Shorea robusta* C.F. Gaertn. forests, mixed deciduous forests, dry deciduous forests, and subtropical pine forests revealed considerable spatial heterogeneity across the Yamunanagar Forest Division (YFD) and Panchkula Forest Division (PFD). The vegetation was stratified into four functional layers (trees, shrubs, herbs, and climbers), reflecting the characteristic patterns of tropical and subtropical forests (Figure 3).

A total of 137 plant species were recorded, including 82 species (38 families) in YFD and 111 species (51 families) in PFD, indicating moderately high species diversity (Table 1 & 2). This level of diversity aligns with earlier studies (Dixit, 1997; Kumar et al., 1999, 2001; Murphy & Lugo, 1986; Raghubanshi & Tripathy, 2009) in tropical dry deciduous forests, where environmental heterogeneity and resource variability promote species coexistence.

The dominance of families such as Poaceae, Asteraceae, and Fabaceae highlights the ecological significance of grasses and forbs in ground vegetation, particularly in nutrient cycling and soil stabilisation. The presence of several families represented by a single species suggests niche specialisation and uneven species distribution within these ecosystems. Mixed deciduous forests exhibited the highest species richness in both divisions, supporting the intermediate disturbance hypothesis, which suggests that moderate levels of disturbance enhance species diversity. In contrast, subtropical pine forests showed comparatively lower tree diversity but higher herbaceous diversity, likely

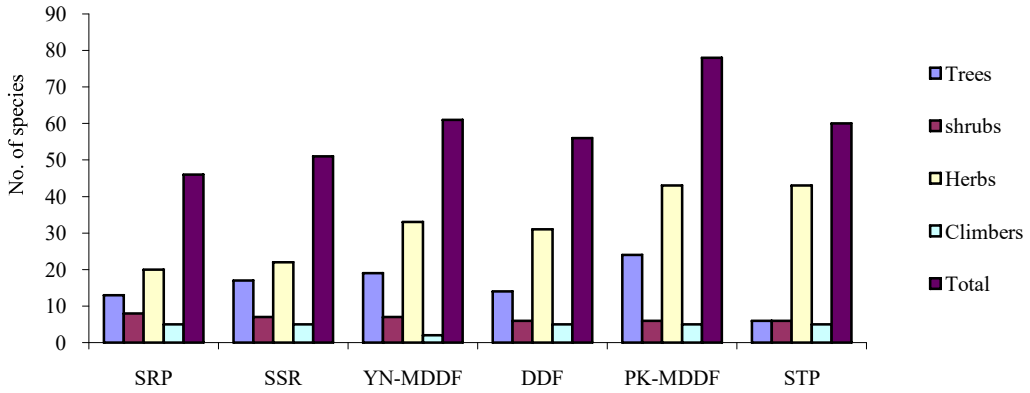


Figure 3. Distribution Patterns of different functional groups in the forests of North-western Himalayan foothills in Haryana. (The figure depicts variation in species richness among functional groups, indicating differential contributions of trees, shrubs, herbs, and climbers to overall forest biodiversity in six types of forest ecosystems studied)

Table 1. Floristic compositions of various functional groups at Yamunanagar Forest Division in Northwestern Himalayan Foothills in Haryana

Plant species	Family	IUCN	SSR	SRP	DDF
Trees					
Senegalia catechu (L.f.) P.J.H. Hurter & Mabb.	Fabaceae	(LC)	-	-	13.47
Aegle marmelos (L.) Corrêa	Rutaceae	(NT)	3.88	1.72	2.54
Terminalia anogeissiana Gere & Boatwr.	Combretaceae	(LC)	-	3.06	29.88
Bauhinia purpurea L.	Fabaceae	(LC)	-	-	6.98
Buchanania lanzan Spreng.	Anacardiaceae	(LC)	2.95	-	-
Casearia tomentosa Roxb.	Salicaceae	(LC)	1.18	3.61	9.28
Cassia fistula L.	Fabaceae	(LC)	9.99	3.51	17.75
Cordia dichotoma G. Forst.	Boraginaceae	(LC)	7.75	3.96	1.42
Diospyros montana Roxb.	Ebenaceae	(LC)	-	-	2.91
Diospyros exculpta Buch.-Ham.	Ebenaceae	(LC)	11.88	-	61.11
Ehretia aspera Willd.	Boraginaceae	(LC)	40.57	50.26	11.28
Ficus racemosa L.	Moraceae	(LC)	1.48	-	-
Ficus religiosa L.	Moraceae	(LC)	-	-	1.58
Garuga pinnata Roxb.	Burseraceae	(LC)	-	-	1.53
Grewia optiva J.R. Drumm. ex Burret	Malvaceae	(LC)	10.95	1.79	-
Holoptelea integrifolia (Roxb.) Planch.	Ulmaceae	(LC)	-	-	18.30
Kydia calycina Roxb.	Malvaceae	(LC)	-	1.80	6.55
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	(LC)	-	-	29.93
Litsea glutinosa (Lour.) C.B.Rob.	Lauraceae	(LC)	4.17	18.14	-
Mallotus philippensis (Lam.) Müll. Arg.	Euphorbiaceae	(LC)	85.27	74.98	6.04
Ougeinia oojeinensis (Roxb.) Hochr.	Fabaceae	(LC)	2.72	-	-
Catunaregam spinosa (Thunb.) Tirveng.	Rubiaceae	(LC)	7.94	-	52.59
Schleichera oleosa (Lour.) ken.	Sapindaceae	(LC)	7.45	5.61	-
Shorea robusta C.F. Gaertn.	Dipterocarpaceae	(LC)	76.69	127.89	19.94
Sterculia villosa Roxb. ex Sm.	Malvaceae	(LC)	12.18	1.91	2.34
Syzygium namosialangense Widodo & E. Lucas	Myrtaceae	(NE)	-	1.86	5.21
Terminalia elliptica Willd.	Combretaceae	(LC)	12.5	-	-
Shrubs					
Justicia adhatoda L.	Acanthaceae	(LC)	8.76	-	21.26
Callicarpa macrophylea Vahl	Lamiaceae	(LC)	4.13	-	41.42
Clerodendrum infortunatum L.	Lamiaceae	(LC)	-	40.02	-
Colebrookea oppositifolia Sm.	Lamiaceae	(LC)	45.60	54.01	12.83
Lantana camara L.	Verbenaceae	(LC)	158.83	141.65	102.66
Mirabilis jalapa L.	Nyctaginaceae	(NE)	-	-	13.56
Bergera koenigii L.	Rutaceae	(LC)	5.20	7.10	-
Nepeta graciflora Benth.	Lamiaceae	(NE)	72.16	50.51	34.12
Pogostemon plectranthoides Desf.	Lamiaceae	(NE)	-	6.71	-
Azanza lampas (Cav.) Alef.	Malvaceae	(LC)	5.32	-	74.15
Herbs					
Abutilon indicum (L.) Sweet	Malvaceae	(LC)	6.33	-	6.34
Achyranthes aspera L.	Amaranthaceae	(LC)	20.60	11.21	20.60
Adiantum capillus-veneris L.	Pteridaceae	(LC)	10.09	19.76	10.09
Aerva javanica (Burm.f.) Juss. ex Schult.	Amaranthaceae	(LC)	-	12.08	8.56
Ageratum conyzoides L.	Asteraceae	(LC)	8.12	-	4.48

Cont...

Table 1. Floristic compositions of various functional groups at Yamunanagar Forest Division in Northwestern Himalayan Foothills in Haryana

Plant species	Family	IUCN	SSR	SRP	DDF
Lysimachia arvensis (L.) U. Manns & Anderb.	Primulaceae	(LC)	12.20	10.19	-
Barleria cristata L.	Acanthaceae	(LC)	4.48	10.18	4.48
Bothriochloa ischaemum (L.) Keng	Poaceae	(LC)	4.12	15.02	-
Senna tora (L.) Roxb.	Fabaceae	(LC)	4.48	4.84	-
Cenchrus setigerus Vahl	Poaceae	(LC)	-	5.53	-
Enteropogon dolichostachyus (Lag.)	Poaceae	(NE)	7.58	13.16	-
Chrysopogon fulvus (Spreng.) Chiov.	Poaceae	(LC)	9.53	12.91	9.53
Commelina paludosa Blume	Commelinaceae	(LC)	4.05	8.23	-
Cynodon dactylon (L.) Pers.	Poaceae	(LC)	19.12	-	15.50
Cyperus rotundus L.	Cyperaceae	(LC)	17.83	-	15.55
Desmostachya bipinnata (L.) Stapf	Poaceae	(LC)	-	17.75	21.94
Eulaliopsis binata (Retz.) C.E. Hubb.	Poaceae	(LC)	23.56	13.81	2.02
Euphorbia hirta L.	Euphorbiaceae	(LC)	-	20.11	6.48
Heteropogon contortus (L.) P. Beauv.	Poaceae	(LC)	30.99	28.19	30.91
Holarrhena pubescens Wall. ex G. Don	Apocynaceae	(LC)	-	10.80	3.58
Mesosphaerum suaveolens (L.) Kuntze	Lamiaceae	(LC)	-	-	2.02
Indigofera heteracantha Wall. ex Brandis	Fabaceae	(LC)	-	23.11	4.48
Launaea procumbens (Roxb.) Ramayya & Rajagopal	Asteraceae	(LC)	-	-	2.02
Vicia lens (L.) Coss. & Germ.	Fabaceae	(NE)	-	-	2.53
Malvastrum coromandelianum (L.) Garcke	Malvaceae	(LC)	-	-	7.99
Parthenium hysterophorus L.	Asteraceae	(LC)	10	-	4.05
Dicliptera paniculata (Forssk.) I. Darbysh.	Acanthaceae	(LC)	20.91	-	6.82
Phyllanthus amarus Schumach. & Thonn	Phyllanthaceae	(LC)	5.54	-	-
Physalis angulata L.	Solanaceae	(LC)	16.73	-	6.82
Pluchea lanceolata (DC.) C.B. Clarke	Asteraceae	(LC)	-	-	5.54
Pupalia lappacea (L.) Juss.	Amaranthaceae	(LC)	-	-	16.73
Tripidium bengalense (Retz.) H.Scholz	Poaceae	(LC)	28.41	-	26.77
Sida ovata Forssk.	Malvaceae	(LC)	9.32	16.03	5.79
Tridax procumbens L.	Asteraceae	(LC)	-	3.48	3.63
Triumfetta romboides Jacq.	Malvaceae	(LC)	-	-	7.87
Urena lobata L.	Malvaceae	(LC)	4.35	-	4.35
Cyanthillium cinereum (L.) H. Rob.	Asteraceae	(LC)	-	5.18	7.57
Chrysopogon zizanioides (L.) Roberty	Poaceae	(LC)	21.56	18.47	21.56
Climbers					
Phanera vahlii (Wight & Arn.) Benth.	Fabaceae	(LC)	32.21	15.36	-
Stephania rotunda Lour.	Menispermaceae	(LC)	30.90	20.33	-
Coccinia grandis (L.) Voigt	Cucurbitaceae	(LC)	36.85	20.87	-
Ichnocarpus frutescens (L.) W.T. Aiton	Apocynaceae	(LC)	57.52	110.54	250.6
Macuna pruriens (L.) DC.	Fabaceae	(LC)	-	-	49.31
Millettia extensa (Benth.) Baker	Fabaceae	(LC)	125.5	109.22	-
Trichosanthes cucumerina L.	Cucurbitaceae	(LC)	17.11	23.56	-

Details & Abbrevations: SSR= Shivalik *Shorea robusta* Forest; SRP= *Shorea robusta* Plain Forest; DDF= Dry Deciduous Forest; (LC) Least Concern; (NT) Near Threatened; (NE) Not Evaluated

Table 2. Floristic compositions of different functional groups at Panchkula Forest division in Northwestern Himalayan Foothills in Haryana

Plant species	Family	IUCN	DDF	MDDF	STP
Tree layer					
Senegalia catechu (L.f.) P.J.H. Hurter & Mabb.	Fabaceae	(LC)	158.32	-	-
Vachellia leucophloea (Roxb.) Maslin, Seigler & Ebinger	Fabaceae	(LC)	-	1.43	-
Terminalia anogeissiana Gere & Boatwr.	Combretaceae	(LC)	12.01	65.16	-
Azadirachta indica A. Juss	Meliaceae	(LC)	16.24	-	-
Bauhinia purpurea L.	Fabaceae	(LC)	-	1.67	-
Bauhinia variegata L.	Fabaceae	(LC)	-	1.64	-
Casearia tomentosa Roxb.	Salicaceae	(LC)	2.37	3.13	12.30
Cassia fistula L.	Fabaceae	(LC)	13.49	15.04	6.02
Cleome viscosa var. *viscosa*.	Cleomaceae	(LC)	-	7.72	-
Diospyros montana Roxb.	Ebenaceae	(LC)	13.03	-	-
Ehretia aspera Willd.	Boraginaceae	(LC)	-	1.06	-
Flacourtia sepiaria Roxb.	Salicaceae	(LC)	-	3.12	-
Garuga pinnata Roxb.	Burseraceae	(LC)	14.46	-	-
Grewia optiva J.R. Drumm. ex Burret	Malvaceae	(LC)	9.42	-	-
Holoptelea integrifolia (Roxb.) Planch.	Ulmaceae	(LC)	-	10.59	-
Kydia calycina Roxb.	Malvaceae	(LC)	-	10.87	6.79
Lagerstroemia parviflora Roxb.	Lythraceae	(LC)	-	7.37	20.75
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	(LC)	18.16	62.83	-
Mallotus philippensis (Lam.) Müll. Arg.	Euphorbiaceae	(LC)	-	5.16	9.98
Mitragyna parviflora (Roxb.) Korth	Rubiaceae	(LC)	2.43	16.70	-
Myristica malabarica Lam.	Myristicaceae	(LC)	-	5.17	-
Naringi crenulata (Roxb.) Nicolson	Rutaceae	(LC)	2.05	1.23	-
Nyctanthes arbor-tristis L.	Oleaceae	(LC)	-	-	7.04
Ougeinia oojeinensis (Roxb.) Hochr.	Fabaceae	(LC)	-	1.33	-
Pinus roxburghii Sarg.	Pinaceae	(LC)	-	-	193.41
Catunaregam spinosa (Thunb.) Tirveng.	Rubiaceae	(LC)	6.87	3.19	-
Searsia parviflora (Roxb.) F.A. Barkley	Anacardiaceae	(LC)	-	6.64	17.42
Falconeria insignis Royle	Euphorbiaceae	(LC)	-	5.21	26.29
Sterculia villosa Roxb. ex Sm.	Malvaceae	(LC)	-	1.15	-
Syzigium cuminii (Linn.) Skeels	Myrtaceae	(LC)	20.1	0.18	-
Terminalia elliptica Willd.	Combretaceae	(LC)	-	57.37	-
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Combretaceae	(LC)	7.28	-	-
Woodfordia fruticosa (L.) Kurz	Lythraceae	(LC)	-	5.04	-
Ziziphus mauritiana Lamk.	Rhamnaceae	(LC)	4.10	-	-
Shrubs					
Justicia adhatoda L.	Acanthaceae	(LC)	6.38	-	-
Colebrookea oppositifolia Sm.	Lamiaceae	(LC)	-	-	69.46
Carrisa spinarum L.	Apocynaceae	(LC)	3.25	34.6	61.59
Lantana camara L.	Verbenaceae	(LC)	217.17	158	-

Cont...

Table 2. Floristic compositions of different functional groups at Panchkula Forest division in Northwestern Himalayan Foothills in Haryana

Plant species	Family	IUCN	DDF	MDDF	STP
Bergera koenigii L.	Rutaceae	(LC)	60.05	78	55.31
Naringi crenulata (Roxb.) D.H. Nicolson	Rutaceae	(LC)	-	-	9.16
Nepeta graciliflora Benth.	Lamiaceae	(NE)	-	10.30	34.75
Sida cordifolia L.	Malvaceae	(LC)	10.40	-	39.96
Girardinia diversifolia (Link) Friis	Utricaceae	(LC)	-	2.11	3.22
Vitex negundo L.	Lamiaceae	(LC)	-	17.3	-
Ziziphus oenophila (L.) Mill.	Rhamnaceae	(LC)	3.20	-	26.55
Herbs					
Achyranthes aspera L.	Amaranthaceae	(LC)	38.11	28.52	22.80
Adiantum capillus-veneris L.	Pteridaceae	(LC)	-	12.25	29.01
Ageratum conyzoides L.	Asteraceae	(LC)	9.71	11.83	2.33
Alysicarpus bupleurifolius (L.) DC.	Fabaceae	(LC)	-	3.87	5.21
Lysimachia arvensis (L.) U. Manns & Anderb.	Primulaceae	(LC)	-	2.95	-
Argemone mexicana L.	Papaveraceae	(LC)	7.30	1.45	3.40
Arisaema concinnum Schott	Araceae	(LC)	-	-	2.31
Asplinium esculentum Retz.	Athyriaceae	(NE)	-	2.64	25.00
Barleria cristata L.	Acanthaceae	(LC)	-	2.40	4.23
Bergenia pacumbis (Buch.-Ham. ex D.Don) C.Y. Wu & J.T. Pan	Saxifragaceae	(LC)	-	-	3.40
Senna tora (L.) Roxb.	Fabaceae	(LC)	13.95	15.34	-
Enteropogon dolichostachyus (Lag.) Keng	Poaceae	(NE)	4.28	1.99	10.23
Chrysopogon fulvus (Spreng.) Chiov.	Poaceae	(LC)	35.31	17.51	-
Commelina paludosa Blume	Commelinaceae	(LC)	13.06	17.55	4.12
Cynodon dactylon (L.) Pers.	Poaceae	(LC)	10.05	-	-
Cyperus rotundus L.	Cyperaceae	(LC)	26.56	10.53	15.24
Desmodium distortum (Aubl.) J.F. Macbr.	Fabaceae	(LC)	-	-	3.76
Desmostachya bipinnata (L.) Stapf	Poaceae	(LC)	-	5.04	8.11
Eclipta prostata (L.)	Asteraceae	(LC)	-	-	2.56
Eulaliopsis binata (Retz.) C.E. Hubb.	Poaceae	(LC)	26.43	8.37	-
Euphorbia hirta L.	Euphorbiaceae	(LC)	1.56	-	12.25
Euphorbia pulcherrima Willd. ex Klotzsch	Euphorbiaceae	(LC)	-	3.50	1.98
Evolvulus alsenoides (L.)	Convolvulaceae	(LC)	-	-	4.24
Girardinia diversifolia (Link) Friis	Utricaceae	(LC)	-	-	0.93
Glycirrhiza glabera L.	Fabaceae	(LC)	-	-	6.39
Heliotropium bacciferum Forsk.	Boraginaceae	(LC)	-	-	7.61
Heteropogon contortus (L.) P. Beauv.	Poaceae	(LC)	31.31	13.23	1.66
Indigofera astragalina DC.	Fabaceae	(LC)	-	-	6.65
Leonotis nepetifolia (L.) R. Br.	Lamiaceae	(LC)	4.62	10.45	1.62
Indigofera astragalina DC.	Fabaceae	(LC)	-	-	4.98
Leucas biflora (Vahl) Sm.	Lamiaceae	(LC)	2.97	6.01	6.02

Cont...

Table 2. Floristic compositions of different functional groups at Panchkula Forest division in Northwestern Himalayan Foothills in Haryana

Plant species	Family	IUCN	DDF	MDDF	STP
Craterostigma sessiliflorum (Benth.) Y.S. Liang & J.C. Wang	Linderniaceae	(NE)	-	2.81	-
Malvastrum coromandelianum (L.)	Malvaceae	(LC)	4.61	9.74	1.62
Mazus pumilus (Burm. f.) steenis	Mazaceae	(LC)	-	7.22	-
Oplismenus compositus (L.) P. Beauv.	Poaceae	(LC)	8.25	-	10.78
Oxalis corniculata L.	Oxilidaceae	(LC)	3.82	3.92	-
Parthenium hysterophorus L.	Asteraceae	(LC)	3.30	6.04	-
Dicliptera paniculata (Forssk.) I. Darbysh.	Acanthaceae	(LC)	-	8.14	-
Phyllanthus niruri auct.	Phyllanthaceae	(LC)	-	2.89	-
Phyllanthus virgatus G. Forst.	Phyllanthaceae	(LC)	-	2.81	7.18
Physalis angulata L.	Solanaceae	(LC)	-	-	2.63
Polygala arvensis Willd	Polygalaceae	(LC)	-	7.41	14.21
Pupalialappacea (L.) Juss.	Amaranthaceae	(LC)	13.62	16.37	6.52
Rungia pectinata (L.) Nees	Acanthaceae	(LC)	-	4.78	1.98
Selaginella wallichii (Hook. & Grev.) Spring	Selaginellaceae	(LC)	-	5.57	4.19
Jacobaea nudicaulis (Buch.-Ham. ex D.Don) B.Nord.	Asteraceae	(LC)	7.81	-	4.02
Cenchrus americanus (L.) Morrone	Poaceae	(NE)	-	8.84	7.72
Sida orientalis Cav.	Malvaceae	(LC)	1.56	-	4.54
Sida ovata Forsk.	Malvaceae	(LC)	2.17	5.39	-
Sonchus brachyotus DC.	Asteraceae	(LC)	-	-	4.84
Taraxacum sect. *Taraxacum* F.H. Wigg.	Asteraceae	(NE)	-	-	2.98
Themeda arundinacea (Roxb.) A. Camus	Poaceae	(LC)	8.09	5.38	-
Tridax procumbens L.	Asteraceae	(LC)	4.07	4.25	8.29
Triumfetta romboides Jacq.	Malvaceae	(LC)	6.72	9.10	-
Urena lobata L.	Malvaceae	(LC)	2.32	2.12	-
Cyanthillium cinereum (L.) H.Rob.	Asteraceae	(LC)	-	4.67	-
Viola canescens Wall.	Violaceae	(LC)	-	-	10.29
Xanthium strumarium L.	Asteraceae	(LC)	2.78	2.57	-
Climbers					
Phanera vahlii (Wight & Arn.) Benth.	Fabaceae	(LC)	-	70.36	-
Stephania rotunda Lour.	Menispermaceae	(LC)	21.76	67.14	-
Cissus repanda (Wight & Arn.) Vahl	Vitaceae	(LC)	70.35	45.92	37.17
Coccinia grandis (L.) Voigt	Cucurbitaceae	(LC)	45.92	21.37	46.69
Ichnocarpus frutescens (L.) W.T. Aiton	Apocynaceae	(LC)	67.14	94.85	162.15
Pueraria tuberosa (Roxb. ex Willd.) DC.	Fabaceae	(NE)	94.85		
Millettia extensa (Benth.) Benth. ex Baker	Fabaceae	(LC)	-	-	16.62
Trichosanthes cucumerina L.	Cucurbitaceae	(LC)	-	-	37.36

Details & Abbreviations: DDF= Dry Deciduous Forest; MDDF= Mixed Dry Deciduous Forest; STP= Subtropical Pine; LC= Least Concern; NE= Not Evaluated

due to increased light penetration and reduced canopy competition, creating favorable microhabitats for understory species (Figure 4). Species dominance patterns based on the importance value index (IVI) varied across forest types and vegetation layers (Table 3). *Shorea robusta C.F. Gaertn.* dominated forests in YFD reflect its strong ecological amplitude and competitive ability under dry deciduous conditions. In PFD, *Acacia catechu* (L.F.) Willd., *Terminalia anogeissiana Gere & Boatwr.* and *Pinus roxburghii* Sarg. dominated respective forest types, indicating adaptation to specific edaphic and climatic conditions.

The shrub layer was largely dominated by the invasive *Lantana camara L.,* which is known to suppress native regeneration and alter vegetation structure. Its widespread dominance across forest types indicates strong ecological plasticity and highlights a major conservation concern. However, *Colebrookea oppositifolia* Sm. dominated in subtropical pine forests, suggesting habitat-specific shrub composition.

The herbaceous layer exhibited high diversity, particularly in open canopy systems, where increased light availability promotes species richness (Figure 4). Dominant herbaceous species included *Heteropogon contortus* (L.) P. Beauv., *Achyranthes aspera* L., and *Adiantum capillus-veneris* L. The dominance of grasses such as *Cenchrus setigerus* Vahl, *Desmostachya bipinnata* (L.) Stapf, and *Saccharum munja Roxb*. underscore their ecological role in soil stabilisation and nutrient cycling. Climber diversity, although relatively low, contributed to vertical stratification and structural complexity, with species such as *Ichnocarpus frutescens* (L.) W.T. Aiton, *Millettia extensa (Benth.) Benth. Ex Baker* and *Pueraria tuberosa* (Roxb. ex Willd.) DC. playing important roles in forest dynamics. Overall, variations in species composition, richness, and dominance patterns reflect the influence of environmental gradients, including soil properties, moisture availability, and topography, as well as anthropogenic pressures. The observed dominance of invasive species such as *Lantana camara* L. poses potential threats to biodiversity and forest regeneration, necessitating targeted management interventions.

3.2. Population Structure and Regeneration Status

The population structure of tree species across forest ecosystems revealed distinct patterns in girth class distribution, providing insights into regeneration dynamics and forest stability (Figure 5-7). In most forest types, a large proportion of individuals were concentrated in lower girth classes, indicating active regeneration and recruitment.

In northern tropical dry deciduous plain *Shorea robusta* C.F. Gaertn. forests, 75.45% of individuals were recorded in lower girth classes (31–90 cm), while only 4.80% occurred in higher girth classes (180–270 cm), suggesting strong regeneration but relatively fewer mature individuals (Figure 8A). Similarly, Shivalik *Shorea robusta* C.F. Gaertn. forests

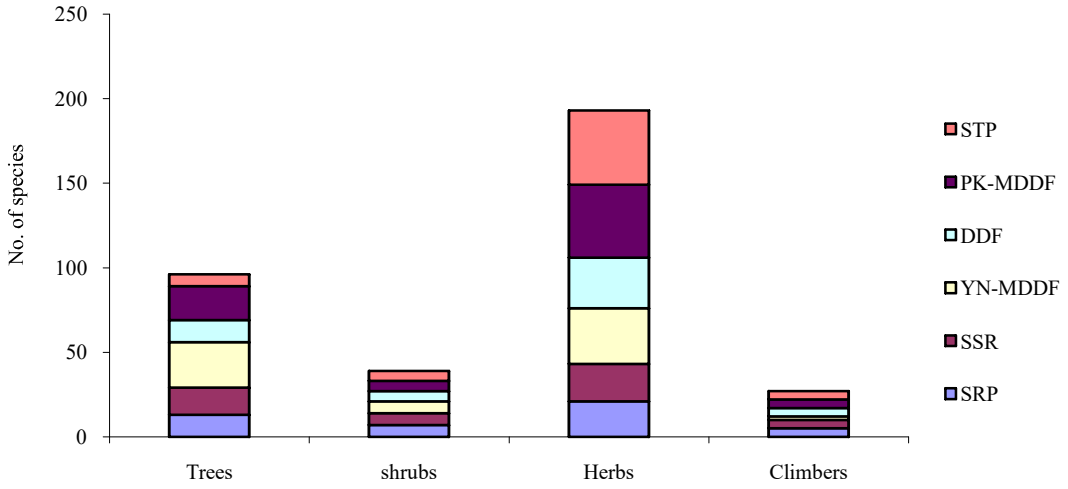


Figure 4. Relative abundance of various functional groups in various forest ecosystems. (The figure shows ecosystem-wise variation in the relative abundance of functional groups, indicating differences in community structure and dominance patterns in six types of forest ecosystems studied)

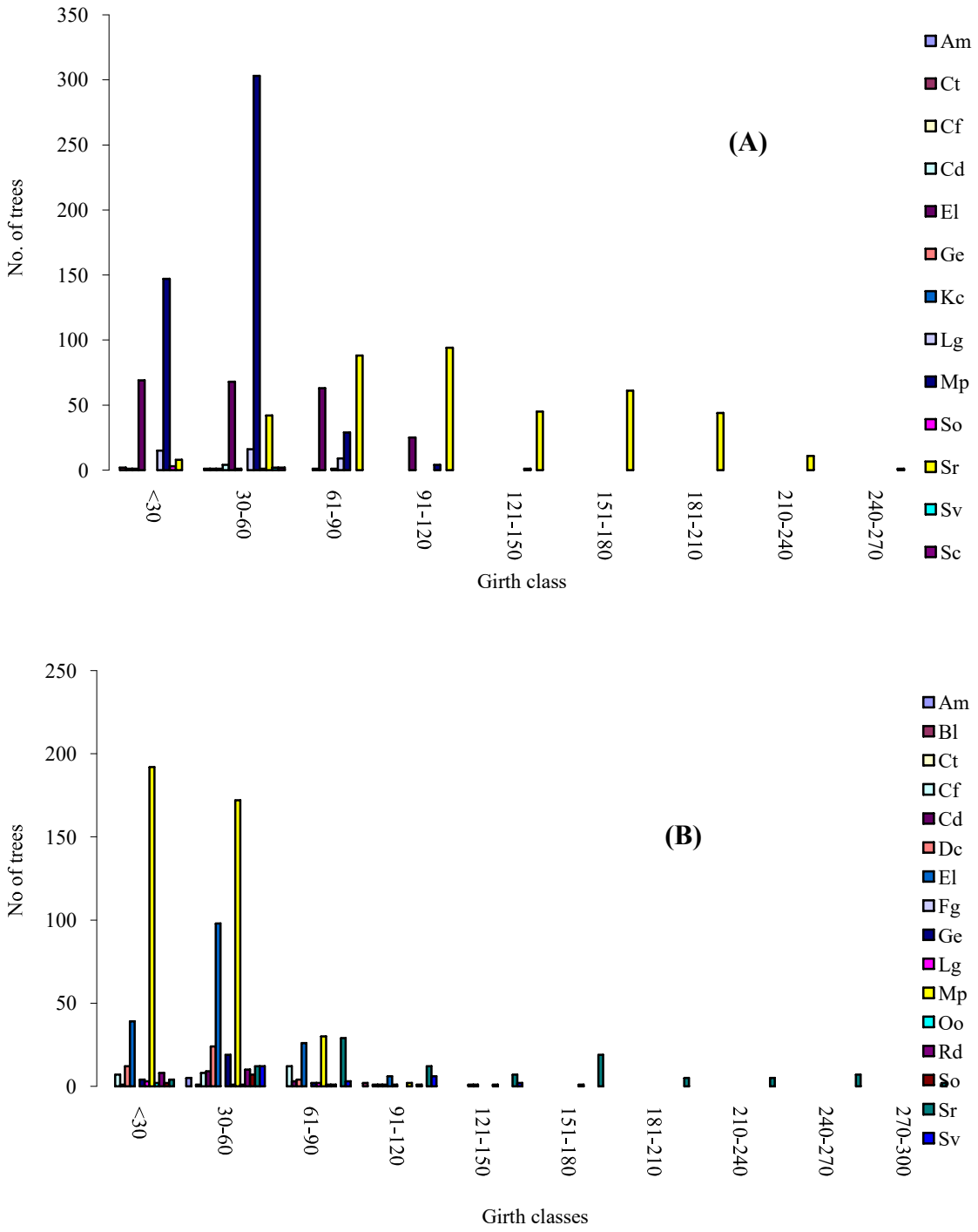


Figure 5. Population structure of tree species in (A) Northern tropical dry deciduous plain *Shorea robusta* forests; (B) Northern tropical dry deciduous Shivalik *Shorea robusta* forests. Abbreviations: Am- *Aegle marmelos*; Ct- *Casearia tomentosa*; Cf- *Cassia fistula*; Cd- *Cordia dichotoma*; El- *Ehretia laevis*; Kc- *Kydia calycina*; Lg- *Litsea glutinosa*; Mp- *Mallotus phillipensis*; So- *Schleichera oleosa*; Sr- *Shorea robusta*; Sv- *Sterculia villosa*; Sc- *Syzygium cumini*; Dc- *Diospyros cordifolia*; Bl- *Buchonarica lanzens*; Oo- *Ougenia oojeinensis*; Rd- *Randia dumetorum; Ficus glomerata* (The figure shows the distribution of tree individuals across girth classes in two *Shorea robusta* forest types, indicating differences in regeneration and population structure)

showed an even greater skew, with 90.12% of individuals in lower girth classes, indicating vigorous recruitment but limited representation of older trees (Figure 8B).

Mixed deciduous forests in YFD also showed dominance of lower girth classes (80.11%), (Figure 9A) whereas in PFD, a relatively balanced distribution was observed

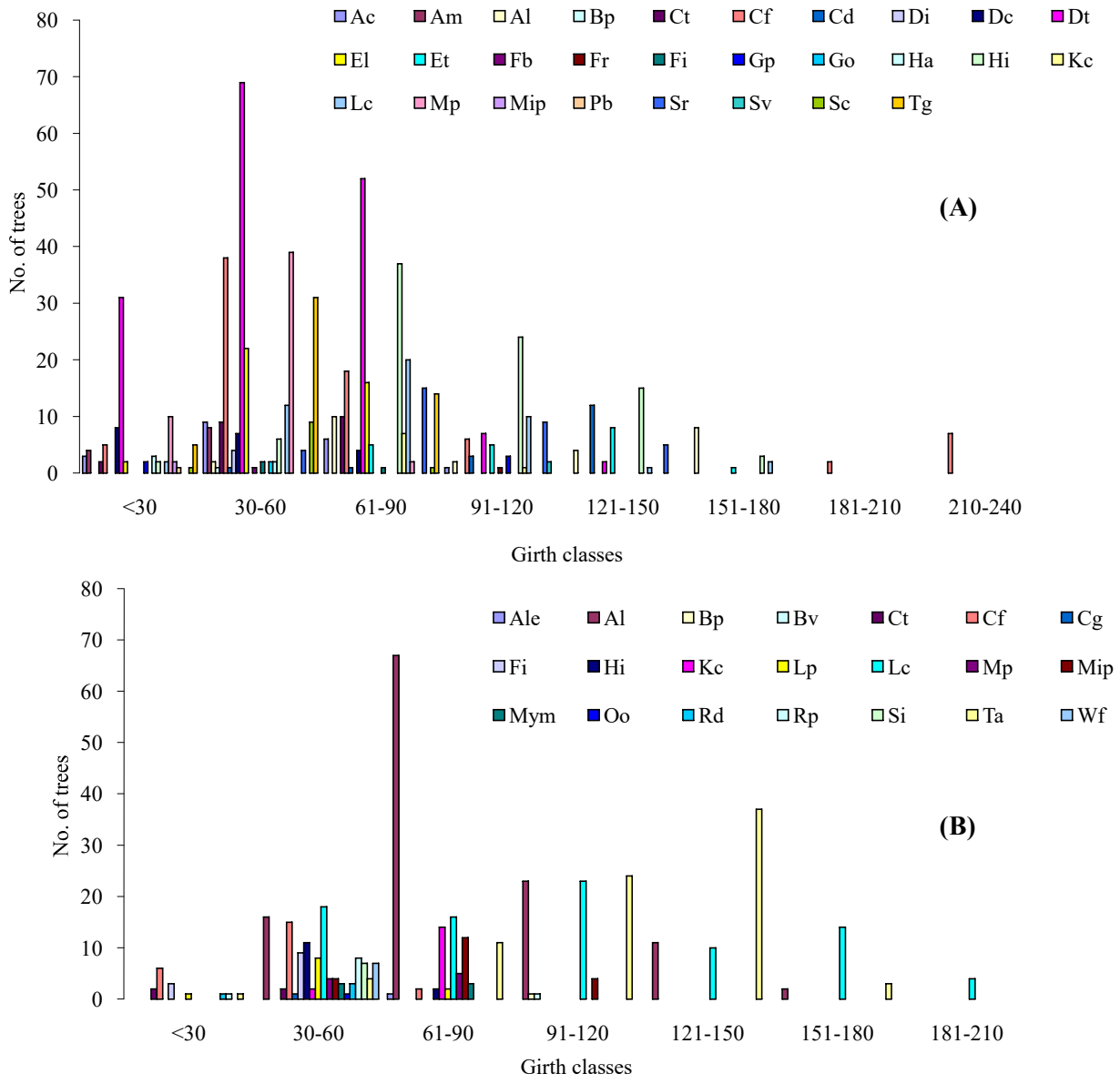


Figure 6. Population structure of tree species in (A) Mixed deciduous forests at Yamunanagar forest division; (B) Northern tropical dry deciduous forests at Panchkula forest division. Abbreviations: Am-*Aegle marmelos*; Ct- *Casearia tomentosa*; Cf- *Cassia fistula*; Cd- *Cordia dichotoma*; El- *Ehretia laevis*; Kc- *Kydia calycina*; Lg- *Litsea glutinosa*; Mp- *Mallotus phillipensis*; So- *Schleichera oleosa*; Sr- *Shorea robusta*; Sv- *Sterculia villosa*; Sc- *Syzygium cumini*; Dc- *Diospyros cordifolia*; Bl- *Buchonarica lanzens*; Oo- *Ougenia oojeinensis*; Rd- *Randia dumetorum; Ficus glomerata;* Ac- *Acacia catechu;* Ale- *Acacia Leuchophloea;* Al- *Anogeissus latifolia;* Bp- *Bauhinia purpurea;* Bv- *Bauhinia varigata;* Fr- *Ficus religiosa;* Fb-*Ficus bengalensis;* Gp- *Garuga pinnata;* Go- *Grewia optiva;* Lc- *Lannea coromendelica;* Mip- *Mitragyna parviflora;* Ha- *Holoptelea integrifolia;* Ta- *Terminalia alata.*

(The figure shows the distribution of tree individuals across girth classes in two forest types, reflecting differences in regeneration patterns and population structure).

(63.48% lower and 35.58% intermediate girth classes), indicating comparatively stable population structure and better representation of different age classes (Figure 10A). In contrast, dry deciduous forests of PFD exhibited an extreme concentration (99.78%) of individuals in lower girth classes, suggesting recent regeneration following disturbance or selective removal of mature trees (Figure 9B).

Subtropical pine forests displayed a more balanced distribution across girth classes, with 45.37% in lower, 43.69% in intermediate, and 10.92% in higher girth classes,

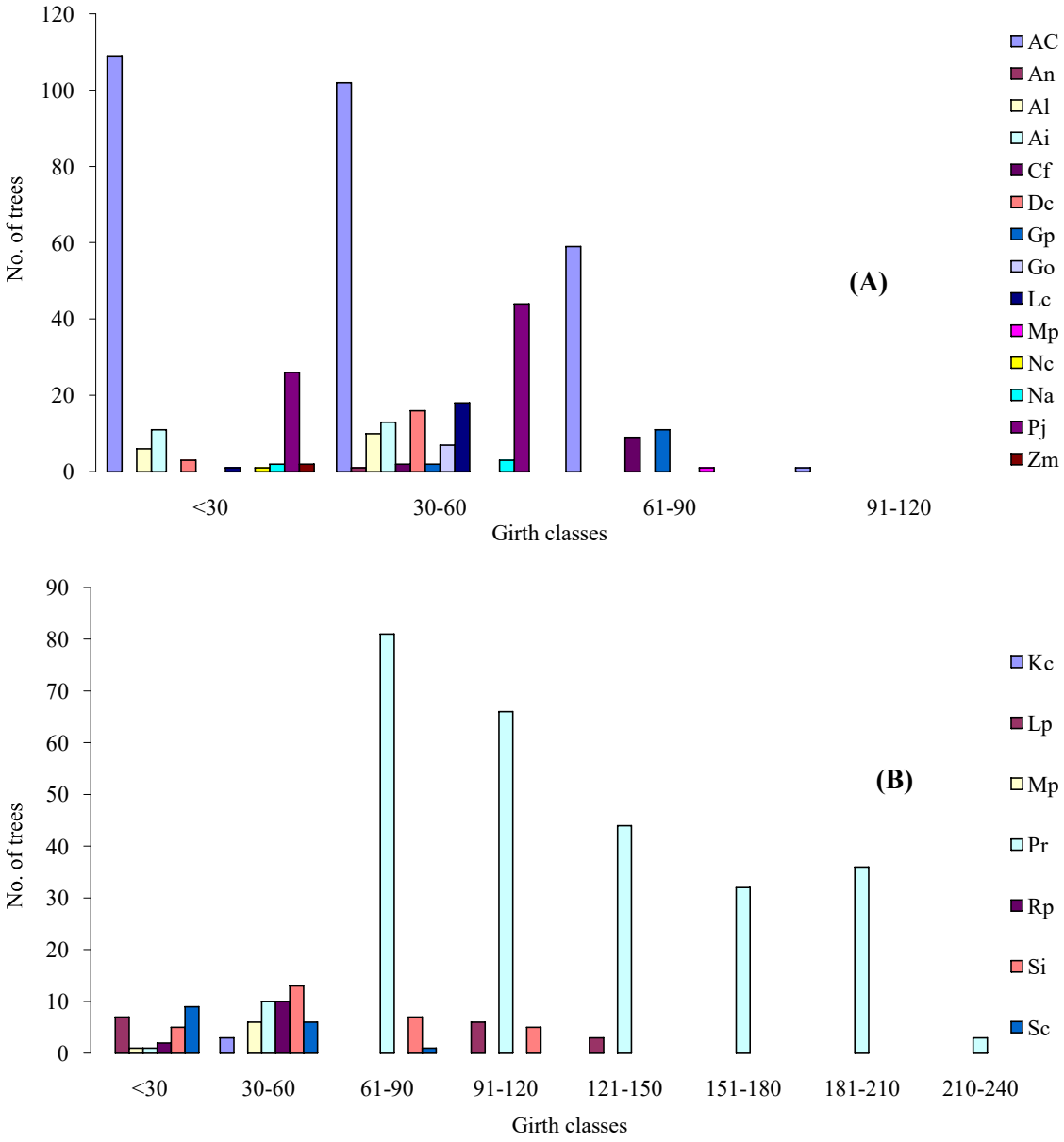


Figure 7. Population structure of tree species in (A) Dry deciduous forests; (B) Sub-tropical pine forests in Panchkula Forest Division. Abbreviations: Ac- *Acacia catechu*; Al- *Anogeissus latifolia*; Ai- *Azadirachta indica*; Cf- *Cassia fistula*; Dc- *Diospyros cordifolia*; Gp- *Garuga pinnata*; Go- *Grewia optiva*; Lc- *Lannea coromendelica*; Mp- *Mallotus phillippensis*; Nc- *Naringi crenulata*; Na- *Nyctanthes arbor-tristis*; Zm-*Ziziphus mauritiana*; Kc- *Kydia calycina*; Lp- *Lagerstromia parviflora*; Pr- *Pinus roxburghii*; Si- *Sapium insinge*; Sc- *Syzugium cumini* (The figure shows the distribution of tree individuals across girth classes in dry deciduous and subtropical pine forests, indicating differences in regeneration and population structure).

indicating relatively stable population dynamics (Figure 10B). The dominance of *Pinus roxburghii* Sarg.in intermediate and higher girth classes reflect its persistence and competitive advantage in these ecosystems.

The predominance of individuals in lower girth classes across most forest types suggests an inverse J-shaped distribution pattern, widely recognised as an indicator of continuous regeneration and sustainable forest dynamics.

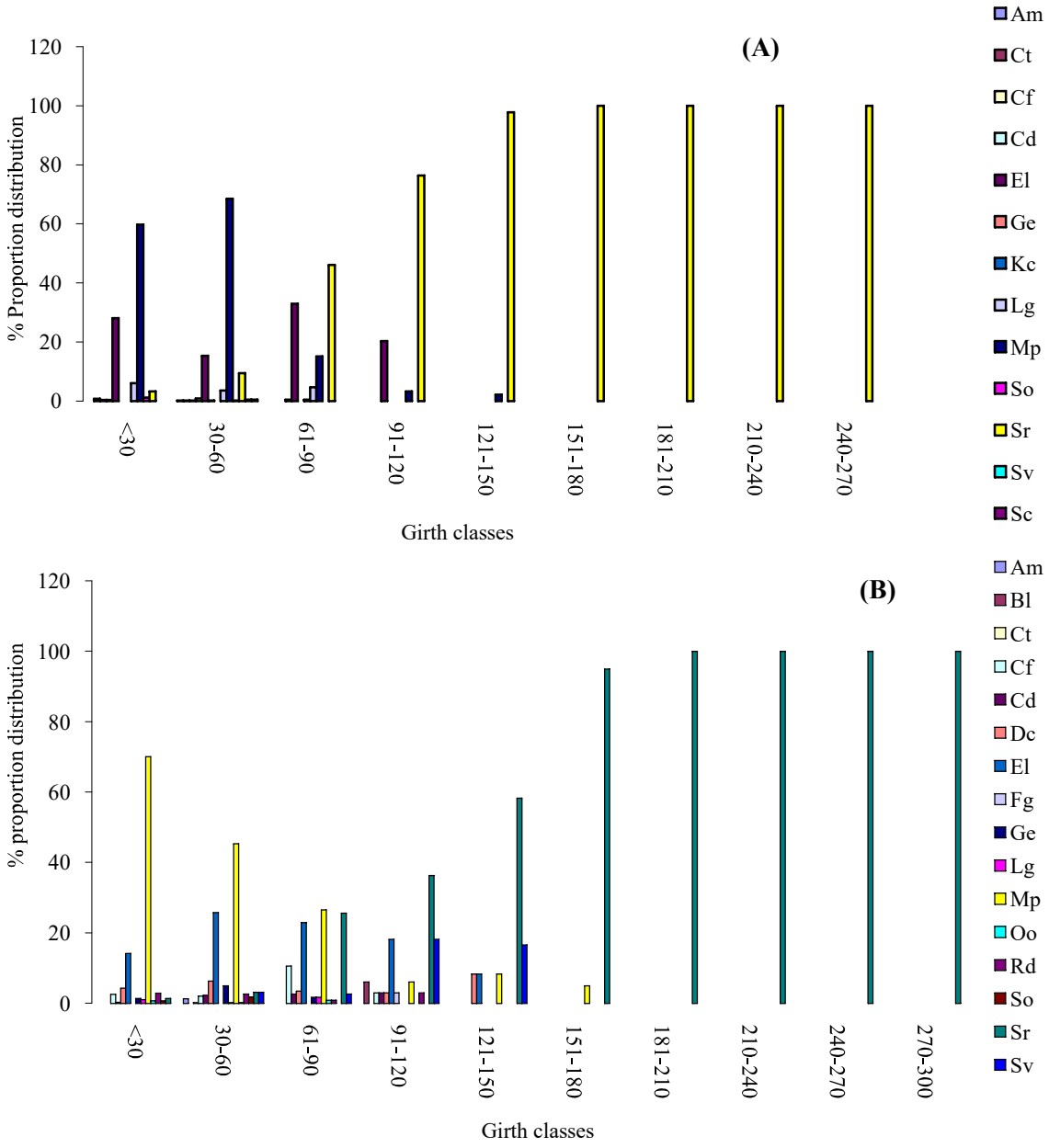


Figure 8. Relative percentage proportion distribution of different tree species in (A) Northern tropical dry deciduous plain *Shorea robusta* forests; (B) Northern tropical dry deciduous Shivalik *Shorea robusta* forests. Abbreviations: Am- *Aegle marmelos*; Ct- *Casearia tomentosa*; Cf- *Cassia fistula*; Cd- *Cordia dichotoma*; El- *Ehretia laevis*; Kc- *Kydia calycina*; Lg- *Litsea glutinosa*; Mp- *Mallotus phillipensis*; So- *Schleichera oleosa*; Sr- *Shorea robusta*; Sv- *Sterculia villosa*; Sc- *Syzygium cumini*; Dc- *Diospyros cordifolia*; Bl- *Buchonarica lanzens*; Oo- *Ougenia oojeinensis*; Rd- *Randia dumetorum; Ficus glomerata* (The figure shows species-wise relative percentage composition of trees in two *Shorea robusta* forest types, indicating differences in species dominance and community composition)

However, the low proportion of larger trees in certain forest types may reflect past disturbances, anthropogenic pressures, or selective harvesting.

3.3. Dominance–Diversity Patterns and Community Organization

Dominance–diversity curves for the trees (Figure 11),

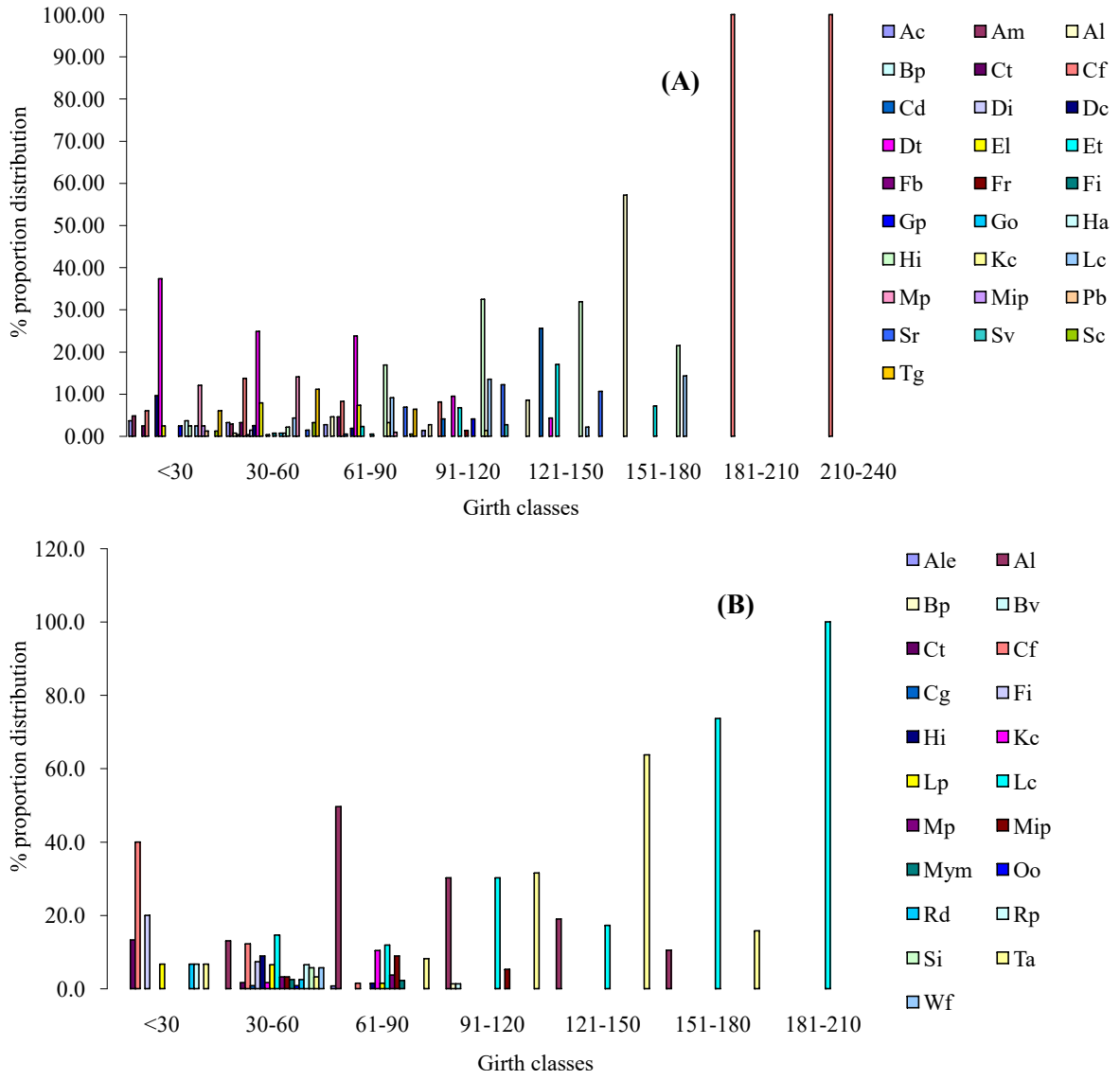


Figure 9. Relative percentage proportion distribution of tree species in (A) Mixed deciduous forests in Yamunanagar Forest Division; (B) Northern tropical dry deciduous forests in Panchkula Forest Division. Abbreviations: Am- *Aegle marmelos*; Ct- *Casearia tomentosa*; Cf- *Cassia fistula*; Cd- *Cordia dichotoma*; El- *Ehretia laevis*; Kc- *Kydia calycina*; Lg- *Litsea glutinosa*; Mp- *Mallotus phillipensis*; So- *Schleichera oleosa*; Sr- *Shorea robusta*; Sv- *Sterculia villosa*; Sc- *Syzygium cumini*; Dc- *Diospyros cordifolia*; Bl- *Buchonarica lanzens*; Oo- *Ougenia oojeinensis*; Rd-*Randia dumetorum;* Fg- *Ficus glomerata;* Ac- *Acacia catechu;* Ale- *Acacia leuchophloea;* Al- *Anogeissus latifolia;* Bp- *Bauhinia purpurea;* Bv- *Bauhinia varigata;* Fr- *Ficus religiosa;* Fb- *Ficus bengalensis;* Gp- *Garuga pinnata;* Go- *Grewia optiva;* Lc- *Lannea coromendelica;* Mip- *Mitragyna parviflora;* Ha- *Holoptelea integrifolia;* Ta- *Terminalia alata* (The figure shows species-wise relative percentage composition of trees in two forest types, indicating variation in species dominance and community structure)

shrubs (Figure 12), and herbaceous layers (Figure 13) across the studied forest ecosystems largely followed a log-normal distribution pattern. This indicates that species abundances are distributed among several species with moderate dominance, reflecting equitable resource partitioning and niche differentiation. Such log-normal distributions are characteristic of structurally complex and relatively stable communities, particularly in mixed deciduous forests where species diversity was highest. The pattern suggests that no single species overwhelmingly dominates the community, allowing the coexistence of multiple species. Similar trends observed in shrub and herbaceous layers further support the heterogeneous and well-structured nature of these ecosystems.

3.4. Species Diversity and Community Indices

Species diversity indices varied significantly among forest types and vegetation layers, reflecting differences in species composition, dominance, and ecological conditions. For the tree layer, Shannon's diversity index ranged from

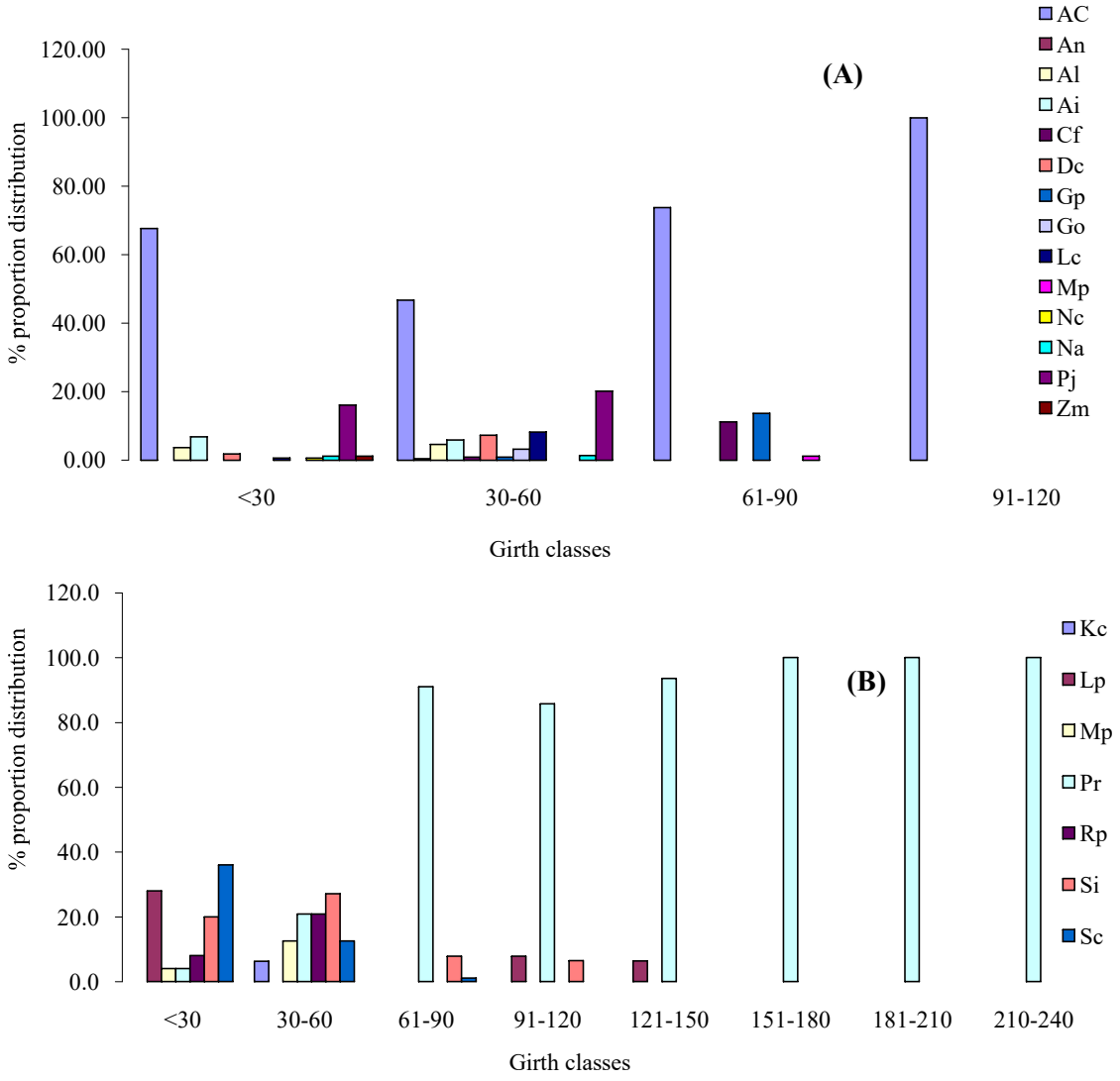


Figure 10. Relative percentage proportion distribution of tree species in (A) Mixed deciduous forests; (B) Sub-tropical pine forests in Panchkula Forest Division. Abbreviations: Ac- *Acacia catechu*; Al- *Anogeissus latifolia*; Ai- *Azadirachta indica*; Cf- *Cassia fistula*; Dc- *Diospyros cordifolia*; Gp- *Garuga pinnata*; Go- *Grewia optiva*; Lc- *Lannea coromendelica*; Mp- *Mallotus phillippensis*; Nc- *Naringi crenulata*; Na- *Nyctanthes arbor-tristis*; Zm- *Ziziphus mauritiana*; Kc- *Kydia calycina*; Lp- *Lagerstromia parviflora*; Pr- *Pinus roxburghii*; Si- *Sapium insinge*; Sc- *Syzugium cumini* (The figure shows species-wise relative percentage composition of trees in mixed deciduous and subtropical pine forests, indicating variation in species dominance and community structure)

1.237 in subtropical pine forests to 2.510 in mixed deciduous forests of PFD, indicating higher diversity in mixed forests and lower diversity in conifer-dominated systems (Table 4). Simpson's concentration of dominance was highest in subtropical pine forests (0.455), reflecting strong dominance by *Pinus roxburghii* Sarg., whereas lower values in mixed forests indicate more equitable species distribution (Table 4).

Species richness (Margalef's index) was highest in mixed deciduous forests in PFD (7.440), further emphasizing their role as biodiversity-rich ecosystems (Table 4). Equitability (Pielou's index) was generally higher in deciduous forests, suggesting balanced species distribution.

In the shrub layer, diversity was highest in subtropical pine forests, while dominance was greatest in mixed deciduous forests of PFD, indicating uneven species distribution. Herbaceous diversity was notably high in subtropical pine forests (H = 3.450), likely due to favorable light conditions and reduced canopy cover (Table 4). Climbers exhibited comparatively lower diversity but contributed to vertical complexity and ecological interactions. Overall, diversity indices suggest that mixed deciduous forests support higher biodiversity and ecological stability, whereas subtropical pine forests exhibit higher dominance and lower diversity due to monospecific tendencies.

3.5. Ecological Implications and Forest Dynamics

The observed patterns of species composition, population structure, and diversity indices collectively reflect the influence of environmental gradients, resource availability, and disturbance regimes on forest ecosystems. The Shannon diversity values (1.237–2.510) fall within the reported range for Indian forests, indicating moderate to high diversity (Table 4).

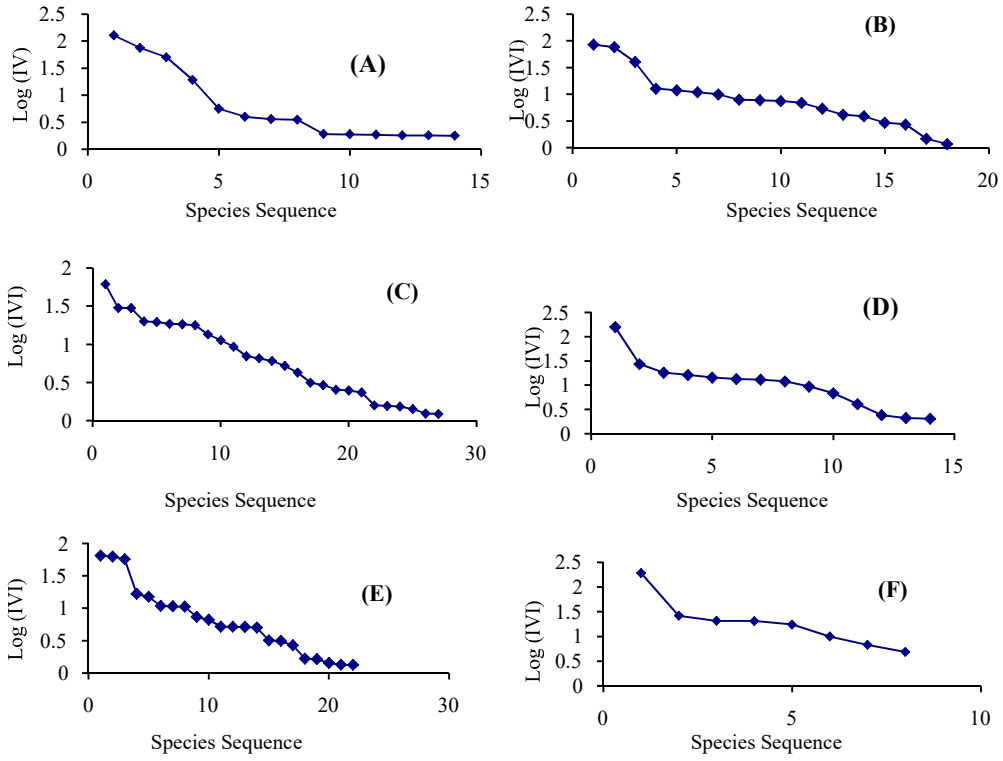


Figure 11. Dominance – diversity curves of Tree species in (A) Northern tropical dry deciduous plain *Shorea robusta* forests (YFD); (B) Northern tropical dry deciduous Shivalik *Shorea robusta* forests (YFD); (C) Northern tropical mixed dry deciduous forests (YFD); (D) Northern tropical dry deciduous forests (PFD); (E) Mixed dry deciduous forest (PFD); (F) Subtropical pine forests (PFD). (The figure shows dominance–diversity relationships of tree species across forest types, highlighting variations in species richness, evenness, and dominance patterns).

Variation in dominance values highlights differences in species interactions and competitive hierarchies across forest types. Higher dominance in subtropical pine forests suggests ecological simplification, whereas lower dominance in mixed forests indicates greater structural complexity and stability.

The widespread presence of invasive species such as *Lantana camara* L. poses a significant ecological challenge, as it can suppress native regeneration and alter community composition. Therefore, effective management strategies should focus on controlling invasive species and promoting native biodiversity to maintain ecosystem resilience.

3.6. Phenology and Regeneration Dynamics of *Shorea robusta* C.F. Gaertn

Phenological observations in *Shorea robusta* C.F. Gaertn. forests revealed distinct seasonal patterns in leaf fall, flowering, fruiting, and regeneration. The species regenerates through both seeds and vegetative means, such as coppicing and root suckers. Seed germination is highly dependent on timely monsoon rainfall, with viability declining rapidly under delayed precipitation. Seedlings typically establish in moist leaf litter under shaded conditions, but their survival is strongly influenced by moisture availability and microhabitat conditions. Leaf fall occurs between February and May, coinciding with the dry

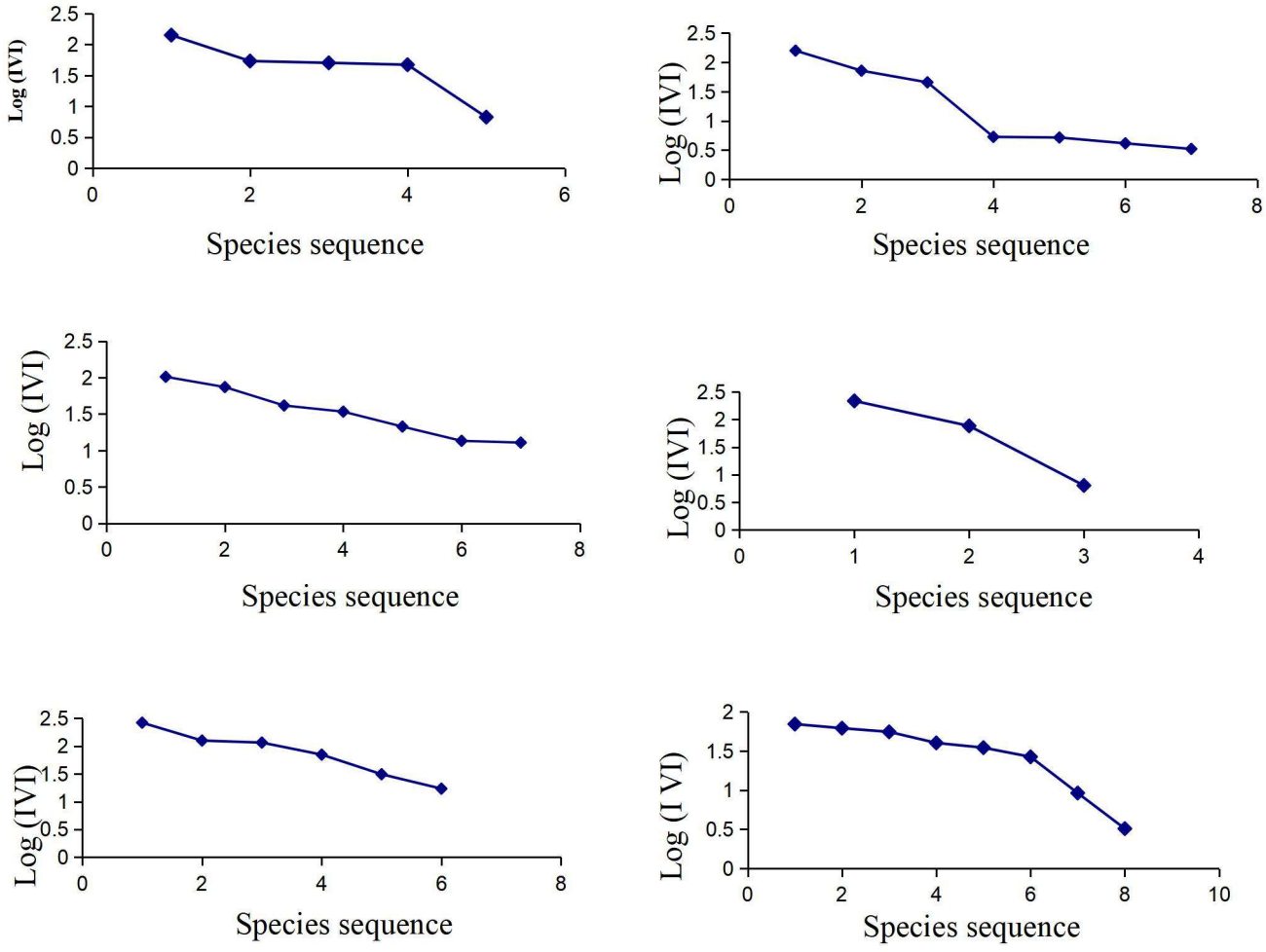


Figure 12. Dominance– diversity curves of Shrub species in (A) Northern tropical dry deciduous plain *Shorea robusta* forests (YFD); (B) Northern tropical dry deciduous Shivalik *Shorea robusta* forests (YFD); (C) Northern tropical mixed dry deciduous forests (YFD); (D) Northern tropical dry deciduous forests (PFD); (E) Mixed dry deciduous forest (PFD); (F) Subtropical pine forests (PFD). (The figure shows rank–abundance (dominance–diversity) patterns of shrub species across forest types, highlighting differences in species richness, evenness, and dominance)

season, while leaf flushing and flowering are triggered by increasing temperature and photoperiod. These phenological patterns reflect adaptive strategies to seasonal climatic variability. Overall, regeneration dynamics in *Shorea robusta* C.F. Gaertn. forests are closely linked to environmental conditions, particularly rainfall and moisture availability, which ultimately influences forest structure and long-term sustainability. The observed variation in community characteristics across forest types highlights the strong influence of edaphic factors and biotic interactions in shaping vegetation structure and diversity. Soil heterogeneity, nutrient availability, and moisture gradients likely contributed to differential species establishment and survival, resulting in distinct diversity patterns across sites. Similar findings have been reported in recent studies, where environmental gradients and site conditions significantly influenced species diversity and forest composition (Kumar et al., 2022; Sharma et al., 2025; Dar et al., 2025).

The studies indicate that the biodiversity of dry deciduous forests is most important because of their vulnerability to habitat loss. The log-normal distribution pattern observed in dominance–diversity curves suggest a relatively stable and mature community structure, where resources are partitioned among a large number of species without extreme dominance by a few taxa. This pattern is generally an indicator of ecological equilibrium and niche

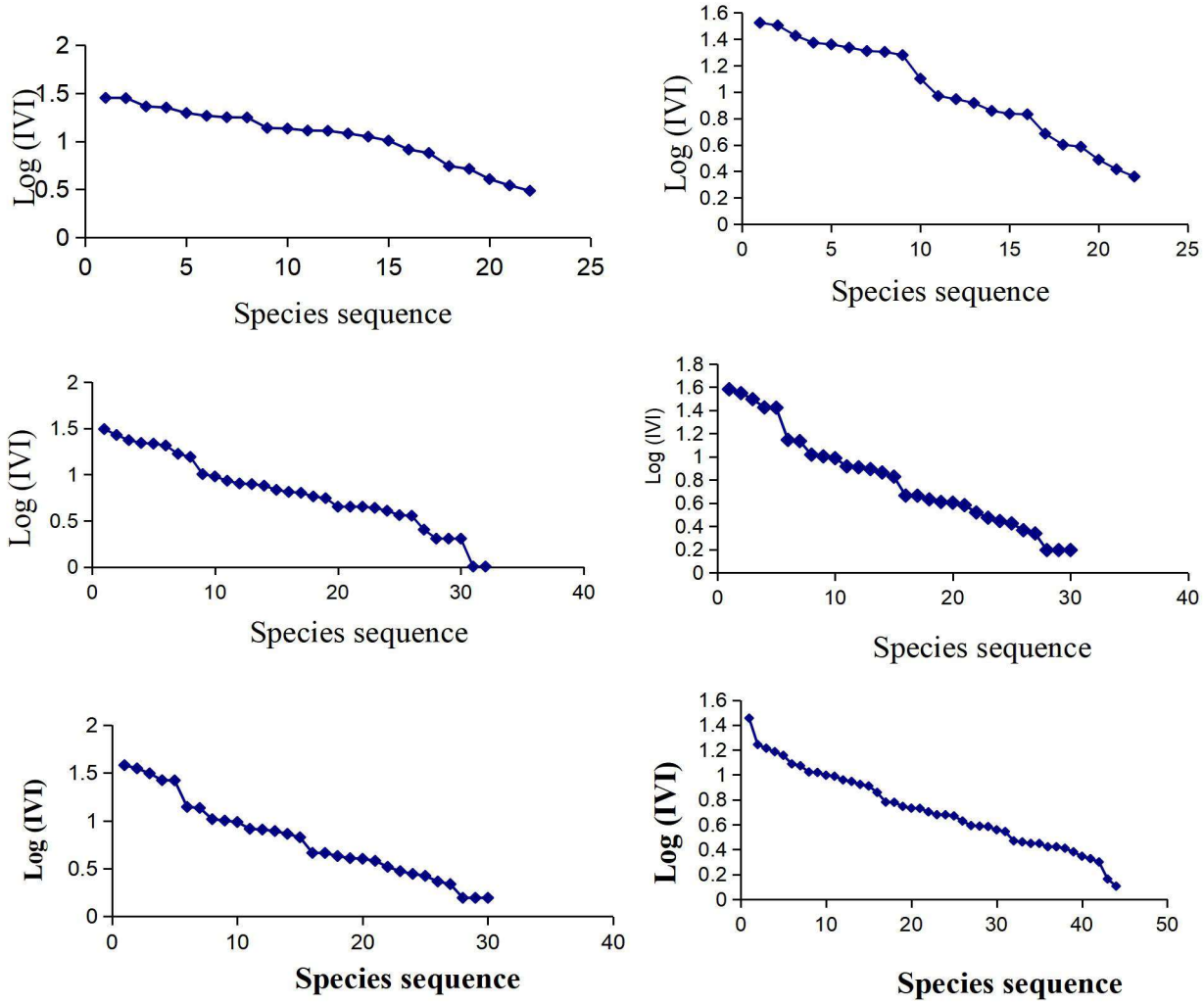


Figure 13. Dominance– diversity curves of Herb species in (A) Northern tropical dry deciduous plain *Shorea robusta* forests (YFD); (B) Northern tropical dry deciduous Shivalik *Shorea robusta* forests (YFD); (C) Northern tropical mixed dry deciduous forests (YFD); (D) Northern tropical dry deciduous forests (PFD); (E) Mixed dry deciduous forest (PFD); (F) Subtropical pine forests (PFD). (The figure shows rank–abundance (dominance–diversity) patterns of herb species across forest types, highlighting variations in species richness, evenness, and dominance).

Table 3. Phyto-sociological analyses of various functional groups in forests of the Northwestern Himalayan Foothills in Haryana

Forest types	Average tree dfensity (trees ha^{-1})	Average basal area (m^{2}ha^{-1})	Plant species (n)	Dominant species (IVI)	IVI variation of rest of species
Yn-SRP	823	55.20	14*	*Shorea robusta* C.F. Gaertn. IVI =127.89)	74.98 to 1.72*
			06**	*Lantana camara* L. (IVI =141.65)	54.01 to 06.71**
			20***	*Heteropogon contortus* (L.) P. Beauv. (IVI =28.41)	23.11 to 03.48***
			06****	*Ichnocarpus frutescens* (L.) W.T.Aiton (IVI =110.54)	109.22 to 15.36****
Yn-SSR	971	23.93	17*	*Mallotus philippensis* (Lam.) Müll.Arg. (IVI = 85.27)	76.69 to 01.18*
			07**	*Lantana camara* L. (IVI = 158.83)	72.16 to 04.13**
			23***	*Heteropogon contortus* (L.) P. Beauv. (IVI =30.99)	28.41 to 04.12***
			06****	*Millettia extensa* (Benth.) Benth. ex Baker (IVI =125.5)	62.92 to 07.11****
Yn-MDDF	518	21.78	20*	*Diospyros exculpta* Buch.-Ham. IVI = 61.11)	52.59 to 01.42*
			07**	*Lantana camara* L. (IVI = 102.66)	74.15 to 12.83**
			31***	*Heteropogon contortus* (L.) P. Beauv. (IVI =30.99)	26.77 to 02.02***
			02****	*Ichnocarpus frutescens* (L.) W.T.Aiton IVI =250.6)	0 to 49.31****
Pk-DDF	502	7.52	15*	*Senegalia catechu* (L.f.) P.J.H.Hurter& Mabb.	20.10 to 02.05*
			06**	(IVI = 158.32)	
			27***	*Lantana camara* L. (IVI = 217.17)	60.05 to 03.20**
			05****	*Achyranthes aspera* L. (IVI = 38.11)	35.31 to 01.56***
				Pueraria tuberosa (Roxb. ex Willd.) DC.(IVI =94.85)	70.35 to 21.76****
Pk-MDDF	378	23.82	25*	*Ceropegia bhatii* S.R.Yadav & Shendage	62.83 to 00.18*
			06**	Wall. (IVI= 65.16)	
			39***	*Lantana camara* L. (IVI = 158.0)	78.00 to 02.11**
			05****	*Achyranthes aspera* L. (IVI = 28.52)	17.55 to 01.45***
				Pueraria tuberosa (Roxb. ex Willd.) DC.(IVI =94.85)	70.35 to 21.37****
Pk-STP	315	34.42	09*	*Pinus roxburghii* Sarg. (IVI = 193.41)	26.29 to 06.02*
			08**	*Colebrookea oppositifolia* Sm. (IVI = 69.46)	61.59 to 09.16**
			41***	*Adiantum capillus-veneris* L.(IVI = 29.01)	25.00 to 00.93***
			05****	*Ichnocarpus frutescens* (L.) W.T.Aiton (IVI = 162.15)	46.69 to 16.62****

Details & Abbrevations: Functional groups:* Trees, **Shrubs, ***Herbs, ****Climbers
Northern tropical dry deciduous *Shorea robusta* forest- (Yn-SRP); Northern tropical dry deciduous Shivalik *Shorea robusta* forest- (Yn-SSR); Mixed dry deciduous forests at Yamunanagar Forest Division- (Yn-MDDF); Dry deciduous Forest at Panchkula Forest Division- (Pk-DDF); Mixed dry deciduous forests at Panchkula Forest Division- (Pk-MDDF); Subtropical Pine- (Pk-STP)

Table 4. Floristic diversity of forests of the Northwestern Himalayan Foothills in Haryana

Forest type	Shannon's index (H)				Margalef's index (D)				Simpson's index (Cd)				Pielou index (e)			
	T	S	H	C	T	S	H	C	T	S	H	C	T	S	H	C
YN-SRP	1.40	1.33	2.93	1.37	4.99	0.69	7.39	3.45	0.21	0.30	0.05	0.29	0.83	0.40	1.71	0.41
YN-SSR	1.61	1.28	2.83	1.42	1.97	1.12	7.28	4.30	0.26	0.36	0.06	0.27	0.70	0.44	1.65	0.43
YN-MDDF	2.44	1.69	3.13	0.44	4.93	0.99	9.55	0.72	0.10	0.21	0.05	0.72	1.24	0.62	2.04	0.05
Pk-DDF	2.15	1.31	2.96	1.51	3.29	0.85	7.91	16.03	0.15	0.35	0.06	0.23	1.07	0.13	1.90	0.46
Pk-MDDF	2.51	1.58	3.28	1.51	7.44	0.81	11.44	16.03	0.12	1.17	0.03	0.23	1.57	0.53	2.34	0.46
Pk-STP	1.23	1.86	3.45	1.30	1.74	1.19	12.93	8.32	0.45	0.16	0.04	0.35	0.45	0.73	2.46	0.39

Details & Abbreviations: Northern tropical dry deciduous *Shorea robusta* forest- (Yn-SRP); Northern tropical dry deciduous Shivalik *Shorea robusta* forest- (Yn-SSR); Mixed dry deciduous forests at Yamunanagar forest Division- (Yn-MDDF); Dry deciduous Forest at Panchkula Forest Division- (Pk-DDF); Mixed dry deciduous forests at Panchkula Forest division- (Pk-MDDF); Subtropical Pine- (Pk-STP); Tree- (T); Shrub- (S); Herb- (H); climber- (C)

diversification. However, it is important to note that such distributions may also arise in moderately disturbed systems where competitive exclusion is reduced, allowing coexistence of multiple species (Ette et al., 2023; Dar et al., 2025). Therefore, while the log-normal model reflects high diversity, it does not necessarily imply the absence of disturbance. The Shannon diversity index values (1.237–2.510) indicate moderate species diversity compared to global tropical forest standards. Although these values are higher than those of some *Shorea robusta* C.F. Gaertn., forests of the Eastern Himalaya (Uma Shankar, 2001), they remain lower than those reported for highly diverse tropical evergreen forests (Knight, 1975). This suggests that the studied forests, while diverse, may be experiencing ecological limitations such as seasonal drought, soil constraints, or anthropogenic pressures including grazing, fuel wood extraction, and fragmentation. Recent studies have similarly emphasized that human-induced disturbances and land-use changes can significantly reduce species diversity and alter community composition (Thounaojam et al., 2021; Chandra et al., 2025; Dar et al., 2025).

Furthermore, the relatively moderate diversity values may also reflect the dominance of *Shorea robusta* C.F. Gaertn., a species known for forming mono-dominant or semi-dominant stands, which can limit under storey diversity through shading and competitive resource uptake. This aligns with the concept that dominance by a single species can reduce evenness, thereby influencing overall diversity indices. Another critical aspect is the scale of observation and methodology used for diversity estimation. Recent advancements using remote sensing and machine learning approaches have shown that diversity estimates can vary significantly depending on spatial resolution and sampling design (Kumar et al., 2022; Ette et al., 2023). Therefore, methodological constraints should also be considered when comparing diversity values across studies. The forest ecosystems of YFD and PFD exhibit heterogeneous vegetation structure, moderate to high species diversity, and active regeneration patterns. Mixed deciduous forests emerge as biodiversity-rich systems with relatively stable community structure, whereas subtropical pine forests exhibit greater dominance and lower diversity. The predominance of lower girth classes indicates ongoing regeneration; however, the limited presence of mature individuals in some forests suggests past disturbances. The invasion of *Lantana camara* L. represents a critical threat to ecosystem stability. These findings emphasize the need for site-specific forest management strategies focusing on invasive species control, conservation of native biodiversity, and maintenance of ecological resilience to ensure long-term sustainability of forest ecosystems.

4. CONCLUSION

The present study demonstrates that the forest ecosystems of the north-western Himalayan Shivalik foothills in Haryana exhibit considerable variation in floristic composition, vegetation structure, and functional group dynamics across different forest types. A total of 137 plant species belonging to 61 families were recorded, highlighting the rich floristic diversity of the region. Mixed deciduous forests supported the highest species diversity and richness with relatively balanced community structure, whereas subtropical pine forests exhibited greater species dominance and lower diversity. The dominance–diversity curves followed a log-normal distribution, indicating structurally organized plant communities with balanced species distribution across most forest types.

The population structure revealed a predominance of individuals in lower girth classes, indicating active regeneration in the majority of forest ecosystems. However, the comparatively low representation of mature trees in certain forest types suggests differences in stand structure and regeneration status. Functional group analysis showed distinct patterns in the dominance and distribution of trees, shrubs, herbs, and climbers among forest types, reflecting the influence of local environmental conditions on community composition. The widespread occurrence and dominance of *Lantana camara* L. in the shrub layer further emphasizes its prominence across the studied forests.

Overall, the study provides a comprehensive assessment of the floristic composition, vegetation structure, diversity patterns, and regeneration status of the major forest ecosystems of the north-western Himalayan Shivalik foothills. These findings establish a scientific baseline for understanding vegetation dynamics and biodiversity patterns in the region and contribute valuable information for future ecological assessment and monitoring of these forest ecosystems.

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Department during the field survey.

CRediT authorship contribution statement

Ravi Kumar: Conceptulization, Data collection, Methedology, software, writing original draft, Formal analysis, Tabulation, Data curation; **Kushal:** Data collection, Tabulation, formal analysis, Data curation; **Subrata Nandy:** Software for Map preparation, Reviewing and editing; **Balkar Singh:** Reviewing and editing.

Conflict of interest

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

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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Phytosociology of Woody Species in the Bagnadi Range of Rajnandgaon Forest Division in Chhattisgarh, India

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ABSTRACT: Chhattisgarh is one of the most forest-rich states of India, with nearly 44% of its geographical area under forest cover, predominantly comprising tropical dry deciduous forests. However, information on the woody species composition and phytosociological characteristics of several forest ranges in central India remains limited. The present study was conducted in the Bagnadi Forest Range of Rajnandgaon Forest Division, Chhattisgarh, to document woody species diversity, community structure and phytosociological characteristics using standard quadrat sampling methods. Parameters including density, abundance, basal area, diversity indices and importance value index (IVI) were evaluated. A total of 43 woody species were recorded, comprising 41 tree species and 2 shrub species. The total woody species density, abundance, and basal area were estimated at 428 ha^{-1}, 4994.45 ha^{-1} and 23568.40 cm^2 ha^{-1}, respectively. Based on IVI, *Cordia myxa, Terminalia elliptica, Anogeissus latifolia, Buchanania latifolia, and Butea monosperma* were the most dominant species in the study area. The Shannon diversity index (1.53) and Simpson's dominance index (0.04) described the diversity and dominance patterns of the woody vegetation within the sampled area. These findings provide baseline information on the woody vegetation composition and phytosociological attributes of the Bagnadi Forest Range, which may support future ecological monitoring and forest management.

Keywords: Frequency, Density, Basal area, Abundance, Dominance, Diversity, Importance Value Index.

1. INTRODUCTION

Chhattisgarh is one of the most forest-rich states of India, with more than 44% of its geographical area under forest cover, predominantly comprising tropical dry deciduous forests dominated by *Shorea robusta* (sal), *Tectona grandis* (teak), bamboo species, and mixed deciduous vegetation. These forests are ecologically significant as they support rich biodiversity, provide habitat for diverse flora and fauna, regulate ecosystem processes and sustain rural livelihoods through the supply of timber and non-timber forest products (NTFPs) (Bardani, 2003). Earlier studies from Chhattisgarh have reported the importance of woody vegetation in maintaining forest structure and productivity, with Bardani (2003) documenting 84 NTFP species, including 38 tree species, from the Bhoramdeo Wildlife Sanctuary.

Species diversity and dominance are widely recognized as important indicators of forest community structure, ecological stability, and ecosystem functioning. Forests with high dominance by a few species often exhibit lower species richness, whereas higher species diversity generally reflects greater ecological resilience and stability (Putman, 1994). Recent studies conducted in tropical dry deciduous forests of Central India have further demonstrated that species composition, tree density, basal area and diversity are influenced by environmental conditions and disturbance regimes, emphasizing the need for periodic phytosociological assessments (Chaturvedi et al., 2019;

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Dugaya et al., 2020; Dar et al., 2025).

Phytosociological investigations provide quantitative information on species composition, density, frequency, abundance, basal area, Importance Value Index (IVI), and community structure, which are essential for evaluating forest health, biodiversity status, and conservation priorities (Sagar & Singh, 2003; Chaturvedi et al., 2017). Such baseline information is increasingly important in tropical dry deciduous forests experiencing anthropogenic disturbances, habitat fragmentation, forest fires and climate variability, as it supports long-term ecological monitoring and sustainable forest management (Mansingh et al., 2025; Ray et al., 2025).

Although several phytosociological studies have been conducted in different forest ecosystems of Central India, published information on the woody species composition and phytosociological characteristics of the Bagnadi Forest Range of Rajnandgaon Forest Division is still limited. The absence of comprehensive baseline ecological information restricts effective biodiversity assessment, long-term vegetation monitoring, and the development of site-specific conservation and forest management strategies. Therefore, systematic phytosociological documentation of the Bagnadi Forest Range is necessary to characterize its woody vegetation, identify dominant species, and generate baseline ecological data for future monitoring, biodiversity conservation, and sustainable forest management.

The objectives of the present study were: (i) to document the woody species composition of the Bagnadi Forest Range; (ii) to evaluate the phytosociological characteristics of woody vegetation using standard ecological parameters; and (iii) to generate baseline ecological information to support biodiversity conservation, ecological monitoring and sustainable forest management.

2. MATERIALS AND METHODS

2.1. Study Area

Rajnandgaon district is situated in the central part of Chhattisgarh, India, between 20°07′08″–21°49′55″ N latitude and 80°23′20″–81°13′05″ E longitude, at an average elevation of 298 m above mean sea level. Tropical dry deciduous forests characterise the Bagnadi Forest Range and fall under the 5A/C3 Southern Tropical Dry Mixed Deciduous Forest type. The climate is sub-humid, with a mean annual rainfall of 1200–1400 mm, about 80% of which is received during the southwest monsoon. May is the hottest month, while December is the coldest. The soils are predominantly Inceptisols and Alfisols.

The study area map (Figure 1) was prepared using Survey of India topographic maps, Google Earth satellite imagery, Forest Department boundary maps, GPS-based field observations, and GIS software (QGIS version 3.34/ArcGIS 10.x) to delineate the study area and sampling locations (Figure 1).

2.2. Vegetation Sampling

The population structure of woody species was studied using a quadrat method following R. Mishra (1968). A total of 150 quadrats of 10 m × 10 m size were laid randomly in the Bagnadi Forest Range. In each quadrat, all woody species were identified and enumerated, and Diameter at Breast Height (DBH) was measured for basal area estimation.

Quadrat locations were selected using a random sampling approach. The forest area was first traversed to identify accessible forest patches, and quadrats were randomly established to obtain representative coverage of the woody vegetation throughout the study area. Sampling was conducted across different forest patches to capture variation in species composition and community structure. Within each quadrat, all woody species (trees and shrubs) were identified and enumerated. The Diameter at Breast Height (DBH) of each tree was measured at 1.37 m above ground level with a diameter tape to estimate basal area. Species identification was carried out using standard regional floras and verified with available botanical literature.

A quadrat size of 10 m × 10 m (100 m²) was adopted following standard phytosociological sampling procedures for woody vegetation (Misra, 1968; Mueller-Dombois & Ellenberg, 1974). This quadrat size is widely used to assess species composition, density, frequency, abundance, and community structure in tropical forest ecosystems. A total of 150 quadrats covering 1.5 ha were sampled to ensure adequate representation of woody species across the study area.

2.3. Data Collection and Phytosociological Analysis

The phyto-sociological parameters such as frequency, density, abundance, basal area, relative frequency, relative density, relative abundance, relative dominance and Importance Value Index (IVI) were calculated using standard as per the following ecological formulas:

Frequency (%)

Frequency = (Number of quadrats in which the species

occurred / Total number of quadrats studied) × 100 Eq. 1

Density (ha^{-1})

Density = Total number of individuals of a species / Total area sampled (ha) Eq. 2

Abundance (ha^{-1})

Abundance = Total number of individuals of a species / Number of quadrats in which the species occurred Eq. 3

Basal cover (cm^{2}ha^{-1})

Individual tree

Basal Area=πD²/ Eq. 4

where D = Diameter at Breast Height (DBH)

Species basal area = $\sum BAi$

Basal area per hectare

BA (cm² ha^{-1}) = ∑BAi/1.5

Relative frequency (%)

Relative Frequency = (Frequency of a species / Sum of frequencies of all species) × 100 Eq. 5

Relative density (%)

Relative Density = (Density of a species / Total density of all species) × 100 Eq. 6

Relative abundance (%)

Relative abundance = (Abundance of individual species/ Abundance of all the species) × 100 Eq. 7

Relative dominance (%)

Relative Dominance = (Basal area of a species / Total basal area of all species) × 100 Eq. 8

2.4. Diversity Indices

IVI = Relative density + Relative frequency + Relative dominance Eq. 9

Simpson (1949) index of Dominance:

The equation used to calculate Simpson's index was

D = Σ pi² Eq. 10

Where,

D = Simpson index of dominance

ni = the IVI of individual species

N= IVI of all the species

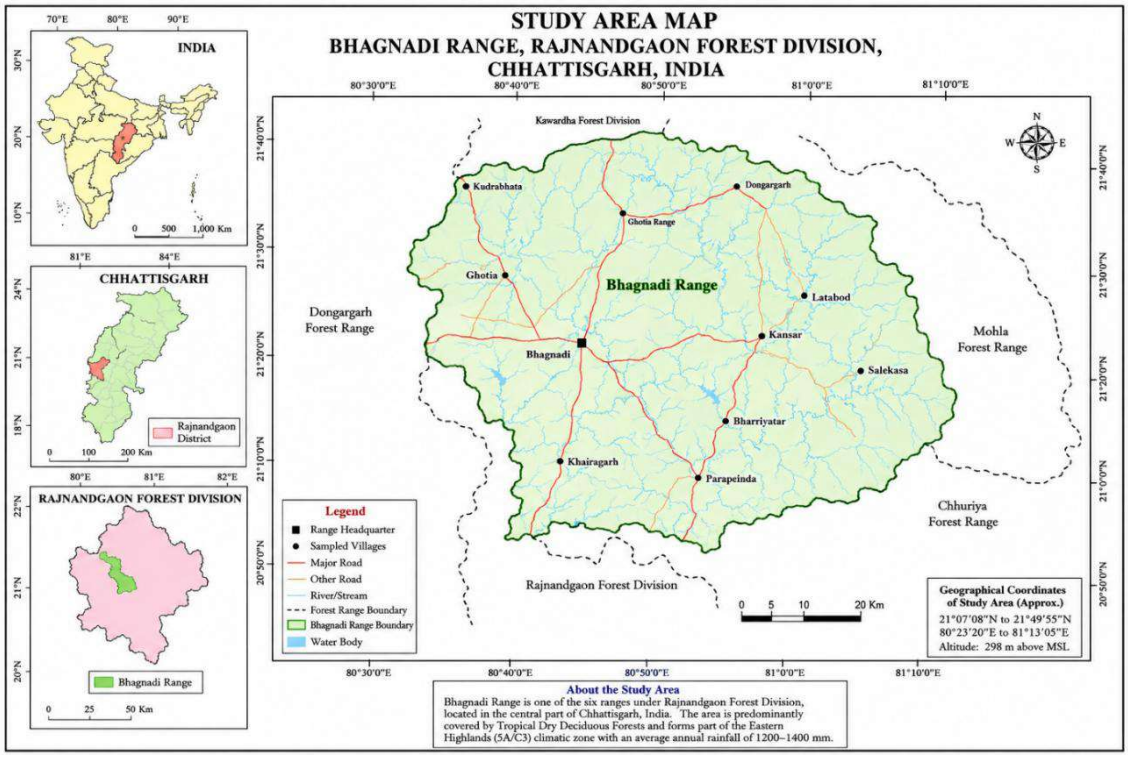
Margalef (1958) Diversity:

R = (S − 1) / ln(N) Eq. 11

where:

S = Total number of species

N = Total number of individuals



Source: Forest Department, Chhattisgarh; Survey of India; Google Earth imagery; author's field survey (2025)

Figure 1. Study area map of the Bagnadi forest range, Rajnandgaon Forest Division, Chhattisgarh, India. The map shows the geographical location and boundaries of the study area

3. RESULTS AND DISCUSSION

The phytosociological analysis of the Bagnadi Forest Range revealed considerable variation in the distribution, density, abundance and dominance of woody species. A total of 43 woody species were recorded, comprising 41 tree species and 2 shrub species, indicating moderate floristic diversity in the tropical dry deciduous forest ecosystem of the study area. Similar vegetation patterns have been reported in other dry deciduous forests of central India (Sagar & Singh, 2003), where a few dominant species contribute significantly to community structure and ecosystem functioning (Table 1).

3.1. Frequency and Relative Frequency

In the Bagnadi forest range, the maximum frequency was found in *Cordia myxa* (44.67%), followed by *Terminalia elliptica* (36.67%) and afterward, the species *Anogeissus latifolia, Buchanania latifolia, Butea monosperma, Madhuca indica, Diospyros melanoxylon, Tectona grandis* and *Semecarpus anacardium* were lodged with a frequency between 15 and 25%. In comparison, species showed a frequency between 10 and 15%: *Holarrhena antdysenteria, Lannea cormandalica, Pterocarpus marsupium, Schleichera oleosa* and *Syzygium cumini.* There were 26 woody species with less than 5% frequency, and among them, the producing woody species were *Aegle marmelos, Emblica officinalis, Anona squamosa* and *Terminalia belirica* (Table 2).

Table 1. Summary of phytosociological structure of woody minor forest species in the Bagnadi forest range of Rajnandgaon forest division in Chhattisgarh, India

Parameters	Bagnadi Forest Range
Total Species	43
Trees	41
-Shrubs	2
Density(ha^{-1})	
-Total Species	428.00
-Trees	425.33
-Shrubs	2.67
Abundance (ha^{-1})	
-Total Species	4994.45
-Trees	4794.45
-Shrubs	200.00
Basal Area (cm^2ha^{-1})	
-Total Species	23568.40
-Trees	23560.29
-Shrubs	8.11
Similarity index (%)	81.25
Dominance	0.04
Diversity	1.53

Source: Author's field survey and analysis

The relative frequency of woody species in the Bagnadi forest range was found to be the highest in *Cordia myxa* (11.96%), followed by *Terminalia elliptica* (9.82%) and between 3–7% in *Anogeissus latifolia, Buchanania latifolia, Butea monosperma, Diospyros melanoxylon, Holarrhena antdysenteria, Madhuca indica, Pterocarpus marsupium, Semecarpus anacardium* and *Tectona grandis.* The relative frequency of the less-than-1 % distribution was recorded in 18 woody species, which accounted for 41.9% of the community's woody species (Table 2).

3.2. Density and Relative Density

In the Bagnadi forest range, the maximum density was found in *Cordia myxa* (56 ha-1), followed by *Terminalia elliptica* (43.33 ha^{-1}), *Madhuca indica* (34 ha^{-1}), *Anogeissus latifolia* (26.67 ha^{-1}), *Buchanania latifolia* (24.67 ha^{-1}), *Semecarpus anacardium* (24.67 ha^{-1}), *Diospyros melanoxylon* (20.67 ha^{-1}), *Butea monosperma* (20.00 ha^{-1}) and *Tectona grandis* (19.33 ha^{-1}). Afterwards, 10 to 15 ha^{-1} population density was recorded in *Cassia fistula, Holarrhena antdysenteria, Lannea cormandalica, Pterocarpus marsupium, Schleichera oleosa* and *Syzygium cumini.* The next-level density of woody species, between 4 and 8 plant ha-1, was found in 11 species, which accounted for 25.5% of the community's woody species contribution: Emblica officinalis, Terminalia chebula, and Terminalia belerica. While population per hectare was found to be less than 4 ha^{-1} for 17 woody species, among them *Azadirachta indica, Pongamia pinnata* and *Zizyphus jujube are the* major ones.

The relative density is the share of an individual species in terms of numerical strength within the total population. In the Bagnadi forest range, the maximum relative density was found in *Cordia myxa* (13.08%), followed by *Terminalia elliptica* (10.12%), *Madhuca indica* (7.94%), *Anogeissus latifolia* (6.23%), *Buchanania latifolia* (5.76%), *Semecarpus anacardium* (5.76%), *Diospyros melanoxylon* (4.83%), *Butea monosperma* (4.67%) and *Tectona grandis* (4.52%). The 22 woody species shared a presence of less than 1.0%, with an aggregated 51.1 % contribution to the community woody species being Shorea robusta, Terminalia belirica and Aegle marmelos (Table 2).

Table 2. Distribution of woody minor forest species in the Bagnadi forest range of Rajnandgaon forest division in CG

Botanical name	Frequency (%)	Density (ha^{-1})	Abundance (ha^{-1})	Basal area (cm^2 ha^{-1})	Relative			IVI
					Frequency	Density	Dominance	
Aegle marmelos (L.) Correa	1.33	1.33	66.67	6.26	0.36	0.31	0.03	0.7
Adina cordifolia (Roxb.) Ridsdale	1.33	1.33	66.67	22.01	0.36	0.31	0.09	0.76
Anogeissus latifolia (Roxb. ex DC.) Wall. Ex Guillem. & Perr.	22.67	26.67	78.43	2380.09	6.07	6.23	10.10	22.4
Annona squamosa L.	2.67	3.33	83.33	101.22	0.71	0.78	0.43	1.92
Azadirachta indica A. Juss.	0.67	1.33	133.33	18.19	0.18	0.31	0.08	0.57
Bauhinia variegata L.	4.00	4.00	66.67	32.71	1.07	0.93	0.14	2.14
Bombax ceiba L.	1.33	2.00	100.00	85.41	0.36	0.47	0.36	1.19
Bridelia retusa (L.) A. Juss.	5.33	6.00	75.00	126.30	1.43	1.40	0.54	3.37
Buchanania latifolia Roxb.	23.33	24.67	70.48	927.56	6.25	5.76	3.94	15.95
Butea monosperma (Lam.) Taub.	17.33	20.00	76.92	583.10	4.64	4.67	2.47	11.78
Capparis decidua (Forssk.) Edgew.	2.00	2.67	88.89	60.53	0.54	0.62	0.26	1.42
Careya arborea Roxb.	3.33	3.33	66.67	24.28	0.89	0.78	0.10	1.77
Casearia graveolens Dalzell	4.67	5.33	76.19	71.88	1.25	1.25	0.31	2.81
Cassia fistula L.	9.33	12.00	85.71	142.59	2.50	2.80	0.61	5.91
Chloroxylon swietenia DC.	4.67	4.67	66.67	54.70	1.25	1.09	0.23	2.57
Cordia myxa L.	44.67	56.00	83.58	4943.82	11.96	13.08	20.98	46.02
Dalbergia paniculata Roxb.	2.67	4.00	100.00	217.67	0.71	0.93	0.92	2.56
Diospyros melanoxylon Roxb.	16.67	20.67	82.67	2441.89	4.46	4.83	10.36	19.65
Ehretia laevis Roxb.	1.33	2.67	133.33	2.92	0.36	0.62	0.01	0.99
Elaeodendron glaucum Pers.	4.00	5.33	88.89	24.59	1.07	1.25	0.10	2.42
Emblica officinalis L.	4.67	6.00	85.71	374.41	1.25	1.40	1.59	4.24
Ficus benghalensis L.	1.33	1.33	66.67	50.52	0.36	0.31	0.21	0.88
Ficus racemosa L.	2.00	2.00	66.67	13.56	0.54	0.47	0.06	1.07
Gardenia latifolia Aiton	0.67	0.67	66.67	1.46	0.18	0.16	0.01	0.35
Holarrhena antidysenterica (L.) Wall. ex A.DC.	11.33	12.67	74.51	92.98	3.04	2.96	0.39	6.39
Lagerstroemia parviflora Roxb.	2.67	4.00	100.00	57.01	0.71	0.93	0.24	1.88
Lannea coromandelica (Houtt.) Merr.	10.67	10.67	66.67	284.71	2.86	2.49	1.21	6.56
Madhuca indica (J. Koenig ex L.) J.F. Macbr.	29.33	34.00	77.27	4109.71	7.86	7.94	17.44	33.24
Pongamia pinnata (L.) Pierre	4.67	4.67	66.67	87.14	1.25	1.09	0.37	2.71
Pterocarpus marsupium Roxb.	11.33	11.33	66.67	444.10	3.04	2.65	1.88	7.57
Schleichera oleosa (Lour.) Oken	10.67	10.67	66.67	386.31	2.86	2.49	1.64	6.99
Semecarpus anacardium L.f.	23.33	24.67	70.48	1070.88	6.25	5.76	4.54	16.55
Shorea robusta Gaertn.	2.00	2.00	66.67	14.94	0.54	0.47	0.06	1.07
Sterculia urens Roxb.	3.33	3.33	66.67	64.82	0.89	0.78	0.28	1.95
Syzygium cumini (L.) Skeels	10.67	10.67	100.00	257.15	2.86	2.49	1.09	6.44

Cont...

Table 2. Distribution of woody minor forest species in the Bagnadi forest range of Rajnandgaon forest division in CG

Botanical name	Frequency (%)	Density (ha^{-1})	Abundance (ha^{-1})	Basal area (cm^2 ha^{-1})	Relative			IVI
					Frequency	Density	Dominance	
Tectona grandis L.f.	16.00	19.33	80.56	788.23	4.29	4.52	3.34	12.15
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	1.33	1.33	66.67	1.71	0.36	0.31	0.01	0.68
Terminalia elliptica Willd.	36.67	43.33	78.79	2918.68	9.82	10.12	12.38	32.32
Terminalia bellirica (Gaertn.) Roxb.	4.00	4.00	66.67	56.96	1.07	0.93	0.24	2.24
Terminalia chebula Retz.	8.00	8.67	72.22	174.37	2.14	2.02	0.74	4.9
Tinospora cordifolia (Willd.) Hook.f. & Thomson	2.67	2.67	66.67	42.95	0.71	0.62	0.18	1.51
Ziziphus jujuba Mill.*	2.00	2.00	66.67	5.65	0.54	0.47	0.02	1.03
Ziziphus rotundifolia (Forsk.) Lam.*	0.67	0.67	66.67	2.46	0.18	0.16	0.01	0.35

Note: The mark * is a shrub (Source: Author's field survey and analysis)

3.3. Abundance and Relative Abundance

The abundance of woody species in the Bagnadi forest range was found in the range of 100–135 plants ha^{-1}, where the maximum abundance was found in Azadirachta indica (133.3 plants ha^{-1}), followed by 100 plants ha-1 in *Bombax ceiba, Dalbergia paniculata, Syzygium cumini,* and *Lagerstroemia parviflora.* The large number of woody species 37 showed their abundance between 100 plant ha^{-1}, where *Anogeissus latifolia, Anona squamosa, Bridelia retusa, Buchanania latifolia, Butea monosperma, Capparis deciduas, Casaria graveolens, Cassia fistula, Cordia myxa, Diospyros melonoxylon, Emblica officinalis, Holarrhena antdysenterica, Madhuca indica* and *Terminalia elliptica* have importance for the production of MFP in the area.

The relative abundance refers to the actual numerical strength of a species among the total species available in a defined community. Here the maximum relative abundance was found in *Azadirachta indica* and *Ehretia laevis* (4.0%), followed by *Bombax ceiba, Dalbergia paniculata* and *Lagerstroemia parviflora* (3.0%), and the rest of the 38 woody species showed between 2 and 2.67% abundance, which aggregated 83% of the community woody species contribution (Table 2).

3.3. Basal area and Relative Dominance

In Bagnadi forest range, the maximum basal area was found in *Cordia myxa* (4943.8 cm2ha-1), followed by *Madhuca indica* (4109.71 cm^2ha^{-1}), *Terminalia elliptica* (2918.7 cm^2ha^{-1}), *Diospyros melonoxylon* (2441.89 cm^2ha^{-1}), *Anogeissus latifolia* (2380.09 cm^2 ha^{-1}), and *Semecarpus anacardium* (1070.88 cm2ha-1), respectively. Basal area was recorded between 100 and 927.56 cm^2ha^{-1} by 13 woody species, and out of them, *Buchanania latifolia* (927.56 cm^2 ha^{-1}), *Tectona grandis* (788.33 cm^2ha^{-1}), *Butea monosperma* (583.10 cm^2ha^{-1}), *Pterocarpus marsupium* (444.10 cm^2ha^{-1}), *Schleichera oleosa* (386.31 cm^2 ha^{-1}) and *Emblica officinalis* (374.41 cm^2ha^{-1}) were major woody species. The basal area occupied ranged from 40 to 100 cm2 ha1 among 10 woody species. While less than 40 cm2 ha-1 basal area was occupied by 13 woody species, among them minor forest-produced woody species, *Aegle marmelose* and *Azadirachta indica* were major ones.

The total land area occupancy of woody species by their basal area was 4994.45 cm^2 ha^{-1} in the Bagnadi forest range, which was contributed highest by *Cordia myxa* (20.98%), followed by *Madhuca indica* (17.44%), *Terminalia elliptica* (12.38%), *Diospyros melanoxylon* (10.36%) and *Anogeissus latifolia* (10.10%), respectively. The basal area contribution of nine woody species ranges from 1 to 4.5%, aggregating 21.7% of the total basal area. The population of 29 woody species occupied 7.04% basal cover in the range of Baghnadi forest (Table 2).

3.5. Importance Value Index (IVI)

The IVI of woody species in the Bagnadi forest range was found to be highest in *Cordia myxa* (46.02), followed by *Terminalia elliptica* (32.32). Afterwards, the importance value index between 10 and 20 occurred in *Anogeissus latifolia, Buchanania latifolia, Butea monosperma, Diospyros melanoxylon, Semecarpus anacardium,*

Madhuca indica and *Tectona grandis.* IVI less than 10 was shared by 23 woody species, which aggregated a (23.2%) contribution in the community woody species, while it was recorded very low (0.35) in *Gardenia latifolia* and *Zizyphus rotundifolia*. The Importance Value Index (IVI) revealed that *Cordia myxa* was the most dominant woody species in the Bagnadi Forest Range, followed by *Terminalia elliptica*. The high IVI of these species indicates their greater ecological importance and successful adaptation to the prevailing environmental conditions of the study area. Their dominance reflects a substantial contribution to the forest community in terms of density, frequency, and basal area, suggesting that they play a key role in maintaining the structural composition of this tropical dry deciduous forest. Similar dominance of *Terminalia elliptica* and *Anogeissus latifolia* has been reported from other tropical dry deciduous forests of Central India, where these species are recognized as characteristic components of relatively stable forest communities (Sagar & Singh, 2003; Chaturvedi et al., 2019).

Species with intermediate IVI values (10–20), including *Anogeissus latifolia, Buchanania latifolia, Butea monosperma, Diospyros melanoxylon, Semecarpus anacardium, Madhuca indica* and *Tectona grandis*, represent important associated species that contribute substantially to overall community structure and species diversity. Several of these species, particularly *Madhuca indica, Diospyros melanoxylon*, and Buchanania latifolia, are economically important non-timber forest products (NTFPs) that provide food, medicine, and livelihood resources for local communities. Their relatively high IVI suggests that the forest continues to support ecologically and socio-economically valuable woody species, highlighting its importance for biodiversity conservation and sustainable resource management. In contrast, twenty-three woody species exhibited IVI values below 10, indicating their comparatively lower abundance and ecological dominance within the community. Species such as *Gardenia latifolia* and *Ziziphus rotundifolia* recorded the lowest IVI values, which may reflect their restricted distribution, low population density, or specific habitat requirements within the study area. Low IVI values do not necessarily indicate poor ecological significance; rather, such species contribute to overall species richness and should be considered in conservation planning because rare and less abundant species are generally more susceptible to habitat alteration and anthropogenic disturbances.

Overall, the observed IVI pattern indicates that the Bagnadi Forest Range is characterized by a few dominant woody species accompanied by a relatively large number of less abundant associated species, a pattern typical of tropical dry deciduous forests. This community structure suggests moderate species diversity with clear dominance by a limited number of species, reflecting the existing environmental conditions and disturbance regime. The baseline information generated through the present study will be valuable for long-term ecological monitoring, biodiversity conservation, and the development of site-specific forest management strategies. The importance value index (IVI) was calculated using Eq. 9. The importance value index (IVI) values of woody species are presented in Table 2 and illustrated in Figure 2.

3.6. Species Diversity and Dominance

The Shannon–Wiener diversity index (H′) was recorded as 1.53*, while Simpson's dominance index (D) was* 0.04*. These values describe* the diversity and dominance characteristics of the woody vegetation in the Bagnadi Forest Range. The observed community was characterized by the dominance of a few woody species with relatively high Importance Value Index (IVI) values. In contrast, many other species occurred with comparatively lower abundance and IVI. Similar diversity and dominance patterns have been reported for tropical dry deciduous forests by Sagar & Singh (2003) and Putman (1994). However, the present study is descriptive and does not evaluate statistical relationships among species diversity, dominance, and density; therefore, no causal relationship is inferred from these observations (Table 1).

The study documented several woody species widely recognised as important non-timber forest products (NTFPs) in Central India. Species such as *Madhuca indica, Diospyros melanoxylon, Buchanania latifolia,* and *Emblica officinalis* are reported in the literature as valuable sources of flowers, fruits, seeds, leaves, and other forest products (Naugraiya & Pathak, 1986; Naugraiya & Pathak, 1993; Tripathi et al., 2000). Their occurrence and relatively high Importance Value Index (IVI) in the Bagnadi Forest Range highlight their ecological significance and their recognized importance in the regional NTFP resource base. However, the present study was limited to the phytosociological assessment of woody vegetation and did not evaluate livelihood dependence, harvesting intensity, extraction pressure or the economic value of these species. Therefore, any socio-economic implications are discussed only in the

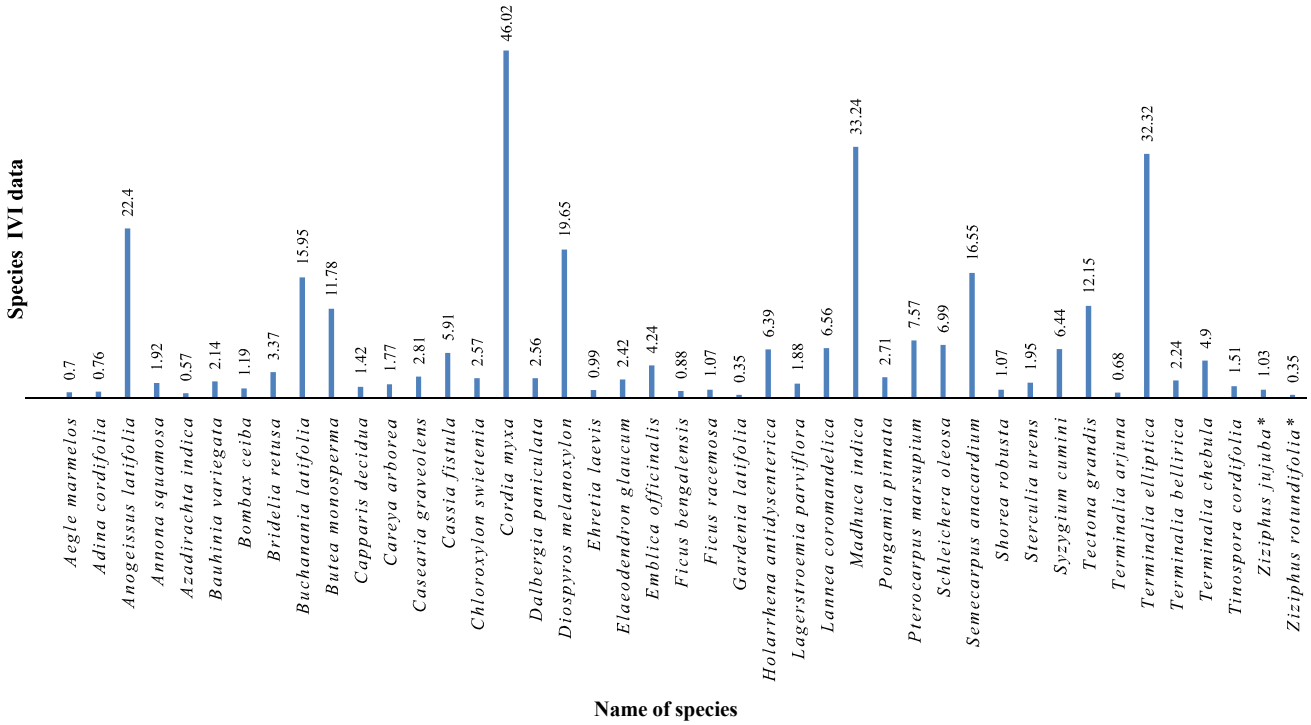


Figure 2. Importance Value Index (IVI) of woody species in the Bagnadi Forest Range, Rajnandgaon Forest Division, Chhattisgarh, India. The figure compares the IVI values of all recorded woody species, indicating their relative ecological importance based on relative density, relative frequency, and relative dominance

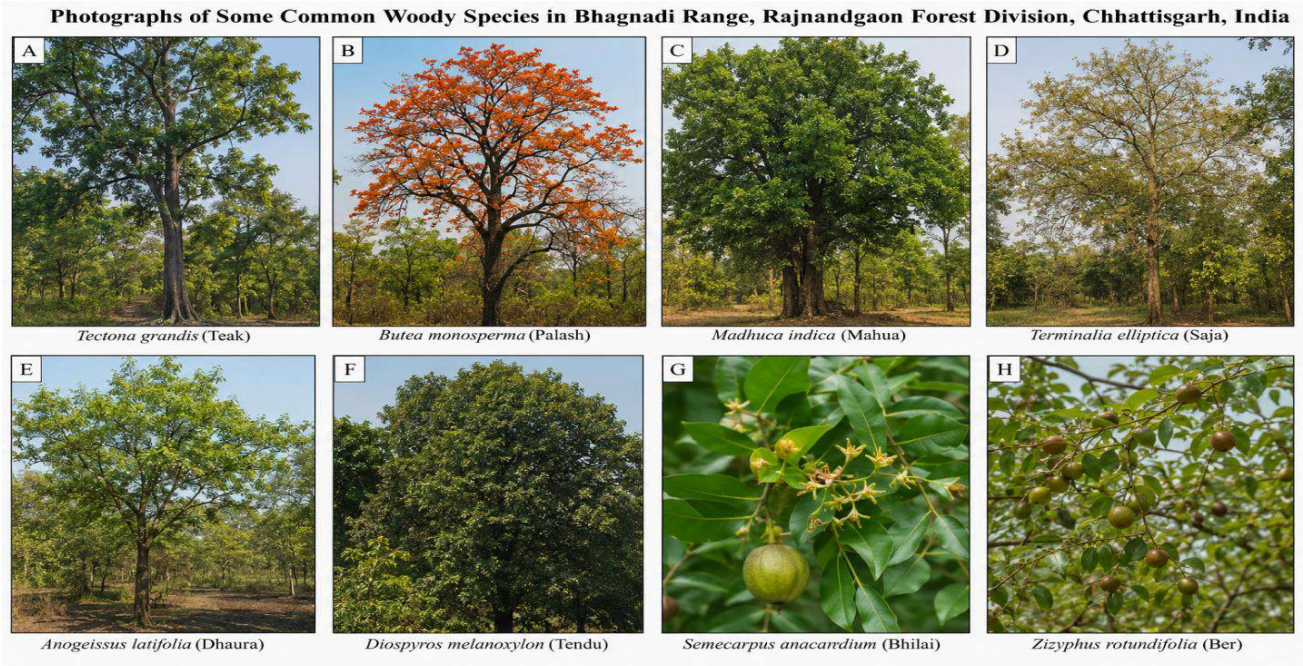


Figure 3. Photographs of representative woody species recorded in the Bagnadi Forest Range, Rajnandgaon Forest Division, Chhattisgarh, India. The photographs illustrate the dominant and ecologically important woody species observed during the phytosociological survey. (A) Cordia myxa, (B) Terminalia elliptica, (C) Anogeissus latifolia, (D) Buchanania latifolia, (E) Madhuca indica, (F) Diospyros melanoxylon, (G) Butea monosperma, and (H) Tectona grandis. These images provide visual identification of representative species contributing to the forest community

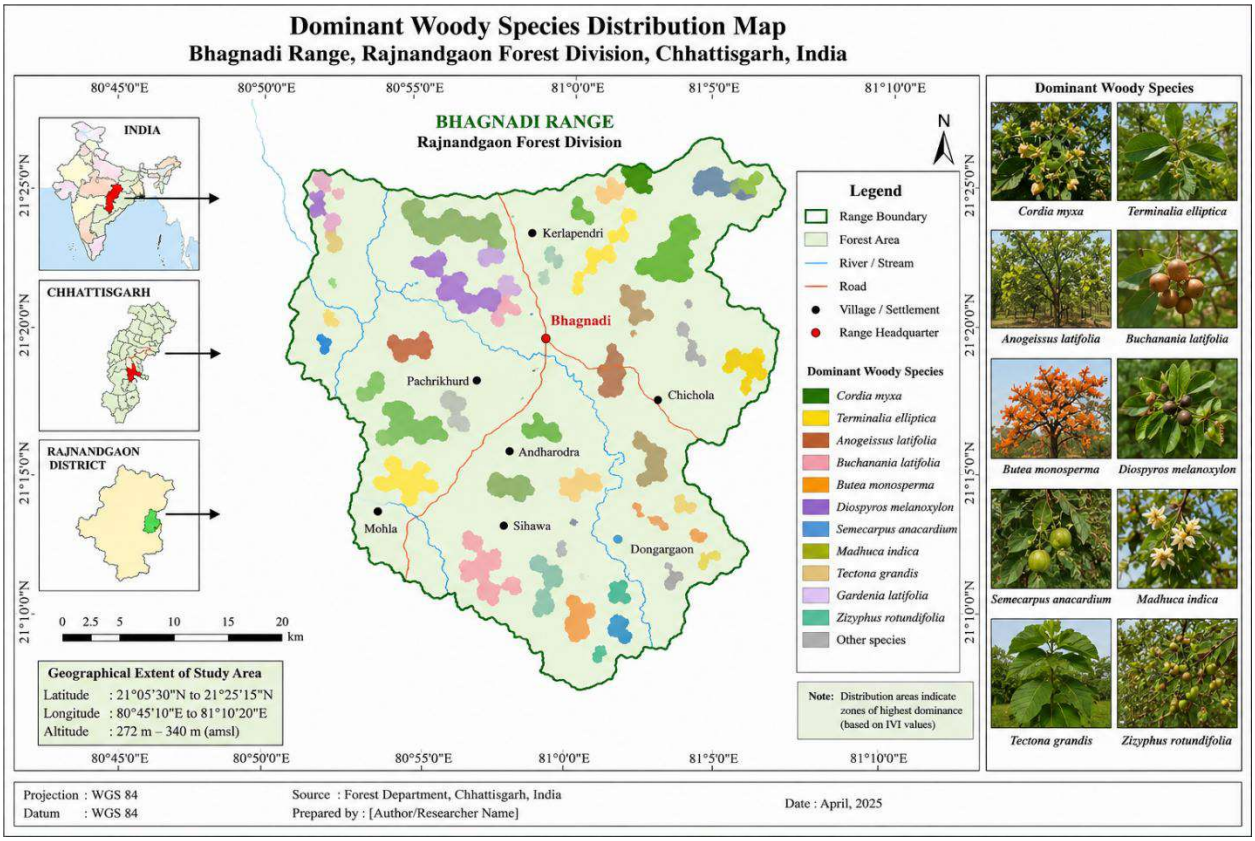


Figure 4. Distribution map of the dominant woody species in the Bagnadi Forest Range, Rajnandgaon Forest Division, Chhattisgarh, India. The map illustrates the spatial distribution of the major woody species recorded during the phytosociological survey, based on field observations. Source**:** Forest Department, Chhattisgarh (2025); Survey and mapping prepared by the authors using GPS field data and GIS analysis

context of published literature and should not be interpreted as direct evidence from the present study (Figures 3 and 4).

4. CONCLUSION

The present study provides baseline information on the woody species composition and phytosociological characteristics of the Bagnadi Forest Range in the Rajnandgaon Forest Division, Chhattisgarh. A total of 43 woody species were recorded, with *Cordia myxa, Terminalia elliptica, Madhuca indica,* and *Anogeissus latifolia* exhibiting the highest Importance Value Index (IVI), indicating their ecological prominence within the woody community. The observed Shannon–Wiener diversity and Simpson's dominance indices indicate a community characterised by the dominance of a few species alongside the presence of several less abundant species. However, the present study did not evaluate statistical relationships among diversity, dominance and density; therefore, no causal relationship is inferred.

The findings provide useful baseline ecological information that may support future biodiversity monitoring, conservation planning, and sustainable forest management in the Bagnadi Forest Range. Although several recorded species are recognised as important non-timber forest products (NTFPs) in Central India, the present study did not assess livelihood dependence, harvesting intensity or economic value; therefore, no direct socio-economic conclusions are drawn. Furthermore, the study was limited to phytosociological assessment and did not include regeneration status, disturbance assessment or detailed statistical analyses of ecological relationships. Future investigations incorporating these aspects, together with long-term vegetation monitoring, will provide a more comprehensive understanding of forest dynamics and strengthen conservation and management strategies.

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CRediT authorship contribution statement

Nalish Kumar Anchal: Conceptualisation, Data collection, Writing original draft, Formal analysis, Data curation; **Mahendra Nath Naugraiya:** Reviewing and editing, Visualization, Tabulation, Data curation. Methodology, Software, Supervision,

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Conflict of interest

The authors declare that they have no competing interests.

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

The authors used AI-assisted tools only for language improvement and grammatical correction during manuscript preparation. All scientific interpretations, analysis and conclusions are solely the responsibility of the authors.

Data availability statement

The data and material are available from the first author.

Ethical approval and consent to participate

This study did not involve human participants or animal experimentation; therefore, ethical approval and consent to participate were not required.

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Insect Visitor Diversity and Relative Abundance on *Cressa cretica* (Linnaeus) - An Important Floral Resource in Coastal Rice-Fallow Ecosystems of Andhra Pradesh, India

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ABSTRACT: The present study was conducted during the summer of 2025 (March–May) to assess the diversity and relative abundance of flower visitors associated with *C. cretica*, a naturally occurring flowering halophyte in the coastal rice-fallow ecosystems of Cheruvu Jammulapalem village, Bapatla district, Andhra Pradesh, India. Flower visitor activity was recorded at different flowering stages during four diurnal observation periods and ecological diversity indices were computed to characterize the flower visitor community. A total of 28 flower- visiting species belonging to five insect orders were recorded. Diptera was the most species-rich order (11 species), followed by Hymenoptera (10 species), Lepidoptera (4 species), Coleoptera (2 species) and Hemiptera (1 species). *Apis mellifera* was the predominant flower visitor during the early flowering stages, whereas Vespidae sp. (1) predominated during the advanced and peak flowering stages. Flower visitor activity exhibited distinct diurnal variation with maximum visitation recorded during the mid-morning (09:30–11:05h, 6.30 ± 0.65) and early afternoon (12:30–14:05h, 5.92 ± 0.57) observation periods. The Shannon–Weaver Diversity Index ranged from 1.61 to 2.18, while Simpson's Diversity Index (1-D) ranged from 0.90 to 0.93, indicating moderate to high diversity of flower visitors. A two-way ANOVA revealed that both flowering stage and observation time significantly influenced overall flower visitor abundance ($P < 0.001$). Dipteran insects also represented a substantial proportion of the flower visitor assemblage throughout the flowering period. These findings indicate that *C. cretica* supports a diverse community of flower-visiting insects and serves as a valuable floral resource in coastal rice-fallow ecosystems, particularly during periods of seasonal floral scarcity.

Keywords: Coastal agroecosystems, *Cressa cretica*, Diversity indices, Flower visitors, Rice fallows

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1. INTRODUCTION

Flower-visiting insects are essential components of terrestrial ecosystems and play a vital role in maintaining ecological balance and supporting agricultural productivity through their interactions with flowering plants. Flowering plants provide essential floral resources, including nectar and pollen, whereas flower-visiting insects facilitate pollen transfer and contribute to plant reproduction. However, the global decline in flower-visiting insect populations, largely driven by habitat loss, land-use change and agricultural intensification, has raised serious concerns about the reduced availability of floral resources and the consequent impacts on ecosystem functioning and agricultural sustainability (Potts et al., 2010; Vickruck et al., 2021). These concerns highlight the importance of identifying and conserving alternative floral resources, particularly during periods of seasonal floral scarcity in agroecosystems.

In agroecosystems, naturally occurring weeds frequently serve as valuable floral resources for a wide range of flower-visiting insects when cultivated crops are not in bloom, thereby supporting insect diversity and maintaining ecological function (Rollin et al., 2013; Altieri et al., 2015). *Cressa cretica* (Convolvulaceae) is a perennial halophytic weed commonly found in coastal rice-fallow ecosystems, salt marshes and saline soils. Following the drainage of rice fields, it emerges profusely during the summer months and flowers abundantly from March to April, a period when alternative flowering plants are relatively scarce. Its prolonged flowering period and accessible floral morphology make it an attractive resource for a diverse assemblage of flower-visiting insects (Subhash, 2025). In addition to its ecological significance, *C. cretica* is well known for its medicinal properties (Priyashree et al., 2010).

Despite its widespread distribution in coastal habitats, information on the diversity, composition and temporal abundance of flower-visiting insects associated with *C. cretica* remains limited. Therefore, the present study was undertaken to document the diversity and relative abundance of flower visitors associated with *C. cretica* and to evaluate its potential role as a floral resource for sustaining flower-visiting insect communities in coastal rice-fallow ecosystems in Andhra Pradesh, India.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was conducted during the summer season of 2025 (March–May 2025) in rice-fallow fields in Cheruvu Jammulapalem village, Bapatla district, Andhra Pradesh, India. The study site was selected based on the natural and abundant occurrence of *C. cretica*. Geographically, the experimental site was located at 15.93° N latitude and 80.44° E longitude, at an altitude of approximately 10 m above mean sea level. The region experiences a tropical coastal climate, receiving an average annual rainfall of approximately 914 mm, most of which occurs during the southwest and northeast monsoon seasons.

2.2. Sampling Methodology

Observations on flower-visiting insects associated with *C. cretica* were recorded during the peak flowering period from 15th March to 15th April 2025 at the experimental site. Sampling was conducted on eight observation days, covering four flowering stages (25%, 50%, 75%, and >90% flowering). The flowering stage was determined by visually assessing the proportion of open flowers relative to the total number of flower buds and open flowers within each sampling quadrat. At each flowering stage, observations were recorded in five randomly selected quadrats (1 m × 1 m), which served as independent sampling units for the study. A five-minute observation period per 1 m² quadrat was adopted following standard flower visitor observation protocols, as this duration provides sufficient time to record actively foraging flower visitors while minimizing observer disturbance and repeated counting of individual visitors.

On each observation day, flower visitors were recorded during four fixed time intervals, namely 06:30–08:05 h, 09:30–11:05 h, 12:30–14:05 h and 15:30–17:05 h following the methodology described by Revanasidda and Belavadi (2019) to capture diurnal variation in visitation activity. During each observation period, all insects visiting the flowers within each quadrat were visually counted and recorded. Only insects that landed on flowers and exhibited active foraging behavior were considered flower visitors. Representative specimens were collected using fine-mesh sweep nets, preserved properly, and later identified with the assistance of taxonomic experts at ICAR–National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru and the Zoological Survey of India (ZSI), Kolkata. In cases where reliable species-level identification was not possible owing to the unavailability of preserved voucher specimens, flower visitors were assigned to the highest confidence determinable taxonomic level based on available field photographs and external morphological characteristics.

The same sampling protocol was followed on each observation day, providing temporal replication across observation dates and spatial replication through multiple quadrats. In total, the study comprised 160 observation units (8 sampling dates × 4 observation periods × 5 quadrats), providing a robust dataset for evaluating variation in flower visitor diversity and abundance across flowering stages and observation periods.

2.3. Diversity Indices

Species diversity, dominance, and evenness of the flower visitor community were assessed using standard ecological diversity indices.

2.3.1 Shannon– Weaver Diversity Index (H′)

The Shannon– Weaver (1963) diversity index was used to estimate species diversity based on the proportion of individuals of each species, and was calculated as (Eq.1):

$H' = -\sum (\ln pi \times pi)$ ……(1)

where pi = Proportion of individuals belonging to the **i**th flower visitor species, and

ln = natural logarithm (base e = 2.718)

2.3.2. Species Evenness (E)

Species evenness was calculated using the Equation 2:

$E = H'/H_{max}$……(2)

where H_{max}=ln S

S = Total number of flower visitor species

Species evenness (E) ranges from 0 to 1, with values approaching *1* indicating a more even distribution of individuals among species.

2.3.3. Simpson's Diversity Index (D)

Simpson's diversity index (D) was used to measure species diversity and dominance within the flower visitor community, and was calculated as (Eq.3):

$D = \sum pi^2$……(3)

Simpson's Index of Diversity was then calculated as 1−D

where pi = proportion of individuals belonging to the **i**th flower visitor species relative to the total number of individuals;

Values of 1−D range from 0 to 1, with values approaching ***1*** indicating greater species diversity and lower dominance and values approaching 0 indicating lower diversity and higher dominance.

2.3.4. Berger–Parker Dominance Index (d)

The dominance of flower visitor species was estimated using Berger–Parker (1970) dominance index, expressed as (Eq.4):

d = ni/N……(4)

where ni = number of individuals of the ith species on the sampling date, and

N = total number of individuals in the sample

A higher *d* value indicates greater dominance of a single species and lower species diversity.

2.4. Relative Abundance of Flower Visitors:

The relative abundance of flower visitors was estimated using pooled observations across all sampling quadrats and the four daily observation periods for each flowering stage. The mean number of individuals of each flower-visiting species recorded per square meter during a five-minute observation period was calculated. Relative abundance was expressed as the percentage contribution of each species to the total number of flower-visiting individuals recorded during the corresponding flowering stage or observation period, using the following formula (Eq.5):

Ra (%) = (ni/N) × 100……..(5)

where, ni –number of individuals of the **i**th flower-visiting species, and

N = Total number of individuals of all flower-visitor species recorded during the corresponding flowering stage or observation period

2.5. Statistical Analysis

The number of flower visitors recorded at different flowering stages and observation periods was expressed as the mean number of individuals per square meter per five-minute observation period. Relative abundance of each visitor species was calculated as described in Section 2.4. Species diversity indices were computed using standard ecological formulae. Descriptive statistics, including means and percentages, were used to summarise the diversity and abundance of flower-visitors. To evaluate the effects of flowering stage and observation time on total flower visitor abundance, a two-way analysis of variance (ANOVA) was performed, followed by Tukey's honestly significant difference (HSD) test for post-hoc mean comparisons. Statistical significance was assessed at the 5% probability level ($P \leq 0.05$). All statistical analyses were performed using IBM SPSS Statistics, Version 25.0.

3. RESULTS AND DISCUSSION

3.1. Flower Visitor Diversity on *C. cretica*

A total of 28 flower-visiting insect species belonging to five insect orders, 16 families and 28 genera were recorded on *C. cretica* during the study period (Table 1 and Figure 1).

Among the recorded taxa, Diptera was the most diverse order, comprising 11 species (39.3%) distributed across seven families, followed by Hymenoptera with 10 species (35.7%) representing three families. Lepidoptera constituted four species (14.3%) belonging to three families, whereas Coleoptera and Hemiptera were represented by two species (7.1%) and one species (3.6%), respectively. The predominance of dipteran flower visitors indicates that *C. cretica* provides suitable floral resources for a wide range of fly species, in addition to hymenopteran visitors.

The diverse assemblage of flower visitors recorded on *C. cretica* suggests that this halophytic species serves as a potential floral resource for a broad spectrum of insects during the summer season, when flowering plants are relatively scarce in coastal rice-fallow ecosystems. The open and easily accessible floral structure of *C. cretica* may facilitate visitation by insects possessing a wide range of mouthpart types and foraging behaviours. Similar observations have been reported for several naturally occurring flowering plants that support diverse assemblages of flower-visiting insects in agricultural ecosystems (Rollin et al., 2013; Altieri et al., 2015).

The predominance of dipteran flower visitors observed in this study is ecologically noteworthy. Dipterans are increasingly recognized as important components of flower-visitor communities, particularly in natural and semi-natural habitats, where they often complement bee visitation (Orford et al., 2015; Rader et al., 2016). Their high representation in *C. cretica* suggests that this species helps maintain flower-visitor diversity within coastal agroecosystems. Similar findings have been reported in other wild flowering plant species, where dipteran visitors constitute a substantial component of the flower-visitor community, particularly in plants with open and easily accessible floral architecture (Ssymank et al., 2015; Strzałkowska et al., 2016; Balfour & Ratnieks, 2022).

3.2. Relative Abundance of Flower Visitors at Different Flowering Stages of *C. cretica*

Figure 3 illustrates the stage-wise changes in the relative abundance of the major flower visitor groups associated with *C. cretica*. During the 25% flowering stage, *A. mellifera* constituted 27.68% of the total flower visitors and was one of the most abundant species along with Crambidae sp. (1) (28.07%), the dark grass blue butterfly (*Zizeeria karsandra*) (14.68%) and *Vespidae* sp. (1) (8.22%). Dipteran visitors were represented at comparatively lower relative abundance during this stage. At 50% flowering, *A. mellifera* remained the predominant flower visitor (35.54%), followed by *Crambidae* sp. (1) (27.62%), dark grass blue butterflies (8.02%), *Vespidae* sp. (1) (6.92%) and muscid flies (6.03%), indicating increased overall flower visitation during the intermediate flowering stage. A noticeable shift in flower visitor composition was observed at 75% flowering, where

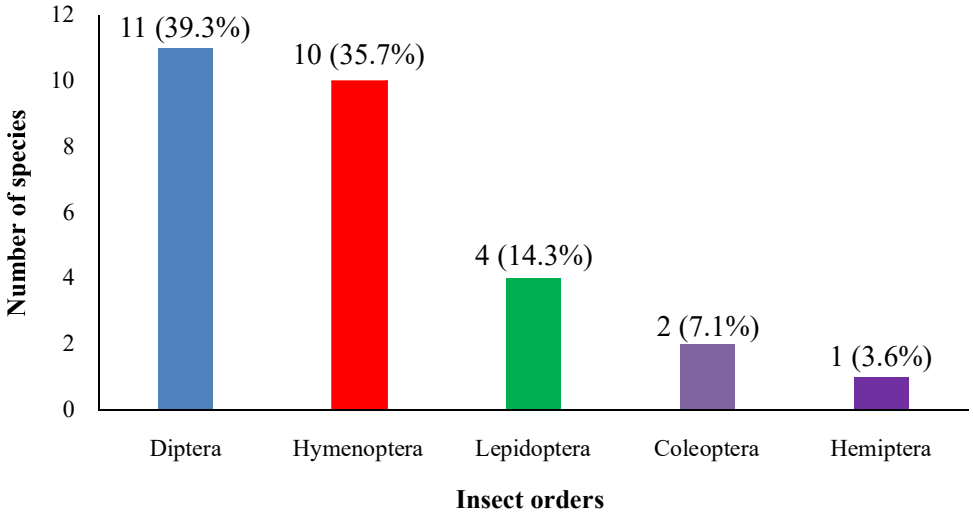


Figure 1. Distribution of flower visitor species among the major insect orders associated with *C. cretica.* Bars indicate the number of species recorded in each insect order and the values in parentheses represent the percentage contribution of each order to the total flower visitor assemblage (28 species).

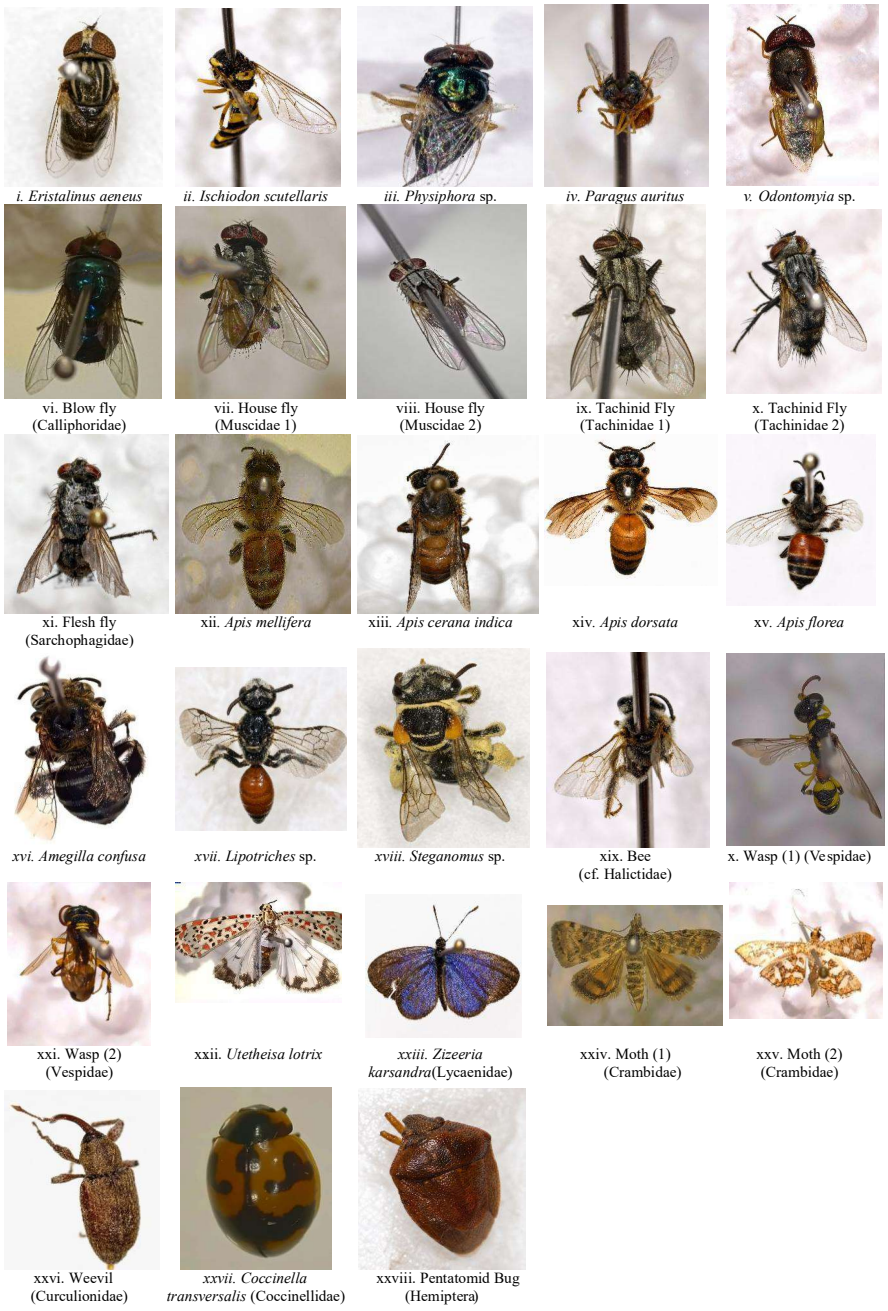


Figure 2. Flower visitors on *C. cretica* during *summer*, 2025 (March–May 2025) in coastal rice-fallow ecosystems of Bapatla district, Andhra Pradesh, India. Within Hymenoptera, the family Apidae was represented by five species *viz*., *Apis mellifera* Linnaeus, *Apis cerana indica* Fabricius, *Apis dorsata* Fabricius, *Apis florea* Fabricius and *Amegilla confusa* Smith (Figure 2, xii–xvi), while Halictidae was represented by *Lipotriches* sp. and *Steganomus* sp. (Figure 2, xvii and xviii) and Vespidae by two vespid morphospecies (Figure 2, xx and xxi). Diptera included representatives of Syrphidae**,** Muscidae**,** Tachinidae**,** Calliphoridae**,** Sarcophagidae**,** Ulidiidae and Stratiomyidae (Figure 2, i–xi), indicating considerable taxonomic diversity within this order. Lepidoptera comprised species belonging to Lycaenidae**,** Crambidae and Erebidae (Figure 2, xxii and xxv), whereas Coleoptera and Hemiptera were represented by genera of Coccinellidae and Curculionidae (Figure 2, xxvi and xxvii) and Pentatomidae (Figure 2, xxviii), respectively. A limitation of the present study is that a small number of flower visitors could only be identified to the family level, reflecting the taxonomic limitations associated with the recorded flower visitor assemblage

Vespidae sp. (1) became the most abundant visitor (29.83%), followed by *A. mellifera* (22.21%). Dipteran visitors, particularly muscid flies (4.84%) and calliphorid flies (3.93%), also showed increased representation, while the relative abundance of lepidopteran visitors declined compared with earlier flowering stages. During the peak flowering stage (>90%), *Vespidae* sp. (1) remained the dominant flower visitor (37.13%), followed by *Crambidae* sp. (1) (19.26%) and *A. mellifera* (17.10%). Other dipteran and hymenopteran species continued to occur at relatively low abundance throughout the flowering period.

Table 1. Diversity of flower visitors on *C. cretica* during s*ummer*, 2025 at Cheruvu Jammulapalem, Bapatla

Flower visitor	Family
Order: Diptera	
Eristalinus aeneus	Syrphidae
Ischiodon scutellaris	Syrphidae
Paragus auritus	Syrphidae
Physiphora sp.	Ulidiidae
Odontomyia sp.	Stratiomyidae
Muscidae sp. (1)	Muscidae
Muscidae sp. (2)	Muscidae
Tachinidae sp. (1)	Tachinidae
Tachinidae sp. (2)	Tachinidae
Calliphoridae sp.	Calliphoridae
Sarcophagidae sp.	Sarcophagidae
Order: Hymenoptera	
Apis mellifera	Apidae
Apis cerana indica	Apidae
Apis dorsata	Apidae
Apis florea	Apidae
Amegilla confusa	Apidae
Lipotriches sp.	Halictidae
Steganomus sp.	Halictidae
Vespidae sp. (1)	Vespidae
Vespidae sp. (2)	Vespidae
Halictidae sp.	cf. Halictidae
Order: Lepidoptera	
Utetheisa lotrix	Erebidae
Zizeeria karsandra	Lycaenidae
Crambidae sp. (1)	Crambidae
Crambidae sp. (2)	Crambidae
Order: Coleoptera	
Coccinella transversalis	Coccinellidae
Curculionid Weevil	Curculionidae
Order: Hemiptera	
Pentatomid Bug	Pentatomidae

The composition of the flower visitor community changed progressively with flowering stage with *A. mellifera* predominating during the early and intermediate flowering stages, whereas *Vespidae* sp. (1) became more abundant during advanced flowering. These observations indicate a stage-dependent variation in flower visitor assemblages on *C. cretica*, suggesting that different groups of flower-visiting insects utilize the floral resources at different phases of flowering. The observed temporal changes in flower visitor composition may reflect differences in foraging preferences, floral display and resource availability associated with flower development. Similar shifts in the composition and abundance of flower-visiting insect communities across flowering phenology have been reported in several wild flowering plant species (Denisow et al., 2014a).

3.3. Diversity Indices of Flower Visitors at Different Flowering Stages of *C. cretica*

The diversity and evenness of the flower visitor community associated with *C. cretica* varied across different flowering stages. The Shannon– Weaver diversity index (H′) ranged from 1.61 at 25% flowering to 2.18 at 75% flowering (Table 2), indicating an increase in species diversity as the flowering period progressed. The highest diversity recorded during the 75% flowering stage suggests the coexistence of a larger number of flower visitor species with a relatively balanced distribution of individuals. In contrast, the comparatively lower diversity at the 25% flowering stage reflects the predominance of a few abundant visitor species during the early phase of flowering.

Species evenness (E) also increased progressively from 0.87 at 25% flowering to 0.89 at 75% flowering before declining slightly (0.85) at the peak flowering stage (>90%) (Table 3). Higher evenness values observed during the intermediate flowering stages (50% and 75%) indicate a more equitable distribution of individuals among the recorded flower visitor species, whereas lower evenness at the peak flowering stage suggests greater dominance by a limited number of taxa.

A similar trend was observed for Simpson's Index of Diversity (1−D), which attained its highest value (0.93) during the 75% and peak (>90%) flowering stages, indicating greater species diversity and lower dominance

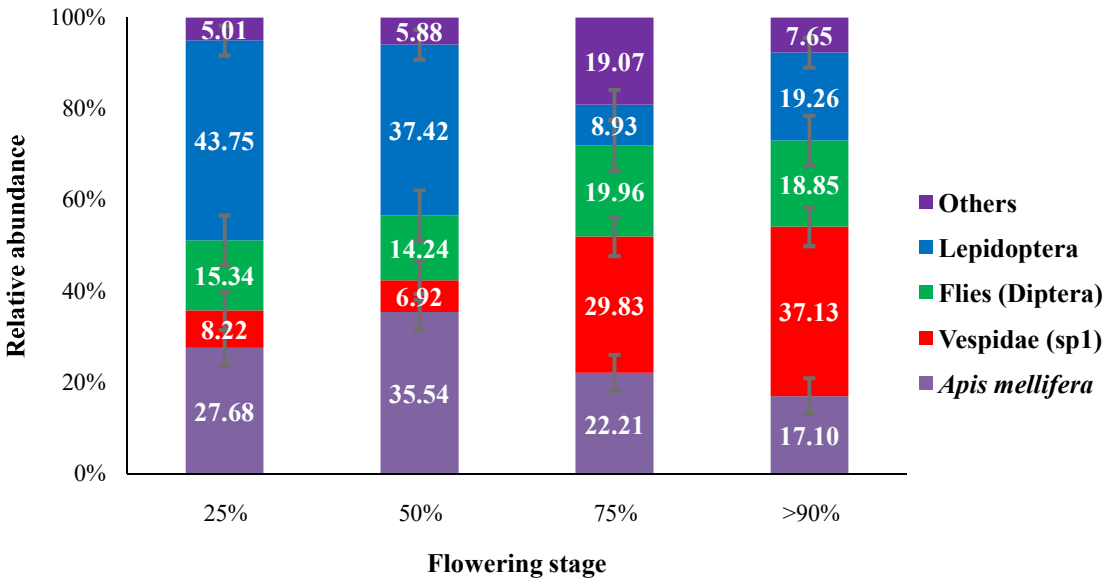


Figure 3. Stage-wise variation in relative abundance (%) of major flower visitors on *C. cretica* at four flowering stages (25%, 50%, 75% and >90% flowering) during summer 2025 at Cheruvu Jammulapalem, Bapatla. Bars represent the percentage contribution of each visitor group to the total flower visitor assemblage recorded at each flowering stage. Values inside the bars represent mean relative abundance (%). Error bars indicate Standard Error (SE) based on replicated observations across quadrats, timer periods and sampling dates

Table 2. Shannon-Weaver Diversity Index (H′) of flower visitors at different flowering stages of *C. cretica* during *summer*, 2025

Hour of the day	25% flowering	50% flowering	75% flowering	>90 % flowering
6.30-08:05	1.82	1.63	2.18	1.78
09.30-11.05	1.95	1.81	2.18	1.86
12:30-14:05	1.87	1.84	2.01	2.00
15:30-17:05	1.61	1.72	1.90	1.56

Table 3. Shannon's Evenness Index (E) and Simpson's Diversity Index (1-D) of flower visitors at different flowering stages of *C. cretica* during *summer,* 2025

Stage of flowering	Evenness Index (E)	Simpson's Diversity Index (1-D)
25% flowering	0.87	0.92
50% flowering	0.89	0.90
75% flowering	0.89	0.93
>90 % flowering	0.85	0.93

(Table 3). The relatively lower values recorded during the initial (25%) and mid (50%) flowering stages reflect the higher contribution of a few dominant flower-visiting species to the overall community.

Across flowering stages, diversity values were generally higher during mid-morning and early afternoon observations, while late afternoon periods showed reduced diversity, possibly reflecting temperature-mediated foraging behaviour. Similar density-dependent patterns of insect visitor diversity have been reported by Reghunath et al. (2024) in *Syzygium parameswaranii*.

Diversity indices consistently indicated that the 75% flowering stage supported the most diverse and evenly distributed flower visitor community. These findings demonstrate that the structure of the flower visitor community varied with flowering phenology, with intermediate flowering stages supporting greater diversity than early flowering stages. Comparable increases in flower visitor diversity with advancing flowering phenology have also been reported for other flowering plant species (Denisow and Bożek, 2008; Denisow et al., 2014a).

3.4. Species Dominance of Flower Visitors at Different Flowering Stages of *C. cretica*

Species dominance estimated using the Berger–Parker dominance index (d), varied across the flowering stages of *C. cretica* (Table 4). During the 25% and 50% flowering stages, *A. mellifera* exhibited the highest dominance, indicating that honey bees constituted the largest proportion of the flower visitor community during the early phase of flowering. In contrast, Vespidae sp. (1) emerged as the dominant flower visitor during the 75% and >90% flowering stages, reflecting a marked shift in community composition as flowering progressed.

Table 4. Dominance of flower visitors at different flowering stages of *C. cretica* during *summer,* 2025 at Cheruvu Jammulapalem, Bapatla

Flower visitor	Flowering stage							
	25% flowering		50% flowering		75% flowering		90% flowering	
	Berger-Parker Dominance Index (d)							
	d	1/d*	d	1/d*	d	1/d*	d	1/d*
Eristalinus aeneus	0.036	27.523	0.020	50.222	0.019	52.021	0.047	21.148
Ischiodon scutellaris	0.000	0.000	0.000	0.000	0.000	0.000	0.011	91.642
Paragus auritus	0.000	0.000	0.000	0.000	0.019	52.021	0.011	91.642
Physiphora sp.	0.000	0.000	0.000	0.000	0.010	104.042	0.000	0.000
Odontomyia sp.	0.000	0.000	0.000	0.000	0.000	0.000	0.005	183.283
Muscidae sp. (1)	0.040	24.771	0.058	17.385	0.043	23.250	0.053	18.960
Muscidae sp. (2)	0.026	38.109	0.033	30.133	0.024	41.617	0.000	0.000
Tachinidae sp. (1)	0.030	33.028	0.020	50.222	0.010	104.042	0.000	0.000
Tachinidae sp. (2)	0.000	0.000	0.000	0.000	0.000	0.000	0.011	91.642
Calliphoridae sp.	0.020	50.812	0.007	150.667	0.048	20.808	0.011	91.642
Sarcophagidae sp.	0.000	0.000	0.000	0.000	0.026	37.833	0.035	28.197
Apis mellifera	0.282	3.544	0.369	2.707	0.233	4.283	0.175	5.728
Apis cerena indica	0.000	0.000	0.000	0.000	0.014	69.361	0.000	0.000
Apis dorsata	0.000	0.000	0.000	0.000	0.060	16.647	0.005	183.283
Apis florea	0.000	0.000	0.000	0.000	0.000	0.000	0.005	183.283
Amegilla confusa	0.000	0.000	0.013	75.333	0.010	104.042	0.000	0.000
Lipotriches sp.	0.012	82.569	0.013	75.333	0.006	156.063	0.005	183.283
Steganomus sp.	0.014	70.773	0.007	150.667	0.056	17.938	0.025	40.730
Vespidae sp.(1)	0.074	13.573	0.076	13.101	0.303	3.306	0.374	2.672
Vespidae sp.(2)	0.012	82.569	0.000	0.000	0.000	0.000	0.038	26.183
Halictidae sp.	0.000	0.000	0.000	0.000	0.005	208.083	0.000	0.000
Utethesia lotrix	0.012	82.569	0.020	50.222	0.000	0.000	0.000	0.000
Zizeeria karsandra	0.150	6.659	0.077	12.914	0.020	49.940	0.000	0.000
Crambidae sp. (1)	0.278	3.591	0.260	3.847	0.062	16.006	0.187	5.336
Crambidae sp. (2)	0.000	0.000	0.000	0.000	0.010	104.042	0.000	0.000
Coccinella transversalis	0.006	165.138	0.000	0.000	0.000	0.000	0.000	0.000
Curculionid Weevil	0.006	165.138	0.027	37.667	0.017	59.452	0.000	0.000
Pentatomid Bug	0.000	0.000	0.000	0.000	0.005	208.083	0.000	0.000

*1/d - Reciprocal of Berger-Parker Dominance Index where higher values indicate lower dominance and greater community evenness

The Berger–Parker dominance values further indicated that species dominance declined progressively from the early to intermediate flowering stages and increased slightly during the peak flowering stage. Correspondingly, the reciprocal Berger–Parker index (1/d) followed the inverse pattern, attaining its highest value during the 75% flowering stage, indicating lower dominance by a single species and a more equitable distribution of flower visitors. Conversely, lower reciprocal values recorded during the 25% and >90% flowering stages suggest comparatively greater dominance of one or a few species within the flower visitor assemblage.

The observed shift in dominance from *A. mellifera* to

Table 5. Overall diurnal pattern of flower visitor abundance on *C. cretica* flowers during *summer*, 2025 at Cheruvu Jammulapalem, Bapatla

Flower visitor	Mean relative abundance (%)				Mean (%)
	06:30-08:05	09:30-11:05	12:30-14:05	15:30-17:05	
Eristalinus aeneus	5.13	2.94	3.46	1.69	3.31
Ischiodon scutellaris	0.00	0.38	0.49	0.00	0.22
Paragus auritus	1.41	0.38	1.37	0.00	0.79
Physiphora sp.	0.00	0.48	0.44	0.00	0.23
Odontomyia sp.	0.00	0.00	0.49	0.00	0.12
Muscidae sp. (1)	8.49	6.18	2.95	2.87	5.12
Muscidae sp. (2)	3.71	1.46	2.51	1.11	2.20
Tachinidae sp. (1)	0.73	1.09	1.69	2.29	1.45
Tachinidae sp. (2)	1.01	0.00	0.49	0.00	0.37
Calliphoridae sp.	0.00	0.88	1.69	4.64	1.80
Sarcophagidae sp.	1.71	0.86	2.10	1.78	1.61
Apis mellifera	12.62	25.68	31.17	33.05	25.63
Apis cerena indica	0.71	0.00	0.44	0.39	0.38
Apis dorsata	2.82	3.74	0.66	0.00	1.81
Apis florea	0.00	0.38	0.00	0.00	0.10
Amegilla confusa	1.01	0.00	0.44	1.11	0.64
Lipotriches sp.	0.00	0.98	1.07	1.23	0.82
Steganomus sp.	2.82	3.34	2.34	1.68	2.55
Vespidae sp.(1)	21.95	19.97	17.98	22.20	20.53
Vespidae sp.(2)	1.74	0.77	2.02	0.59	1.28
Halictidae sp.	0.00	0.48	0.00	0.00	0.12
Utethesia lotrix	0.00	2.77	0.00	0.00	0.69
Zizeeria karsandra	6.11	6.47	6.47	5.49	6.13
Crambidae sp. (1)	25.90	17.52	19.24	18.45	20.28
Crambidae sp. (2)	0.00	0.96	0.00	0.00	0.24
Coccinella transversalis	0.73	0.00	0.00	0.00	0.18
Curculionid Weevil	1.41	1.81	0.51	1.44	1.29
Pentatomid Bug	0.00	0.48	0.00	0.00	0.12
Total visitor abundance (Mean ± SE)	*2.73 ± 0.35^{a}*	*6.30 ± 0.65^{b}*	*5.92 ± 0.57^{b}*	*5.32 ± 0.50^{b}*	-

Upper rows show mean relative abundance (%) of each visitor taxon (columns sum to 100% within each time interval). The bottom row (in italics, separated by a rule) reports the mean number of individual visitors (not %) recorded at each time interval, compared by Tukey's HSD following two-way ANOVA (Table 6); means sharing a common superscript letter do not differ significantly (P>0.05)

Vespidae sp. (1) across flowering stages indicates temporal variation in the relative contribution of different flower-visiting taxa during the flowering period. Similar changes in species dominance across flowering phenology have been reported for other naturally occurring flowering plant species (Denisow et al., 2014a). Overall, the Berger–Parker dominance index complemented the diversity indices by demonstrating that no single flower visitor species remained dominant throughout the entire flowering period.

3.5. Diurnal Pattern of Flower Visitor Abundance on *C. cretica* Flowers

The abundance of flower visitors varied throughout the day, indicating distinct diurnal visitation patterns (Table 5). Overall flower visitor abundance was significantly lower during the early morning period (06:30–08:05 h; 2.73 ± 0.35) compared to the remaining three-time intervals (09:30–11:05 h, 6.30 ± 0.65; 12:30–14:05 h, 5.92 ± 0.57 and 15:30–17:05 h, 5.32 ± 0.50; Tukey's HSD, P<0.05), which did not differ significantly among themselves. This pattern suggests that flower visitor activity increased sharply after early morning and remained comparatively stable through midday and afternoon.

Among the recorded species, *A. mellifera* exhibited the highest relative abundance during the late afternoon (15:30–17:05 h; 33.05%), followed by the early afternoon (31.17%) and mid-morning (25.68%) periods, while the lowest activity was recorded during the early morning (12.62%). Overall, *A. mellifera* was the most abundant flower visitor, with a mean abundance of 25.63%. *Vespidae* sp. (1) remained active throughout the day and exhibited relatively consistent visitation across all time intervals, with a mean abundance of 20.53%. Crambidae sp. (1) was most abundant during the early morning (25.90%) and continued to visit flowers throughout the day, although its relative abundance declined slightly during later observation periods, contributing an overall mean abundance of 20.28%. Among the dipteran visitors, muscid flies, *Eristalinus aeneus* and sarcophagid flies exhibited moderate but consistent visitation throughout the day. In contrast, several taxa, such as *Utetheisa lotrix*, *Odontomyia* sp., Halictidae sp. and pentatomid bugs were recorded only occasionally, contributing relatively little (≤0.12%) to the overall flower visitor assemblage.

Total flower visitor abundance was significantly influenced by both flowering stage ($F = 16.83$; $df = 3, 84$; $P < 0.001$) and observation time ($F = 14.30$; $df = 3, 84$; $P < 0.001$). In addition to this overall pattern, the interaction between flowering stage and observation time was not significant ($F = 1.59$; $df = 9, 84$; $P = 0.130$), indicating that the effect of observation time on visitor abundance remained broadly consistent across flowering stages (Table 6).

The observed diurnal variation in flower visitor activity is consistent with previous studies reporting that the abundance and composition of flower-visiting insects vary throughout the day in response to changes in floral display (Denisow and Bożek, 2008; Denisow et al., 2014b). The present findings demonstrate that flower visitor activity on *C. cretica* is influenced by both flowering stage and time of observation, resulting in distinct temporal patterns of visitation during the flowering period.

The present findings highlight the ecological importance of naturally occurring halophytic vegetation in agroecosystems. During the summer months, when flowering crops and other herbaceous vegetation are relatively scarce in coastal rice-fallow ecosystems, *C. cretica* may serve as a valuable floral resource for a wide range of insects that visit flowers. The consistently high frequency and diversity of flower visitors observed suggest that this species may contribute to sustaining insect

Table 6. Two-way ANOVA showing the effects of flowering stage and observation time on total flower visitor abundance on *C. cretica*

Source of variation	df	SS	MS	F-value	P-value
Flowering stage	3	228.77	76.26	16.83	<0.001**
Observation time	3	194.32	64.77	14.30	<0.001**
Flowering stage × Observation time	9	64.97	7.22	1.59	0.130 ns
Error	84	380.50	4.53		
Total	99	868.56			

SS = Sum of Squares; MS = Mean Square; **P<0.01; ns = not significant (p=0.05). Error term based on N=100 sampling observations (days × time intervals) across the four flowering stages

communities during periods of seasonal floral scarcity. The findings also have practical implications for the management of coastal rice-fallow ecosystems. The conservation of naturally occurring flowering weeds such as *C. cretica* may help sustain diverse flower-visiting insects during periods of limited floral availability, thereby contributing to biodiversity conservation and ecological sustainability. However, this investigation was conducted during a single flowering season at one location; therefore, the results should be interpreted within the scope of the present study. Future investigations across multiple seasons and locations would provide a more comprehensive understanding of the ecological significance of *C. cretica*.

4. CONCLUSION

The present study documented a diverse assemblage of 28 flower-visiting insect species associated with *C. cretica* in the coastal rice-fallow ecosystems of Andhra Pradesh with *A. mellifera***,** *Vespidae* sp. (1)*,* dipteran flies and lepidopteran species constituting the major visitor groups. Flower visitor composition, abundance and diversity varied significantly across flowering stages and observation times, with the highest diversity recorded during the intermediate (75%) flowering stage and a shift in dominance from *A. mellifera* during early flowering to Vespidae sp. (1) during later stages. These results confirm that flower visitor communities on *C. cretica* are structured by both floral phenology and diurnal timing. The findings provide baseline ecological information on an under-studied halophytic weed and its associated insect fauna, which may support future comparative studies across seasons and locations in coastal agroecosystems.

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CRediT authorship contribution statement

Subhash T: Data curation, Formal analysis, Investigation, Project administration, Software, Writing - original draft; **Suneel Kumar G V:** Supervision, Validation, Visualization, Writing - review & editing; **Koteswara Rao S R:** Conceptualization, Methodology, Resources, Writing - review & editing; **Vijaya Kumar G:** Writing - review & editing

Conflict of interest

The authors declare no conflicts of interest.

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

The authors reviewed and edited the content as required and take full responsibility for the accuracy, integrity and originality of the manuscript. No AI tools were used for data collection, analysis and interpretation of results.

Data availability

The datasets generated and analysed for the current study are available from the corresponding author.

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Conjoint Application of Different Nutrient Sources and Biofertilizers for Micronutrient Enrichment and Energy Budgeting in Maize under Rainfed Conditions

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ABSTRACT: Exclusive focus on primary nutrients has reduced the availability of micronutrients to plants, posing a serious threat to nutritional security. Integrated nutrient management (INM) represents a sustainable strategy to improve soil micronutrient availability and crop yield. A study was carried out during *kharif* (summer) 2022 at the research farm of the Department of Soil Science, CSKHPKV, Palampur to evaluate the influence of INM on maize productivity along with micronutrient concentration in the soil. The experiment comprised of nine treatments with NPK at graded doses (100, 75, and 50%), FYM (7, 10 t ha^{-1}), vermicompost (3.5, 5 t ha^{-1}), and biofertilizers (*Azotobacter* + *Bacillus*), replicated thrice under a randomised block design. Maize variety Kanchan (K-25) was opted as the test crop. The experimental soil was acidic (pH 5.63) with silty clay loam soil texture and adequate available micronutrients (Fe, Mn, Zn and Cu). Soil samples were analysed for DTPA-extractable micronutrients, while grain and stover samples were analysed for micronutrient concentration and total uptake. The synergistic application of 75% NPK along with 5 t vermicompost (VC) ha^{-1} (T_7) and biofertilizers recorded the highest grain and stover yields, improved soil micronutrient availability, and enhanced micronutrient concentration and uptake in maize. Considering the energy indices of different treatments, energy use efficiency was highest under treatment T_9 (50% NPK + 5 t VC t ha^{-1} + biofertilizers) while other indices were highest under treatment T_7. Overall, INM proved effective in improving maize productivity, soil micronutrient status and energy efficiency, highlighting its potential for sustainable crop production.

Keywords: Biofertilizers, Energy budgeting, Integrated nutrient management, Sustainability.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most important cereal crops, serving as a staple food, livestock feed and industrial raw material. With the growing demand for food, feed and bio-based products, India's maize requirement is projected to reach about 65 million tonnes by 2050 (IIMR, 2022), and this increase in production should preferably come from an increase in productivity rather than an increase in area. Productivity enhancement particularly revolves around the application of primary nutrients, neglecting secondary and micronutrients. Although a lot of focus has

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now been shifted towards secondary nutrients, micronutrient still lacks sufficient attention. Moreover, with the increasing cases of malnutrition, there is a need of not only food security but also of nutritional security. Micronutrient deficiency in soil directly translates into poor nutrient content in plants, posing a serious impact on human and animal health, thus highlighting the importance of optimum micronutrient content in cereal crops, particularly maize.

Micronutrients, such as zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu), though required in trace amount, play a fundamental role in plant physiological and biochemical processes, viz., enzyme regulation, chlorophyll synthesis, protein metabolism, and carbohydrate translocation (Kaur et al., 2023) and increase tolerance to various stresses (Pandey, 2020). These benefits establish micronutrients as essential elements for plants to boost their grain quality and ensure nutritional security; therefore, managing these nutrients is of immense importance. However, current nutrient management practices lack sufficient supplementation of micronutrients, thereby demanding a paradigm shift towards a holistic approach.

Integrated nutrient management (INM) offers a strategic framework where nutrients are made available through the synergistic application of various chemical, organic manures and biofertilizers, thus optimizing crop production and ensuring quality grains (Kushwah et al., 2024). However, most studies on INM have primarily focused on improving soil macronutrient availability; for instance, Shekhawat et al. (2021) reported significantly higher total N, P and K uptake by the test crop (maize) under the conjoint application of 75% RDF using chemical fertilizer and 25% RDF through vermicompost, *Azotobacter* and VAM. Nevertheless, research evaluating the potential of INM for enhancing micronutrient content in plant biomass is still limited, indicating a substantial knowledge gap for biofortification of maize grains and warranting future investigation.

Moreover, beyond productivity, sustainable agricultural intensification requires assessment of resource-use efficiency and energy budgeting, to ensure optimization of nutrient management practices by quantifying energy dynamics (Imamsaheb et al., 2026). Since, the manufacture and application of mineral fertilizers are energy-intensive processes, implementing INM practices can potentially reduce external energy inputs while improving output in terms of crop yield. Considering the above rationale, the present investigation was performed to identify a nutrient management strategy capable of enhancing maize productivity and quality while improving energy use efficiency and contributing to sustainability.

2. MATERIALS AND METHODS

2.1. Experimental Site

The study was conducted at the Research Farm of the Department of Soil Science during the *kharif* season, 2022. The experimental site is situated at 32.11° N latitude and 76.53° E longitude at an altitude of about 1290 metres amsl (above mean sea level). The site is located in the Palam valley of the North-Western Himalayas of Kangra district and falls under the mid-hills sub-humid agro-climatic zone of Himachal Pradesh. These soils have evolved from fluvo-glacial parent material and belong to the order Alfisol and subgroup "*Typic Hapludalf"* (Soil Survey Staff, 1975). The experimental area received a total of 1847.4 mm rainfall, whereas the weekly maximum and minimum temperature ranged between 24.2 and 34.2 °C and 11.8 and 21.8 °C, respectively, throughout the crop duration.

2.2. Experimental Details and Treatments

The experiment was carried out in a randomized block design with a plot size of 15 m^2 comprising nine treatments all replicated thrice, supplemented with recommended nitrogen (N), phosphorus (P) and potassium (K) levels (120:60:40) applied at graded rates of 100, 75 and 50% integrated with either farmyard manure (FYM @ 7, 10 t ha^{-1}), vermicompost (VC @ 3.5, 5 t ha^{-1}) and biofertilizers (*Azotobacter* and *Bacillus* sp.). The FYM showed 45.6% moisture and a mean nutrient content of 0.89, 0.48, and 0.76% of N, P, and K, respectively, whereas vermicompost contained about 27.8% moisture with 1.27, 0.85, and 0.72% of N, P, and K, respectively, on a dry weight basis. The sources of N, P and K were urea, single super phosphate, and muriate of potash, respectively, in the specified treatments. Half dose of N and full doses of P and K were applied at the time of sowing in the treatments with NPK. The remaining half dose of nitrogen was top-dressed in two equal splits at the knee-high and pre-tasselling stages of maize. Kanchan (K-25) variety of maize was grown as test crop and after harvest both grain and stover yields were recorded. Further, post-harvest soil samples were collected, processed and analysed for available micronutrient content. The DTPA extraction method was employed for analysing Fe, Mn, Zn and Cu as given by Lindsay and Norvell (1978). The soil of

the studied field was classified as Typic Hapludalfs according to the USDA Soil Taxonomy system (Soil Survey Staff, 2022), with a silty clay loam texture comprising 16.42% sand, 42.51% silt, and 35.26% clay, and was acidic (pH 5.63) in reaction (1:2.5; soil: water). The organic carbon (OC) content was moderate at 7.89 g kg^{-1}. The cation exchangeable capacity of the soil was 10.80 c mol (p^{+}) kg^{-1}. Further, the soil was low in N (255.2 kg ha^{-1}), medium in P (17.5 kg ha^{-1}) and available K (188.6 kg ha^{-1}). Soil was high in DTPA-extractable Fe (25.16 mg kg^{-1}), Mn (14.64 mg kg^{-1}), Cu (0.98 mg kg^{-1}), and Zn (1.09 mg kg^{-1}).

Similarly, the plant samples were collected from each plot at harvesting. These samples were cleaned, air-dried, and further dried in an oven at a temperature of 60 ± 5°C. Once dried, the samples were processed using a steel grinder and were then preserved in paper bags for further analysis. The digestion process as outlined by Piper (1950) was used for the determination of micronutrient content, both in grain and stover. After digestion, the content in plant samples was analysed by the procedure as stated by Jackson (1973). Finally, the nutrient uptake was calculated by multiplying the content by yield for respective treatments as detailed below (Eq.1):

Nutrient uptake (g ha^{-1}) = (% concentration of nutrient x Grain or Stover yield (q ha^{-1}))/10 ….(1)

Total nutrient uptake was derived as follows (Eq.2):

Total uptake = Uptake by stover + Uptake by grains….(2)

2.3. Energy Budgeting

Energy input under the maize crop was estimated following the methodology suggested in the study by Paramesh et al. (2023) for each treatment by multiplying the quantity of inputs and their respective energy equivalents (Table 1). Then, the energy output was determined by multiplying grain and stover yield with their respective energy equivalents. However, the energy input associated with biofertilizers was considered negligible due to their low application rate and minimal embodied energy compared with chemical fertilizers and organic amendments. Furthermore, different energy indices, including energy productivity (EP), specific energy (ES), energy use efficiency (EUE), and net energy gain (NEG) for maize, were calculated using the following equations (Eqs. 3, 4,5 and 6) :

EUE = (Energy output (MJ ha^{-1})) / (Energy input (MJ ha^{-1}))…….(3)

NEG = (Total energy output (MJ ha^{-1})) − (Total energy input (MJ ha^{-1}))……(4)

ES (MJ kg^{-1}) = (Energy input (MJ ha^{-1})) / (Total yield (kg ha^{-1}))……..(5)

EP (kg MJ^{-1}) = (Total yield (kg ha^{-1})) / (Energy input (MJ ha^{-1}))…….(6)

2.4. Statistical Analysis

The data generated through field and laboratory analysis were subjected to the Shapiro-Wilk test using the Statistics Kingdom software and further analysed using one-way analysis of variance (ANOVA), following the standard procedures described by Gomez and Gomez (1984) to determine the significance of treatment effects. Further, Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was performed for comparative analysis of treatment means

Table 1. Quantity of nutrients applied in maize crop and respective energy equivalents

Treatments	Quantity applied (kg ha^{-1})					Energy equivalent (MJ ha^{-1})				
	FYM	N	P	K	VC	FYM	N	P	K	VC
T_1: 100% NPK	0	120	26.16	33.2	0	0	7932	324.38	368.52	0
T_2: 100% NPK + 10t FYM ha^{-1}	10000	120	26.16	33.2	0	4700	7932	324.38	368.52	0
T_3: 100% NPK + 5t VC† ha^{-1}	0	120	26.16	33.2	5000	0	7932	324.38	368.52	1750
T_4: 100% NPK + 7t FYM ha^{-1}	7000	120	26.16	33.2	0	3290	7932	324.38	368.52	0
T_5: 100% NPK + 3.5t VC ha^{-1}	0	120	26.16	33.2	3500	0	7932	324.38	368.52	1225
T_6^{*}: 75% NPK + 10t FYM ha^{-1} + ‡Biofertilizers	10000	90	19.62	24.9	0	4700	5949	243.29	276.39	0
T_7^{*}: 75% NPK + 5t VC ha^{-1} + Biofertilizers	0	90	19.62	24.9	5000	0	5949	243.29	276.39	1750
T_8^{*}: 50% NPK + 10t FYM ha^{-1} + Biofertilizers	10000	60	13.08	16.6	0	4700	3966	162.19	184.26	0
T_9^{*}: 50% NPK + 5t VC t ha^{-1} + Biofertilizers	0	60	13.08	16.6	5000	0	3966	162.19	184.26	1750

†VC: Vermicompost; ‡Biofertilizers: *Azotobacter* + *Bacillus* sp.; N: Nitrogen; P: Phosphorus; K: Potassium; FYM: Farm Yard Manure; *Energy input from biofertilizer was not considered

significant at 95% probability using the Web Agri Stat Package (WASP) platform, CCARI (https://ccari.res.in/wasp2.0/index.php).

3. RESULTS AND DISCUSSION

3.1. Influence of INM on Maize Grain and Stover Yield

The maize grain yield recorded a significant increase with the conjoint use of NPK fertilizers along with FYM, vermicompost and biofertilizers (*Azotobacter* and *Bacillus* sp.) over the sole application of 100% NPK (T_1) (Table 2). The maximum grain yield was recorded under treatment T_7 (4.08 t ha^{-1}) comprising 75% NPK along with 5 t VC ha^{-1} and biofertilizers, which had statistical parity with treatment T_6 (75% NPK + 10 t FYM ha^{-1} + biofertilizers) and treatment T_3 (100% NPK + 5 t VC ha^{-1}), recording grain yields of 3.89 t ha^{-1} and 3.85 t ha^{-1}, respectively. This enhancement in crop yield can be attributed to the synergistic influence of chemical fertilizers, ameliorated soil properties by the incorporation of organic amendments, and improved nutrient mobilisation by biofertilizers. The readily available N, P, and K from inorganic fertilizers fulfilled initial nutrient requirements of the crop during early growth stages, while the gradual mineralization of organic manures ensured a sustained release of nutrients in synchrony with crop demand throughout the growing season. Furthermore, grain yield under treatments T_8 and T_9 exhibited an increment of 5.6 and 8.5%, respectively, over treatment T_1. The maize stover yield varied from 5.12 to 6.25 t ha^{-1}, where treatment T_7 showed the highest yield. However, reduced NPK application to 50% (T_8 and T_9) supplemented with FYM or vermicompost along with biofertilizers reported reduced stover yield in comparison to 100 and 75% NPK. The enhancement in crop yield can be attributed to the synergistic influence of readily available NPK through chemical fertilizers, ameliorated soil properties by the incorporation of organic amendments, and improved nutrient mobilisation by biofertilizers. These amendments may have promoted nutrient availability by reducing their losses and improving synchronization of nutrient supply as per crop demand, resulting in better crop yield even under reduced NPK application (Rao et al., 2025; Biswasi et al., 2025). Additionally, higher nutrient content, greater microbial activity, and porous structure of vermicompost enabled it to outperform FYM-based treatments at the corresponding NPK fertilizer levels.

Moreover, the soil application of *Azotobacter* and *Bacillus* sp. in addition to chemical and organic inputs also increased the grain yield, which could be credited to the enhanced fixation of nitrogen, solubilization and mineralization of phosphorus, potassium and micronutrients, synthesis of hydrolytic enzymes and better resistance to environmental stresses (Goswami et al., 2016). The proliferation of soil microbes ultimately improves nutrient availability to plants, which can be corroborated through increased crop yield. On the other side, the relatively small yield increase despite a 50% reduction in chemical fertilizer input depicts better fertilizer-use efficiency due to combined nutrient application with organic and bio-fertilizers and also represents a significant advantage in terms of input cost savings as well as environmental sustainability (Ahirwar et al. , 2025).

Table 2. Effect of integrated nutrient management practices on maize grain and stover yield

Treatment	Grain yield (t/ha)	Stover yield (t/ha)
T_1: 100% NPK	3.04	5.12
T_2: 100% NPK + 10t FYM ha^{-1}	3.63	5.51
T_3: 100% NPK + 5t VC† ha^{-1}	3.85	5.74
T_4: 100% NPK + 7t FYM ha^{-1}	3.43	5.36
T_5: 100% NPK + 3.5t VC ha^{-1}	3.58	5.66
T_6: 75% NPK + 10t FYM ha^{-1} + ‡Biofertilizers	3.89	6.07
T_7: 75% NPK + 5t VC ha^{-1} + Biofertilizers	4.08	6.25
T_8: 50% NPK + 10t FYM ha^{-1} + Biofertilizers	3.21	5.22
T_9: 50% NPK + 5t VC t ha^{-1} + Biofertilizers	3.30	5.38
CD ($p \leq 0.05$)	4.0	5.3

†VC: Vermicompost; ‡Biofertilizers: *Azotobacter* + *Bacillus* sp.; N: Nitrogen; P: Phosphorus; K: Potassium; FYM: Farmyard Manure

3.2. Influence of INM on Micronutrient Content in Maize Grain

INM practices significantly influenced the concentration of Fe, Mn, Zn and Cu in maize grain (Figure 1). Among the treatments, T_7 (75% NPK + 5 t vermicompost ha^{-1} + *Azotobacter* + *Bacillus* sp.) recorded the highest concentrations for all four micronutrients, registering 61.9 mg kg^{-1} Fe, 39.5 mg kg^{-1} Mn, 37.5 mg kg^{-1} Zn, and 28.1 mg kg^{-1} Cu in grain, respectively (Supplementary Table 1 and Table 2). The values obtained under T_7 were followed by T_6 (75% NPK + 10 t FYM ha^{-1} + *Azotobacter* + *Bacillus* sp.).

Compared with the sole application of 100% NPK (T_1), T_7 enhanced Fe concentration by 28.4%, Mn by 20.8%, Zn by 23.4%, and Cu by 31.3% in grain and stover, respectively. The integrated application of organic manures with the recommended dose of fertilizers (T_2–T_5) also improved micronutrient concentrations over T_1, although the increase was lower than that achieved with the combined application of organics and biofertilizers. Under reduced fertilizer application (50% NPK), vermicompost-based treatment (T_9) consistently recorded higher micronutrient concentrations than the corresponding FYM treatment (T_8), indicating greater efficiency of vermicompost in maintaining micronutrient availability under lower fertilizer inputs.

The improved concentration of micronutrient cations under INM can be attributed to the synergistic effects of inorganic fertilizers, organic amendments and beneficial microorganisms in improving nutrient availability and their uptake. Organic manures, particularly vermicompost, release humic substances, low-molecular-weight organic acids and other chelating compounds during decomposition, which reduce fixation and maintain micronutrients in plant-available forms. Simultaneously, improved soil biological activity by *Azotobacter* and *Bacillus* spp. through production of organic acids and growth hormones promotes root proliferation and facilitates greater nutrient absorption as well as translocation to above-ground plant parts, resulting in higher concentrations both in grain and stover. The superior performance of vermicompost-based treatments over FYM may be attributed to its higher nutrient density, greater degree of humification, richer microbial population and faster mineralization rate, which ensure a sustained supply of available micronutrients throughout crop growth. The comparatively lower micronutrient concentrations observed under T_8 and T_9 indicate that although the integration of organic manures and biofertilizers partially compensated for the reduction in mineral fertilizer application, a 50% reduction in NPK was insufficient to maximize micronutrient accumulation in plant tissues. Overall, the findings demonstrate that integrating 75% of the recommended NPK with vermicompost or FYM and microbial inoculants improved the nutritional quality and agronomic biofortification potential of maize without the need for the complete recommended dose of mineral fertilizers.

3.3. Influence of INM on Micronutrient Uptake by Maize Crop

The data revealed that application of 75% NPK + 5 t vermicompost ha^{-1} + biofertilizers reported the highest total

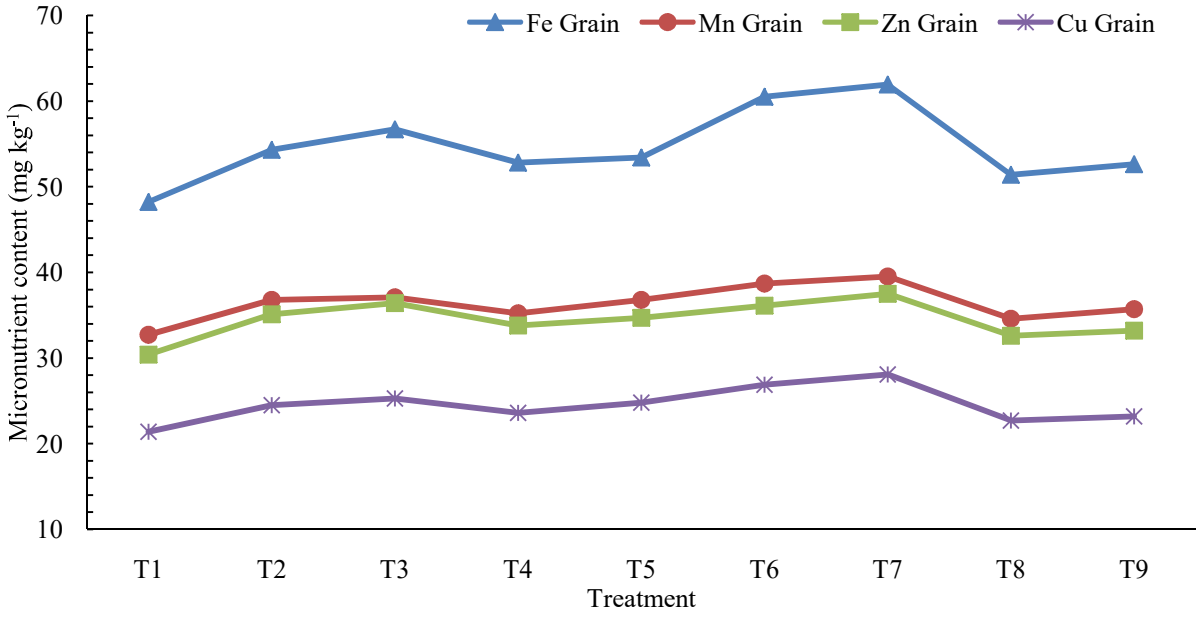


Figure 1. Effect of INM treatments on the concentration of Fe, Mn, Zn and Cu in maize grain after harvest. Treatments (T_1–T_9) comprised NPK levels (100, 75 and 50%), FYM, vermicompost and biofertilizers (*Azotobacter* + *Bacillus*). Among the treatments, T_7 (75% NPK + 5 t VC ha^{-1} + biofertilizers) recorded the highest concentration of all four micronutrients

Fe uptake, which was found statistically at par with 75% NPK + 10 t FYM ha^{-1} along with biofertilizers (Figure 2). The per cent increase in total Fe uptake under treatment T_7 and T_6 was 39.6 and 33.6% over 100% NPK. Further analysis revealed that the application of T_7 reported the highest total Mn and Zn uptake (Figure 3) and increased by 47.9 and 63.7% over T_1 (100% NPK). Similarly, the highest total Cu uptake by the maize crop was under T_7 (Figure 2). The lowest micronutrient uptake was under control, *i.e.*, 100% NPK.

The highest uptake of micronutrients in treatment T_7 might be due to the application of balanced inputs, which results in maximum yield under this treatment, thereby increasing uptake (Thakur et al., 2022). The application of vermicompost prevents nutrient losses through chelation and increases their concentration in soil solutions.

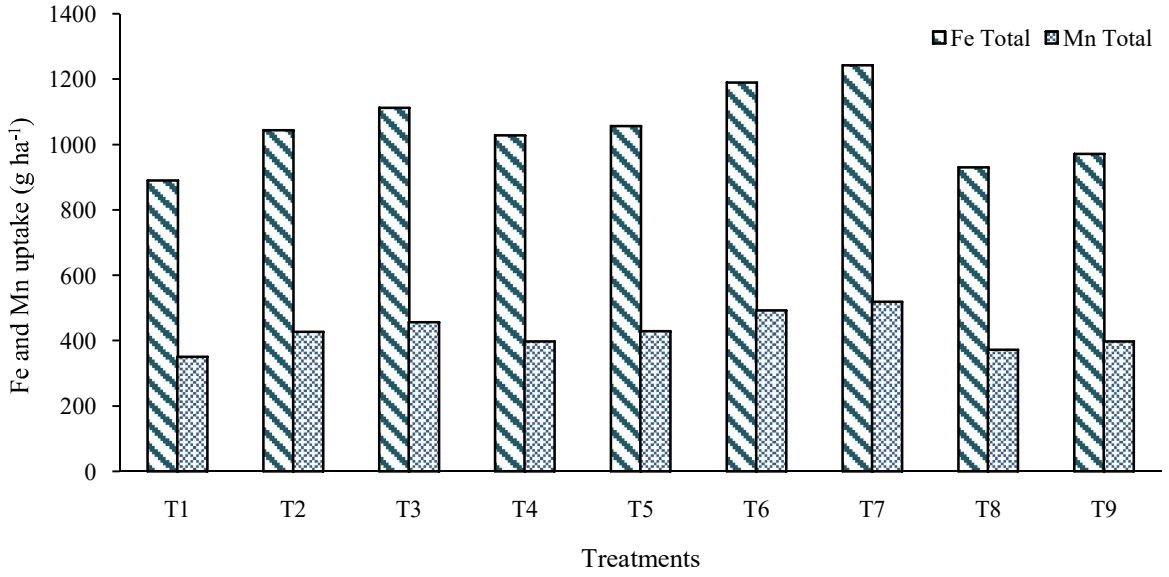


Figure 2. Effect of INM treatments on total Fe and Mn uptake (g ha^{-1}) by maize (grain + stover) at harvest. The integrated application of 75% NPK + 5 t VC ha^{-1} + biofertilizers (T_7) recorded the highest total Fe and Mn uptake

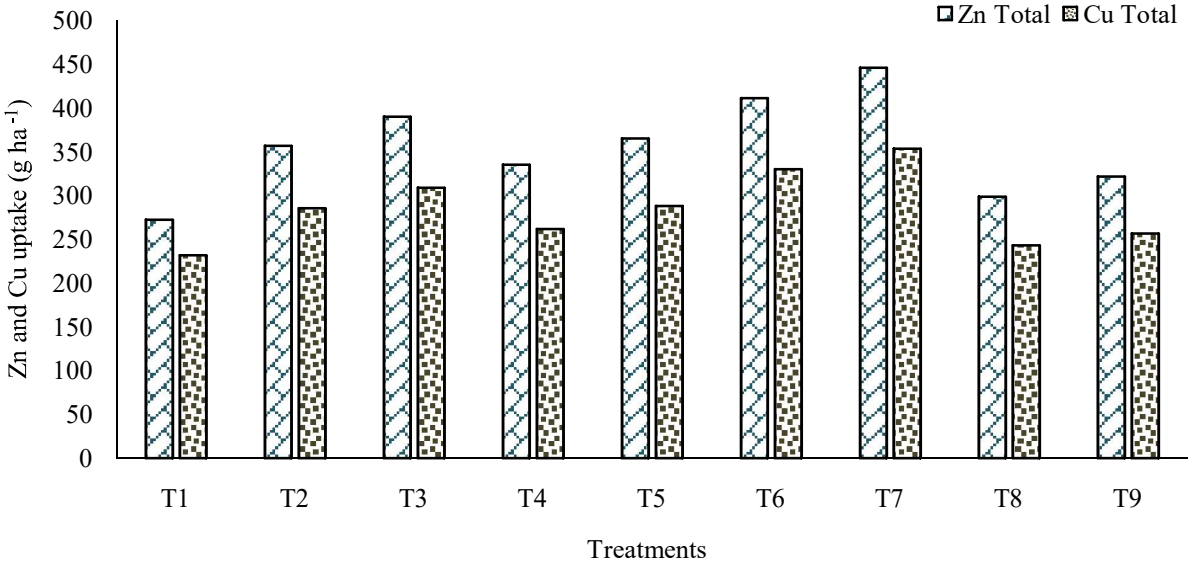


Figure 3. Effect of INM treatments on total Zn and Cu uptake (g ha^{-1}) by maize (grain + stover) at harvest. The integrated application of 75% NPK + 5 t VC ha^{-1} + biofertilizers (T_7) recorded the highest total Fe and Mn uptake

Moreover, conducive rhizosphere conditions by organic manures for the proliferation of *Azotobacter* and *Bacillus* result in mobilisation of micronutrients, accelerating their availability (Gao et al., 2020). Although the addition of organic manures and biofertilizers in T_8 and T_9 improved soil biological activity, the reduction of fertilizer dose to 50% restricted overall nutrient supply required for optimum crop growth. Consequently, lower biomass production and poor root development limited the absorption and accumulation of micronutrients, resulting in lower total micronutrient uptake comparable to other integrated treatments (T_2-T_5).

3.3. Influence of INM on Available Micronutrients

Under different implemented INM practices, the treatment comprising 75% NPK + 5 t vermicompost ha^{-1} + *Azotobacter* + *Bacillus* sp. (T_7) recorded the highest availability of all the tested micronutrients, which was statistically alike with T_6 (75% NPK + 10 t FYM ha^{-1} + biofertilizers) (Figure 4). Fe, Mn, Zn and Cu increased by 31.9, 70.5, 16.7, and 26.3%, respectively, under treatment T_7 compared to the sole application of chemical fertilizers (T_1). Among organic sources, vermicompost integrated treatments showed slightly higher micronutrient content than FYM. This can be attributed to the higher degree of decomposition and humification of vermicompost, which increases the concentration of readily available micronutrients. Vermicompost also contains abundant humic and fulvic substances that form organo-metallic complexes (chelates) with micronutrients, reducing their precipitation and fixation in soil (Rehman et al., 2023). In contrast, FYM decomposes more slowly and releases nutrients gradually, making their availability comparatively lower.

Concurrently, treatments receiving reduced NPK levels (50%) along with organic and biological inputs also improved micronutrient availability compared to 100% NPK alone, emphasizing the synergistic influence of organic and biological inputs on micronutrient dynamics. The decomposition of organic amendments releases organic acids, maintaining soil pH, which enhances micronutrient solubility and reduces their adsorption onto soil colloids (Dhaliwal et al., 2019). In addition, biofertilizers stimulate mineralization and mobilization of native soil micronutrients (Janardhan et al., 2023). Consequently, the combined application of organic and biological inputs partially compensates for the reduced mineral fertilizer and improves micronutrient availability as compared to sole application of inorganic fertilizers. These results were in agreement with Singh et al. (2024), who stated that available micronutrients (Fe, Zn, Cu and Mn) increased significantly with the application of FYM in combination with inorganic

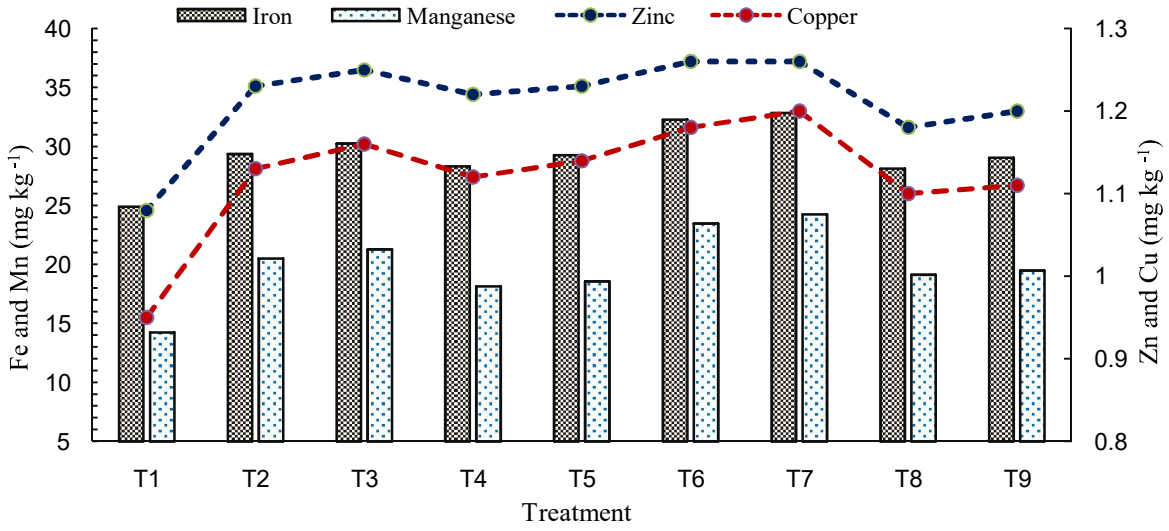


Figure 4 Effect of INM treatments on available soil micronutrients. Fe and Mn concentrations (mg kg^{-1}) are represented by bars, whereas Zn and Cu concentrations (mg kg^{-1}) are represented by dotted and dashed lines, respectively. Treatments (T_1–T_9) comprised different combinations of recommended NPK levels (100, 75 and 50%), FYM, vermicompost and biofertilizers (*Azotobacter* + *Bacillus*). The integrated application of 75% NPK + 5 t VC ha^{-1} + biofertilizers (T_7) recorded the highest DTPA-extractable micronutrients

Table 3. Impact of different nutrient management practices on energy dynamics

Treatment	Input energy (MJ/ha)	Output energy (MJ/ha)	EUE	Net energy (MJ/ha)	Specific energy (MJ/kg)	Energy productivity (kg/MJ)
T_1: 100% NPK	8625	108188	12.54	99563	1.06	0.94
T_2: 100% NPK + 10t FYM ha^{-1}	13325	122486	9.19	109161	1.46	0.68
T_3: 100% NPK + 5t VC† ha^{-1}	10375	128970	12.43	118595	1.08	0.92
T_4: 100% NPK + 7t FYM ha^{-1}	11915	115171	9.67	103256	1.34	0.74
T_5: 100% NPK + 3.5t VC ha^{-1}	9850	123276	12.52	113426	1.08	0.92
T_6*: 75% NPK + 10t FYM ha^{-1} + ‡Biofertilizers	11126	132308	11.89	121182	1.12	0.89
T_7*: 75% NPK + 5t VC ha^{-1} + Biofertilizers	8218	139351	16.96	131133	0.96	1.04
T_8*: 50% NPK + 10t FYM ha^{-1} + Biofertilizers	9012	112437	12.48	103425	1.21	0.83
T_9*: 50% NPK + 5t VC t ha^{-1} + Biofertilizers	6062	115010	18.97	108948	0.97	1.03

†VC: Vermicompost; ‡Biofertilizers: *Azotobacter* + *Bacillus* sp.; N: Nitrogen; P: Phosphorus; K: Potassium; FYM: Farm Yard Manure

*Energy input from biofertilizer was not considered

fertilizers in relation to the control.

3.4. Energy Budgeting

The energy indices such as EP, ES, EUE and NEG were analysed for each treatment, and the results clearly demonstrated significant variations in input–output relationships as well as EUE (Table 3). Total energy input was highest under treatment T_2 *i.e.,* 100% NPK + 10 t FYM ha^{-1} (13325 MJ/ha), due to the cumulative contribution of inorganic fertilizers and organic inputs. However, reduced fertilizer levels integrated with organics, such as under treatment T_7 (75% NPK + 5 t VC ha^{-1} + biofertilizers), exhibited comparatively lower input energy, while maintaining higher productivity. Consequently, energy output was maximized under T_7, mainly due to higher grain and stover yields, resulting in the highest NEG (131133 MJ/ha) and EP (1.04 kg/MJ), along with the lowest ES (0.96 MJ/kg) requirement. Considering EUE, treatment T_9 reported maximum EUE (18.97) due to its lowest input energy requirement (6062 MJ/ha) and comparatively higher output energy (115010 MJ/ha) as compared to other treatments. Although T_9 was more energy efficient, T_7 provided a better balance between crop productivity and energy efficiency, resulting in higher overall agronomic performance, making it more suitable than other treatments. Additionally, EUE increased although fertilizer dose decreased because the synergistic application of organic manures and biofertilizers sustained higher crop yield per unit input compared to control.

The superior performance of INM treatments highlights the role of conjunctive use of nutrients from different sources in optimizing energy dynamics under agricultural systems (Paramesh et al., 2023). This synergistic effect enhanced output energy without any proportionate increase in input, thereby improving overall energy efficiency (Singh et al., 2016). Thus, the conjunctive use of fertilizer sources offers an energy-efficient and resource-conserving nutrient management approach for sustainable maize production.

4. CONCLUSION

The conjoint addition of organic, inorganic and bio-fertilizers resulted in elevated maize grain and stover yield, under treatment T_7 (75% NPK + 5 t vermicompost ha^{-1} + biofertilizers), which was statistically comparable with treatment T_6 (75% NPK + 10 t FYM ha^{-1} + biofertilizers) and T_3 (100% NPK + 5 t VC ha^{-1}). Concurrently, the availability of micronutrients as well as their uptake by plants and EUE also exhibited significant increases with the integration of inorganic and organic inputs along with bio-fertilizers compared to the control. Although the study effectively demonstrates the combined influence of nutrient sources, the absence of treatment comprising vermicompost or FYM and reduced NPK fertiliser without biofertilizers limits the precise interpretation of the individual contribution of each source. Collectively, these findings underscore that partial substitution of synthetic fertilizers with organic and biological inputs sustains soil fertility, ensures better crop production and helps maintain maximum EUE, crowning INM as a viable strategy for sustainable crop production and nutrient enrichment. However, as the investigation was restricted to a single crop and location, future research

should focus on evaluating INM across different field conditions, crops and soil types to refine micronutrient management strategies for better crop production and sustainable soil health.

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CRediT authorship contribution statement

Mansi Bahman: Data collection, Methodology, Software, Writing –original draft, Formal analysis, Data curation; **Dhanbir Singh:** Conceptualization, Supervision and editing; **Pardeep Kumar:** Visualisation, reviewing and editing; **Praveen Thakur:** Reviewing and editing; **Shilpa Kumari:** Tabulation and editing

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Conflict of interest statement

The authors declare no conflict 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 writing process of this manuscript.

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Economic Evaluation of Positive Externalities from Honeybee Pollination: Evidence from Punjab, India

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ABSTRACT: Pollination is a fundamental ecosystem service that underpins agricultural productivity, biodiversity conservation and economic sustainability. The present study assesses the economic contribution of pollination (EVP) in Punjab agriculture using secondary data for 2024–25 using the dependence ratio method. Pollination services contributed Rs 3399 million (4%) to the total agricultural economic value of Rs 80286 million. Though cereals dominate the state's agricultural economy, they do not benefit from pollination due to their self- or wind-pollinated nature. In contrast, fruits, vegetables, oilseeds, and fibre crops exhibit significant dependence on animal pollination, with fruits accounting for the largest share (69.8%) of EVP, followed by vegetables and oilseeds. Crop-wise analysis reveals that about 61% of crops rely on pollinators to varying degrees, with the highest economic gains concentrated in crops with great and moderate dependence, including rapeseed & mustard, sunflower, guava, tomato, and cotton. The results showed that pollination services contribute to farm income and nutritional security by significantly enhancing yield and quality, particularly in high-value crops. Despite having a relatively small share in total agricultural value, pollination remains an indispensable input for achieving sustainable agricultural development. The study emphasises the need of targeted policy interventions, including the promotion of scientific beekeeping, conservation of pollinator habitats, reduced pesticide use, and increased awareness, to harness the full ecological and economic potential of pollination in the state.

Keywords: Dependence, Economic value, Ecosystem services, Pollination, Positive externalities.

1. INTRODUCTION

Pollination is one of the most essential ecosystem services that sustain biodiversity; it plays a crucial role by supporting agricultural productivity and linking natural ecosystems with crop production. Pollination also generates significant economic benefits by improving crop yield, quality, and farmers' incomes (Giannini et al., 2015; Gill et al., 2016; Hristov et al., 2020). As a vital regulating ecosystem service, pollination supports food security, poverty alleviation, biodiversity conservation, climate resilience, and sustainable agricultural production, thereby contributing to the achievement of the United Nations Sustainable Development Goals (SDGs) (Montoya et al., 2020). Further, either directly or indirectly, pollinators are linked to 58 of the 169 SDGs across 14 of the 17 Goals, which shows that progress towards the SDGs is underscored by the close relationship between the conservation and sustainable management of pollinators and their habitats. Pollination is often regarded as the "third production factor" in agriculture after land and labour due to its indispensable role in crop production.

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In addition to its ecological role, pollination provides considerable economic significance, particularly through its contribution to agriculture and food security. Globally, animal-mediated pollination benefits nearly two-thirds of major food crops, with bees serving as the dominant pollinators (Klein et al., 2007). The crops which rely on pollinators have substantially higher economic value than the crops that do not depend on pollinators (Gallai et al., 2009) and the contribution of animal-mediated pollination to annual global agricultural production is estimated at USD 235–577 billion (Potts et al., 2016). Conversely, the absence of animal pollination would cause global crop production to shrink by 5 to 8% (Aizen et al., 2009), highlighting its critical role in ensuring food security and agricultural sustainability.

The Western honeybee (*Apis mellifera* L.), with large colony populations, strong floral constancy, adaptability to diverse agro-climatic conditions, and ease of management, is the most widely managed and economically valuable species among all pollinators (Calderone, 2012; Tylianakis, 2013; Papa et al., 2022). Although some wild bee species may exhibit greater pollination per visit, their relatively low abundance and restricted geographical distribution limit their overall contribution. In contrast, bees are considered the dominant providers of pollination services in agricultural ecosystems as it provide the honeybee is considered the dominant provider of pollination services in agricultural ecosystems, as they provide consistently high visitation rates (Woodcock et al., 2013; Rader et al., 2009). As a result, honeybees are instrumental in enhancing the productivity and quality of numerous field, fruit and horticultural crops worldwide.

The economic significance of honeybees extends far beyond honey production, as their pollination services have generated substantial agricultural value and have significantly increased crop yield, fruit quality and farm profitability across different production systems (Breeze et al., 2011; Calderone, 2012; Geslin et al., 2017; Hall et al., 2020; Stanley et al., 2020). In India, the yield and quality of several crops, including guava, coconut, citrus, cotton, rapeseed and other horticultural crops, have improved through managed honeybee pollination, demonstrating its importance for sustainable agricultural development (Rajagopal et al., 2005). A study from South India highlighted the dominance of bees in crop pollination, reporting that they accounted for 73 % of all pollinating insects, far exceeding the contributions of flies, bats, wasps, beetles, birds, and butterflies (Pannure et al., 2016).

Despite the substantial contribution of honeybees to agricultural productivity as well as farm income, the economic value derived from their pollination services is still inadequately recognised in agricultural planning and policy. Quantification of an economic value to these ecosystem services is essential for accurately reflecting their contribution to agricultural productivity and sustainability. In this context, the present study seeks to quantify the economic contribution of pollination as a critical agricultural input and to estimate its significance to the economy of the agrarian state of Punjab, thereby providing evidence for the inclusion of pollination services in agricultural development and policy decisions.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was undertaken between 29°30′–32°32′ N latitude and 73°55′–76°50′ E longitude in the state of Punjab, located in north-western India. Punjab is one of India's most agriculturally productive states due to its characteristics like fertile alluvial soils, a well-developed canal irrigation system and a subtropical climate. It has been instrumental in strengthening the country's food security since the Green Revolution so it's widely known as the "Food Bowl of India". The state is an ideal region for assessing the economic value of pollination services because it is a leading producer of wheat (*Triticum aestivum*), rice (*Oryza sativa*), cotton (*Gossypium hirsutum*), oilseeds, fruits, and vegetables.Figure 1 illustrates the geographical location of Punjab state in north-western India and the study area considered in the present study.

2.2. Data Sources

The study was based entirely on secondary data collected from published reports, research articles, statistical abstracts and official government databases relevant to agriculture, beekeeping, and crop production in Punjab.

The estimation of the economic value of pollination services (EVP) for each crop required three key parameters: (i) annual crop production (kg), (ii) the crop-specific pollinator dependence ratio (DR), and (iii) the farm harvest price (Rs kg^{-1}). From the Statistical Abstract of Punjab 2025 published by the Government of Punjab, data on the production of agricultural crops for the agricultural year 2024–25 were obtained, and production data for fruits and vegetables for the same study period were sourced from the Department of Horticulture, Punjab, Mohali.

The crop-specific pollinator dependence ratios used in this study were adopted from Chaudhary and Chand (2017), based on the internationally accepted pollination dependence categories proposed by Klein et al. (2007). To estimate the economic contribution of managed pollination for selected crops in Punjab during the agricultural year 2024–25, these dependence ratios were applied (Table 1). Farm harvest prices for the selected crops were taken from the AGMARKNET portal maintained by the Directorate of Marketing and Inspection, Ministry of Agriculture and Farmers Welfare, Government of India. The estimated economic value reflects only the contribution of honeybee pollination and excludes pollination services provided by wild pollinators.

2.3. Calculation of Economic Value of Pollination for Crops

Several approaches developed to estimate the economic value of pollination services (EVP) include a) using the market prices method, b) the damage cost method, and c) the production factor method (Baylis et al., 2021; Lippert et al., 2021; Zhang et al., 2022). However, these approaches generally require farm-level primary data, controlled field experiments or econometric production functions, making them data-intensive, time-consuming and costly to implement across a large number of crops. In the present study, the dependence ratio (DR) method, which represents a simplified bio-economic form of the production factor approach, was adopted. This approach assumes that the role of pollinators in agricultural production can be measured through crop-specific dependence ratios, indicating the proportionate decline in output that would occur in the

Table 1. Dependence rate on animal pollination across different crop groups

Crop group	Dependence rate (DR)
Essential	0.95
Great	0.65
Modest	0.25
Little	0.05
No increase	0.00

Source: Chaudhary and Chand, 2017

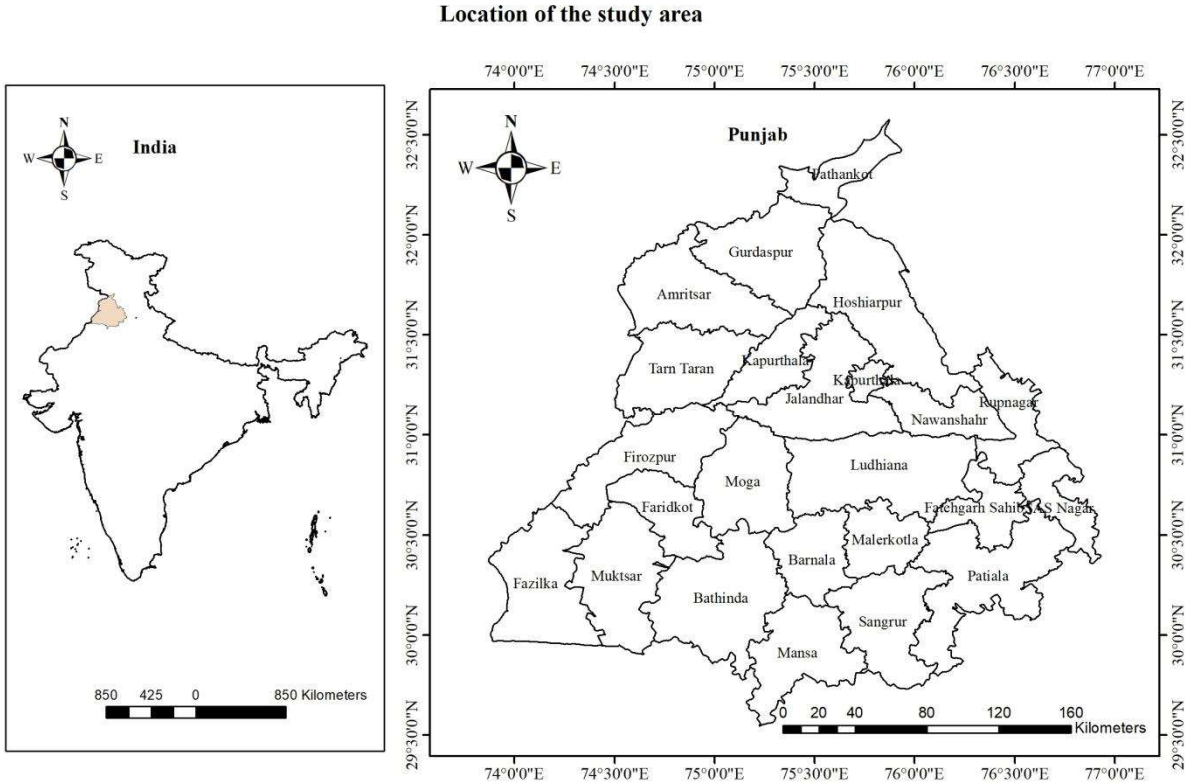


Figure 1. Location of the study area: Punjab state, India.
Source: ArcGIS

absence of pollination services and estimates the economic contribution of pollination by applying crop-specific DR to the total economic value of crop production. The dependence ratios are based on internationally accepted pollination dependence categories developed by Klein et al. (2007) and subsequently quantified by Gallai et al. (2009) and Eilers et al. (2011). These dependence values (Table 1) have also been adopted in the earlier national-level study in India by Chaudhary and Chand (2017) and other international-level studies (Mulatu, 2019; Borges et al., 2020). However, crops that were wind-pollinated (particularly cereals) or those reproduced through vegetative propagation or parthenocarpy were placed in a separate category termed "no increase," and were assigned a DR value of zero.

Further, the selected crops were classified into seven groups: cereal crops i.e. barley *(Hordeum vulgare),* bajra (*Pennisetum glaucum*), maize (*Zea mays*), rice and wheat, oilseeds i.e. groundnut (*Arachis hypogaea*), rapeseed (*Brassica rapa*) & mustard (*Brassica juncea*), sunflower (*Helianthus annuus*) and sesamum (*Sesamum indicum*), pulses i.e. gram (*Cicer arietinum*) and moong (*Vigna radiata*), fibres (cotton), sugarcane (*Saccharum officinarum*), fruits and vegetables.

2.4. Analytical Framework

As the first step, the Economic Value of production (EV) for each crop was computed by multiplying the total production by the corresponding farm harvest price, as represented in the following equation (Eq. 1):

EV = Q X FHP…….. (1)

EV = Economic value of the yearly output of a crop/ commodity

Q = Total annual production quantity of the crop/commodity

FHP = Farm harvest price of the crop/commodity.

Subsequently, the EVP service was calculated by multiplying the economic value of crops and commodities (EV) thus obtained with the corresponding dependence rate (DR from Table 1), as represented in the following equation (Eq. 2). The estimates were subsequently classified into different categories for further analysis.

EVP = EV X DR …….. (2)

where DR = Dependence rate.

3. RESULTS AND DISCUSSION

3.1. Bee Diversity and Pollination Services in India

Pollination is an essential ecological process that sustains both natural and agricultural systems. According to the Zoological Survey of India, harbouring nearly 4 % of the global bee diversity (21000 species), with a total of 827 species of bees belonging to 6 families that aid in the pollination of at least 108 food and cash crops (Angmo et al., 2024) and several other species of medicinal plants with cultural significance. The spatial distribution of bee diversity across states and union territories of India (Figure 2) revealed that Himachal Pradesh accounts for a higher share of the country's bee diversity (9.29%) due to its wide altitudinal variation, diverse vegetation zones and extensive forest cover in mountainous regions that support rich bee fauna, followed by Uttarakhand (7.72%) and Maharashtra (7.21%). Punjab, with 89 species of bees, holds a 4.52% share in the bee diversity.

3.2. Trends in Pollination-Attributed Economic Value Across Major Crop Categories in India

Figure 3 presents the pollination-attributed Economic Value (EVP) disaggregated by major crop categories and commodities at the national level. It was observed that the EVP of all the categories increased consistently over the decade, rising from about Rs 1154.1 ('000 million) in 2012-13 to Rs 2663.3 ('000 million) in 2021-22, indicating a widening economic footprint of animal-mediated pollination in crop output over time. The observed increase in the EVP over the study period reflects the combined influence of changes in the cultivated area, crop productivity and prevailing market prices of pollination-dependent crops. Among categories, fruits & vegetables contributed the largest share (Rs 117.25 '000 million), indicating that horticulture consistently accounts for the largest portion of pollination-attributable value. Oilseeds (Rs 85.06 '000 million) were observed to be the second-largest contributor during the time period. Fibres and spices had moderate shares, while pulses, medicinal and narcotic plants, and other crops contributed only small amounts.

3.3. Economic Significance of Pollination in Punjab Agriculture

Further analysis of the data for Punjab state revealed that the economic value (EV) of major crops and commodities in the state during 2024–25 was estimated at Rs 80286 million (Table 2). Of this, the economic value credited to pollination services was about Rs 3399 million accounting for about 4 % of the total EV.

In terms of economic value (EV), the share of cereals was the highest among the 7 crop/commodity categories i.e.

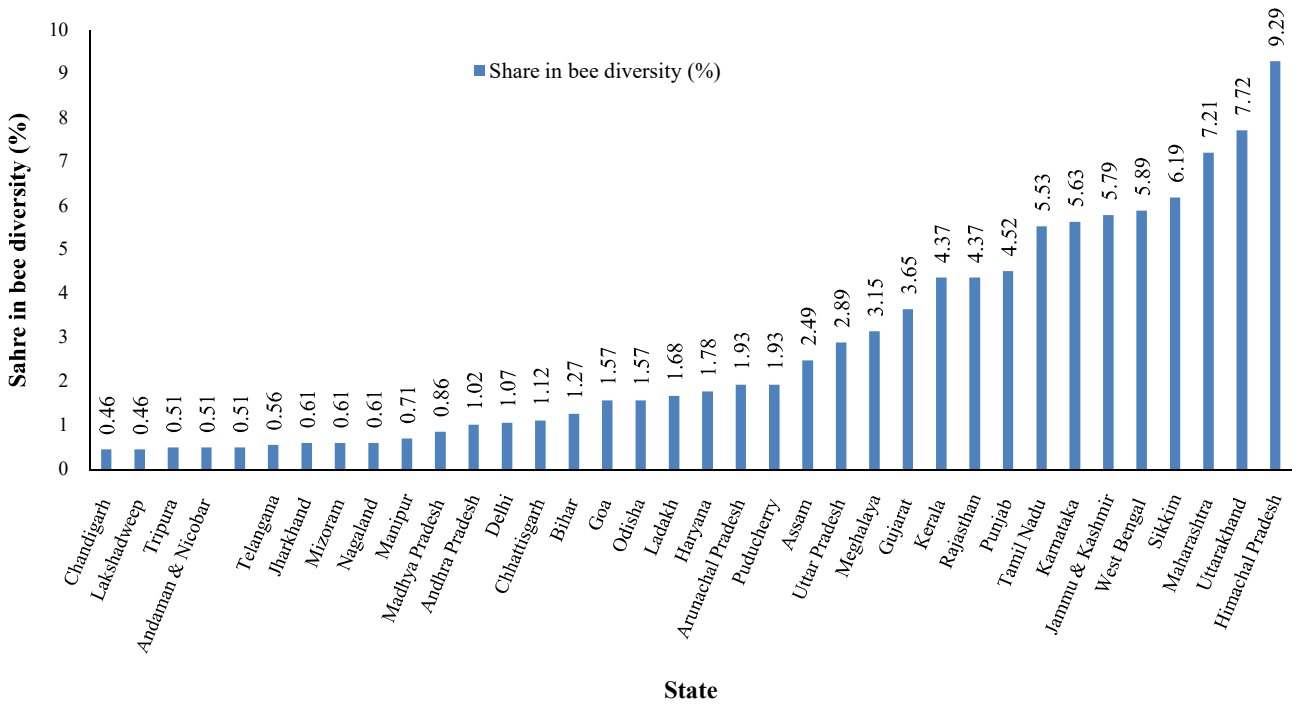


Figure 2. Statewise diversity of bee pollinators in India
Source: Government of India (GoI), 2025

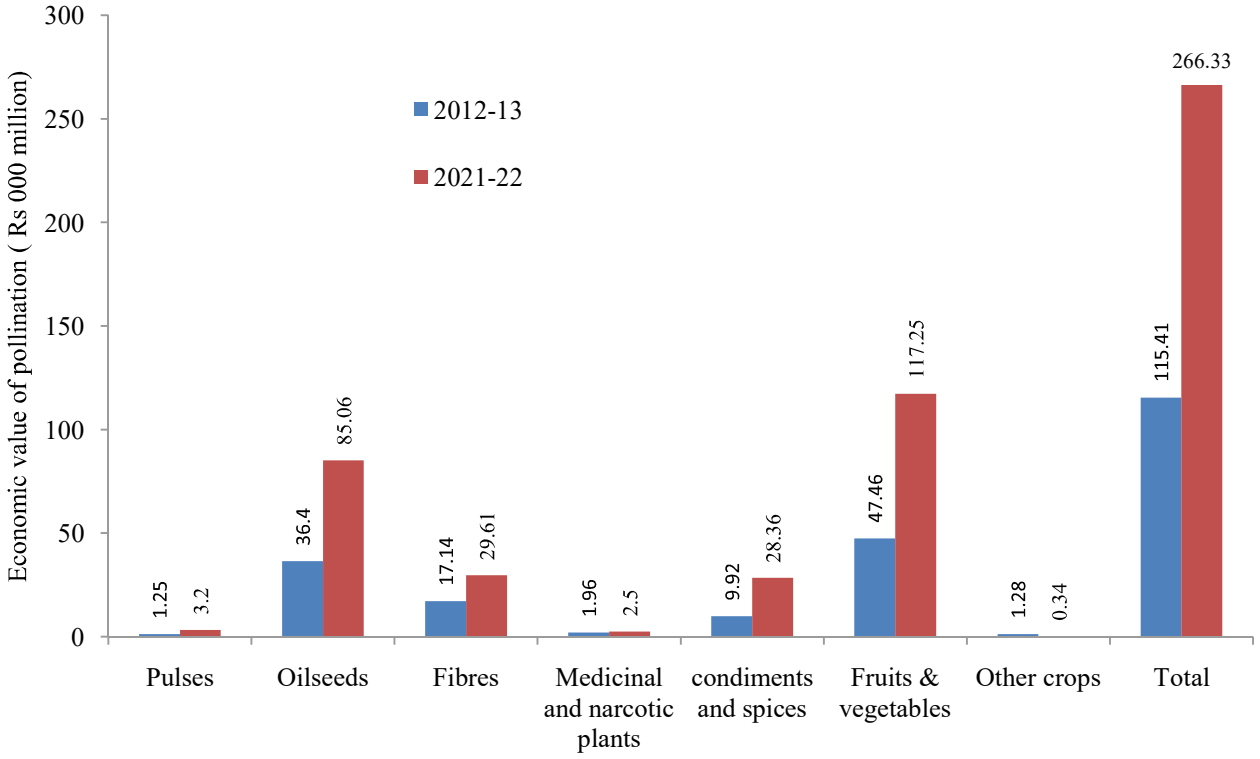


Figure 3. Pollination-attributed value of output across crop categories in India
Source: Government of India (GoI), 2025

66.84 % followed by vegetables (16.03%), fruits (12.11%), sugarcane (3.55%), cotton (0.96%), oilseeds (0.46%) and pulses (0.04%) as shown in Figure 4. On the other hand, the share of EVP was the highest in fruits (69.82%) followed by vegetables (17.89%), oilseeds (6.57%), fibres (5.69%) and pulses (0.03%). Cereals being self- or wind pollinated, and sugar being vegetative propagated does not rely on pollinators for commercial production which resulted in no EVP for cereals and sugarcane.

Out of the 41 major crops and commodities analysed in the study, more than half of them, i.e. 25 (60.98%), were found to be dependent on animal pollination with varying degrees to produce seeds, fruits, nuts, and similar reproductive products (Table 3a &3b). The remaining 16 crops did not show dependence on animal pollination, as they were primarily self-pollinated, wind-pollinated, parthenocarpic or harvested and consumed at the vegetative stage.

Table 3a presents a detailed crop-wise assessment of the

Table 2. Economic value and contribution of pollination to Punjab agriculture, 2024–25

Commodities	Economic value (EV) (Rs million)	% share of EV to total	Economic value due to pollination (EVP) (Rs million)	% share of EVP to total	Increase in production due to pollination
Cereals	53663.5	66.84	0	0	0
Pulses	33.2	0.04	1.0	0.03	3.01
Oilseeds	367.8	0.46	223.4	6.57	60.74
Fruits	9723.0	12.11	2373.3	69.82	24.41
Sugarcane	2853.8	3.55	0	0	0
Vegetables	12871.8	16.04	608.3	17.89	4.73
Fibre crops	773.1	0.96	193.3	5.69	25.0
Total	80286.2	100.0	3399.2	100.0	4.23

Source: Authors' calculations

Table 3a. Crop-wise economic value and contribution of pollination in Punjab (Pulses, oilseeds and Fibres)

Commodities	Economic value EV (Rs million)	Economic value due to pollination EVP (Rs million)	Increase in production due to pollination (% share)
Pulses			
Gram	14.0	0.00	0.00
Moong	19.2	1.0	5.00
Sub total	33.2	1.0	2.89
Oilseeds			
Rapeseed and mustard	326.4	212.2	65.00
Sunflower	11.1	7.2	65.00
Sesamum	12.6	3.2	25.00
Groundnut	17.7	0.9	5.00
Sub total	367.8	223.4	60.74
Fiber crops	0.00	0.00	
Cotton (American)	766.8	191.7	25.00
Cotton (Desi)	6.3	1.6	25.00
Sub total	773.1	193.3	25.00
Cereals			
Wheat	45335.2	0.00	0.00
Paddy	7573.2	0.00	0.00
Maize	717.2	0.00	0.00
Bajra	1.4	0.00	0.00
Barley	36.4	0.00	0.00
Sub total	53663.5	0.00	0.00
Sugarcane	2853.8	0.00	0.00

EV and the contribution of animal pollination to selected crop groups in Punjab, namely pulses, oilseeds, fibre crops, cereals and sugarcane. The results indicated significant variation in pollination dependence across these commodities. Pulses, with a total EV of around Rs 33 million, showed minimal dependence on pollination, as only Rs one million is attributed to pollinator activity, resulting in a modest (2.89%) increase in production. Among pulses, gram exhibited no dependence, whereas moong showed a slight benefit from pollination. On the other hand, oilseeds were observed as the most pollinator-dependent group, with a total EV of about Rs 368 million, of which Rs 223 crore was derived from pollination services, leading to a substantial 60.74 % increase in production. Within this group, rapeseed and mustard contributed the largest share, followed by sunflower, both exhibiting high dependence (around 65%), while sesamum and groundnut had moderate to low dependence. Similar findings have been reported in Nepal, where bee pollination significantly increased mustard production compared with production in the absence of insect pollination (Devkota et al., 2016). Fibre crops, primarily cotton, also demonstrated considerable reliance on pollinators, with a total economic value of Rs 773 million and Rs 193 million attributed to pollination, resulting in a 25 % increase in production for both American and desi cotton. On the other hand, cereals, which dominated the agricultural economy with a total value of around Rs 53664 million, were predominantly self-or wind-pollinated. Similarly, sugarcane, with an economic value of Rs 2854 million, exhibited no dependence on animal pollination due to its vegetative mode of propagation.

A considerable variation in pollination dependence within and across the fruits and vegetables in the state was also observed (Table 3b). Fruits, with a total economic value of Rs 9723 million, showed a substantial contribution from pollination services amounting to Rs 2373 million, resulting in an overall 24 % increase in production. However, this dependence varied widely among individual fruit crops. The crops that rely highly on pollinator include musk melon and watermelon, each showing about 95 % increase in production followed by guava, sweet lime, pear, peach and plum with around 65 % dependence. Fruits such as ber, orange, and litchi exhibited moderate dependence of around 25 % whereas kinnow (Citrus reticulate), lemon (Citrus limon), and mango (Mangifera indica) show relatively low dependence of about 5 %. On the other hand, banana and grapes showed no contribution from pollination.

In the case of vegetables, the total EV was higher at Rs 12872 million, but the contribution from pollination was comparatively low at Rs 608 million, resulting in only a 4.73 % increase in production. Several major vegetables such as cabbage, cauliflower, garlic, onion, green peas, potato and carrot show no dependence on pollination, as they are either

Table 3b. Crop-wise economic value and contribution of pollination in Punjab (Fruits & vegetables)

Commodities	Economic value EV (Rs million)	Economic value due to pollination EVP (Rs million)	Increase in production due to pollination (% share)
Fruits			
Ber	102.1	25.5	25.00
Banana	80.8	0.00	0.00
Lemon	144.5	7.2	5.00
Kinnow	4795.6	239.8	5.00
Sweet lime	144.4	93.9	65.00
Orange	210.4	52.6	25.00
Guava	1250.2	812.6	65.00
Grapes	63.1	0.00	0.00
Litchi	604.1	151.0	25.00
Mango	1080.1	54.0	5.00
Musk melon	329.5	313.1	95.00
Peach	206.8	134.4	65.00
Pear	561.8	365.1	65.00
Plum	60.5	39.4	65.00
Water melon	89.2	84.7	95.00
Sub total	9723.0	2373.3	24.41
Vegetables	0.00	0.00	
Cabbage	176.9	0.00	0.00
Cauliflower	1525.9	0.00	0.00
Brinjal	365.5	91.4	25.00
Garlic	648.1	0.00	0.00
Okra	203.9	51.0	25.00
Onion	463.8	0.00	0.00
Green Peas	3581.6	0.00	0.00
Potato	3232.7	0.00	0.00
Sweet potato	1.4	0.1	5.00
Tomato	645.9	419.9	65.00
Carrot	1842.0	0.00	0.00
Green chillies	184.1	46.0	25.00
Sub total	12871.8	608.3	4.73

Source: Authors' calculations

self-pollinated or propagated vegetatively. However, certain vegetables demonstrated moderate to high reliance on pollinators. Tomato exhibited a significant dependence, with a 65 % increase in production, i.e. Rs 420 million, attributed to pollination. Similarly, brinjal, okra, and green chillies exhibited moderate dependence (around 25 %), while sweet potato showed minimal dependence (5 %).

Dwelling further into the degree of dependence, the largest share of EVP services belongs to crops that fall under the "great dependence" category (Rs 2085 million), followed by modest (Rs 614 million), essential (Rs 398 million) and little dependence (Rs 303 million). Among the 25 entomophilous crops, insect pollination was found to be essential for only two fruit crops (Musk melon and

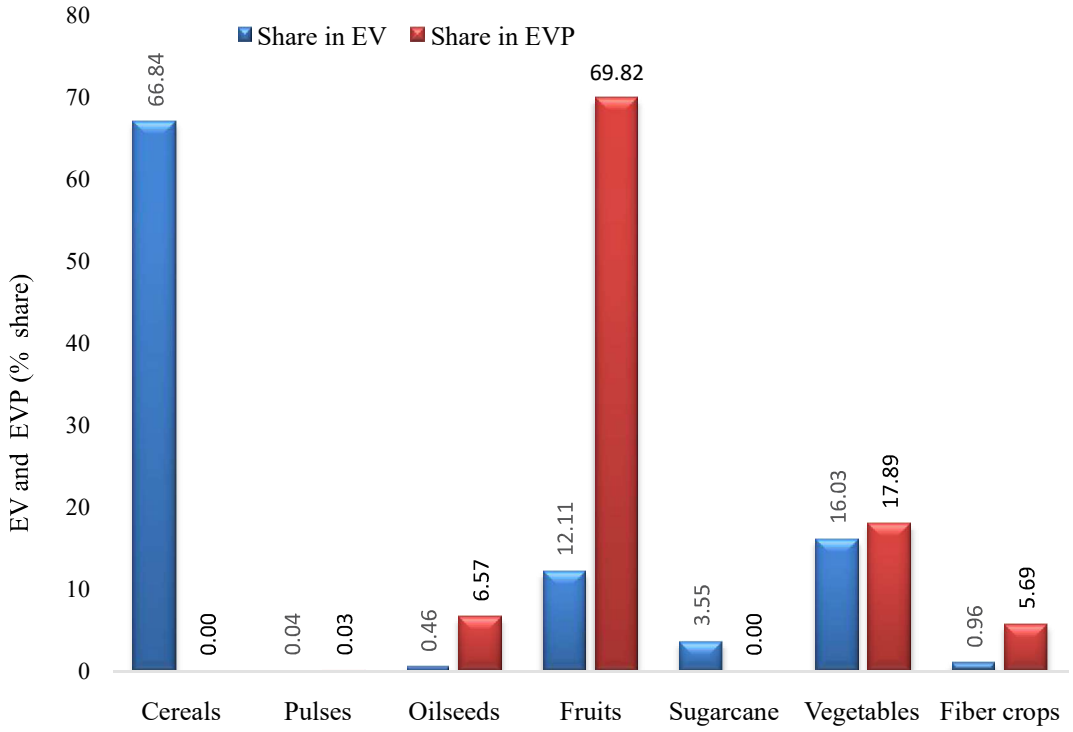


Figure 4. Category-wise contribution to economic value (EV) and economic value due to pollination (EVP) in Punjab

Table 4. Category-wise distribution of economic value of pollination services by degree of dependence in Punjab agriculture

Category/Degree of	Essential	Great	Modest	Little	Total
Cereals	0.00	0.00	0.00	0.00	0.00
Pulses	0.00	0.00	0.00	1.0	1.0 (0.03)
Oilseeds	0.00	219.4	3.2	0.9	223.4 (6.58)
Fruits	397.8	1445.4	229.1	301.0	2373.3 (69.82)
Vegetables	0.00	419.9	188.4	0.00	608.2 (17.0)
Sugar	0.00	0.00	0.00	0.00	0.00
Cotton	0.00	0.00	193.3	0.00	193.3 (5.69)
Total	397.8	2084.7	614	302.9	3399.2 (100.0)

Note: Figures in parentheses indicate percentage of total

watermelon); in their absence, a reduction in yield of 90-100% takes place. Another 8 crops were greatly dependent, including oilseeds like rapeseed & mustard, sunflower, along with fruits: sweet lime, guava, peach, pear, plum, and vegetable tomato. Ten crops were found to be moderately dependent on pollination, while pollinators were of little importance to five crops (moong, lemon, kinnow, mango, and sweet potato); without animal-assisted pollination, a reduction of up to 10% in yield was observed. The findings indicate that the economic value of pollination services is highly concentrated in crops with high and moderate dependence, particularly fruits, oilseeds, and vegetables; conversely, staple crops like cereals and sugarcane do not benefit from pollination. The results highlighted the need for targeted pollinator conservation strategies, especially for high-value crops that depend on pollinators, where the economic returns are substantial.

Besides individual variations in insect pollination benefits, oilseeds as a category are extremely dependent on pollination for their production and quality improvement and the contribution to the tune of 6.58 % in yield, valued at Rs 223.4 million, is an indication of this fact. Further, fruits, which are an important source of quality nutrition and a vital component of Punjab's agricultural economy, derived the highest benefits from insect pollination. The economic value of pollination in fruits was estimated at Rs. 2373 million representing about 70 % of the state's total EVP, highlighting the indispensable role of pollinators in sustaining fruit production and thus enhancing farm income.

3.4. Limitations of the Study

- The present study estimates only the contribution of managed honeybees to crop pollination and excludes the pollination services provided by wild bees and other insect pollinators, which may also contribute substantially to agricultural production.
- Further, the estimation is based on secondary data for a single agricultural year (2024–25) for Punjab state and does not capture inter-annual variations arising from changes in weather, crop area, productivity, market prices or abundance of pollinators.
- The economic valuation of pollination services (EVP) by honeybees has been done using the dependence ratio (DR) method, which relies on generalised crop-specific dependence ratios reported in the literature rather than field-based measurements of pollination services. Consequently, the estimated values should be interpreted as indicative estimates of the economic contribution of managed honeybee pollination rather than precise measurements of actual pollination benefits under field conditions.

4. CONCLUSIONS

Honeybee pollination is an indispensable ecological service and an under-recognized and under-appreciated economic driver in Punjab agriculture. It plays a significant role in improving productivity as well as quality of high-value crops such as fruits, vegetables, oilseeds, and cotton, even though its overall share in total agricultural value appears modest due to the dominance of non-pollinator-dependent cereals. With the highest economic gains concentrated in those with high and moderate dependence, a large proportion of crops depend on pollinators, underscoring the need for focused attention on pollinator management. Therefore, promoting scientific beekeeping, conserving natural habitats, encouraging crop diversification towards pollinator-dependent crops and integrating pollinator-friendly practices such as reduced use of pesticides, flowering field margins, and adoption of integrated farming systems are essential. And to fully realize the ecological and economic potential of pollination, there is a need for increased research, farmer awareness and supportive policies, ensuring sustainable agricultural growth and improved farm incomes in Punjab.

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CRediT authorship contribution statement

Sangeet Ranguwal: Conceptualisation, methodology, supervision, formal analysis and writing the original draft; **Japneet Kaur:** Data collection, tabulation, and manuscript drafting; **Amit Guleria:** Literature review, manuscript review and editing.

Conflict of interest

The authors declare that they have no conflicts of interest related to the content of this manuscript.

Statement on generative AI and AI-Assisted technologies in the writing process

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YouTube as an Agricultural Extension Tool: Lessons Learned from Viewers' Reactions in Punjab, India

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ABSTRACT: YouTube is used in agriculture for a variety of purposes, from increasing awareness to teaching about agricultural advances. The study was conducted to analyse viewers' responses to the videos of Punjab Agricultural University, Ludhiana. A total of 150 farmers and 64 Agriculture Development Officers (ADOs) were included in the study to ascertain viewers' responses to selected videos. The majority of respondents perceived that the video's language was simple, the duration of the message was sufficient, and they were highly satisfied. The majority of the farmers and ADOs (86 %) reported the information as factual. The analysis of viewer responses indicated that content relevance was the most important factor driving audience engagement, as farmers demonstrated greater interest in information that directly addressed their immediate field problems. The credibility of subject experts significantly influenced viewers' trust and acceptance of the information provided. Presentation style played a crucial role, with visually supported explanations enhancing comprehension over purely verbal communication. The use of simple and farmer-friendly language further improved clarity and learning outcomes. High factual accuracy strengthened the institutional credibility of the digital platform, thereby reinforcing farmers' confidence in its advisory services.

Keywords: Content analysis, Digital agricultural extension, Social media, Viewers' reactions, YouTube.

1. INTRODUCTION

Information has always been considered a major influence on human behaviour. When information is received, processed, and retained in the human mind, it passes through stages of thinking and decision-making that ultimately result in behavioural change (Tamizhkumaran & Saravanan, 2021). In the digital era, social media has become one of the most powerful sources of information dissemination and interaction. These platforms facilitate discussion, relationship building and opinion formation among users. YouTube has emerged as a major medium for sharing information, education and entertainment worldwide. It allows users to create, upload, view, comment on and share videos, making it a highly interactive platform. In agriculture, YouTube has become an important e-extension tool, providing farmers with visual and practical knowledge related to crop production, pest and disease management, marketing, mechanization and new technologies (Thakur & Chander, 2018). The visual nature of YouTube makes complex agricultural practices easier to understand and adopt (Gharis et al., 2014; Doyle & Briggeman, 2014). Recent evidence indicates that digital agricultural advisory services are increasingly complementing conventional extension systems by providing farmers with rapid and cost-effective access to

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agricultural information, particularly in low- and middle-income countries (Porciello et al., 2022). Among digital platforms, YouTube has emerged as an effective medium for agricultural education because it enables the large-scale dissemination of multilingual and visually rich educational content at relatively low cost (Reeves et al., 2024). Studies have further demonstrated that the quality, credibility and presentation of agricultural YouTube videos significantly influence audience interaction and engagement (Chakma et al., 2022).

Agricultural YouTube channels not only disseminate information but also create opportunities for interaction between content creators and viewers. Viewer responses in the form of likes, dislikes, comments and shares reflect how the audience perceives the usefulness, clarity, credibility and relevance of the uploaded videos (Snelson, 2011). These responses provide immediate feedback and help determine whether the information provided is accepted, trusted, and applied by viewers. Viewer responses indicate the level of engagement and interest generated by the videos and help assess their influence on farmers' awareness, learning, and decision-making (Ma et al., 2016), providing valuable insights into the acceptance, usefulness, and impact of the videos (Khan, 2017). By analysing these responses, researchers can assess whether the videos meet farmers' information needs and influence their knowledge and farming decisions. Recent research has highlighted that evaluations of digital agricultural videos have largely focused on technology adoption, intervention outcomes and scalability. In contrast, relatively little attention has been paid to understanding how users perceive, engage with and respond to the videos themselves. Researchers have argued that examining users' experiences and engagement provides deeper insights into the effectiveness of video-based extension and helps identify opportunities for improving digital communication strategies (Coggins et al., 2025).

Digital extension through YouTube can contribute to agroecological sustainability by improving the resource use efficiency of agricultural information dissemination. Unlike conventional extension approaches, which depend on printed extension literature, repeated field visits, farmer meetings, and travel by extension personnel, YouTube enables research-based recommendations to reach a large audience through a single digital platform (Bentley et al., 2022). Video-based extension has been recognized as an effective mechanism for delivering agricultural knowledge at scale, improving the timeliness and accessibility of advisory services while complementing conventional extension systems. Consequently, YouTube can reduce material inputs, transport-related energy consumption, and the environmental footprint associated with extension delivery while supporting more sustainable and resource-efficient agricultural advisory systems (Sen et al., 2025).

Punjab Agricultural University (PAU) serves the farming community of Punjab through research, teaching and extension services. To strengthen its extension activities in the digital era, PAU uses several social media platforms, including YouTube. The official PAU YouTube channel (https://www.youtube.com/channel/UCa3bxtjJAu3jUnUvV1BxhXQ), started on 10 December 2015, uploads videos related to crop management, pest and disease control, water management, farm mechanization, *kisan melas* (farmers' fairs) and new agricultural technologies. (These videos aim to provide timely, reliable and practical information to farmers and other stakeholders (PAU 2023; PAU 2023). While agricultural YouTube channels are increasingly used for information dissemination, the effectiveness of these videos cannot be judged only by their availability; it is essential to understand how viewers respond to them. Therefore, this study was undertaken to analyse viewer reactions to the PAU Ludhiana YouTube channel. The aim was to assess the responses of both farmers and extension experts to the channel's videos, evaluate their content across key dimensions, and identify the factors that contribute to a video's overall reaction score, ultimately drawing lessons for improving YouTube-based extension efforts. The study has systematically analysed viewers' reactions across multiple dimensions to understand what makes agricultural videos effective from farmers' perspectives.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in five agroclimatic zones of Punjab. These five zones were purposively selected to represent diverse agricultural zones of Punjab, i.e. Sub-mountain Undulating Zone, Undulating Plain Zone, Central Plain Zone, Western Plain Zone, and Western Zone. Distinct geographical features, cropping patterns, rainfall, and cropping intensity characterize each zone. The sub-mountain undulating zone, covering Gurdaspur and Hoshiarpur, receives about 1,000 mm of rainfall and supports crops like wheat, paddy (unhusked rice), and sugarcane, with a cropping intensity of 177%. The undulating plain zone of Rupnagar (Ropar) and SBS Nagar

(Nawanshahr) also receives around 1,000 mm of rainfall but has a higher cropping intensity of 187%, growing similar crops. The central plain zone, which includes major districts such as Amritsar, Ludhiana, and Patiala, receives comparatively low rainfall (630–700 mm) and primarily focuses on wheat and paddy cultivation, with a cropping intensity of 189%. Moving towards the southwest, the western plain zone (Firozpur and Faridkot) and the broader western zone (including Bathinda, Mansa, and Muktsar) have similar rainfall levels (630–700 mm) but differ slightly in cropping intensity, recorded at 190% and 193% respectively. These western regions diversify cropping of including cotton along with wheat and paddy (Punjab Revenue, 2012).

2.2. Sampling Design

A multistage sampling design was adopted for the study. The respondents of the study were categorised into two groups: farmers and extension professionals.

2.2.1. Selection of district and villages

In the first stage, five zones were purposively selected. In the second stage, from each zone, one district was selected randomly. i.e., Hoshiarpur (Sub-mountain Undulating Zone), Nawanshahr (Undulating Plain Zone), Sangrur (Central Plain Zone), Firozpur (Western Plain Zone), Mansa (Western Zone). The selected districts include a total of 1416 villages (Hoshiarpur), 468 (Nawanshahr), 571 (Sangrur), 1000 (Firozpur), and 240 (Mansa). In the third stage, one village was selected randomly from each selected district. This sampling approach was adopted to obtain geographically distributed responses from different regions of Punjab while maintaining a manageable field survey. The selected village served as the sampling unit for farmer respondents.

2.2.2. Selection of farmers

A list of farmers was obtained from each selected village. Farmers were selected using simple random sampling and invited to participate in the study. As participation was voluntary, some initially selected farmers were unavailable or declined to participate. In such cases, additional farmers were randomly selected from the same sampling frame until 30 willing participants were obtained from each village, resulting in a total sample of 150 farmers. The selected farmers were invited to a common venue within the village, where the purpose of the study was explained. Participation was entirely voluntary, and only those who were willing were included in the study. An equal number of respondents (30) was selected from each sampled village to ensure balanced representation of all agro-climatic zones and facilitate comparison of viewer reactions across regions. The objective of the study was a comparative analysis of viewer reactions under uniform viewing conditions, rather than the estimation of population-level parameters.

2.2.3. Selection of extension experts

A total of 64 Agriculture Development Officers (ADOs) were posted across the selected districts (Sangrur: 12, Mansa: 11, Firozepur: 18, Nawanshahr: 4, and Hoshiarpur: 19). All ADOs were included in the study, constituting a census of extension professionals.

2.3. Research Design

The study followed an exploratory research design to assess viewer responses to YouTube videos. An exploratory research design is the initial research conducted to gain familiarity with a phenomenon or to achieve new insights into it (Kothari & Garg, 2019).

2.4. Selection of Videos

A total of 731 videos available on the YouTube channel of PAU Ludhiana up to 1st March 2023 were considered. These videos were classified into two categories:

Scripted videos: pre-planned content such as demonstrations, dialogues, dramas, interviews, stories, and talks.

Non-scripted videos: Content including coverage of events such as kisan melas, virtual events, panel discussions, PAU live sessions, and question–answer sessions.

Out of 731 videos, 501 were identified as scripted videos, and all scripted videos were selected for further analysis. For analysing viewer reactions, the scripted videos were further categorized into four major thematic areas based on their content. The categories included Pest Management (77 videos), Crop Management (44 videos), Water Management (16 videos), and Weed Management (13 videos). From each thematic area, two videos were selected randomly to ensure representation while maintaining manageable interview duration and minimizing respondent fatigue. Thus, a total of 8 videos were selected.

2.5. Data Collection

The selected videos were personally shown to all respondents (farmers and extension experts), and their responses were recorded. To ensure that all participants viewed the same content under similar conditions, the

videos were shown to farmers in small groups within each selected village. After viewing the videos, each farmer's reactions were recorded independently using a structured interview schedule developed specifically for the study. Individual interviews were conducted to ensure independent responses and to minimize the possibility of peer influence on respondents' perceptions. The interview schedule was developed based on the objectives of the study and relevant literature. It was examined by a panel of experts in Agricultural Extension Education for content validity. As the interview schedule consisted of predefined evaluation dimensions and response categories rather than psychometric statements, its content validity was established through expert review. A panel of ten experts evaluated the relevance, clarity, and adequacy of the selected dimensions and their corresponding continuum with respect to the study objectives. Based on their suggestions, the instrument was revised before final administration.

2.6. Operational Definitions

The operational definitions and measurement of the

Table 1. Measurement framework for viewer response

Dimension	Operational definition	Measurement scale (Score)
Video length	Length of time a particular video or a segment of a video runs.	Hours, minutes, and seconds.
Views	Number of times a video is watched. It can also be used to count the number of times a video is started.	View (%) = $\frac{\text{Number of views of a category}}{\text{Total number of views of all categories}} \times 100$
Category of video	Type of presentation used in the video.	The categories are talk, demonstration and interview.
Viewer reaction	Viewer reaction was operationalized as the opinion or feedback of respondents regarding the content, presentation, and effectiveness of the videos, along with suggestions for improvement.	Viewer responses were assessed across fifteen dimensions as shown below:
Suitability of content with title	Degree to which the video content matches its title	Suitable (3) / Somewhat suitable (2) / Not suitable (1)
Clarity	Reception of the message without noise or disturbance	Very clear (3) / Clear (2) / Not clear (1)
Utility	Perceived usefulness of the message	Very useful (3) / Useful (2) / Not useful (1)
Completeness	Thoroughness of information provided in the video	Complete (3) / Just complete (2) / Incomplete (1)
Compatibility	Consistency of information with previous farm practices	Compatible (3) / Just compatible (2) / Not compatible (1)
Specificity	Degree of brevity and to-the-point description	Very specific (3) / Specific (2) / General (1)
Understandability	Ease with which audience grasps the message	Very easy (3)/ Easy (2)/ Difficult (1)
Language spoken	Simplicity of language used in the message	Very simple (3)/ Simple (2)/ Difficult (1)
Duration of message delivery	Adequacy of time taken for message delivery	Highly sufficient (3)/ Sufficient (2)/ Insufficient (1)
Relevance	Suitability of message to farmers' needs	Highly relevant (3)/ Relevant (2)/ Not relevant (1)
Satisfaction	Level of gratification of information need	Highly satisfied (3)/ Satisfied (2)/ Not satisfied (1)
Use of English/ technical words	Extent of English/technical words used	Too many (3)/ Many (2)/ Not at all (1)
Motivational value	Extent to which message stimulates action	Highly motivational (3)/ Motivational (2)/ Not motivational (1)
Factual content	Degree of fact-based information in message	Completely factual (3)/ Partially factual (2)/ Not factual (1)
Total reaction scores	It was employed to identify the video with the best/least overall viewer response.	Ranks were assigned to each selected video for each dimension on the basis of mean score. Ranks were then converted to scores and total reaction score was calculated for each video.

study variables are presented in Table 1.

2.7. Statistical Analysis

To analyse viewer responses, a total reaction score was computed by summing the scores across all fifteen dimensions, with a minimum possible score of 15 and a maximum possible score of 45. Based on the total reaction scores, the selected videos were ranked to identify those with the highest and lowest viewer response. Descriptive statistical tools, such as the mean and ranking, were used to interpret the data. An independent-samples t-test was employed to compare the mean reaction scores of farmers and agriculture development officers (ADOs) of the Department of Agriculture in Punjab. Farmers and Agriculture Development Officers were included as two stakeholder groups in agricultural extension. Since both groups viewed the same videos under identical conditions and responded to the same interview schedule, their viewer reactions could be meaningfully compared. The comparison was limited to their perceptions of the videos and did not imply equivalence in their demographic or professional characteristics. For data analysis, the Statistical Package for the Social Sciences (SPSS) version 23 was used.

3. RESULT AND DISCUSSION

3.1. Descriptive Elements of Published Video

Table 2 presents the descriptive elements of published videos. It provides a classification of eight selected videos into different categories and subjects. The subjects are "crop management", "pest management", "water management" and "weed management". The categories are talk, demonstration and interview. Talk referred to a monologue presentation in which information on a specific topic was delivered by an individual. Interview referred to a two-person interaction initiated by the interviewer to obtain information about the expert and his/her experience on a particular theme. Demonstration referred to a presentation mode in which all the steps involved in making or performing a task were shown to viewers through videos.

Table 2. Descriptive elements of published video

Code	Title of video	Subject of video	Category of video	Year	Video length (min)	No. of views	No. of likes
A	If DAP was applied to wheat in the same field, then do not apply more DAP to paddy; there will be no difference in yield. (ਜੇ ਇਸੇ ਖੇਤ ਵੱਚਿ ਕਣਕ ਨੂੰ ਡੀਏਪੀ ਪਾਈ ਸੀ ਤਾਂ ਹੁਣ ਝੋਨੇ ਨੂੰ ਹੋਰ ਡੀਏਪੀ ਨਾ ਪਾਓ ਝਾੜ ਵਿੱਚ ਕੋਈ ਫਰਕ ਨਹੀ ਪਵੇ)	Crop management	Talk	2018	01:41	810	5
B	Direct sowing of paddy (ਝੋਨੇ ਦੀ ਸੱਧੀ ਬਜਾਈ)	Crop management	Demonstration	2022	05:29	5663	93
C	Importance of seed treatment in paddy (ਝੋਨੇ ਵਚਿ ਬੀਜ ਸੋਧ ਦੀ ਮਹੱਤਤਾ)	Pest management	Demonstration + Talk	2020	02:04	859	29
D	Rice pests and their control (ਝੋਨੇ ਦੇ ਕੀੜੇ ਮਕੌੜੇ ਅਤੇ ਉਹਨਾਂ ਦੀ ਰੋਕਥਾਮ)	Pest management	Talk	2019	09:31	1982	61
E	Super Seeder Questions Answers, Yellowing of crop after first irrigation (ਸੁਪਰ ਸੀਡਰ ਦੇ ਸਵਾਲ ਜਵਾਬ| ਪਹਲਿੀ ਸਿੰਚਾਈ ਤੋਂ ਬਾਦ ਫਸਲ ਦਾ ਪੀਲਾਪਣ)	Water management	Talk	2021	01:43	124	4
F	Information regarding drip irrigation (ਤੁਪਕਾ ਸਿੰਚਾਈਸਬੰਧੀ ਜਾਣਕਾਰੀ)	Water management	Demonstration	2021	04:02	119	11
G	Prevention of Phalaris minor in wheat (ਕਣਕ ਵਿੱਚ ਗੁੱਲੀ ਡੰਡੇ ਦੀ ਰੋਕਥਾਮ)	Weed management	Talk	2018	07:17	13232	343
H	How to prevent weeds in wheat - Dr. Makhan Singh Bhullar (ਕਣਕ ਵਿੱਚ ਨਦੀਨਾਂ ਦੀ ਰੋਕਥਾਮ ਕਿਵੇਂ ਕਰੀਏ - ਡਾ. ਮੱਖਣ ਸਿੰਘ ਭੁੱਲਰ)	Weed management	Interview	2022	08:39	4032	210

* Notes: Text in parentheses is the original Punjabi titles of the respective YouTube videos.
Link for the videos: https://www.youtube.com/channel/UCa3bxtjJAu3jUnUvV1BxhXQ

[1]DAP= Diammonium phosphate fertilizer

Crop management videos provide information on how to grow and manage crops. A video entitled "If DAP[1] was applied to wheat in the same field, then do not apply more DAP to paddy; there will be no difference in yield" was a talk about crop management, while a video on "Direct sowing of paddy" was a demonstration of direct-seeded rice. Videos on pest management provide information on how to identify, prevent, and control pests. The video entitled "Importance of seed treatment in paddy" was a demonstration and talk about pest management, while the video entitled "Rice pests and their control" was a talk about pest management in rice crops. Videos related to water management provide information on how to irrigate crops. The video entitled "Super-seeder questions answers, yellowing of crop after first irrigation" was a talk about water management. In contrast, the video entitled "Information regarding drip irrigation" was a demonstration of the drip irrigation technique. Videos related to weed management provide information on how to identify, prevent, and control weeds. The video entitled "Prevention of Phalaris minor in wheat" was a talk about management of Phalaris minor weed in wheat crop, while the video entitled "How to prevent weeds in wheat - Dr Makhan Singh Bhullar" was an interview with an expert on weed management in wheat crop.

The videos were published between 2018 and 2022. The most views (13232) were on the video entitled, "Prevention of *Phalaris minor* in wheat", whereas the fewest views (119) were on the video entitled, "Information regarding drip irrigation". The most likes (343) were on the video entitled, "Prevention of *Phalaris minor* in wheat", whereas the fewest likes (4) were on the video entitled, ″Super seeder questions answers, yellowing of crop after first irrigation″. The longest video among all was "Rice pests and their control", with a video length of 09:31 minutes and the shortest video, with a video length of 01:41 minutes, was "If DAP was applied to wheat in the same field, then do not apply more DAP to paddy; there will be no difference in yield."

3.2. Viewer Response Analysis: Based on Total Reaction Score

Table 3. Distribution of selected videos on the basis of total reaction score

Title of video	Total reaction scores ADOs (n=64)	Rank	Total reaction scores farmers (n=150)	Rank	t value	Overall reaction score (n=214)	Rank
If DAP was applied to wheat in the same field, then do not apply more DAP to paddy; there will be no difference in yield. (ਜੇ ਇਸੇ ਖੇਤ ਵਿੱਚ ਕਣਕ ਨੂੰ ਡੀਏਪੀ ਪਾਈ ਸੀ ਤਾਂ ਹੁਣ ਝੋਨੇ ਨੂੰ ਹੋਰ ਡੀਏਪੀ ਨਾ ਪਾਓ ਝਾੜ ਵਿੱਚ ਕੋਈ ਫਰਕ ਨਹੀਂ ਪਵੇ*)	36.64	6	37.35	4	2.18	36.99	5
Direct sowing of paddy (ਝੋਨੇ ਦੀ ਸਿੱਧੀ ਬਿਜਾਈ*)	37.80	3	36.35	5	3.06	37.07	4
Importance of seed treatment in paddy (ਝੋਨੇ ਦੀ ਬੀਜ ਸੋਧ ਦੀ ਮਹੱਤਤਾ*)	37.20	5	36.34	6	1.81	36.77	6
Rice pests and their control (ਝੋਨੇ ਦੇ ਕੀੜੇ ਮਕੌੜੇ ਅਤੇ ਉਹਨਾਂ ਦੀ ਰੋਕਥਾਮ*)	37.30	4	38.01	2	1.16	37.65	3
Super Seeder Questions Answers, Yellowing of crop after first irrigation (ਸੁਪਰ ਸੀਡਰ ਦੇ ਸਵਾਲ ਜਵਾਬ/ਪਹਿਲੀ ਸਿੰਚਾਈ ਤੋਂ ਬਾਅਦ ਫਸਲ ਦਾ ਪੀਲਾਪਣ*)	35.88	7	36.11	7	0.49	35.99	7
Information regarding drip irrigation (ਤੁਪਕਾ ਸਿੰਚਾਈ ਸੰਬੰਧੀ ਜਾਣਕਾਰੀ*)	35.23	8	34.92	8	0.52	35.07	8
Prevention of Phalaris minor in wheat (ਕਣਕ ਵਿੱਚ ਗੁੱਲੀ ਡੰਡੇ ਦੀ ਰੋਕਥਾਮ)	37.88	2	38.61	1	1.58	38.24	2
How to prevent weeds in wheat - Dr. Makhan Singh Bhullar (ਕਣਕ ਵਿੱਚ ਨਦੀਨਾਂ ਦੀ ਰੋਕਥਾਮ ਕਿਵੇਂ ਕਰੀਏ– ਡਾ. ਮੱਖਣ ਸਿੰਘ ਭੁੱਲਰ)	39.41	1	37.96	3	3.33	38.68	1

Note: Text given in parentheses is the original Punjabi titles of the respective YouTube videos

Data in Table 3 shows the distribution of total reaction scores for eight selected videos. For the ADOs, the video entitled "How to prevent weeds in wheat – Dr. Makhan Singh Bhullar" recorded the highest total reaction score (39.41), followed by "Prevention of *Phalaris Minor*" (37.88), while "Information regarding drip irrigation" obtained the lowest score (35.23). For the farmers, "Prevention of *Phalaris minor* in wheat" achieved the highest total reaction score (38.61), followed by "Rice pests and their control" (38.01), whereas "Information Regarding Drip Irrigation" again received the lowest score (34.92). Overall, "How to prevent weeds in wheat - Dr. Makhan Singh Bhullar" emerged as the most highly rated video with a total reaction score of 38.68, indicating that it was well appreciated by both ADOs and farmers. The videos "How to prevent weeds in wheat – Dr. Makhan Singh Bhullar" and "Prevention of *Phalaris minor*" achieved superior reaction scores because they addressed highly relevant weed-management issues, presented factually reliable information in clear and simple language, and were delivered by experts at a farmer-friendly pace, which enhanced viewers' satisfaction, clarity, trust, and engagement.

"During interviews, several farmers commented that videos featuring Dr. Makhan Singh Bhullar were particularly trusted because 'he explains like he is sitting with us in the field'."In contrast, "Information regarding drip irrigation" recorded the lowest overall reaction score (35.07) because it was more technical, less clear, and less immediately relevant to the majority of farmers than weed- and pest-management videos. The limited use of simple language, lower practical applicability, and weaker audience engagement reduced clarity and satisfaction, resulting in lower overall appreciation of this video.

The *t*-values presented in Table 3 indicate the extent of difference between the mean total reaction scores of farmers and agriculture development officers (ADOs) for each selected video. Higher *t*-values indicate greater differences in perception between the two respondent groups, whereas lower t-values suggest minimal or negligible differences, indicating that both groups perceived the videos similarly. The highest and most significant differences were observed for "How to prevent weeds in wheat – Dr. Makhan Singh Bhullar" ($t = 3.34$) and "Direct sowing of paddy" ($t = 3.05$), showing that farmers and ADOs differed more noticeably in their evaluations of these videos. In both cases, ADOs generally rated the videos higher, possibly due to their stronger appreciation of expert delivery, technical depth, and scientific accuracy.

A moderate level of difference was found for "If DAP was applied to wheat…" ($t = 2.18$) and "Prevention of *Phalaris minor* in wheat" ($t = 1.59$), suggesting some variation in perception between the two groups, although their overall responses remained largely similar. In contrast, very low and statistically non-significant differences were observed for "Super Seeder Questions–Answers" ($t = 0.50$), "Information regarding drip irrigation" ($t = 0.52$), and "Rice pests and their control" ($t = 1.16$), indicating that both farmers and ADOs held almost identical opinions regarding these videos, particularly in terms of clarity, usefulness, and satisfaction.

Overall, the *t*-value analysis reveals that farmers and extension experts generally shared similar perceptions across most videos; however, a few expert-led, technically detailed videos produced greater differences in their evaluations. This suggests the need to balance scientific rigour with farmer-friendly presentation in order to ensure broader acceptance and uniform understanding across different categories of viewers.

3.3. Lessons Learned from Viewers' Reactions

The systematic analysis of viewer responses to the PAU Ludhiana YouTube channel revealed several important lessons for strengthening digital agricultural extension. Content relevance emerged as the strongest driver of engagement, as the video *"*Prevention of *Phalaris minor* in wheat*"* recorded the highest number of views (13,232), likes (343), and the highest reaction score among farmers (38.61), indicating that farmers actively seek solutions to their immediate field problems. In contrast, *"*Information regarding drip irrigation*"* received the lowest number of views (119) and the lowest overall reaction score (35.07), showing that topics perceived as less urgent or less aligned with farmers' current practices generated weaker engagement. Expert credibility also played a crucial role, as videos featuring Dr. Makhan Singh Bhullar obtained the highest overall reaction score (38.68), reflecting that a trusted and relatable expert enhanced viewers' confidence and acceptance of technical information.

Presentation style and content delivery further influenced the effectiveness of learning. Videos that combined demonstration with verbal explanation, such as *"*Importance of seed treatment in paddy*"*, were better received because they enabled farmers to both visualize and understand the practice, whereas purely talk-based videos

were less effective for complex topics. Video duration was also closely linked to content complexity; while most videos were considered adequate in length, nearly one-third of respondents felt that the DAP application video required more explanation, suggesting that technical subjects need more time for clarity. In addition, the use of simple, farmer-friendly language significantly improved understandability, whereas technical or English terms created barriers for many viewers. Finally, factual accuracy strongly supported institutional credibility, as 86 per cent of respondents rated the information in the videos as completely factual, confirming PAU's reliability as a digital extension source and highlighting the importance of maintaining scientific accuracy to sustain farmer trust.

The positive response of viewers suggests that YouTube can complement conventional extension by providing a resource-efficient and environmentally sustainable way of disseminating agricultural information, reducing the need for repeated travel, printed extension literature, and field visits, and farmer meetings, while maintaining wide outreach.

3.4. Limitation of the Study

This study has certain limitations. First, the study was conducted in only one randomly selected village from each selected district, so the results may not represent the views of all farmers in Punjab. Findings should be interpreted as representative of the sampled respondents and not as estimates for the entire farming population of Punjab. Second, respondents watched only a selected set of PAU YouTube videos, so the findings may not apply to all agricultural videos or other types of social media content. Third, respondents' reactions were measured immediately after viewing the videos under controlled conditions rather than in the natural YouTube environment, where people choose what to watch and may interact differently with the content. Future studies should include a larger and more diverse sample, a wider range of digital content, and an assessment of the long-term impact of social media on farmers' knowledge and adoption.

4. CONCLUSION

The study clearly revealed that the PAU Ludhiana YouTube channel plays a significant role in disseminating agricultural information to farmers and extension personnel. Among the selected videos, "*Prevention of Phalaris minor in wheat*" recorded the highest number of views (13,232) as well as the maximum number of likes (343), indicating its high relevance and usefulness to the farming community. In contrast, the video "*Information regarding drip irrigation*" received the lowest number of views (119), suggesting relatively lower interest or need for improvement in presentation and content delivery. The highest overall reaction score was observed for the video "*Prevention of* Phalaris minor *in wheat*", reflecting its comprehensive coverage and viewer engagement. Almost all respondents reported that the duration of message delivery in most of the videos was sufficient. Particularly, the video *"Super Seeder Questions–Answers, yellowing of crop after first irrigation"* was rated as having adequate duration by 95.31 per cent of ADOs and 94 per cent of farmers. However, about one-third of the ADOs and farmers felt that the video "*If DAP was applied to wheat in the same field…*" needed to be longer to provide a better explanation. In terms of satisfaction, both ADOs and farmers expressed satisfaction, or high satisfaction, with most of the selected videos. A large proportion of ADOs (79.69%) reported being highly satisfied with the content of *"How to prevent weeds in wheat – Dr. Makhan Singh Bhullar*". On the other hand, a notable proportion of ADOs (29.69%) and farmers (42.67%) were not satisfied with the video "*Information regarding drip irrigation*", indicating a need for improvement in its clarity, coverage, or presentation. Regarding the credibility of information, only a very small proportion of respondents (4.69% ADOs and 0.67% farmers) felt that some information in a few videos was not factual. Overall, a large majority (86%) of both ADOs and farmers considered the information provided in all selected videos to be completely factual, confirming the reliability and trustworthiness of PAU's YouTube content. However, the findings of this study are constrained by its geographical scope (five districts of Punjab) and its focus on only eight scripted videos, which may limit the generalizability of the results to other regions or content formats.

4.1. Recommendations and Implications

Based on the findings of this study, it is recommended to use the PAU YouTube channel as an effective medium of extension advisory services. As the PAU is using its YouTube channel to deliver advisory extension services, it is necessary to develop YouTube videos based on farmers' information needs and developed by experts who understand farmers' needs, language, and farming context to achieve maximum impact.

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Credit authorship contribution statement

Davinder Singh: Data collection, Writing – original draft, tabulation, data curation; **Davinder Singh:** Conceptualization, methodology, supervision, formal analysis; **Harmanpreet Singh:** Reviewing and editing.

Data availability

The data generated and analysed during the study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Generative AI or the use of AI tools in writing, figures/ images and artwork

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Resource Economics of Groundwater Irrigation in the Malnad Region of Karnataka, India

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ABSTRACT: The study was conducted to assess the resource economics of groundwater irrigation in the Malnad region of Karnataka, India. Primary data on cropping pattern, labour and resource use pattern of the crops grown by the farmers, returns realised, investment made on groundwater irrigation structures, yield of irrigation borewell, water used on the farmwere elicited from 30 groundwater irrigation farms of Sagara, Thirthahalli and Hosanagara taluks of Shivamogga district in 2024-2025. Enterprise budgeting, duly incorporating the cost of groundwater, was conducted to assess profitability. The results indicated that the average age of the functioning borewell was 7.20 years and the average depth of the borewell was 423.62ft. The farmers witnessed initial failure of 41.49 % and premature failure of 4.26%. The Groundwater extracted per farm and per functioning borewell was 63.11 and 34.42 acre-inches, respectively. The estimated average variable cost of groundwater on the sample farms was Rs. 1408, and the annual negative externality was Rs. 21269.The economic efficiency of groundwater, as reflected in gross and net returns per rupee of groundwater, was substantial at Rs. 11.06 and Rs. 6.07, respectively. The cost of groundwater and annual negative externality reflected the unsustainable extraction of groundwater. Hence, farmers, rather than being myopic, should strike an effective balance between the economic efficiency of groundwater use and its sustainability. Groundwater, which was hitherto believed to be a free resource, has become a scarce resource; hence, farmers while using groundwater in farming should judiciously use it to realise better economic returns and sustain groundwater for the next generation.

Keywords: Groundwater, Sustainability, Malnad region, Negative externality

1. INTRODUCTION

Groundwater accounts for the largest share of 30.10% of freshwater in the World (U.S. Geological Survey, 2025). It is considered as the cornerstone of the world's agricultural productivity and economic growth, supporting livelihoods and ensuring water security for a vast population. An increase in demand for agricultural and horticultural crops and dairy products has exponentially increased reliance on groundwater resources, since surface water resources are limited by their proximity and seasonal nature. India is the World's largest pumper of groundwater, extracting approximately 25.00 % of the global total each year to meet both agricultural and domestic water demands (India Observatory, 2024).Currently, the country utilizes nearly 60.50% of its total extractable groundwater resources. Of the total extracted groundwater, 87% is used for irrigation, 11% for domestic purposes, and only 2% for industrial purposes (Annual report 2023-2024, CGWB, GOI).Nearly 70% of India is characterized by hard rock formations. The

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substantial variations in climate and rainfall in peninsular India and northwestern regions have made water availability increasingly critical.

In Karnataka, the stage of groundwater extraction increased from 66.26 % in 2023 to 68.44 % in 2024, while recharge decreased from 18.93bcm to 18.74bcm, with 45 out of 237 assessed groundwater units classified as over-exploited(Annual report of Groundwater Department of Karnataka 2024-25).This heavy dependence on groundwater has led to over-exploitation of water from the aquifer and caused a continuous deterioration in groundwater quality, decline in water levels, drying of wells, decline of well yields, and increase in the cost of energy required to lift water from a greater depth, indirectly rising production costs (Meenu, 2025). However, numerous initial and premature well failures in hard-rock areas of Karnataka have resulted in the virtual disappearance of dug/open wells. Farmers in Karnataka continue to invest heavily in drilling deep borewells to ensure a successful one, given the very high probability of well failure. To tackle groundwater overuse, technologies like drip, sprinkler, and other micro-irrigation methods have been introduced. They support the aim of the Pradhan Mantri Krishi Sinchayee Yojana -"Per Drop More Crop" and "Har Khet Ko Pani" (Deshpande et al., 2024). But in reality, these methods often increase groundwater use by expanding farm areas and extensive cultivation instead of saving water (Singh et al., 2024). The extraction of groundwater in hard-rock areas of the Central and Eastern dry zones of Karnataka was found to be unsustainable. However, the situation in the Malnad region of Karnataka, classified as a hard rock area, was found to be safe in terms of groundwater extraction, as reported by CGWB, 2024. Even then, the farmers in the Malnad region are experiencing rampant failures of borewells in the recent past, adding to the total investment on irrigation borewells.

2. MATERIALS AND METHODS

The study was undertaken in the Malnad region of Karnataka. From the Malnad region, Shivamogga district was considered (Figure 1). From Shivamogga district, farmers were randomly selectedfrom taluks such as Sagara (2494.50 mm), Hosanagara (3070.00 mm), Thirthahalli (2866.80 mm) (Annual weather report,2024) receiving high rainfall. From the above taluks, sample farmers using groundwater irrigation either through borewell or open well were randomly selected. The study was conducted on a pilot basis since, farmers in and around Shivamogga district, coming under the Malnad region, were expressing the predicament of borewell failure. The failure of borewells has increased in the region in recent years due to changes in

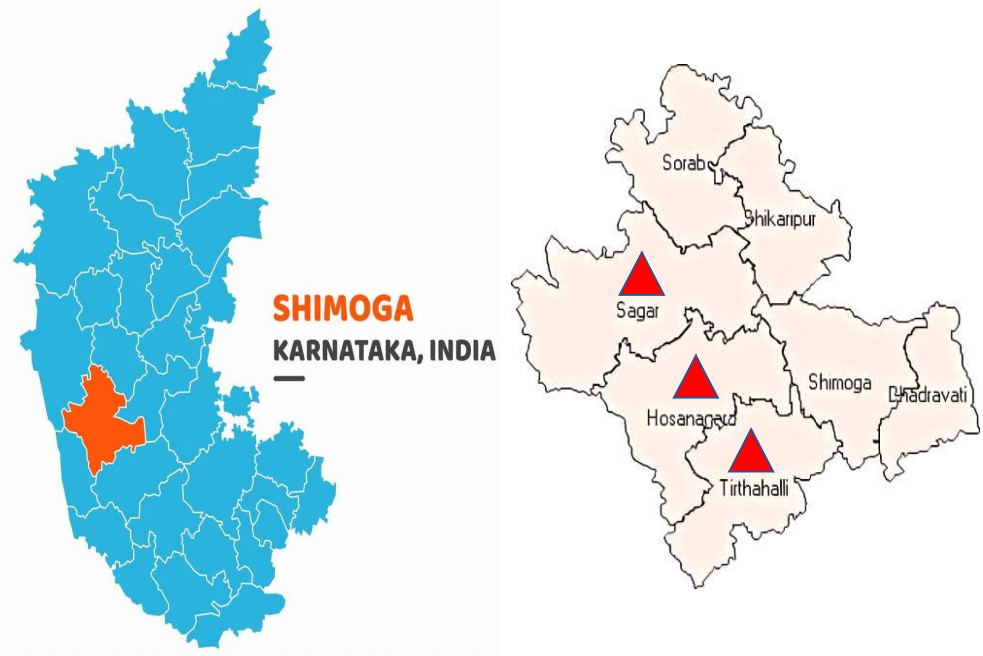


Figure 1. Map showing study area. Red coloured area on the left-hand side of the figure indicates the sample district i.e., Shivamogga chosen from the Malnad region and triangles on the right-hand side of the figure indicates the talukas selected from the sample district. The sample respondents for the study were chosen from the talukas indicated by the red coloured triangles

cropping patterns. Farmers have shown a transition from traditional paddy to commercial crops like arecanut and ginger due to their profitability. In order to meet their water requirement, farmers have started relying on groundwater. Since no studies have focused on groundwater economics in the Malnad region of Karnataka, this study was conducted to ascertain the extent of groundwater use in the Malnad region and its repercussions on the profitability of farming in the region.

2.1. Sampling Framework

Out of 1000 farmers, a sample of 30 from the Shivamogga district who rely on groundwater for farming was randomly selected. This study was done on a pilot basis to find out the unsustainable use of groundwater. The average operational holding of the sample farmers was 3.88acres. The study provides a clue about the groundwater extraction situation and its repercussions on the profitability of farming in the study region in particular and the Malnad region in general. The condition imposed at the time of sample selection was that the farmer under consideration should be a user of groundwater for farming. Farmers who were performing farming solely under rainfed situation were excluded. Farmers who have used groundwater and realised returns for at least one year were considered to estimate the influence of water cost on their profitability.

2.2. Data

The primary data on the number of borewells drilled or open wells dug, year of drilling, depth of the borewell, number of functioning wells, investment made on drilling and casing, investment on irrigation pumpset, water conveyance structure, drip/sprinkler irrigation structure were collected using an interview schedule. In addition, information on resource and labour use patterns of major crops grown on their farmland across seasons, output realised across crop enterprises, and selling prices of outputs was collected. Special emphasis was given to the number of irrigations per crop per year, the time to irrigate an acre per irrigation, the discharge rate per hour from drippers or sprinklers, and the discharge rate by the flooding method, to estimate the groundwater used on the farm.

2.3. Definition of Borewell Failure

Initial failure of a borewell refers to a borewell which did not yield any groundwater at the time of drilling and thereafter. Premature failure refers to the borewell which served below the subsistence life or the Pay Back Period (PBP). Economic life/age of borewell refers to the number of years during which a borewell yielded groundwater beyond the PBP. Subsistence life of borewell refers to the number of years a borewell yielded groundwater for the PBP. The PBP is obtained by dividing the sum of investment made on drilling, casing, IP set, conveyance structure, storage structure, drip/sprinkler structure and electrification charges of the borewell by the life/age of the irrigation borewell. The hypothesis was that an irrigation borewell is considered to have served its purpose if it has at least paid back the total investment made for the purpose. This implies that PBP indicates the period in which a borewell recovered the investment made.

2.4. Negative Binomial Probability of Borewell Failure

The investment on a borewell thus includes the investment on functioning as well as failed borewell/s. Given the high probability of borewell failure in hard rock areas, a farmer drills 'n' number of borewells considered as 'n' Bernoulli trials comprising 'x' number of failed borewells preceding 'r' number of successful borewells. Hence, the (r-1) successful borewells will be drilled in (n-x) trials and the r^{th} successful borewell will be drilled in the n^{th} trial. Here X, the number of failed borewell/s before the r^{th} successful borewell occurs as a random phenomenon, follows the negative binomial distribution (NBD). The NBD is a special case of binomial distribution. The vital properties of NBD are that the mean of the distribution is lower than the variance, and the number of successes in the random experiment is prefixed, while the number of trials is random. The NBD probability function with probability p of a success and q of a failure is given as (Eq.1):

$$P_x\{nb(x;r,p)\}=\binom{x+r-1}{r-1}p^r q^x \text{ for x =0,1,2,3,….,}$$

r-1 r≥0 ; 0 < p<1…….(1)

Mean = $\frac{rq}{p}$ and Variance = $\frac{rq}{p2}$

Where the NBD probability of borewell success (r >1)

is p = $\frac{r}{(r+mean)}$ = $\frac{mean}{var}$ and q = (1- p) is the probability of failure.

2.5. Economics of Groundwater Irrigation

The cost of cultivation of the crop was obtained as the sum of cost of human labour, bullock labour, machine labour, seeds and fertilisers, manure, plant protection chemicals, cost of irrigation, interest on working capital at the rate of 4%, risk premium at 2% and management cost at

5% on variable cost. Gross return for each crop was the value of the output and the by-product at the prices realised by farmers.Net returns from borewell irrigation are the gross returns from gross irrigated area minus the cost of production of all crops. The cost of cultivation of all crops in this study accordingly included the cost of irrigation explicitly since volumetric measurements of groundwater applied were made for all crops (Patil & Chandrakanth, 2016).

2.6. Costing of Irrigation Water

In the case of groundwater, the shrinking life/age of wells, increasing drilling depths and costs, reduced groundwater output, increasing pumping costs, increased pump capacities, and associated costs of micro irrigation are posing challenges in valuation. For example, groundwater in open wells / tube wells used to serve for 30 to 40 years is now serving for two to three years or even less. Thus, depreciation on fixed investment is no longer relevant, since investment is becoming a variable cost. Thus, amortized investment on both functioning and non-functioning wells (due to externalities) enters as the marginal cost influencing decision making rather than fixed cost, since depreciation is a mere replacement cost not used in decision making process. Hence, studies conducted on estimation of cost of cultivation of crops by the Directorate of Economics and Statistics – CACP, and Farm Management Surveys had virtually no consideration for the cost of groundwater.

Paradoxically, even with an innumerable number of organizations on water – such as Central Water Commission, Ministry of Water Resources, the Central Groundwater Board, National Water Development Authority, State Water Resource Departments, State Departments of Mines and Geology, urban and rural water supply development boards, efforts towards volumetric measurement of water applied are still crude and approximate. Thus, water cost is not properly accounted for in any of the costing procedures, including the Commission on Agricultural Costs and Prices (CACP), which has inadequate information on water use in the RT forms. The costing methodology of cultivation by DES (Directorate of Economics and Statistics) / CACP (Commission for Agricultural Costs and Prices) ignores the cost of groundwater by treating depreciation on all items of expenditure on irrigation well and irrigation pump (IP) set as fixed cost (for an unspecified number of years). Even the yield of the well and number of hours of operation of the IP set for different crops are not properly accounted for in the record-type forms, where cost of cultivation is assessed on a daily basis from thousands of sample farmers all over India. Thus, the DES/CACP is yet to take adequate steps to properly account for the cost of groundwater irrigation in the Cost Concepts (of Cost A1, A2, B1, B2, C1, C2, C3). However, in the CACP / Farm management Surveys, irrigation cost often does not include cost of water in general and the cost of groundwater irrigation in particular. The increasing rate of initial and premature failure of borewells has made it indispensable to consider investment in drilling and casing of irrigation wells as variable cost rather than fixed cost, as marginal cost enters into decision making process.

Therefore, the total cost of groundwater irrigation was divided into two components: variable and fixed costs. As the farmers do not pay for the electricity to pump groundwater for irrigation, the variable cost of groundwater was considered as the amortized cost of drilling and casing of the borewell for the economic life of the irrigation well, plus the Operation and Maintenance costs (O&M). The variable cost per acre-inch was obtained by dividing the amortized investment on casing and drilling plus O & M cost by the volume of groundwater extracted in the year of collecting field data (2024-25). However, farmers can use the irrigation pump sets and accessories for at least ten years, irrespective of failure of irrigation wells. Hence, the fixed cost of groundwater was the amortized cost (of pump sets, conveyance structure, drip irrigation, water storage structure, and electrification charges)over a period of ten years. The amortized fixed investment was divided by the volume of groundwater extracted in the year (2024-25) to obtain the fixed cost of groundwater per ha-cm (acre-inch). The labour cost of irrigation was considered along with labour costs of other cultural operations. Thus, the annual cost of irrigation per borewell was added across all borewells on the farm. This total cost of irrigation was then apportioned for each crop according to the volume of groundwater used in each crop (Chandrakanth & Patil, 2018; Patil et. al., 2016; Rohith et. al., 2015).

Cost of irrigation per acre-inch = [Total annual cost of irrigation]/ [volume of water used for the crop in acre-inches of groundwater used].

2.7. Amortized Cost of Borewell

To obtain the annual share of groundwater irrigation cost, the borewell investment has been amortized with the

assumption that investment on drilling and casing is no longer a fixed cost, given the increasing probability of well failure, which forces farmers to continue investing to irrigate crops through new borewells/drillings. The amortized cost varies with the amount of capital investment, age of the borewell, discount rate, and year of construction of the borewell. The amortization methodology employed by Diwakara & Chandrakanth (2007) was used in the present study.

Choice of discount rate

From the sample, the investment on the earliest well (IEW) and the investment on the latest well (ILW) were compared using the formula IEW $(1+i)^n$ = ILW and interest rate 'i' was solved to obtain approximately2%. Accordingly, a 2% discount rate was used. The same analogy was used by Patil (2015), Chandrakanth & Patil (2019), and Anitha & Chandrakanth (2022).

Amortized cost [1] of borewell was worked out using following formulae:

Amortized cost of irrigation = (Amortized cost of Borewell + Amortized cost of pump set + Amortized cost of conveyance + Amortized cost of over ground structure + Operation and maintenance cost of pump set and accessories) given by eq.2.

$$\text{Amortized cost of BW} = (\text{Compounded cost of BW}) \times \frac{(1+i)^{AL} \times i}{(1+i)^{AL} - 1]} \quad \ldots\ldots\ldots\ldots (2)$$

where,

AL= Average Age or life of borewell i = discount rate considered = 2%

Compounded cost of B=(Historical investmenton BW) $\times(1+i)^{(2024\text{-yearofdrilling})}$

The working life of Pumpsets and Accessories (P and A) was considered to be 10 years, since farmers consider 10 years to be their economic life.

where,

i = discount rate considered at 2%

Compounded cost of Pumpset and Accessories= (Historical cost of P and A) $\times(1+i)^{(2024\text{-yearofinstallationofPandA})}$

$$\text{Amortized cost of P and A} = (\text{Compounded cost of P and A}) \times \frac{(1+i)^{10} \times i}{(1+i)^{10} - 1]} \quad \ldots\ldots\ldots (3)$$

The working life of conveyance structure (CS) was also considered to be 10 years. The usual mode of conveyance of groundwater is through PVC pipe

where,

i = Discount rate considered at 2 %

Compounded cost of CS=

(Historical cost of CS) $\times(1+i)^{(2024\text{-yearofinstallationofCS})}$

$$\text{Amortized cost of conveyance structure (CS)} = (\text{Compounded cost of CS}) \times \frac{(1+i)^{10} \times i}{(1+i)^{10} - 1]} \quad \ldots\ldots\ldots\ldots (4)$$

The working life of micro (drip) irrigation structure (MIS) was considered as10 years since farmers usually replace them after 10 years

where,

i = Discount rate considered at 2%

Compounded cost of MIS=

(Historical cost of MIS) $\times(1+i)^{(2024\text{-yearofinstallationofMIS})}$

$$\text{Amortized cost of micro irrigation structure} = (\text{Compounded cost of MIS}) \times \frac{(1+i)^{10} \times i}{(1+i)^{10} - 1]} \quad \ldots\ldots\ldots (5)$$

The amortized cost of over ground storage structure was estimated as under

$$\text{Amortized cost of overground storage structure} = (\text{Compounded cost of OSS}) \times \frac{(1+i)^{10} \times i}{(1+i)^{10} - 1]} \quad \ldots\ldots\ldots (6)$$

where,

i = Discount rate considered at 2 %

Compounded cost of OSS=

(Historical cost of OSS) $\times(1+i)^{(2024\text{-yearofconstructionofOSS})}$

2.8. Borewell Yield

The field measurements of the groundwater yield of borewells were made by recording the number of seconds taken to fill a bucket or overground structure of groundwater of known volume. Initially, the borewell was run for ten minutes to avoid the initial pump-yield bias. This was linearly extrapolated to obtain the groundwater yield in gallons per hour.

2.9. Groundwater Use in Conventional Irrigation System

The acre-inches (or ha cm) of groundwater used for each crop in each season (summer, kharif, rabi) in conventional system of irrigation was calculated as = [(area irrigated in each crop) * (frequency or number of irrigations per month) * (number of months of crop) * (number of hours for one irrigation for the cropped area in question) * (Average yield of borewell in Gallons Per Hour)] /22611gives groundwater use for each crop in acre inches.

[1]Diwakar and MG Chandrakanth (2007)

2.10. Groundwater use in drip irrigation system

The groundwater used for irrigation in each crop (acre inches) in Drip irrigation = {Number of drips or emitters for the cropped area X groundwater discharged per emitter per hour (liters per hour) X No. of hours to drip irrigate the cropped area for one irrigation X frequency of irrigations per month (in number) X Duration of crop irrigated in months /4.54/22611}.

2.11. Annual Cost of Irrigation

Farmers are not paying for electricity because it is subsidised. Studies conducted in the Department of Agricultural Economics indicated that in order to pump an acre-inch of water, 80 kilowatt-hours of electricity were used (Chandrakanth et al., 2001). According to WM Shivakumar (Karnataka Electricity Board, Research Wing, Bangalore, lecture presented to Department of Agricultural Economics, UAS Bangalore, dated 19/10/2004), the electricity used by an irrigation well was 6532.00 kilowatt-hours per year. Usually, an irrigation well yields around 1500 gallons per hour. With an estimated 6 hours of pumping per day for about 250 days per year, the total water extracted is around 100 acre-inches per well. Thus, 65 kilowatt-hours were used to lift around 100 acre-inches of water, according to this estimate. Thus, the electricity used to lift one acre-inch of groundwater ranges from 42 to 65 kilowatt-hours. The cost of power generation varies according to the source, ranging from Rs. 4.98 per kilowatt-hour from hydroelectric power to Rs. 5.80 per kilowatt-hour from Thermal, with an average of Rs. 5.40 per kilowatt-hour. Considering an average of 50 kilowatt-hours to lift one acre-inch of groundwater, it costs Rs.270.

2.12. Reciprocal Externality

In the case of groundwater, due to violation of isolation (inter-well) distance, a farmer drilling borewell(s) has an unintended side effect on the neighbouring farmer's well, resulting in an increase in cost of extraction due to an increase in the number of well/s drilled, inefficiency and welfare loss. Further, as there is no mechanism to compensate for the loss inflicted and no institution governs the behaviour of farmers, the externality persists. This is a classic case of a reciprocal externality, as it is difficult to identify which well or borewell is responsible for over-extraction and/or the failure of neighbouring well (s), similar to traffic pollution externalities. Unless there is a regulation/institution on extraction, use, recharge, reuse the externalities persist resulting in inefficiency as well as welfare loss. In hard rock areas, each one's groundwater extraction is not independent, but is interdependent with that of neighbouring well(s), both at a given time and over time. This results in a reciprocal externality, as all groundwater users impose external costs on one another simultaneously and over time. In the case of a unidirectional externality, a farmer, by drilling deeper and/or increasingly extracting groundwater, inflicts an externality on others and on himself at a given time and over time due to interference of well/s. Over time, all farmers pumping groundwater impose external costs on all others, including themselves due to cumulative interference, a phenomenon of reciprocal externality (Dasgupta, 1982).

The externality per borewell = (Amortized investment on drilling and casing of borewells over the subsistence life of borewell/s or economic life of borewell/s whichever is relevant) divided by (number of borewells which served PBP + serving economic life) minus (Amortized investment on drilling and casing of borewells over the subsistence life of borewell/s or economic life of borewell/s whichever is relevant) divided by all the borewells on the farm.

3. RESULTS AND DISCUSSION

3.1. Cropping Pattern

The cropping pattern of farmers having borewell irrigation in the Malnad region of Karnataka presented in Table 1, indicates that arecanut is the dominant crop of the region cornering 88.99% of the Gross Cropped Area. It was followed by paddy cultivated under rainfed conditions. The cropping intensity was 189.94%, and the irrigation intensity was 201.36%. Farmers in the Malnad region are heavily dependent on arecanut for their livelihood. Area under arecanut is expanding at an exponential rate due to remunerative prices and ease of management compared to other annual crops. The other possible reason is the suitability of the crop to the Malnad region, with its natural undulation and heavy rainfall. The Malnad region is considered the traditional belt of arecanut cultivation. The studies on supply response of arecanut have clearly indicated that farmers are highly price responsive (Gajanana 1985; Patil et.al., 2014). The prices of arecanut have always hovered around Rs. 50000.00 per quintal in the recent past, and it has enabled farmers to expand the area under cultivation of arecanut. The other major crop was paddy grown under rainfed conditions mainly for the sake of subsistence. Farmers, by using lesser or no chemical

fertilizers, cultivate paddy for self-consumption. Area under paddy crop in the recent past has declined in the region due to its labour-intensive and less remunerative nature. Arecanut demands water for better growth and yield. The water requirement was relatively higher during the summer season. This requirement was met through groundwater, either from an open well or a borewell, by the farmers. Area expansion under arecanut and other commercial crops, such as ginger, has been considered one of the prime reasons for reliance on groundwater.

3.2. Profile of Irrigation Wells on Sample Groundwater Irrigation Farms

In the Malnad region of Karnataka, borewells dominated groundwater irrigation. On the selected groundwater-irrigation farms, a total of 94 borewells were drilled and 8 open wells were dug. Of the total number of borewells drilled, 54.26% of the borewells were found to be functioning at the time of data collection. In comparison, 41.49% exhibited initial failure, and the remaining 4.26% exhibited premature failure. The average age of the functioning borewell among the selected borewell irrigation farms in the Malnad region of Karnataka was 7.20 years. The prima facie of the table clearly indicates that the farmers have shown a transition in groundwater utilization pattern from open well to borewells. The average age of borewells in CDZ and EDZ ranged from 4.75 to 7 years (Patil, 2015 and Anitha, 2022). The negative binomial probability of borewell failure is 0.45. On average around 3.13 borewells were drilled by the farmers in the study region to irrigate their crops. Out of the drilled borewells, two borewells were found to be functioning. Initial failure was rampant in the Malnad region due to the fact that borehole walls collapse if not properly cased, leading to the burial of equipment or the sealing of the water source. The initial failure forces farmers to drill an additional borewell until they get a successful borewell to safeguard their arecanut garden. Conversely, the percentage of initial and premature failure of borewells in the Central Dry Zone was estimated to be 49% and 3%, while it was 25- 32% and 14-34 % in the Eastern Dry Zone of Karnataka, respectively (Patil, 2015 and Anitha, 2022). The magnitude of failure adds to the total cost of irrigation and hence, the failure percentage was quite higher on farms in the dry zone compared to the Malnad region.

Table 1. Cropping pattern sample of groundwater irrigation farms in the Malnad region

(n=30)

Crops	Area (acres)	Proportion of gross cropped area
Paddy (rainfed)	11.75	5.45
Maize (rainfed)	0.50	0.23
Ginger	2.50	1.16
Sugarcane	1.50	0.70
Perinneals		0.00
Arecanut	192.00	88.99
Coconut	1.50	0.70
Cashew	6.00	2.78
Gross cropped area	215.75	
Net cropped area	113.58	
Cropping intensity	189.94	
Gross irrigated area	197.5	
Net irrigated area	98.08	
Irrigation intensity	201.36	

3.3. Investment on Irrigation Borewells

The depth of the borewell in the study region hovered around 423.62ft, while in dry regions of Karnataka, it has exceeded 600 ft in the Central Dry Zone of Karnataka and 804 ft in the Eastern Dry Zone of Karnataka (Anitha, 2022; Patil,2014). The majority of the farmers were found to be using of 5 hp irrigation pump set to lift groundwater. The modal horsepower of irrigation pump sets was found to be of 8hp in CDZ and 20 hp in EDZ. The functioning wells in the study regions were found to yield 1885.00 gallons of water per hour across three seasons. The cropping pattern followed in the region forced farmers to adopt micro irrigation technologies such as drip irrigation on their farms. The investment made on the farm on micro irrigation infrastructure came to Rs. 82941.00. Arecanut, the major crop grown by the farmers in the study region, demands water during the summer season, as it is highly sensitive to dry spells. Yield drastically reduces in arecanut if not properly irrigated. On average, farmers have drilled 3 borewells, and the nominal investment across all borewells came to 0.40 million.

The sensitivity analysis was performed at social discount rate of 5% and 8% to obtain amortized cost of drilling and casing, amortized cost on fixed structures, annual negative externality, and total amortized cost of irrigation per borewell. The results indicated that higher the social discount rate higher the Amortized cost of drilling and casing that ranged from Rs. 35121 & Rs. 42289, Amortized cost of

drilling and casing per functioning well (Rs. 64732 & Rs.77945), Annual negative externality (Rs. 29612 & Rs. 35655), Amortized cost on ip, drip and conveyance per well (Rs. 11267 & Rs. 14179), Amortized cost on ip, drip and conveyance per functioning borewell (Rs. 20765 & Rs. 26133), Total amortized cost per borewell (Rs.46388& Rs.56468) and Total amortized cost per functioning borewell (Rs.854978 & Rs.104078) at 5% and 8% social discount rate, respectively.

Table 2. Profile of irrigation borewells in the Malnad region

(n=30)

Particular	Value
Number of borewells among sample farms	94
Number of borewells per farm	3.13
Number of initially failed borewells in the sample	39.00
Percentage of initial failure	41.49
Number of prematurely failed borewells in the sample	4
Percentage of premature failure	4.26
Number of functioning borewells in the sample	51
Percentage of functioning borewells	54.26
Average age of functioning borewells	7.20
Range of drilling of borewells	1990 to 2024
Number of open wells among sample farms	08
Number of functioning open wells in the sample farms	04
Percentage of open wells yielding water	50
Negative binomial probability of borewell failure	0.43

Investment in an irrigation borewell depends on the depth of the borewell, the horsepower of the irrigation pump set used to pump groundwater, and the number of initial and prematurely failed borewells. The stock concept of investment made on irrigation borewell indicated by the nominal investment made on all wells per acre of gross irrigated area was Rs. 61423.40. The flow concept of investment at current prices reflected in the ratio of variable component of amortized cost per functioning well to gross irrigated area was Rs. 7366.72. The investment made on functioning borewell came to 0.22 million. The annual negative externality in groundwater irrigation in the Malnad region of Karnataka, estimated by taking the difference of Amortized cost of drilling and casing per borewell and Amortized cost of drilling and casing per functioning borewell, came to Rs. 21269.00. This reflects the magnitude of exploitation of groundwater in the study region. If the difference was found to be zero, groundwater extraction of groundwater could be considered sustainable. The total of Amortized cost of irrigation per functioning borewell came to Rs. 62383.00. The extent of negative externality was found to be lower in the Malnad region receiving assured rainfall of 1949.60mm at Rs. 21269.00 compared to dry regions of Karnataka at Rs.47580.58 receiving rainfall of 540 to 600mm (Patil,2014).

3.4. Costing of Groundwater Irrigation in the Malnad Region

The average holding size of the farm in the study region

Table 3. Investment on irrigation borewell in the Malnad region

(n=30)

Particular	Unit	Value (Rs.)
Depth of borewell in feet	feet	423.62
Horse power of Irrigation pump set	hp	05
Yield of functioning borewell per hour	gallons	1885.00
Investment on micro irrigation per farm in current prices	Rs.	82941.00
Nominal investment on all borewells per farm	Rs.	404166.00
Nominal investment per functioning borewell	Rs.	220454.00
Amortized cost of drilling and casing per borewell	Rs.	27204.00
Amortized cost of drilling and casing per functioning borewell	Rs.	48473.00
Annual negative externality per borewell	Rs.	21269.00
Amortized cost on IP set, drip, conveyance, storage structures per borewell	Rs.	7807.00
Amortized cost on IP set, drip, conveyance, storage structures per functioning borewell	Rs.	13910.00
Total of amortized cost of irrigation per borewell	Rs.	35011.00
Total of amortized cost of irrigation per functioning borewell	Rs.	62383.00

was found to be 3.88 acres. The Gross irrigated area per farm was 6.58 acres, and Gross irrigated per functioning well was half of the Gross irrigated area, clearly indicating that on every farm there were two functioning borewells. Gross irrigated area indicates the utilization potential of groundwater resources in absolute terms. Groundwater extracted per farm and per functioning borewell was 63.11 and 34.42 acre-inches. The utilization of groundwater depends on its availability and requirement. Availability reflects the supply factor being influenced by a gamut of factors like natural recharge, aquifer capacity, extent of positive externality through recharge efforts by the neighbouring farmers, amount of rainfall, extent of negative externality exhibited by the neighbouring farmers etc. Requirement is the demand factor influenced by the crop taken up by the farmer, nature of the crop and the number of crops taken per year influence the utilization of groundwater. It also depends on the irrigation methods adopted, viz., conventional flood irrigation or modern irrigation methods such as drip/sprinkler irrigation. It may further be influenced by the water conservation measures adopted by the farmers. In the study region, farmers were found utilizing groundwater for the cultivation of arecanut. Arecanut cultivated following flood irrigation required a relatively larger amount of groundwater @ 17-22 acre-inches, while arecanut cultivated using drip required 10 to 12 acre-inches. Out of the 30 sample farmers, only three were reliant on flood irrigation, while the majority relied on drip irrigation. Drip irrigation conserves water and improves water use efficiency (Narendra et.al., 2021). The groundwater, hitherto treated as a free gift of nature, has become an economic good in the recent past due to negative externality. The costing of groundwater was earlier considered as fixed cost even by the methodology followed by the CACP in estimation of costs of principal crops. That methodology was apt then since borewell or well failure was minimal. But now, farmers are facing rampant borewell failures, forcing users to consider variable costs. Variable costs of groundwater include Amortized drilling cost plus casing cost per acre-inch of groundwater. As the number of initial failure/s, premature failure/s increases, the amortized cost of drilling and casing increases and adds to the variable cost of groundwater. The estimated variable cost of groundwater for sample farms in the study region was Rs. 1408.00. Variable costs have a greater bearing on marginal cost, which plays a crucial role in decision-making. While, fixed costs are estimated by dividing amortized costs on irrigation pumpsets, accessories, irrigation technologies, overhead water storage structure, water conveyance structure and volume of groundwater used. It was estimated to be Rs. 404.00 per acre inch. The total cost of irrigation in the study region per acre inch came to Rs.1812.00 which was on par with the cost of groundwater after duly accounting for the inflation factor on farms in the Central Dry Zone of Karnataka of Rs.1461.52 practising farming with drip for broad based crops and Rs.3141.08 in narrow-spaced crops (Patil,2015). The cost of groundwater is a clear indication of the existence of a negative externality in groundwater irrigation. The rising cost of groundwater alarms groundwater users regarding its unsustainable use. Compared to the dry zone, the cost of groundwater was relatively lower but substantial; hence, farmers, while using groundwater, should judiciously use it through adoption of modern technologies to halt further pressure on groundwater. The cost of groundwater in the dry zone was relatively higher due to deeper depth of borewell, increasing number of initial and premature failures, shorter life of borewell, relatively higher investment on irrigation pumpsets of higher horsepower, electrical cable and other accessories compared to the Malnad region.

3.5. Economics of Groundwater irrigation in the Malnad region of Karnataka

The returns accrued to the farmers from groundwater irrigation in the Malnad region of Karnataka, depicted in Table 5, indicate that sample farmers have realized returns of Rs.1.26 million from the average size of land holding of 3.88 acres. Gross returns and net returns per functioning borewell came to Rs.0.69 million and Rs.0.39 million. A total of 63.11 acre-inches of groundwater was pumped by the sample farmers to irrigate the crops grown by them and realized gross returns of Rs. 20040 per acre-inch and net returns of Rs.11005 per acre-inch. The economic efficiency of groundwater, reflected in terms of Gross returns per rupee of groundwater and net returns per rupee of groundwater, was Rs. 11.06 and Rs. 6.07, respectively.

The magnitude of gross returns or net returns realized per acre-inch or per rupee of groundwater was substantial, indicating economic efficiency with which farmers in the Malnad region are using groundwater. The higher returns were due to the type of crops farmers cultivate using groundwater. Farmers in the Malnad region cultivate commercial crops like arecanut, ginger, pineapple, pepper, banana, etc., using groundwater. All the crops grown by the farmers are highly lucrative. The efficiency should not be at

the cost of sustainability. Since efficiency and sustainability are mutually exclusive, both cannot be attained at the same time. Farmers should be cautious in using groundwater a precious natural resource giving due consideration for intergenerational equity. Farmers have given due consideration only for the demand side of groundwater

Table 3.1. Sensitivity analysis of choosing higher social discount rate of 5% and 8%

Particular	5%	8%
Amortized cost of drilling and casing per well	35120.61	42289.01
Amortized cost of drilling and casing per functioning well	64732.10	77944.46
Annual negative externality per borewell	29611.49	35655.44
Amortized cost on ip, drip and conveyance per well	11266.57	14178.77
Amortized cost on ip , drip and conveyance per functioning borewell	20765.83	26133.42
Total Amortized cost per borewell	46387.18	56467.79
Total Amortized cost per functioning borewell	85497.94	104077.90

Table 4. Cost of groundwater irrigation in the Malnad region

(n=30)

Particular	Unit	Value
Average size of holding	Acre	3.88
Gross irrigated area per farm	Acre	6.58
Gross irrigated area per functioning borewell	Acre	3.59
Net irrigated area per farm	Acre	3.27
Net irrigated area per functioning borewell	Acre	1.78
Groundwater extracted per farm	Acre-inches	63.11
Groundwater extracted per functioning borewell	Acre - inches	34.42
Variable cost of groundwater	Rs.	1408
Fixed cost of groundwater	Rs.	404

Table 5. Economics of groundwater irrigation in the Malnad region of Karnataka

(n=30)

Particular	Value (Rs)	Range (Rs)
Gross returns per farm	1264634 (353727.40)	254897-3850000
Gross returns per acre	326357.30 (75250.61)	169931-470500
Gross returns per borewell	387133 (284741.50)	127448-1155000
Gross returns per functioning well	689800.60 (353727.40)	254897-1634500
Gross returns per acre inch	20040.03 (6322.32)	12984-40657
Gross returns per rupee of water	11.06 (13.60)	1.33-47.49
Net returns per farm	694493 (220894.20)	123165-2249512
Net returns per acre	179224 (54295.07)	82109-333834
Net returns per borewell	212599.9 (194667.40)	61582-1001502
Net returns per functioning well	378814.4 (220894.20)	(123164-1001502)
Net returns per acre inch	11005.28 (4721.48)	5029-25270
Net returns per rupee of water	6.07 (7.94)	0.63-30.11

Note: Figures in the parentheses are standard deviations

Table 5.1. Returns realized on groundwater farms across holding size

Particular	Marginal farmers[b]	Small farmers[b]	Large farmers[a]
Size of land holding (acres)	1.87[b]	3.56[b]	7.78[a]
Gross returns per farm (Rs.)	663694.08[b]	1101064.09[b]	2551857.14[a]
Net returns per farm (Rs.)	347370.85[b]	630918.97[b]	1389461.71[a]

Small and Marginal farmers were on par with each other with respect to Gross and net return per farm with superscript 'b' and significantly differed from large farmers with superscript 'a'

irrigation and highly neglected and denied the supply side of groundwater. Hence, farmers should give utmost importance to the supply side of groundwater irrigation by indulging in artificial recharge of groundwater. The gross and net return realized per farm across holding size was significantly different. It was profound and substantial in case of large farms while it was on par in case of marginal and small farms. The gross and net returns estimated on a per-farm basis were found to be highest in large farms, followed by small farms and marginal farms (Table 5.1).

3.6. Limitations of the Study

Since the study was conducted on a pilot basis, a sample of 30 farmers relying on groundwater irrigation to meet the condition of a large sample size in statistical parlance was considered. Among 30 sample respondents, only three sample farmers were found to be following flow irrigation; hence, groundwater economic parameters were compared with previous studies conducted in the central dry zone. The investment cost on borewell and estimation of irrigation cost was done considering the data provided by the sample farmers based on memory recall basis and hence results may be influenced to some extent by that bias.

4. CONCLUSION AND POLICY IMPLICATION

The Malnad region of Karnataka, receiving assured rainfall data (2341.90 mm), is not devoid of groundwater exploitation as a result of changes in cropping patterns practised by farmers. The reliance on groundwater in the Malnad region has increased due to the cultivation of commercial crops like arecanut, pineapple, and ginger. The reliance has started exerting pressure on groundwater, reflected in the number of failures of borewells, depth of borewells, disappearance of open wells, magnitude of negative externality and variable cost of groundwater. The economics of sample groundwater irrigation farms clearly indicated that sample farmers of the Malnad region were economically efficient with respect to groundwater use, as reflected in net returns per rupee of groundwater of Rs.6.07. But the cause of concern is the magnitude of initial failure, which accounts for 41.49 %, negative externality of Rs. 21269, and variable cost of Rs. 1408, which, in comparison to dry zones of Karnataka, was found to be lower but substantial. Hence, this study though done on pilot basis alarms farmers about ill effect of unsustainable use of groundwater. Farmers in this region should judiciously use groundwater giving due consideration for intergenerational equity and sustainability.

The probability of well failure, negative externality and cost of groundwater signalled the groundwater predicament in the Malnad region. Thus, farmers in the Malnad region should give importance to supply-side interventions in groundwater, like scientific recharge efforts in a collective mode, on-farm conservation measures to improve the recharge rate and use scientific methods of identifying aquifers to overcome the problem of false aquifers, which can add to the initial failure of a borewell.

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Credit authorship contribution statement

Kiran Kumar R Patil: Conceptualization; Data collection; Methodology; Software; Writing – original draft; Supervision; Formal analysis; Data curation; **Meghana S.V.:** Formal analysis; Tabulation; **Mallikarjuna M.C.:** Data collection; Tabulation, Reviewing, **Aravinda Kumar M.K**.: Reviewing; Editing

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 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 analysed during the current investigation are available from the corresponding author upon reasonable request.

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Single-Visit Pollen Deposition Provided by Bees Reveals Trait-Mediated Differences in Pollen Transfer Across Crop Species

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ABSTRACT: Insect-mediated pollination is an important ecosystem service, and bees are among the most effective pollinators. The effectiveness of pollination varies considerably among bee species because of differences in their morphology and foraging behaviour. We quantified the number of conspecific pollen grains deposited during a single bee visit for 14 bee species visiting mustard, brinjal, and chilli flowers in an experimental garden. Virgin flowers were exposed to a single bee visit, after which deposited pollen grains were counted to estimate one component of pollination effectiveness. Associations between bee traits and single-visit pollen deposition were evaluated using generalized linear mixed models that accounted for crop identity. Single-visit pollen deposition varied significantly among bee species and crop types. *Apis dorsata* Fabricius, 1793, *Apis cerana* Fabricius, 1793, and *Xylocopa* sp. 1 recorded the highest single-visit pollen deposition in mustard, chilli and brinjal, respectively. Inter-tegular distance and scopal area were positively associated with pollen deposition, indicating that larger bees with greater pollen-carrying capacity deposited more pollen. Dense body hairiness, early peak foraging activity, and vibration-based pollen collection were also associated with greater pollen deposition, whereas smaller bees, including *Braunsapis puangensis* Cockerell, 1929, and *Lasioglossum* sp. 3, deposited fewer pollen grains. These findings demonstrate that pollen deposition differs among bee species and crops and is associated with bee functional traits. These findings improve our understanding of how bee functional traits influence pollen transfer during individual flower visits and may support the development of pollinator-friendly management strategies.

Keywords: Bee traits, Crop pollination, Single-visit pollen deposition, Buzz pollination, Pollen transfer.

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1. INTRODUCTION

Around three-quarters of global crop species partially depend on animal-mediated pollination for the production of fruits, vegetables, and other crops (Klein et al., 2007). According to Gallai et al. (2009), pollinators play a substantial role in gaining economic benefits from crop production. Among pollinators, bees are considered the most important group for sustainable agricultural systems, contributing up to 75% of total pollination services (Velthuis & van Doorn, 2006; Klein et al., 2007). However, not all bee species contribute equally to crop pollination. Their effectiveness varies considerably because of differences in morphology, foraging behaviour, and visitation frequency (Sahli & Conner, 2007; Hoehn et al., 2008; Chang et al., 2023). Along with visitation frequency, the quantity and quality of pollen deposited during each visit are important for successful fruit set, although floral maturity and other factors also influence reproduction (Tscharntke et al., 2025). Therefore, understanding the pollination efficiency of different bee species is essential for improving crop production and maximizing the gains from pollination in agricultural systems.

Single-visit pollen deposition (hereafter SVD), defined as the number of conspecific pollen grains deposited on a stigma during a single visit by a pollinator, is increasingly used to assess pollinator effectiveness because it directly measures pollen transfer to the female reproductive organs of flowers (King et al., 2013; Ballantyne et al., 2015). In contrast to visitation frequency, which only reflects how often pollinators visit flowers, SVD provides an estimate of the quality of a visit in terms of its potential contribution to plant reproduction. The number of pollen grains deposited during a visit can be influenced by pollinator behaviour, including foraging activity and pollen collection strategies, as well as by morphological traits of the visitor (Ivey et al., 2003; King et al., 2013). However, studies examining how these traits affect SVD across different crop systems remain limited, restricting our understanding of the factors that determine pollination effectiveness. This also may vary among crop species because differences in floral morphology, such as anther accessibility and stigma position, influence how pollinators contact reproductive structures and transfer pollen (Nagano et al., 2014; Kuriya et al., 2015). Consequently, a single bee species may differ in the amount of pollen deposition per visit across different crops.

Studies have shown that solitary bees can be more effective pollinators than honey bees, exhibiting higher single-visit efficiency and visiting more flowers per unit time (Eeraerts et al., 2020). Bumble bees have also been reported to deposit more pollen and enhance fruit development in several crops compared with honey bees (Thomson & Goodell, 2001; Artz & Nault, 2011; Zhang et al., 2015). Variations in morphology and foraging behaviour largely drive these differences. Inter-tegular distance (ITD), a widely used proxy for bee body size, together with traits such as body hair density, scopal area, and foraging behaviour, may influence pollen transfer. Larger bees generally carry greater pollen loads, make greater contact with floral reproductive structures, and often forage over longer distances, making body size an important determinant of pollen transfer (Goulson et al., 2002; Greenleaf et al., 2007; Garibaldi et al., 2013; Howlett et al., 2018; Kendall et al., 2019). In crops with poricidal anthers that depend on buzz pollination, such as brinjal and chilli, body size may further influence pollination success by determining how effectively bees contact anthers and receptive stigmas during floral visits, thereby enhancing pollen transfer by vibration (Armbruster & Muchhala, 2009; De Luca & Vallejo-Marín, 2013; Vallejo-Marín & Russell, 2024).

Mustard (*Brassica juncea* (L.) Czern., 1859), brinjal (*Solanum melongena* L. 1753), and chilli (*Capsicum annuum* L. 1753) are economically important crops widely cultivated in India. The rapeseed-mustard group contributes 28.6% of total oilseed production and accounts for 27.8% of India's oilseed economy (Shekhawat et al., 2012). Brinjal is an important vegetable crop, with annual production of approximately 12.68 million tonnes (Ministry of Horticulture and Farmers Welfare, 2018), while chilli is an important commercial and export-oriented crop contributing to farmer livelihoods and India's spice industry (Reddy et al., 2014; Singh et al., 2021; Jalgaonkar et al., 2024), for their edible seeds and fruits. Production of these crops depends on successful pollen transfer to receptive stigmas, which is largely mediated by animal pollinators (Garibaldi et al., 2013; Drummond, 2019). Because these crops are cultivated over extensive areas, artificial pollination is not a practical option, making pollinator visits essential for fruit and seed production. In addition, flowers remain receptive for only a limited period, so pollen must be delivered within a relatively short time window for successful fertilization. Recent work suggests that pollen deposition on stigmas provides a more informative measure of pollination service than visitation frequency alone

because it is often more closely associated with reproductive success and crop yield (Ramírez-Mejía et al., 2024). The importance of effective pollination is evident in high bush blueberry, where honeybee pollination contributes an estimated USD 400 million annually to the blueberry industry in the United States (Ramírez-Mejía et al., 2024). These findings highlight the need to assess not only how often pollinators visit flowers, but also how effectively they transfer pollen during each visit.

Despite growing evidence that pollinator traits influence pollen transfer, pollination effectiveness is shaped by multiple behavioural and morphological characteristics that remain poorly understood across crop systems. Most previous studies evaluating the factors of pollinator effectiveness have focused on single-crop systems or on comparisons among a limited number of pollinator species. However, less is known about how combinations of bee traits influence pollen deposition across different crop species with contrasting floral characteristics.

We hypothesized that (1) bee species with larger body size, greater scopal area, dense body hair, early peak activity, and vibration-based pollen collection behaviour would deposit more conspecific pollen per visit; and (2) we expected pollen deposition to differ among crop species. To test these hypotheses, we quantified single-visit pollen deposition in mustard, brinjal, and chilli and evaluated the association between functional traits and pollen transfer.

2. MATERIALS AND METHODS

2.1. Study Site

The study was conducted in an experimental garden located in Alipurduar district, West Bengal, India (26°27′55.7″ N, 89°35′09.2″ E; Figure 1). Alipurduar district is situated in the northern part of the Indian state of West Bengal, within the Eastern Terai-Dooars region, in the foothills of the eastern Himalayas. The experimental garden was established to quantify SVD in three pollinator-dependent crops: mustard (*Brassica juncea*), brinjal

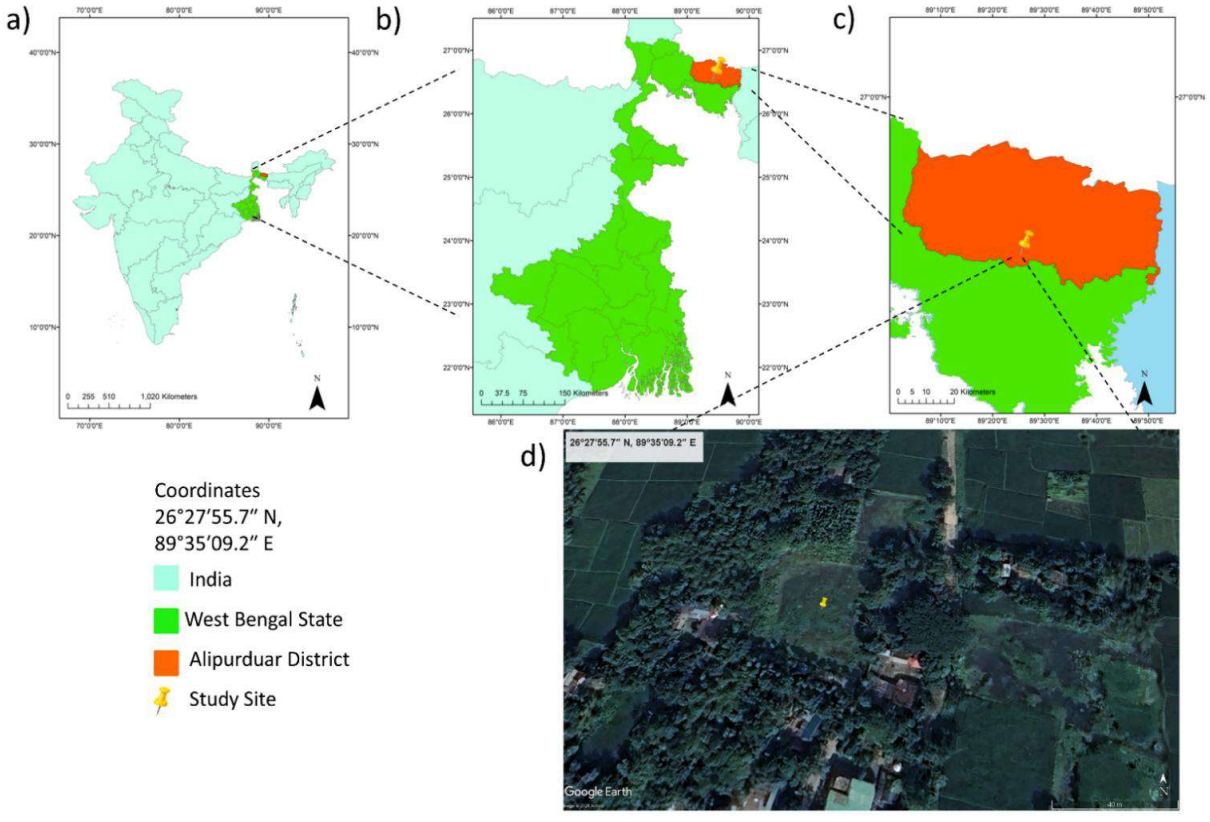


Figure 1. Geographic location of the experimental garden in a) India, b) West Bengal State, c) Alipurduar district; d) the inset satellite image shows the exact location of the study site where single-visit pollen deposition experiments were conducted on mustard, brinjal, and chilli

(*Solanum melongena*), and chilli (*Capsicum annuum*) under similar management practices. Field experiments were conducted from November 2024 to March 2025.

Bhutan borders Alipurduar district to the north, Assam to the east, Cooch Behar district to the south, and Jalpaiguri district to the west (Saha & Das, 2024). The landscape consists of forest areas, including Buxa Tiger Reserve, Rajabhatkhawa, Chilapata, and Raidak forests. The district experiences a tropical monsoon climate, receiving approximately 3100 mm of annual rainfall, with temperatures ranging from below 10 °C during winter to above 35 °C during summer (Ghosh et al., 2025).

Rice (*Oryza sativa* L., 1753) is the dominant crop during the monsoon season (kharif), whereas mustard is the principal oilseed crop cultivated during the winter season (rabi), coinciding with the study period. Among the pollinator-dependent vegetable crops widely grown in the district are chilli and brinjal, together with tomato (*Solanum lycopersicum* L., 1753), cabbage (*Brassica oleracea* L., 1753), cauliflower (*Brassica oleracea* var. *botrytis* L.), and cucumber (*Cucumis sativus* L., 1753) (Basak et al., 2023). The Alipurduar district is a heterogeneous transition zone that contains mosaic patches of forests, agricultural fields, tea gardens, and fallow lands that support diverse pollinator communities. The landscape is also experiencing increasing agricultural intensification and land-use change, making it an ideal region for studying pollination services and pollinator effectiveness in agricultural systems.

2.2. Crop Selection

Three economically important and widely cultivated crops in India were selected for the present study: mustard (*Brassica juncea*), brinjal (*Solanum melongena*), and chilli (*Capsicum annuum*) (Basak et al., 2023). These crops were chosen because they represent two major crop families (Brassicaceae and Solanaceae), differ in floral morphology and pollination biology, and exhibit varying degrees of dependence on insect-mediated pollination (Klein et al., 2007). Based on pollinator dependency categories proposed by Chaudhary and Chand (2017), mustard is highly dependent on insect pollination (40-90%), whereas brinjal and chilli are moderately dependent (10-40%). Mustard produces small, open flowers with exposed anthers and stigmas that are readily accessible to a wide range of pollinators (Chandrashekar et al., 2013). In contrast, brinjal and chilli possess poricidal anthers; however, brinjal bears larger, more conspicuous flowers with a prominent anther cone and depends more strongly on buzz pollination for pollen release, whereas chilli is less dependent on bee-induced vibrations (De Luca & Vallejo-Marín, 2013; Jayasinghe et al., 2017). These differences in floral architecture, pollen presentation, stigma accessibility, pollination mechanism, and pollinator dependence provide an ideal system for comparing SVD among crops. A single uniform variety of each crop was used throughout the experiment to ensure consistency in plant growth and floral characteristics.

2.3. Single-visit Pollen Deposition (SVD)

SVD was used to estimate one component of pollination effectiveness, which quantifies the number of conspecific pollen grains deposited on a receptive stigma during a single pollinator visit, providing a standardized per-visit measure of pollination effectiveness (Cresswell, 2000; Rader et al., 2013).

The study was conducted in a 33-dec experimental garden containing 60 chilli and 60 brinjal plants, with adjacent plants spaced at least 1.5 m apart. Mustard was cultivated in a separate 23-dec experimental field located approximately 40 m from the experimental garden during the same study period. Pollination observations were conducted throughout the flowering period of each crop, between November 2024 and March 2025. For each bee species within each crop, single-visit pollen deposition (SVD) was estimated from 8-12 independent visits to newly opened virgin flowers. These observations were distributed across the flowering season, with at least one observation obtained per month for each bee species whenever the species was active. Observations were conducted only under favourable weather conditions between 07:00 am and 12:00 noon (India Standard Time). Sampling was avoided during rainfall and periods of strong wind to minimize the influence of environmental variation on pollinator activity. The study was carried out during the winter cropping season. During the study period, sunrise occurred between approximately 05:40 and 06:20 am; therefore, observations were conducted approximately 1-6 h after sunrise, coinciding with the principal period of bee foraging activity, floral anthesis, and peak stigma receptivity across the three study crops (Free, 1993).

To prevent insect visitation before observations, flower buds expected to reach anthesis the following morning were individually enclosed overnight in fine cotton-mesh bags (mesh size 0.8-1.0 mm). The mesh allowed adequate ventilation while preventing insect access, thereby ensuring stigma virginity (Figure 2).

At anthesis, each flower was carefully uncovered and monitored until it received a single bee visit. To maximize the independence of observations, flowers were selected from different plants whenever possible; however, in a few instances, multiple flowers from the same plant were used when no additional suitable flowers from other plants were available. Bees were allowed to complete their foraging bout without disturbance to ensure natural pollen transfer. The visited stigma was then excised and mounted in a drop of 30% aqueous glycerol solution on a clean glass slide. After gently crushing the stigma beneath a coverslip to release the deposited pollen grains, they were counted under a compound microscope at 10× magnification using a modified stigma squash protocol based on Kearns and Inouye (1993) (Figure 3).

Conspecific pollen grains were distinguished from heterospecific pollen by comparison with a reference pollen library prepared from freshly collected pollen of the three focal crop species. Pollen grains were identified under a compound microscope by their characteristic size, shape, aperture type, and exine ornamentation, and only conspecific grains were included in the single-visit pollen deposition counts.

To account for background autonomous self-pollen deposition, 15 flowers of each crop were bagged before anthesis and maintained free from insect visitation until stigma collection. The pollen grains present on the stigmas of these unvisited flowers were counted to estimate autonomous self-pollen deposition. The mean pollen count of these control flowers was subsequently subtracted from



Figure 2. Workflow for estimating SVD, in the experimental garden, brinjal, mustard and chilli flowers were bagged with fine-mesh bags before flower opening to prevent prior insect visitation and subsequently unbagged and plucked after a single visitation. Pollinator exclusion images of (a) mustard, (b) brinjal, and (c) chilli. Panels (d & e) show the experimental garden where SVDs were conducted

the pollen deposition recorded after a single pollinator visit to estimate the net single-visit pollen deposition.

2.4. Bee Species Collection and Traits Estimation

Following each single-visit pollen deposition observation, the focal bee visitor was captured immediately after completing its visit to the flower using a sweep net. Each bee individual contributed to only one single-visit pollen deposition observation before being captured, thereby preventing repeated observations of the same individual. Specimens were preserved individually in 70% ethanol for subsequent identification and morphometric analyses (Figure 4). Bees were identified to species or morphospecies level using the taxonomic keys of Michener (2007).

To assess associations between bee functional traits and single-visit pollen deposition, we compiled morphological and behavioural traits for each bee species (Table 1). Morphological traits included inter-tegular distance (ITD), body hairiness, and scopal area (AOS), whereas behavioural

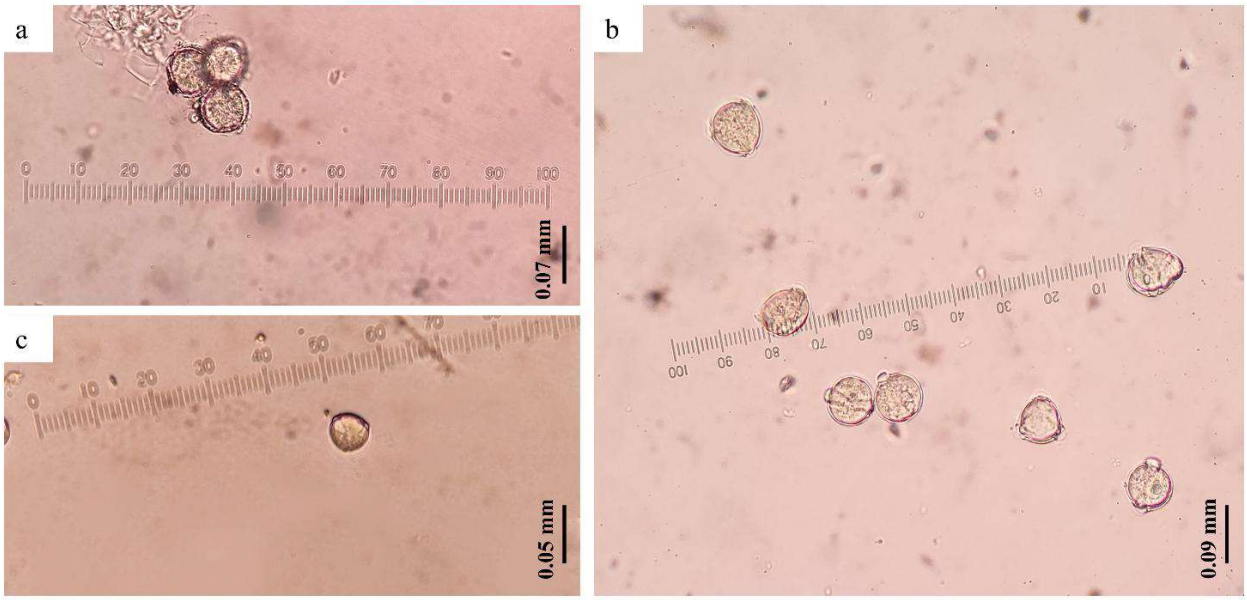


Figure 3. Following a single bee visit, stigmas were excised and mounted in 30% glycerol. Conspecific pollen grains were identified by comparison with a reference pollen library using pollen size, shape, aperture type, and exine ornamentation under a compound microscope before counting. Pollen grains of (a) mustard, (b) brinjal, and (c) chilli were observed during SVD analyses. Scale bars indicate pollen size for each crop species

Table 1. Morphological and behavioural traits of bee pollinators and their description

Trait	Trait type	Categories/Description	Source
Inter-tegular distance (ITD)	Continuous variable	____	Measurement from samples
Area of scopa (AOS)	Continuous variable	____	Measurement from samples
Mean pollen transport (MPT)	Categorial variable	Corbiculae (MPT1); Ventral scopa (MPT2); Scopa (hind trochanter–basitarsus) (MPT3); Scopa (tibia and basitarsus) (MPT4).	Michener (2000); Coutinho et al. (2021)
Mean pollen uptake (MPU)	Categorial variable	First leg (MPU1); Vibration (MPU2); First leg and mandible (MPU3); First leg and vibration (MPU4).	Michener (2000); Coutinho et al. (2021)
Peak activity time (PAT)	Categorial variable	(1) Within 0–3 h after sunrise (PAT1); (2) Within 3–6 h after sunrise (PAT2)	Data from the field study
Hairiness (density of hair) (HAI)	Categorial variable	Dense (HAI1) / Sparse (HAI2).	Michener (2000); Coutinho et al. (2021)

traits included peak activity time (PAT), pollen uptake behaviour, and pollen transport behaviour.

Body size was estimated from the inter-tegular distance (ITD), measured as the shortest linear distance between the outer margins of the tegulae on the dorsal thorax following Chakraborty et al. (2025). Measurements were made under a stereomicroscope fitted with a calibrated ocular micrometre. Scopal area (AOS) was estimated under the same stereomicroscope using the calibrated ocular micrometre by measuring the maximum length and width of the scopal region. The scopal area was approximated as an ellipse using the formula: Area = $\pi \times (\text{length}/2) \times (\text{width}/2)$, following Chakraborty et al. (2025).

Body hairiness (HAI) was classified into two categories (sparse and dense) based on the extent of hair coverage on the thorax and abdomen following the functional trait classification of Coutinho et al. (2021) and the morphological descriptions of Michener (2000). Species with relatively low hair coverage and clearly visible cuticle were classified as sparsely haired, whereas species with extensive hair coverage over the thorax and abdomen were classified as densely haired. Body hairiness was included as a functional trait because greater hair coverage enhances pollen adhesion and transport, thereby influencing pollen deposition on stigmas (Stavert et al., 2016).

Peak activity time (PAT) was assigned based on field

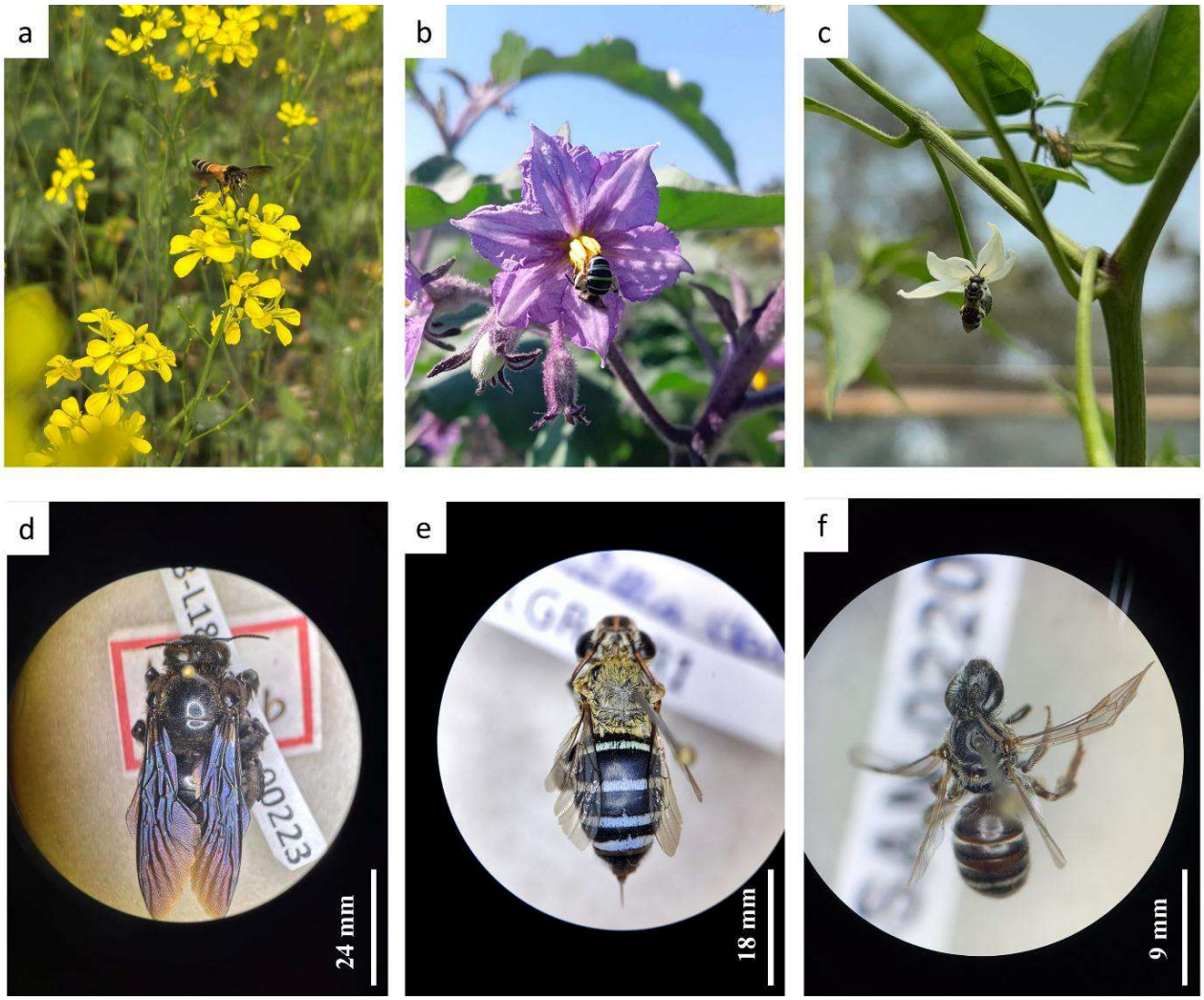


Figure 4. Representative bee pollinators identified from the study crops, include: (a) *Apis dorsata*, (b) *Hoplonomia* sp. 1, (c) *Lasioglossum* sp. 2, (d) *Xylocopa* sp. 1, (e) *Amegilla* sp. 1, and (f) *Lasioglossum* sp. 1. The images in panels (a-c) depict flower-visiting bees observed in the field, while panels (d-f) feature voucher specimens used for trait measurement. Scale bars indicate the body size of each bee species

observations during the single-visit pollen deposition study. For each bee species, the time interval during which the majority of flower visits were recorded was considered its peak activity period. Activity was classified into two categories: 0-3 h and 3-6 h after sunrise. During the study period (November 2024-March 2025), sunrise in Alipurduar district occurred between approximately 05:40 am and 06:20 am; therefore, observations conducted between 07:00 am and 12:00 noon encompassed both activity categories. Sampling was distributed throughout the observation period to ensure representation of both activity periods.

Information on pollen collection behaviour (MPT, MPU) was obtained from field observations and supplemented with published literature (Michener, 2000; Martins et al., 2015; Coutinho et al., 2021; Chakraborty et al., 2025).

For continuous traits, at least five female specimens per species (or morphospecies when species-level identification was not possible) were measured. Species-level mean values were subsequently calculated for continuous traits, whereas categorical and behavioural traits were assigned at the species level. These species-level trait values were used to examine associations between bee functional traits and single-visit pollen deposition.

2.5. Statistical Analysis

To investigate whether bee morphometric traits influenced single-visit deposition of pollen grains, we fitted generalized linear mixed models (GLMMs) using the glmmTMB package in R version 4.4.1 (2024). SVD was modelled as the response variable, and six bee traits were analysed separately as fixed effects: inter-tegular distance (ITD), area of scopa (AOS), hairiness (HAI), mean pollen uptake (MPU), peak activity time (PAT), and mean pollen transport (MPT). Crop identity was included as a random intercept in all models to account for non-independence among observations within crops. Because SVD represented overdispersed count data, all models were fitted using a negative binomial error distribution (nbinom2) with a "log" link function. Model assumptions were evaluated using simulation-based residual diagnostics implemented in the DHARMa package (Hartig, 2017). For categorical predictors with more than two levels (MPU and MPT), pairwise differences among trait categories were examined using estimated marginal means with Tukey-adjusted post hoc comparisons implemented in the emmeans package. Model fit was assessed using Akaike's Information Criterion (AIC). Because bee traits were measured at the species level, repeated observations within each species improve the precision of SVD estimates but do not represent independent trait values. Therefore, the results indicate associations between bee traits and SVD across bee species.

3. RESULTS AND DISCUSSION

3.1. Variation in SVD Among Bee Species and Crops

A total of 11, 9, and 6 bee species were evaluated for single-visit pollen deposition on mustard, brinjal, and chilli, respectively (Table 2). SVD varied significantly among bee species and crop types (Figure 5). In mustard, SVD ranged from 76 ± 37 SD pollen grains per visit in *Braunsapis puangensis* to 589 ± 183 SD pollen grains per visit in *Apis*

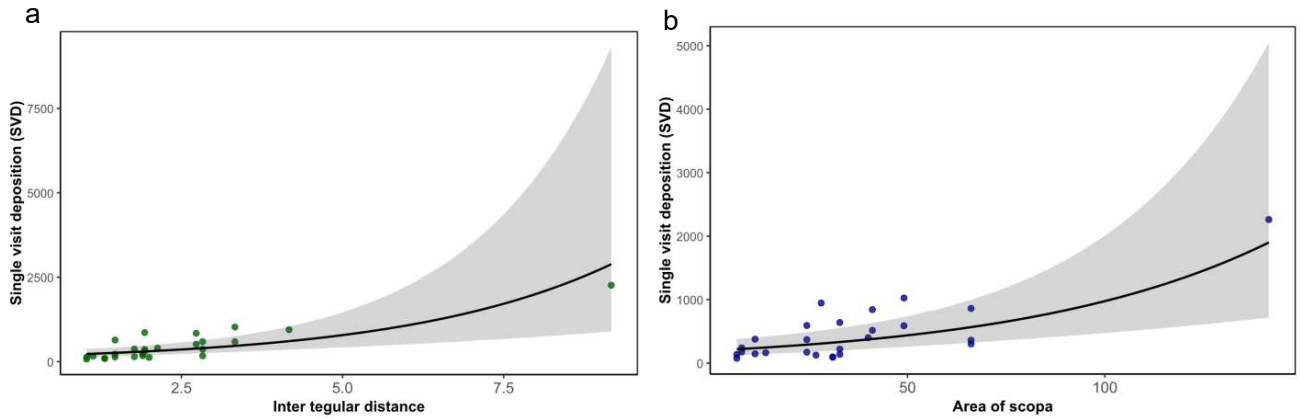


Figure 5. Graphical representation of SVD of different bee species visiting brinjal, chilli, and mustard flowers. SVD represents the average number of conspecific pollen grains deposited on a stigma during a single pollinator visit. The y-axis represents different bee species visiting different crops. The x-axis represents mean SVD. Error bars represent standard error of mean

dorsata. The second- and third-highest SVD values were recorded for *Hoplonomia* sp. 1 (517 ± 122 SD) and *Andrena* sp. 2 (401 ± 98 SD), respectively. In contrast, *Lasioglossum* sp. 3 (98 ± 50 SD) and *Apis florea* Fabricius, 1787 (126 ± 32 SD) recorded the second- and third-lowest SVD values.

In brinjal, SVD ranged from 137 ± 35 SD pollen grains per visit in *Braunsapis puangensis* to 2263 ± 468 SD pollen grains per visit in *Xylocopa* sp. 1. The second- and third-highest SVD values were recorded for *Apis dorsata* (1026 ± 305 SD) and *Amegilla* sp. 1 (947 ± 229 SD), respectively, followed by *Lasioglossum* sp. 2 (862 ± 55 SD) and *Hoplonomia* sp. 1 (843 ± 253 SD). *Ceratina sutepensis* Cockerell, 1929 (377 ± 45 SD) exhibited the second-lowest SVD value among the bee species recorded on brinjal. Brinjal exhibited the highest SVD values among the three crops.

In chilli, SVD ranged from 93 ± 45 SD pollen grains per visit in *Lasioglossum* sp. 3 to 370 ± 162 SD pollen grains per visit in *Apis cerana*. The second-highest SVD value was recorded for *Lasioglossum* sp. 2 (361 ± 162 SD), whereas *Lasioglossum* sp. 1 (138 ± 73 SD), *Ceratina sutepensis* (147 ± 32 SD), and *Ceratina* sp. 1 (176 ± 77 SD) recorded comparatively lower SVD values.

3.2. Association of Bee Functional Traits with SVD

Inter-tegular distance (ITD) significantly influenced SVD ($\beta = 0.312 \pm 0.080$ SE, $P < 0.001$) (Figure 6. a, Table 3). Area of scopa (AOS) also significantly influenced SVD ($\beta = 0.016 \pm 0.004$ SE, $P < 0.001$) (Figure 6. b, Table 3).

The mechanism of pollen uptake (MPU) positively impacted SVD. Those employing the first leg and mandible mechanism (MPU3) deposited fewer pollen grains per visit compared to bees collecting pollen using the first leg (MPU1) ($\beta = -0.634 \pm 0.289$ SE, $P = 0.028$) (Figure 7. a, Table 3), Tukey-adjusted pairwise comparisons revealed that bees using vibration alone deposited significantly more pollen grains than those using the first leg and mandible mechanism ($\beta = 1.238 \pm 0.360$ SE, $P = 0.003$) and those using first leg combined with vibration ($\beta = 0.935 \pm 0.343$ SE, $P = 0.033$).

Peak activity time (PAT) significantly affected SVD. Bees exhibiting peak activity within 3-6 h after sunrise deposited fewer pollen grains per visit than those active within the first 0-3 h after sunrise ($\beta = -0.861 \pm 0.212$ SE, $P < 0.001$) (Figure 7. b, Table 3).

The mean pollen transport (MPT) also influenced SVD. Relative to bees carrying pollen in corbiculae (MPT1), bees transporting pollen using scopa located on the tibia, basitarsus, and first tarsal segment (MPT4) exhibited higher SVD ($\beta = 0.913 \pm 0.463$ SE, $P = 0.049$) (Figure 7. c, Table 3). However, Tukey-adjusted pairwise comparisons indicated that the only significant contrast occurred between MPT3 and MPT4, with bees transporting pollen on the tibia, basitarsus, and first tarsal segment depositing more pollen

Table 2. SVD values of different bee species across mustard, chilli, and brinjal crops

Bee species	Mustard SVD (Mean ± SD (n))	Brinjal SVD (Mean ± SD (n))	Chilli SVD (Mean ± SD (n))
Xylocopa sp. 1	ND	2263 ± 468 (10)	ND
Apis dorsata	589 ± 183 (10)	1026 ± 305 (10)	ND
Amegilla sp. 1	ND	947 ± 229 (12)	ND
Lasioglossum sp. 2	302 ± 155 (10)	862 ± 55 (10)	361 ± 162 (10)
Hoplonomia sp. 1	517 ± 122 (11)	843 ± 253 (12)	ND
Lasioglossum sp. 1	221 ± 228 (11)	640 ± 117 (10)	138 ± 73 (11)
Apis cerana	172 ± 94 (10)	593 ± 175 (9)	370 ± 162 (10)
Andrena sp. 2	401 ± 98 (11)	ND	ND
Ceratina sutepensis	ND	377 ± 45 (10)	147 ± 32 (8)
Ceratina sp. 1	240 ± 52 (10)	ND	176 ± 77 (11)
Tetragonula sp. 1	164 ± 28 (11)	ND	ND
Braunsapis puangensis	76 ± 37 (10)	137 ± 35 (11)	ND
Apis florea	126 ± 32 (10)	ND	ND
Lasioglossum sp. 3	98 ± 50 (11)	ND	93 ± 45 (12)

Notes: n = number of samples taken, and ND = Not determined, SD = Standard deviation

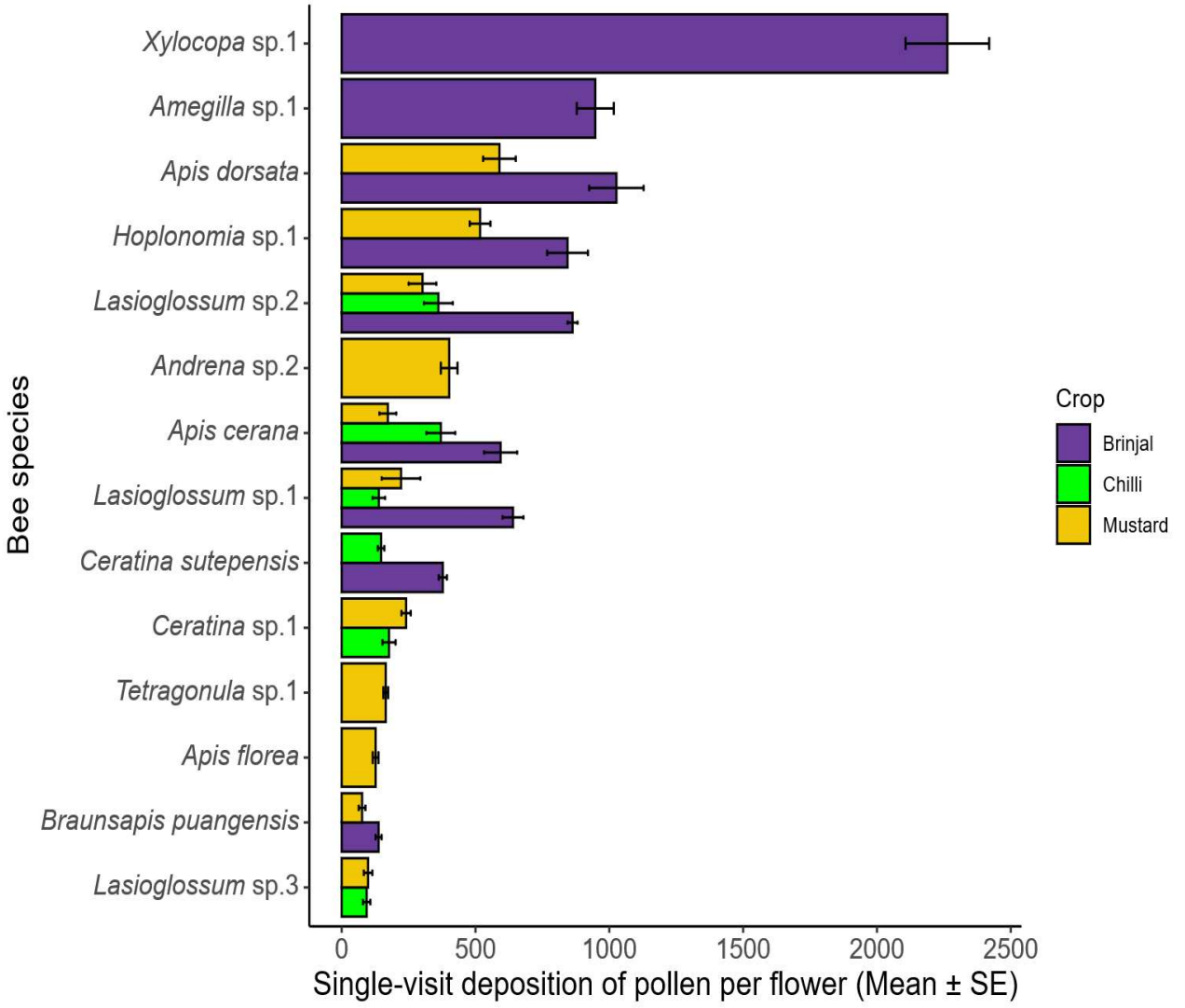


Figure 6. SVD depends on bee morphometry and the graph showing the relationship between (a) inter-tegular distance (ITD) (X-axis) and (b) area of scopa (AOS) (X-axis) on SVD (Y-axis)

Table 3. Significant relationships between bee traits (predictor variables) and SVD (response variable). Generalized linear mixed models (GLMMs) were fitted using a negative binomial error distribution with a log-link function, with crop species included as a random effect. Only significant trait effects are presented. AIC values indicate the relative fit of each model

Response variable (Y)	Predictor variable (X)	Distribution (link)	Estimate (β ± SE)	z-value	p-value	AIC
SVD	ITD	Negative binomial (log)	0.312 ± 0.080	3.88	<0.001	349.14
SVD	AOS	Negative binomial (log)	0.016 ± 0.004	4.04	<0.001	350.18
SVD	PAT (pat2)	Negative binomial (log)	-0.861 ± 0.212	-4.06	<0.001	352.84
SVD	HAI (hai2)	Negative binomial (log)	-0.831 ± 0.292	-2.84	0.004	358.26
SVD	MPU (mpu3)	Negative binomial (log)	-0.634 ± 0.289	-2.19	0.028	358.45
SVD	MPT (mpt4)	Negative binomial (log)	0.913 ± 0.463	1.97	0.049	359.86

grains per visit than those transporting pollen on the hind femurs and tibias (β = −1.291 ± 0.445 SE, P = 0.019).

Hairiness (HAI) significantly affected SVD. Compared with densely haired bee species (HAI1), sparsely haired species (HAI2) deposited significantly fewer pollen grains per visit (β = −0.831 ± 0.292 SE, P = 0.004) (Figure 7. d, Table 3).

3.3. Bee Traits, Floral Characteristics, and Implications for Pollination

Our findings demonstrate that SVD varied among bee species within and across the three study crops, reflecting differences in both crop identity and bee assemblages. Bee species with larger body size, such as *Xylocopa* sp. 1 and *Apis dorsata*, generally exhibited higher SVD than smaller-bodied species where they occurred (Figure 5). Moreover, inter-tegular distance, scopal area, dense body hairiness, early peak activity, and specific pollen collection and transport mechanisms were positively associated with increased pollen deposition. Together, these findings indicate that variation in SVD is closely associated with bee functional traits across the study crops.

Clear differences in SVD among bee species are supported by previous studies showing that not all flower visitors contribute equally to pollination (King et al., 2013; Ballantyne et al., 2015), and body size influences pollen loads and pollination success (Goulson et al., 2002; Földesi et al., 2021). The positive relationships between SVD and both inter-tegular distance (ITD) (Figure 6. a) and scopal area (Figure 6. b) suggest that larger bees can be more effective pollinators. Larger bees can carry greater quantities of pollen and contact a larger portion of the floral reproductive structures (Greenleaf et al., 2007; Kendall et al., 2019). Our findings extend this evidence by

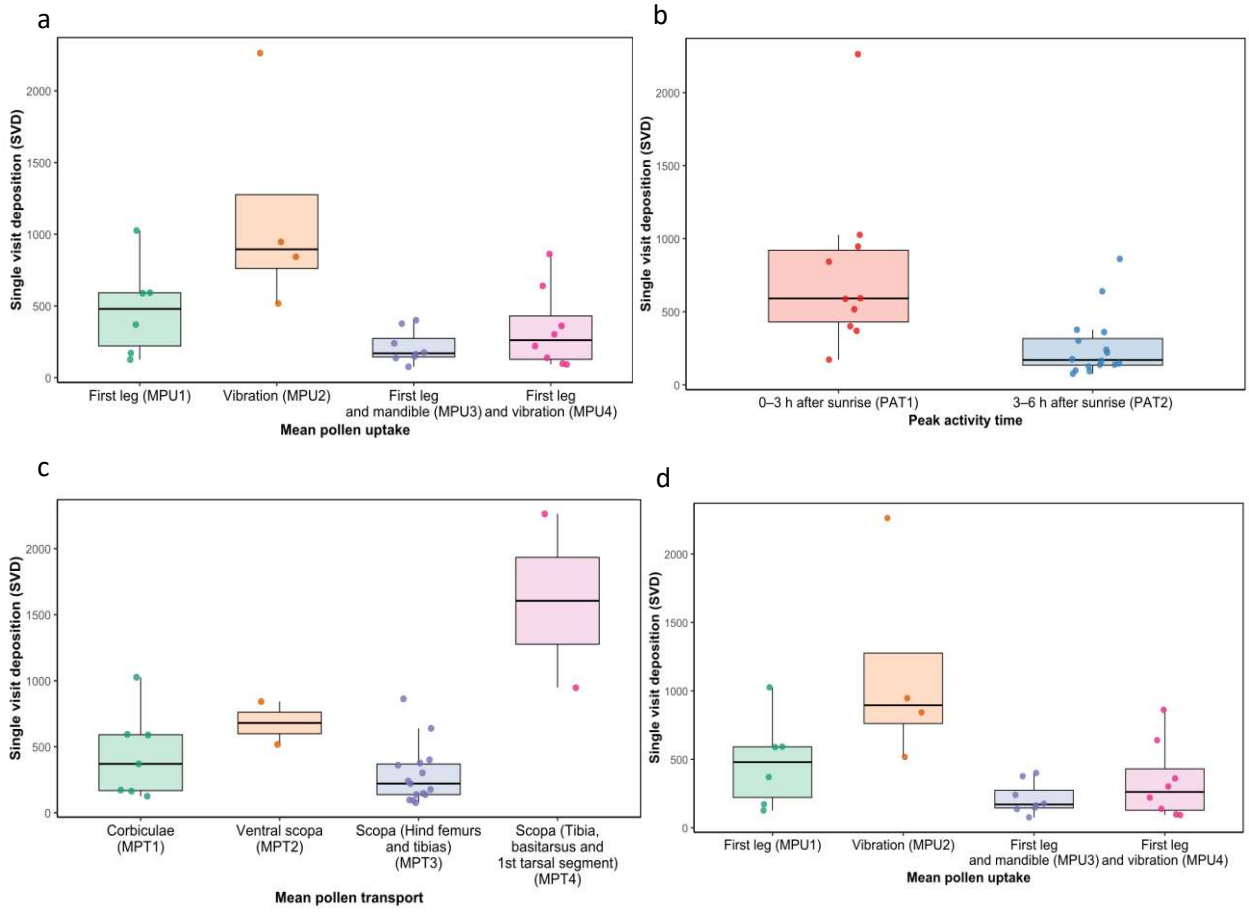


Figure 7. Effects of multiple traits on SVD by bee pollinators. Boxplots show variation in SVD (Y axis) according to (a) mean pollen uptake (MPU) (X axis), (b) peak activity time (PAT) (X axis), (c) mean pollen transport (MPT) (X axis), and (d) body hairiness (HAI) (X axis)

demonstrating that body size can predict the actual delivery of pollen to stigmas across multiple crop systems. The positive effect of scopal area further suggests that traits associated with pollen-transport capacity can substantially contribute to pollination efficiency. In brinjal, *Xylocopa* sp. 1 deposited more pollen grains, reflecting its large body size and its ability to perform buzz pollination, a behavioural trait that efficiently releases pollen from the poricidal anthers characteristic of Solanaceae (De Luca & Vallejo-Marín, 2013). Similarly, *Amegilla* sp. 1 and *Apis dorsata* also exhibited relatively high SVD values, suggesting that large body size and effective contact with floral reproductive structures and pollen-carrying capacity enhance pollen deposition in flowers. In contrast, smaller bees such as *Braunsapis puangensis* and *Lasioglossum* sp. 3 deposited fewer pollen grains, possibly because their smaller body size limits contact with stigmas and reduces the amount of pollen deposited in flowers (Chang et al., 2023). Although larger bees generally deposited more pollen, the substantially higher pollen deposition by *Apis dorsata* in brinjal than in mustard indicates that bee body size alone does not fully explain variation in SVD. Pollination outcomes likely arise from interactions between bee functional traits and crop-specific floral characteristics, including floral architecture, the spatial arrangement of reproductive organs, and the degree of morphological fit between the pollinator and the flower (Armbruster & Muchhala, 2009; Nogueira et al., 2018). Because floral traits were not quantified in the present study, these mechanisms remain speculative and warrant further investigation.

The higher SVD observed in brinjal compared with mustard and chilli may be attributed, at least in part, to differences in floral architecture that influence the fit between flowers and pollinators and, consequently, pollen transfer (Nogueira et al., 2018). A closer fit between floral reproductive structures and the pollinator's body can enhance contact with anthers and stigmas, thereby increasing pollen transfer, whereas a poor fit may reduce pollen deposition even when visitation occurs (Nagano et al., 2014; Kuriya et al., 2015). Experimental evidence from buzz-pollinated systems further demonstrates that pollen deposition can be influenced by the match between pollinator size and the spatial arrangement of floral reproductive structures (Solís-Montero & Vallejo-Marín, 2017; Willmer et al., 2017). The relatively high SVD recorded for large-bodied bees such as *Xylocopa* sp. 1 and *Amegilla* sp. 1 on brinjal may therefore reflect differences in bee-flower interactions. However, because floral architecture was not quantified in the present study, the contribution of specific floral traits to the observed differences in SVD among crops remains uncertain.

Bee species classified as densely haired exhibited greater pollen deposition than those classified as sparsely haired, indicating that body hair density contributes to pollen transfer. Dense body hair on the thorax and abdomen provides a larger external surface area for pollen grains to adhere to during floral visits, increasing pollen retention and the amount of pollen available for transfer to receptive stigmas. This interpretation is consistent with previous studies showing that bee body hair plays an important role in pollen capture and transport (Stavert et al., 2016). Behavioural traits also significantly influenced SVD. Bees active during the early hours after sunrise deposited more pollen than those with later peak activity, possibly because freshly dehisced anthers provide abundant pollen resources during early foraging periods (Rader et al., 2013; Zhang et al., 2019). In addition, bees that used vibration-based pollen collection deposited more pollen than those that relied primarily on the first leg and mandible. Vibrational pollen extraction may promote greater pollen release and increase pollen availability on the body surface, thereby enhancing pollen deposition during flower visits (Willmer et al., 2017).

Collectively, our findings suggest that maintaining diverse bee assemblages containing species with complementary morphological and behavioural traits may enhance pollen transfer and crop pollination services. Reliance only on managed pollinators may overlook the important contributions of wild bee taxa, some of which exhibited substantially higher single-visit pollen deposition. Conservation of multiple resources that support functionally diverse bee communities could therefore improve crop pollination and contribute to sustainable agricultural production.

3.4. Limitations and Future Research

Our results demonstrate that bee functional traits are significant predictors of SVD, and that crop-specific floral characteristics may contribute to variation in pollination effectiveness. This study focused on only three economically important crop species; floral traits were not quantified separately, and crop identity was included as a random effect to account for crop-specific variation. The limited number of crop species also constrained our ability to conduct robust crop-specific analyses, and this should be

considered a limitation of the present study. Future studies incorporating a broader diversity of crop species or cultivars, together with quantitative measurements of floral traits (e.g., flower size, corolla depth, anther-stigma separation, and floral display), would enable a more comprehensive evaluation of the joint effects of floral and pollinator traits on pollen deposition. Although this study was conducted at a single experimental location, the consistency of the observed relationships across multiple bee species and repeated observations throughout the flowering season supports the robustness of the findings. Validation across additional locations and seasons would further establish the generality of these relationships. Finally, SVD should be considered an indirect measure of pollination effectiveness rather than a direct measure of reproductive success. Although pollen deposition on the stigma provides a useful indicator of potential pollination success, this study did not assess subsequent outcomes such as fruit set or seed production. Future studies linking SVD with fruit and seed production would therefore provide a more direct assessment of the reproductive consequences of bee visitation.

4. CONCLUSION

Our study demonstrates that SVD varies substantially among bee species and crop systems and is strongly associated with bee morphological and behavioural traits. Larger-bodied bees with greater scopal area, dense body hairiness, early foraging activity, and vibration-based pollen collection generally deposited more pollen per visit. Furthermore, the same bee species often exhibited different levels of SVD across crop species, indicating that pollen transfer is context-dependent and likely influenced by crop-specific floral characteristics in addition to bee functional traits. These findings highlight that crop pollination cannot be evaluated solely on the basis of visitation frequency, as pollinator quality differs markedly among species. From a broader management perspective, conserving bee communities with diverse functional traits and considering crop-specific pollination requirements may help maintain effective pollination services and contribute to sustainable agricultural production. Future studies integrating SVD with visitation frequency, quantitative floral trait measurements, fruit and seed set, and crop yield across a broader range of crop species and environmental conditions would provide a more comprehensive understanding of pollinator contributions to agricultural ecosystems.

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CRediT authorship contribution statement

Dona Roy: Data collection, Methodology, Software, Writing original draft, Formal analysis, Tabulation, Data curation; **Manobrata Das:** Conceptualization, Reviewing and editing, Visualization; **Indranil Samajpati:** Conceptualization, Formal analysis, Reviewing and editing, Visualization; **Ankan Bhandari:** Reviewing and editing, Visualization; **Debaditya Kumar:** Reviewing and editing, Visualization; **Parthiba Basu:** Methodology, Conceptualization; **Rita Kundu:** Supervision, Reviewing and editing, Visualization.

Conflict 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 artificial intelligence tools were used to write this manuscript.

Data availability statement

All data collected and analysed during this study are included in this article. Additional raw data will be made available from the corresponding author upon reasonable request.

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Comparative Study on Temporal Dynamics and Plant-pollinator Networks of Hover Flies (Diptera: Syrphidae) in Agricultural and Horticultural Ecosystems, Karaikal, Puducherry, India

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ABSTRACT: Global declines in pollinator populations have shifted research focus toward non-bee pollinators, especially hover flies (Diptera: Syrphidae), which provide dual ecosystem services as adult pollinators and larval biocontrol agents. Despite their ecological significance, hover fly diversity and associated plant interactions had remained undocumented in the coastal agricultural and horticultural landscapes of Karaikal, Union Territory of Puducherry, India. This study aimed to assess the species richness and the temporal dynamics of hover flies at the Pandit Jawaharlal Nehru College of Agriculture and Research Institute between October 2024 - June 2025. The investigation recorded a total of 10 hover fly species in the agricultural ecosystem and 9 in the horticultural ecosystem, with interactions involving 15 different flowering plant species. The results revealed higher species diversity (1.07), evenness (0.61) and dominance (0.77) within the horticultural ecosystem. Furthermore, plant-pollinator network analysis showed higher nestedness (29.36), connectance (0.29) and linkage diversity (3.73) in the horticultural ecosystem when compared to the agricultural ecosystem. The Principal Coordinates Analysis demonstrated that the hover fly assemblage in the agricultural ecosystem was influenced by crop phenology, whereas assemblages in the horticultural ecosystem were driven by floral resource pulses. Most of the hover flies showed a strong negative correlation with maximum and minimum temperatures, while showing a positive correlation with relative humidity and sunshine hours. These findings highlight the ecological importance of weed flowers in supporting the hover fly community. The study concludes that maintaining high floral diversity is essential for the conservation of hover fly populations and ensures the delivery of sustainable pollination services.

Keywords: Conservation, Diversity, Flower, Hover fly, Plant- Pollinator, Pollination, Yield.

1. INTRODUCTION

Pollination is one of the important mechanisms in plant reproduction and is mainly carried out by abiotic (wind, water) and biotic factors (animals, birds, insects, including bees, flies, butterflies and beetles). Insect pollination improves fruit quality and increases the yield of economic crops (Eilers et al., 2011); the global decline in honey bees has necessitated research into non - bee pollinators to ensure food security (Requier et al., 2023). Among these, hover flies (flower flies or syrphid flies) belonging to the family of true flies are the most colourful and valuable keystone insect, which emerged as the second most important pollinator group after bees, pollinating nearly 72 % of economic crops - a service valued at nearly $300 billion per year (Doyle et al., 2020; Rehermann et al., 2026). Syrphidae consist of 200

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genera and 6009 species belonging to three families globally except Antarctica and remote oceanic islands (Larson et al., 2001). The two major subfamilies of flower flies, Syrphinae (1800 species), have larvae that are terrestrial predators and Eristalinae (3800 species) larvae act as decomposers, fungus feeders, plant feeders and aquatic filter-feeders (Rotheray & Gilbert, 2011; Skevington et al., 2019). Sengupta et al. (2016) reported a total of 355 hover fly species belonging to 69 genera recorded in India. In India, Ghorpade (2019) recorded a total of 131 hover fly species belonging to 49 genera in 10 Tribes and 3 Subfamilies from the southern Indian sub-continent. The ecological significance of syrphids is highlighted by their unique role as 'dual - service' provider in the ecosystem (Li et al., 2023). The brightly coloured adult flies hover around the flowers, while the maggots of syrphid flies (aphidophagous) are predatory on soft-bodied insects like aphids, thrips and scale insects, making them an effective biocontrol agent (Jiang et al., 2025). It also plays an important role in nutrient recycling by decomposing organic matter (Ghahari et al., 2008; Skevington et al., 2019). The adult feeds on nectar and pollen to improve the fecundity rate, thereby contributing to pollination services to a wide range of economic crops, usually considered as a generalist pollinator, the floral preferences are altered based on the availability of plants (Matouskova et al., 2023). Syrphid flies maintain ecological stability by visiting a greater number of plants than bee pollinators (Olsson et al., 2021). This functional diversity allows them to provide stable ecological services in agroecosystems where bees and butterflies were less active in highly elevated places due to harsh weather and low pressure (Sengupta et al., 2019a). Their high sensitivity makes them an excellent bioindicator of landscape health and climate change. In Karaikal, the incidence of soft bodied sucking pests like thrips and aphids was most prevalent during the summer season (March - May), causing yield loss in crops like cotton (*Gossypium hirsutum)*, black gram (*Vigna mungo)* and chilli (*Capsicum annuum)* etc. In the horticultural ecosystem, the majority of crops are highly cross-pollinated; hoverflies can be used in pollination. Despite their immense ecological value, there is no previously documented research on syrphid diversity within coastal agro-ecosystems. Studying the diversity of flies, abundance and associated floral resources enables to efficiently utilise them in pollination and conservation practices (Ela et al*.,* 2021). So, the present investigation was undertaken to fill the research gap by studying hover fly diversity and their plant interactions within Karaikal region. By elucidating the temporal dynamics of these hover flies, this study provided a pathway for conservation and effective utilisation in sustainable pollination services.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was carried out to record the diversity of hover flies during October 2024 to June 2025 (*rabi/Winter* 2024 - 2025; Summer 2025) at Pandit Jawaharlal Nehru College of Agriculture and Research Institute (PAJANCOA and RI), Karaikal, U. T. of Puducherry, India (Figure 1). The study area situated at 10°52′ North latitude and 79°52′ East longitude, with an elevation of 4 meters above mean sea level. Minimum and maximum temperature ranged from 20 to 27°C and 30 to 38°C, respectively. Relative humidity was in the range from 53 to 95 per cent. The average annual rainfall of the study area was 1260 mm. The hover flies and their associated floral crops were recorded from two ecosystems (agricultural and horticultural ecosystems).

2.2 Survey and Data Collection

The hover flies were surveyed by systematic transect method every week over two different seasons between 0800 - 1600 hrs. Following Sengupta et al. (2019b), Aerial net was commonly used to collect hover flies over the flowers from various case of field crops, vegetable crops, tree crops and weeds. Sampling was focused on active floral availability; in both ecosystems, multiple linear transects (10 meters) were established wherever flowering was present and hover flies were collected by walking along the transects at a constant speed, covering each transect over a period of 5 minutes. Twenty-five sweeps were made diagonally across each plot and samples were placed in separate plastic sachets (Rekha et al., 2009; Hussain et al., 2012). The floral availability data were recorded at weekly intervals for identification of plant species and their association with the hover flies. For each flowering plant specific parameters like floral colour, shape and blooming period were recorded to evaluate their influence on hover fly attraction and diversity. The adult hoverflies were photographed *in situ* with a DSLR camera (Canon 770d) for behavioural and morphological reference.

2.3. Specimen Preservation and Identification

The collected specimens were properly killed (using ethyl acetate), dried, properly pinned in insect boxes, and identified specimens were deposited in the insect repository of the Department of Agricultural Entomology, PAJANCOA and RI. The hover flies were sent to Mr S. S. Anooj, Assistant

Professor at Kerala Agricultural University, Kerala, for species-level identification.

2.4. Statistical Analysis

Data were analysed by using PAST version 5.1 (Hammer et al., 2001) and R 4.5 software (R Core Team, 2026). Diversity indices were calculated using PAST software. Plant - pollinator interaction (network, group and species level) was analysed using bipartite graph. Principal Coordinates Analysis (PCoA) applied to weekly abundance data, shows community composition over time. A ternary plot was used to illustrate the dominance of hover fly species over different time periods. A correlation heatmap was analysed to find out the relationship between hover flies and meteorological parameters *viz*., maximum and minimum temperature, relative humidity (RH), rainfall (RF), wind speed and sunshine.

3. RESULTS AND DISCUSSION

3.1 Relative Abundance of Hover Flies across Two Ecosystems

Hover flies were recorded from October 2024 to June 2025 in Agricultural and Horticultural ecosystems of PAJANCOA and RI (Table 1). The Agricultural ecosystem was represented by trees such as Badam and Jamun, and a diverse of weed flora including False buttonweed, Justicia, Sessile joyweed, Indian vicoa, Coatbuttons, Flat-stemmed buttonweed, and Indian mallow. In contrast the Horticultural ecosystem characterized by crops such as Bittergourd, Chilly, and Brinjal, as well as trees like Mango, Sandal wood and Yellow bell, accompanied by various weed plants. A total of 10 species were collected from the agricultural ecosystem, and nine species from the Horticultural ecosystem belonged to 10 genera and three subfamilies (Figure 2). In the agricultural ecosystem, the hover fly subfamily Eristalinae (57%) dominated, followed by Syrphinae (43%), whereas Syrphinae (57%) was the most abundant subfamily in the horticultural ecosystem followed by Eristalinae (43%). The taxonomic composition of the current study aligns with the findings of Maruthadurai and Anooj (2024) who reported a total of 19 hover fly species which belonged to two subfamilies (Eristalinae and Syrphinae) in Goa. *Mesembrius quadrivittatus* was most abundant with 37.66 % in agricultural ecosystem. In contrast *Paragus crenulatus* (37.18 %) was most dominant species in horti ecosystem. Mondal et al., (2026) reported a total of 7



Figure 1. Location map of study area: India (left), Karaikal (middle), PAJANCOA and RI (Right) (Source: Google Earth Pro, 2026)

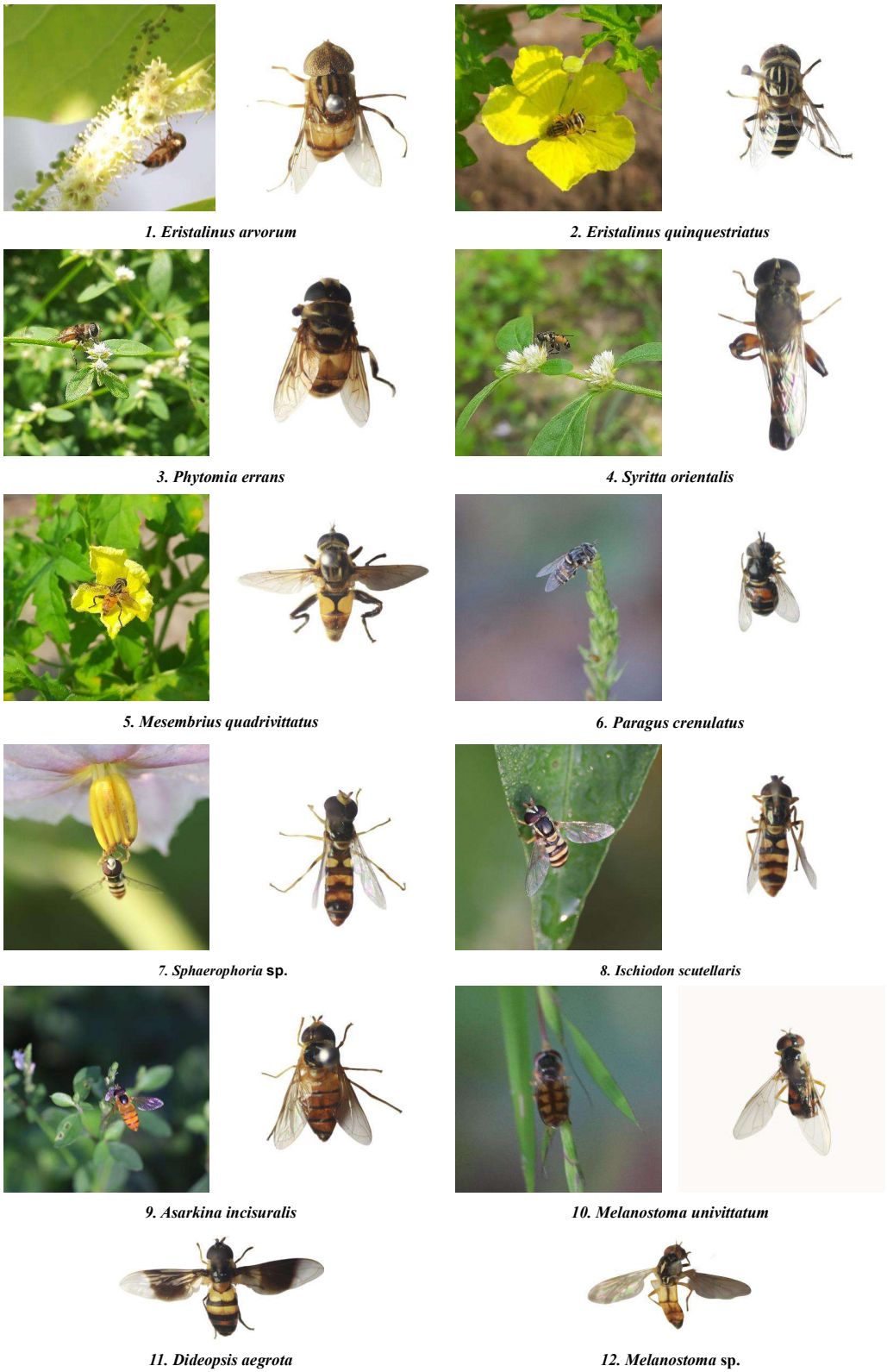


Figure 2. Diversity of hover flies in PAJANCOA and RI, Karaikal. Specimens 1- 6, 8-10 and 12 were recorded from the agricultural ecosystem, while 1- 8 and 11 were recorded in the horticultural ecosystem

hover fly species from rural and suburban areas of West Bengal, two species such as *E. arvorum* and *I. scutellaris* that were also recorded in the present study. Hover fly mostly foraged on Sessile joy weed (*Alternanthera sessilis)* (6 species) in both ecosystems followed by Justicia (*Justicia* sp.) and Mango (*Mangifera indica*) flowers each with five species. The sessile joy weed flowering season present from November to February which supported the population. The flies preferred majorly white flowers (6 species) followed by yellow flowers (5 species) and attracted to star shaped flowers like Chilly, brinjal (*Solanum melongena).* This finding correlated with Klecka et al. (2018) study where syrphids mostly preferred yellow and white flowers dominantly. The hover flies pollinate economic valuable crops like mango, brinjal, chilly, bitter gourd (*Momordica charantia)*, jamun (*Syzygium cumini*) and sandal wood (*Santalum album)* (Table 2). In comparing temporal dynamics of hover flies in two ecosystems, showed that horticultural ecosystem showed early hover fly activities (4th - 8th Standard week (SW)) and the highest abundance recorded during sixth week, dominated by *E. arvorum.* In agricultural ecosystem abundance peaked during eighth standard week dominated by *P. crenulatus* (Figure 3). Similarly, Budhathoki et al. (2021) reported that hover flies were most abundant during the spring season (March - May), while they were recorded less during the winter season (December - February), mainly associated with flowering plants and the aphid population in Nepal. Kanher et al. (2021) recorded the largest number of hover fly individuals in the month of March (128), lowest during summer (May) and winter (December) in Pakistan, which aligns with the present study.

3.2. Biodiversity Indices of Hover Flies

A total of 702 individuals belonging to 9 species were collected from the horticultural ecosystem, and 393 individuals belonging to 10 species were collected from the agricultural ecosystem. The comparative analysis of hover flies across ecosystems showed that the horticultural ecosystem showed more species diversity, evenness and less dominance when compared to the agricultural ecosystem (Shannon: 1.70; 1.58; Evenness: 0.61; 0.48; Simpson: 0.77;0.72) (Table 3). Similar to the present finding, Thangjam et al. (2019) reported that the horticultural ecosystem recorded the greater diversity index and evenness (2.84 and 0.95) when compared to the agricultural ecosystem (2.52 and 0.87) in Assam. Naderloo and Rad (2014), recorded the highest hover fly richness during the

Table 1. Inventory hover flies and associated floral resources

Species name	Agri	Horti	Individual RA (%)		Subfamily	Subfamily RA (%)		Associated flowering plants
			Agri	Horti		Agri	Horti	
Eristalinus arvorum Fabricius	+	+	3.82	15.67	Eristalinae	57	43	Mango, Sandal wood tree, Badam tree, Sessile joyweed, Jamun tree
Eristalinus quinquestriatus Fabricius	+	+	6.36	4.13				Bitter gourd, Mango, Sandal wood tree, Badam tree, Sessile joyweed
Phytomia errans Fabricius	+	+	1.27	3.13				Mango, Sessile joyweed
Syritta orientalis Macquart	+	+	7.89	20.80				Sessile joyweed, Justicia
Mesembrius quadrivittatus Wiedemann	+	+	37.66	13.11				Coatbuttons, Bitter gourd, Sessile joyweed, Indian vicoa
Paragus crenulatus Thomson	+	+	34.61	37.18	Syrphinae	43	57	Flat-stemmed buttonweed, False buttonweed, Justicia, Sessile joyweed
Sphaerophoria sp.1	-	+	-	4.84				Brinjal
Ischiodon scutellaris Fabricius	+	+	0.76	0.57				Mango, Yellow bell, Chilly
Asarkina incisuralis Macquart	+	-	6.36	-				Justicia, Indian Mallow
Melanostoma univittatum Wiedemann	+	-	0.76	-				Justicia
Dideopsis aegrota Fabricius	-	+	-	0.57				Mango, Yellow bell
Melanostoma sp.1	+	-	0.51	-				Justicia

spring season. Their richness and diversity majorly coincide with the greatest blooming period and aphid incidence (McCravy et al., 2024). This structural pattern showed that diversified horticultural ecosystems provided complex habitat with different floral resources, which increased hover fly evenness when compared to and agricultural ecosystem (Heiniger et al., 2025).

3.3. Plant - Syrphid Interaction Dynamics

Plant-pollinator analysis was used to map the relationship between the hover flies and floral resources (Figure 4). Network-level plant-pollinator interaction

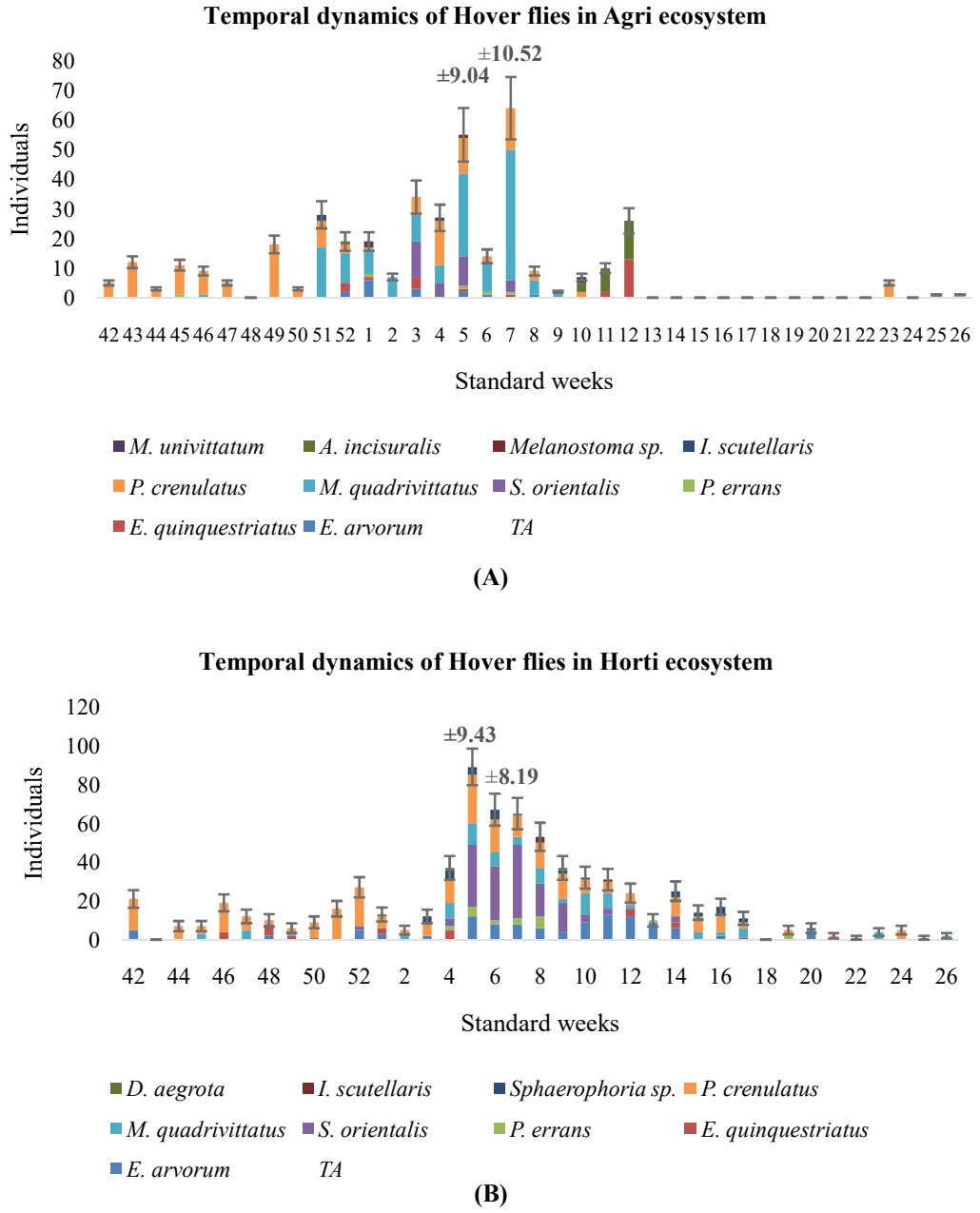


Figure 3. Temporal dynamics of hover flies in A) agricultural populations peak around standard week 7 and B) horticultural ecosystems peak around standard week 4. Column heights correspond to cumulative weekly collection totals. Error bars represent the calculated standard error (SE) of the total community abundance per standard week (SE= √Total Count), with manually annotated experimental peak variances highlighted over the main seasonal shifts

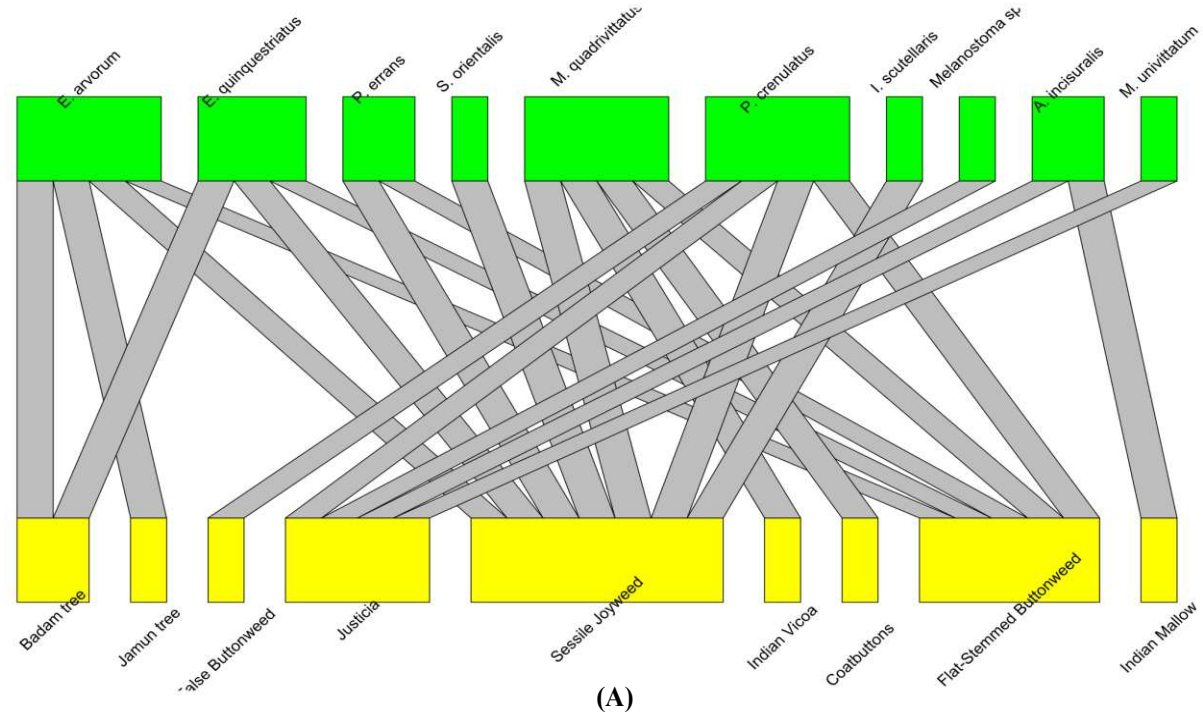
showed that connectance, linkage density and modularity are moderate in both ecosystems. Nestedness (29.36) and cluster coefficient (0.30) was higher in horticultural ecosystem when compared to agricultural ecosystem (18.42; 0.22) (Table 3). Klecka et al. (2018) noted a significant nestedness between syrphid and plant interactions, which indicated that specialist hover flies visit a subset of plants visited by generalist species. This showed that horticultural ecosystems are enriched by a diverse array of flowering crops, which tend to support more interconnected ecological

Table 2. Floral characteristics (Hover flies forage)

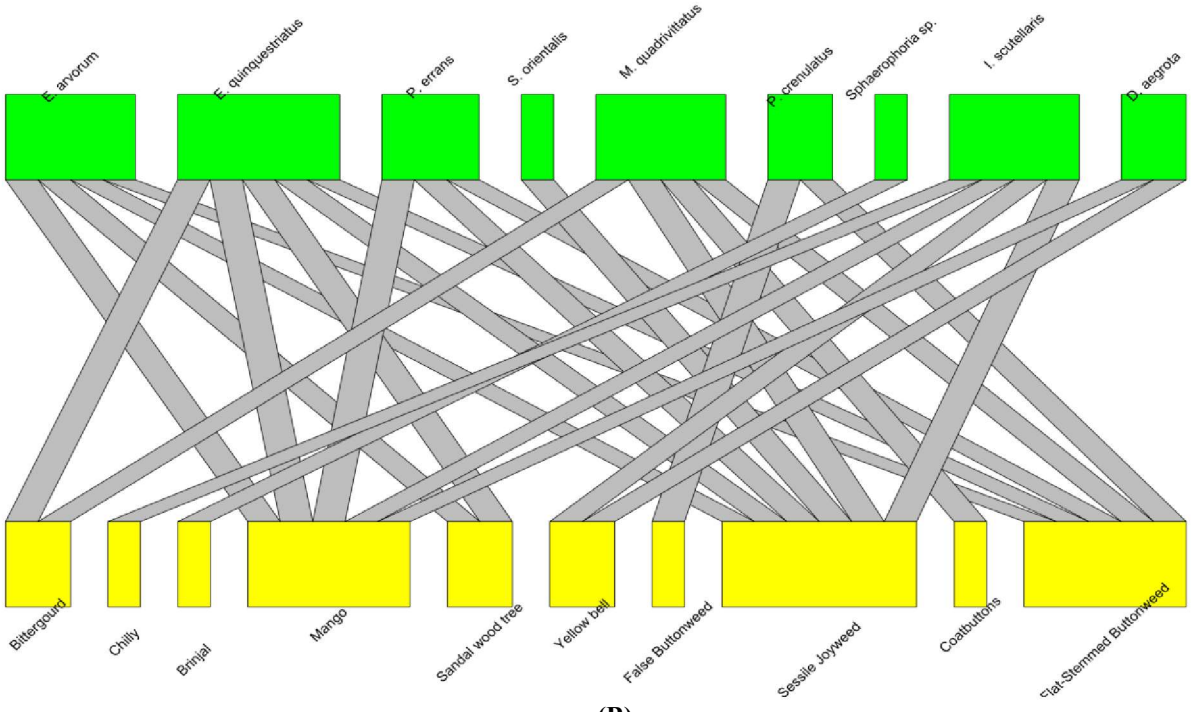
Common name	Scientific name	Family	Flower colour	Flower shape	Season
Mango	*Mangifera indica*	Anacardiaceae	Creamy	Panicle	Nov–Dec
Sandalwood tree	*Santalum album*	Santalaceae	Reddish	Cluster	Oct–Nov, March
Badam (Indian almond)	*Terminalia catappa*	Combretaceae	White	Raceme	March
Sessile joyweed	*Alternanthera sessilis*	Amaranthaceae	White	Spike	Dec–Feb
Jamun tree	*Syzygium cumini*	Myrtaceae	White	Panicle	April
Bitter gourd	*Momordica charantia*	Cucurbitaceae	Yellow	Funnel	March – April
Justicia	*Justicia* sp.	Acanthaceae	Purple	Tubular	Nov- Feb
Coatbuttons	*Tridax procumbens*	Asteraceae	Yellow	Daisy	Oct, Dec–Mar, May
Indian vicoa	*Vicoa indica*	Asteraceae	Yellow	Head	
Flat-stemmed buttonweed	*Oldenlandia* sp.	Rubiaceae	White	Star	Nov – Feb
False buttonweed	*Spermacoce* sp.	Rubiaceae	White	Cluster	Oct–Dec
Brinjal	*Solanum melongena*	Solanaceae	Purple	Star	April – May
Yellow bell	*Tecoma stans*	Bignoniaceae	Yellow	Trumpet	Oct–Nov, Feb, March, April
Chilly	*Capsicum annuum*	Solanaceae	White	Star	April – May
Indian Mallow	*Abutilon indicum*	Malvaceae	Yellow	Hibiscus	Nov- Dec

Table 3. Biodiversity indices of hover flies across ecosystems

Parameters	Agricultural ecosystem		Horticultural ecosystem	
Biodiversity indices				
Taxa_S (Richness)	10		9	
Individuals (Abundance)	393		702	
Simpson (1-D)	0.72		0.77	
Shannon (H')	1.58		1.70	
Evenness (e^H/S)	0.48		0.61	
Network level interaction				
Connectance	0.26		0.29	
Modularity Q	0.43		0.37	
Nestedness	18.42		29.36	
Cluster coefficient	0.22		0.30	
Linkage density	3.65		3.73	
Group level interaction	**HL (High level)**	**LL (Low level)**	**HL (High level)**	**LL (Low LEVEL)**
Mean number of links	3.00	4.30	3.54	3.92
Partner diversity	0.99	1.24	1.18	1.19
Niche overlap	0.34	0.20	0.31	0.21
Functional complementarity	11.76	13.59	14.63	16.36
Extinction slope	1.83	2.13	2.04	2.89



(A)



(B)

Figure 4. Plant - pollinator interaction networks. Comparison of species interaction in A) an agricultural ecosystem and B) horticultural ecosystem. Nodes indicate plant and pollinator species, lines indicate visitation frequency. The horticultural network exhibits significantly higher nestedness (29.36) than the agricultural ecosystem (18.42). This increased nestedness reflects a diverse array of flowering crops supporting interconnected networks where specialist hover flies visit plant subsets frequented by generalists, enhancing overall network stability

networks. This pattern was similar to the findings of Olsson et al. (2021), who observed that urban landscapes with higher floral diversity support more pollinator communities when compared to rural areas. This network structure aligns with the recent evidence showed that agricultural landscapes improved the floral distribution, boosting hover fly network integration and abundance (Requier et al*.,* 2023). Among pollinator assemblages, hover fly-flower interactions were higher where bee activity was less, thereby exhibiting their role in stabilising food webs. The present finding is in support of the fact that Deeksha et al. (2022) found that a cultivated land site has higher connectance (0.23) and Links per species (1.97) when compared to an uncultivated area. At the group level, Low-level (LL) hoverflies were more connected, with mean links of 4.30 in agricultural and 3.92 in horticultural ecosystems, compared to high-level (HL) hoverflies (Agri: 3.00; Horti: 3.54). HL hoverflies exhibit greater niche overlap (Agri: 0.34; Horti: 0.31) than LL hoverflies (Agri: 0.20; Horti: 0.21), which showed shared resources. This high level of resource sharing indicated functional redundancy, which protects the network against single-plant extinction within diverse communities (Grassi et al*.,* 2024). LL hover flies showed more functional complementarity (vulnerable to extinction) compared to HL hover flies (Table 3). At the species level, *M. quadrivittatus* showed higher strength and diversity in the agricultural ecosystem, while *E. quinquestriatus and I. scutellaris* showed higher degree and strength in the horticultural ecosystem. In the case of the plant network, sessile joyweed showed the highest strength in the agricultural ecosystem, while mango showed the highest degree in the horticultural ecosystem (Table 4 and 5).

3.4. Temporal Dynamics and Hover Fly Abundance

Temporal dynamics of hover fly showed that in agricultural ecosystem it is based on crop phenology which can be effectively used in pollination and biocontrol services while in horticultural, assemblage peaked with floral resource pulse (Figure. 5). More balance structure was recorded in horticultural ecosystem (PCoA1 = 28.83%, PCoA2 = 24.43%) when compared to agricultural ecosystem (PCoA1 = 35.56%, PCoA2 = 22.38%) . Li et al. (2023) reported that synchronisation of hover fly activity with the flowering period is important for maximising ecological services, as larvae provide effective pest suppression, while adults provide pollination services, providing a sustainable ecosystem. In both ecosystems, the earlier weeks showed tight clusters of generalist pollinators (42nd - 46th SW), during the middle weeks (47th - 18th SW) peak diversity was recorded, and during the later weeks (19th- 26th SW) the assemblage declined due to reduced floral resources. Budhathoki et al. (2021) recorded the principal component 1 (75.1%) due to floral resource richness and weather-related abundance, whereas principal component 2 (24.90%) reflected habitat specific differences. This seasonal trend demonstrated that agricultural ecosystem hover fly communities depend on short-blooming floral resources; the higher PCoA clustering in the

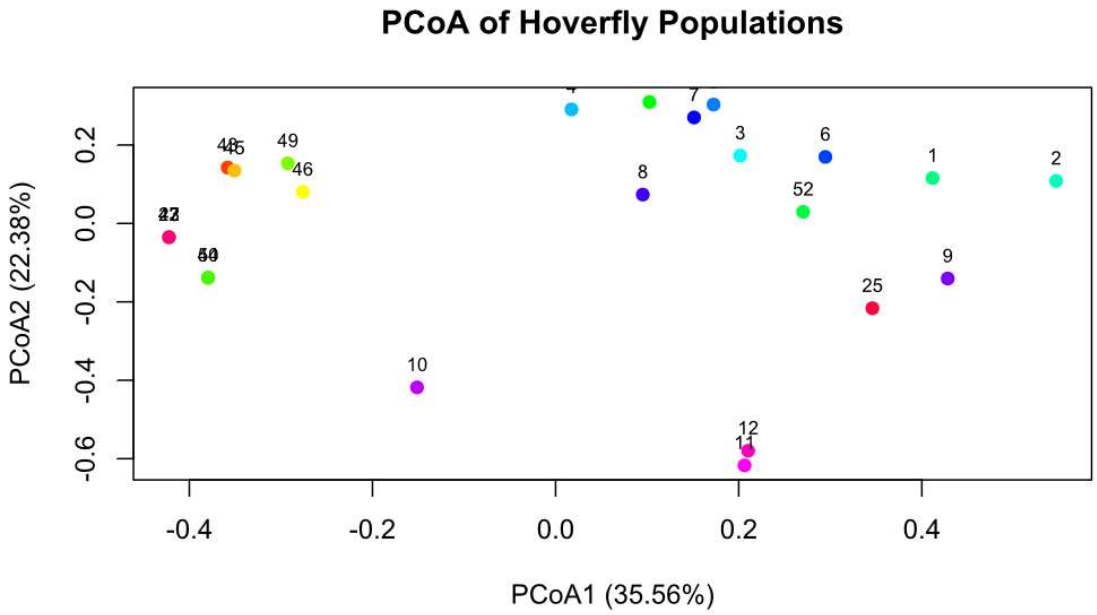
Table 4. Species-level interaction between two ecosystems (Hover flies – Higher level)

Species	Degree		Strength		Partner diversity		Betweenness		Nested rank	
	Agri	Horti	Agri	Horti	Agri	Horti	Agri	Horti	Agri	Horti
E. arvorum	4	4	1.84	1.07	1.39	1.39	0.00	0.27	0.00	0.13
E. quinquestriatus	3	5	0.84	1.57	1.10	1.61	0.00	0.27	0.33	0.00
P. errans	2	3	0.34	0.57	0.69	1.10	0.00	0.27	0.44	0.50
S. orientalis	1	1	0.14	0.17	0.00	0.00	0.00	0.00	0.67	0.88
M. quadrivittatus	4	4	2.34	1.87	1.39	1.39	0.00	0.10	0.11	0.25
P. crenulatus	4	2	1.59	1.20	1.39	0.69	1.00	0.00	0.22	0.63
I. scutellaris	1	4	0.14	1.87	0.00	1.39	0.00	0.10	0.78	0.38
Melanostoma sp.	1	–	0.25	–	0.00	–	0.00	–	0.89	–
A. incisuralis	2	–	1.25	–	0.69	–	0.00	–	0.56	–
M. univittatum	1	–	0.25	–	0.00	–	0.00	–	1.00	–
Sphaerophoria sp.	–	1	–	1.00	–	0.00	–	0.00	–	1.00
D. aegrota	–	2	–	0.70	–	0.69	–	0.00	–	0.75

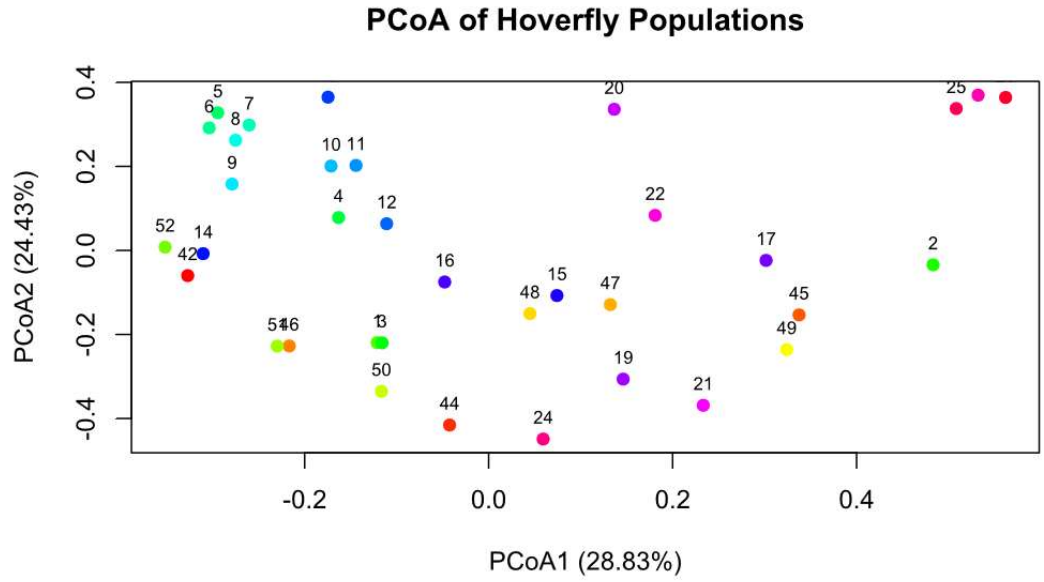
horticultural ecosystem reflected sustained floral resource availability. The mixed orchard designs and non-crop plants successfully bridged the seasonal food gaps, stabilizing the hover fly population over time (Herz et al., 2024)

3.5. Temporal Shifts in Species Dominance

Ternary plot showed the dominant species assemblage across weeks in both ecosystems (Figure 6). Temporal succession was observed in the agricultural ecosystem, with



(A)



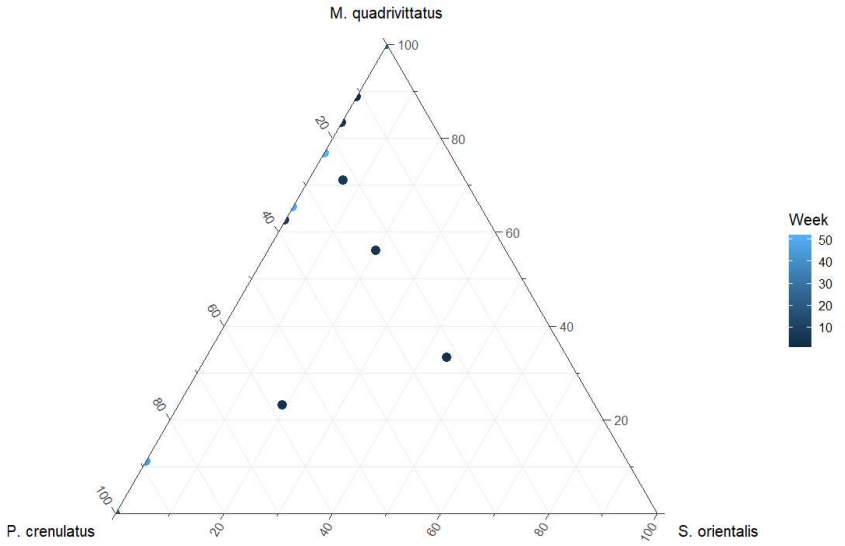
(B)

Figure 5. Temporal dynamics of hover fly communities. (A) Agricultural ecosystem dynamics driven by crop phenology, optimizing pollination and pest suppression services (PCoA1 = 35.56%, PCoA2 = 22.38%) and (B) Horticultural ecosystem dynamics showing a more balanced assemblage structure tied to seasonal floral pulses (PCoA1 = 28.83%, PCoA2 = 24.43%). Tight clustering shows a stable community, whereas wide dispersion indicates high species turnover

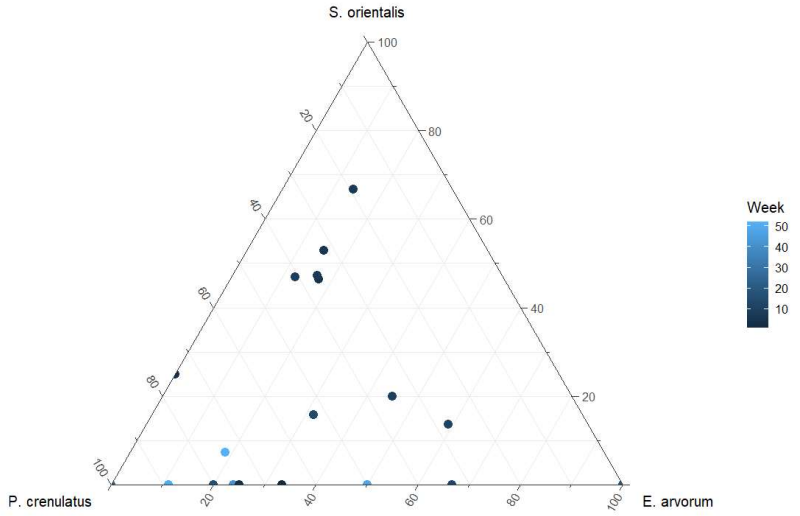
P. crenulatus persistent across different weeks; *M. quadrivittatus* was dominant in mid-season. In the horticultural ecosystem, structural succession was observed, where one species was replaced by another in a sequence; *S. orientalis* was dominant during mid-season, followed by *E. arvorum* and *P. crenulatus* was persistent across weeks. This variation showed that a complex horticultural ecosystem exhibited sequential species turnover, providing temporal niche partitioning which reduced interspecific competition among different species when compared to an agricultural ecosystem (Olsson et al., 2021).

3.6. Impact of weather factors on hover fly abundance

Hoverflies showed a strong positive correlation with humidity in the horticultural ecosystem when compared to the agricultural ecosystem, Morning RH (Horti: 0.54; Agri: 0.36)



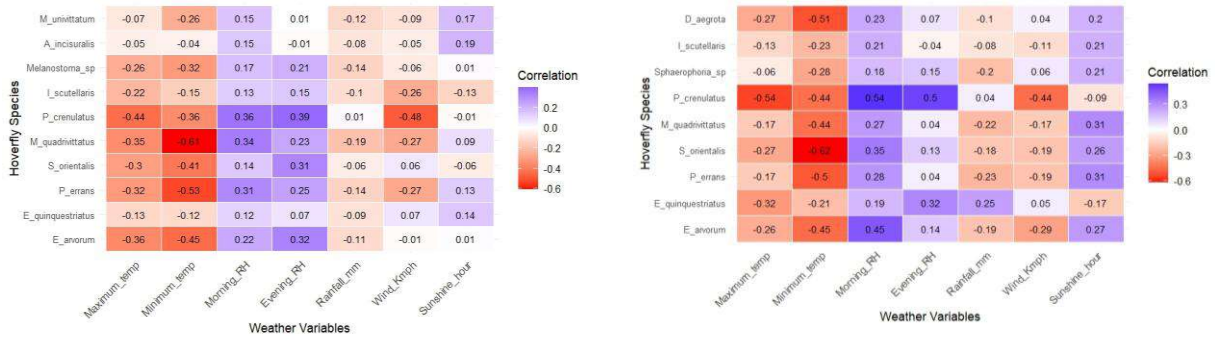
(A)



(B)

Figure 6. Distribution of dominant hover flies. Ternary plot represents the relative abundance of dominant Syrphid species across standard weeks in (A) agricultural and (B) horticultural ecosystems. The proximity of points in any vertex indicates higher dominance of the species

and Evening RH (Horti: 0.50; Agri: 0.39) (Figure 7). The activity increased with bright sunshine in the horticultural ecosystem. Most of the hover fly species showed a negative correlation with the Maximum temperature (Agri: -0.05 to -0.44; Horti: -0.06 to -0.54) and minimum temperature. Wind showed strong negatively correlation with the activity in agricultural ecosystem (-0.48). The rainfall does not impact the abundance hover flies. Similar to the above finding, Sanchez et al. (2022) reported that Eristalinus aeneus is active under a wide range of conditions, such as temperature, sunshine and relative humidity, in mango flowers. Ansari and Memon's (2017) study showed that hover fly abundance was negatively correlated with temperature, rainfall, relative humidity, and cloudiness. Understanding their relationship with weather parameters is an important tool for effective pest control and yield stability.



(A) **(B)**

Figure 7. Correlation heatmap between hover fly abundance and weather parameters in A) agricultural ecosystem and B) horticultural ecosystem (n = 37). The colour intensity and scale represent the positive (blue) and negative (Red) correlation coefficient (r). In the agricultural ecosystem (A), highly significant negative correlations were observed between minimum temperature and *M. quadrivittatus* (r = -0.61, p < 0.001), *P. errans* (r = -0.53, p < 0.001), and *E. arvorum* (r = -0.45, p < 0.01). In the horticultural ecosystem (B), *S. orientalis* was highly negatively correlated with minimum temperature (r = -0.62, p < 0.001), while *P. crenulatus* exhibited highly significant correlations with maximum temperature (r = -0.54, p < 0.001) and morning relative humidity (r = 0.54, p < 0.001)

Table 5. Species-level interaction between two ecosystems (Plants – Lower level)

Species	Degree		Strength		Partner diversity		Betweenness		Nested rank	
	Agri	Horti	Agri	Horti	Agri	Horti	Agri	Horti	Agri	Horti
Badam tree	2	–	0.58	–	0.69	–	0.00	–	0.38	–
Jamun tree	1	–	0.25	–	0.00	0.00	0.00	0.00	0.50	–
False Buttonweed	1	1	0.25	0.50	0.00	0.00	0.00	0.00	0.63	0.89
Justicia	4	–	2.75	–	1.39	–	0.30	–	0.25	–
Sessile Joyweed	7	6	3.58	2.28	1.95	1.79	0.35	0.35	0.00	0.00
Indian Vicoa	1	–	0.25	–	0.00	–	0.00	–	0.75	–
Coatbuttons	1	1	0.25	0.25	0.00	0.00	0.00	0.00	0.88	1.00
Flat-Stemmed Buttonweed	5	5	1.58	1.53	1.61	1.61	0.35	0.40	0.13	0.22
Indian Mallow	1	–	0.50	–	0.00	–	0.00	–	1.00	–
Bittergourd	–	2	–	0.45	–	0.69	–	0.04	–	0.33
Chilly	–	1	–	0.25	–	0.00	–	0.00	–	0.67
Brinjal	–	1	–	1.00	–	0.00	–	0.00	–	0.78
Mango	–	5	–	1.53	–	1.61	–	0.21	–	0.11
Sandal wood tree	–	2	–	0.45	–	0.69	–	0.00	–	0.44
Yellow bell	–	2	–	0.75	–	0.69	–	0.00	–	0.56

4. CONCLUSION

Based on the findings of the present study, hover fly abundance is mainly influenced by floral resources and weather parameters. The horticultural ecosystem consisted of a robust plant-pollinator network when compared to the agricultural ecosystem, though both rely significantly on weed flora to sustain syrphid populations. Further surveys should be done to understand the life cycle and habitat preferences of the flies. Future research should utilize DNA bar coding to effectively map hover fly diversity. Applying the results to field management suggests that mass culturing of hover flies can provide dual benefits: natural aphid population suppression and enhanced pollination. Evaluating pollination efficiency in relation to temporal dynamics provides an accurate contribution of hover flies to the productivity of economic crops can be precisely measured. Furthermore, it is necessary to study the impact of insecticides on hover fly populations and how strategic landscape planning can help them move from wildflowers into field crops. Conservation of wild native flowers is essential to sustain the hover fly community, which in turn provides pollination services, leading to increased yield in economic crops and maintaining ecological balance.

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Credit authorship contribution statement (both authors)

T. Yuvasri: formal analysis, writing—original draft; **M. Kandibane:** Investigation, validation, reviewing.

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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.

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.

Data availability

The data will be made available on request.

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New Distributional Records of Six Species of Aquatic Hemiptera from West Bengal, India

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ABSTRACT: The diversity and distribution of aquatic Hemiptera in the southern part of West Bengal remain poorly documented despite of their ecological significance in freshwater ecosystems. Existing state records are largely restricted to the Himalayan and sub-Himalayan regions of northern West Bengal. In contrast, the southern part, comprising the Gangetic Plain Biogeographic Zone, remains poorly explored. To address this knowledge gap, the present study documented a total of six species of the aquatic Hemiptera belonging to five genera and four families from the southern West Bengal, India. All six species are reported for the first time from the state of West Bengal, and four of them are reported for the first time from the Gangetic Plain Biogeographic Zone. Additionally, one species is reported for the first time from mainland India, having previously been known only from the Andaman and Nicobar Islands. Detailed diagnostic characters of each species are provided to facilitate accurate identification, along with notes on their habitat and distribution. Additionally, color photographs of all recorded species and a distribution map depicting their occurrence across different states of India are provided.

Keywords: New records, Biogeographic Zone, Range extension, Hemiptera distribution, Freshwater insects.

1. INTRODUCTION

Aquatic and semi-aquatic Hemiptera, commonly known as water bugs, belong to the suborder Heteroptera of the order Hemiptera. Owing to their diverse ecological adaptations, aquatic Hemiptera constitute an important component of freshwater ecosystems. They play significant ecological roles as predators, scavengers, and prey, thereby contributing to the maintenance of aquatic food webs and ecosystem balance (Hazarika & Goswami, 2010). Several taxa are effective predators of mosquito larvae and have been recognized as potential biological control agents (Ohba & Nakasuji, 2006; Saha et al., 2007). Globally, approximately 4,000 species of aquatic and semi-aquatic Heteroptera have been described (Dudgeon, 1999; Spence & Andersen, 1994). In India, the fauna comprises 346 species belonging to 91 genera and 18 families (Dubey et al., 2025).

West Bengal, encompassing the Himalayan, sub-Himalayan, Gangetic Plain, and coastal biogeographic zones, provides a wide range of freshwater habitats that support a rich diversity of Aquatic Hemiptera. Basu et al. (2018) documented 61 species representing 34 genera and 14 families from the Himalayan and sub-Himalayan regions of the state. In southern West Bengal, Das et al. (2014) recorded 28 species belonging to 17 genera and 11 families from the Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah. Subsequently, Mitra et al. (2016) reported 18 species representing 14 genera and 7 families from the

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Sundarban Biosphere Reserve, while Chandra et al. (2020) documented 32 species under 16 genera and 10 families from the East Kolkata Wetlands. More recently, Khanra and Dash (2024) recorded 14 species belonging to nine genera and six families from the Mundeswari River.

In the present study, six species recorded newly for the state West Bengal. Detailed distributional information for each species, including their occurrence in different Indian states and countries outside India, is provided to enhance current knowledge of their geographic range and distribution patterns.

2. MATERIALS AND METHODS

Aquatic Hemiptera were collected from different aquatic habitats such as rivers, ponds, canals, and lakes in South Bengal using a hand-operated D-framed net. Collected specimens were preserved in 70% alcohol in glass vials and labelled with the date of collection, locality, geographic coordinates and name of collector. The morphological studies were carried out using a Leica M205A stereo binocular microscope. Specimens were identified using standard taxonomic literature available for the group. Male genitalia were dissected and examined for identification in accordance with the standard literature: Hungerford & Matsuda, 1958; Jehamalar et al., 2018; Nesemann & Sharma, 2013; Lansbury, 1973; Sareein et al., 2019. All the examined specimens have been deposited in the National Zoological Collection, Hemiptera Section, Zoological Survey of India, Kolkata, India.

3. RESULTS AND DISCUSSIONS

3.1. Taxonomy

Order: Hemiptera

Suborder: Heteroptera

Infraorder: Nepomorpha Popov, 1968

Family: Belostomatidae Leach, 1815

Genus: *Lethocerus* Mayr, 1853

3.1.1. *Lethocerus patruelis* (Stål, 1855)

Material examined: 1♂, 1♀: A big Pond, Chhandra, Hooghly, 22.832785°N, 87.895198°E, 11,v.2025 Coll: S. Khanra; 1 ex (♀), Press quarter lake, Jagacha, Santragachi, Howrah, 22.5918075°N, 88.2827053°, 16.x.2025, Coll: S. Khanra

Diagnostic characters: Body length of male 70-89 mm and female 78-92 mm. Body elongated and dorsoventrally flattened. Pronotum broad and wide, and its anterior margin slightly convex. Two light stripes on the pronotum narrow, and the area between the two light stripes densely covered with dark brown spots. Markings on the mesosternum and metasternum are pale blackish or absent. Claw of fore tarsomere long and slender, with the length ratio of the claw to the combined length of fore tarsomeres II and III approximately 1:2. Female genital segment terminating in two protuberances, without externally visible hairs, and spiracle VII positioned more distally in relation to the apex of the genital operculum. Ventral diverticulum of the male genitalia is widely separated, forming a distinct median space.

Distribution: India: As per Figure 1A

Elsewhere: Afghanistan, Albania, Bulgaria, Croatia, Italy, Greece, Iraq, Iran, Israel, Kuwait, Macedonia, Montenegro, Myanmar, Nepal, Pakistan, Romania, Serbia, Syria, Saudi Arabia, Thailand, Turkey, United Arab Emirates, Yemen,

Remarks: This species generally inhabits submerged vegetation in ponds and lakes. The species is predaceous in habit and exhibits effective camouflage within its hunting grounds, enabling it to ambush prey efficiently.

Family: Nepidae Latreille, 1802

Genus: *Cercotmetus* Amyot & Serville, 1843

3.1.2. *Cercotmetus brevipes* Montandon, 1909

Material examined: 1♀: Water body near rice field, Bhuta, West Midnapore, 22.571527°N, 87.770243°E, 21.x.2025, Coll: S. Khanra.

Diagnostic characters: Body length of female 36-39 mm, length of respiratory siphon 7.4-8 mm. Vertex elevated between the eyes but lacking a prominent tubercle. Interocular space about 1.5 times the width of an eye. Prothorax ventrally tricarinate, with central keel originating between fore coxa and becoming evenly rounded and raised posterior of the transverse groove; lateral keels on the prothorax slightly shorter than median keel. Anterior margin of mesosternum bearing a pair of small nodules. Mesosternum longitudinally rounded and lacking prominent keel. Metasternum with a distinct median keel. Fore coxa less than half as long as prothorax. Fore femur with a central tooth. The midfemur is clearly shorter than the prothorax. Membrane large, reaching about halfway along the sixth tergite, which is slightly expanded distally. Female genitalia not extending beyond the apex of abdomen.

Distribution: India: As per Figure1B

Remarks: This species is recorded for the first time from the Gangetic Plain biogeographic province. Previously, it was known only from the Western Ghats, Semi-arid, and North-

East biogeographic zones of India (Jaiswal & Banerjee, 2023). Among the known species of the genus, it is one of the smallest in terms of body size.

Infraorder: Gerromorpha

Family: Veliidae Amyot & Serville, 1843

Genus: *Neoalardus* Distant, 1912

3.1.3. *Neoalardus typicus* (Distant, 1903)

Material examined: 2♀: Pond in Narayan Pakuria, East Midnapore, 22.401408°N, 87.784099°E, 29.x.2025, Coll: S. Khanra.

Diagnostic characters: Body length 4.5-4.7 mm in males and 4.8-5.0 mm in females; body elongated and slender with long appendages. Entire body, including antennae, densely covered with a double layer of long, erect hairs. Head posteriorly produced, extended well behind posterior margin of eyes. Rostrum reaching the anterior margin of the mesocoxa. Antennae very long and slender. Pronotum broader than long, lateral margins subparallel along approximately three-fourths of their length and posteriorly rounded. Metanotum visible only on lateral side. Abdomen very long and elongated. Legs unmodified, male foreleg lacking grasping comb. Parameres long, symmetrical, upwardly curved, middle portion wide, bearing a distinct dorsal tooth, distal area tapering and apically truncate with ventrolateral margin rounded.

Distribution: India: As per Figure 1C

Elsewhere: Singapore, China, Malaysia.

Remarks: The species can be readily distinguished from its congeners by the absence of a grasping comb on the male protibia. In India, it has been recorded from only a few states, indicating a scattered and possibly localized distribution. Previously, the species was known only from the Semi-arid, North-East, and Island biogeographic zones (Jehamalar & Chandra, 2020). The present record represents its first report from the Gangetic Plain biogeographic province, thereby extending its known distribution in India.

Family Gerridae Leach, 1815

Genus: *Limnometra* Mayr, 1865

3.1.4. *Limnometra minuta* Mayr, 1865

Material examined: 2♂, 3♀: Muri Ganga Ghat, Bankimnagar, South 24 Pargana, West Bengal, 21.682210°N, 88.152124°E, 30. iv.2025, Coll: S. Khanra.

Diagnostic characters: Body length 7.2-7.6 mm in males, 7.8-8.5 in females. Body elongate and slender, pale yellow in colouration. Head longer than broad. Tylus nearly black. A pair of light brown longitudinal lines originates posterior and lateral to the tylus, converging anterior to the eye emarginations to form a median stripe. Another brown line runs along the inner margin of each eye and joins the mediolateral stripe anteriorly, forming a distinct M-shaped marking on the dorsal surface of the head. Antennae filiform, first and third segment sub-equal in length, and fourth segment longest and second segment shortest. Basal part of first antennal segment marked with a dark spot. Pronotum brownish yellow with a central median line and brown lateral stripes that become faint medially. Mesonotum with a brown fascia along the lateral margins. Legs pale yellow; mesocoxa without any spine-like projection. Sixth abdominal segment laterally produced into a distinct triangular flattened plate on each side.

Distribution: India: As per Figure 1D

Remarks: This species is primarily associated with lentic habitats and slow-flowing streams. Previously, it was known only from its type locality in the Nicobar Islands. The present record from Sagar Island, West Bengal, represents the first report of the species from mainland India. During the present study, specimens were collected from several irrigation canals on Sagar Island, where they were observed inhabiting slow-moving waters with marginal vegetation.

3.1.5. *Limnometra ciliata* Mayr, 1865

Material examined: 1♀, canal connected to Matla river, Gopalganj, South 24 pargana, 22.052426 °N, 88.603998°E, 26. iv.2025, coll: S. Khanra.; 2♂, 1 ♀: aquatic body connected to Bidyadhari river, Minakhan, Malancha, North 24 Pargana, 22.507151 N, 88.776650 E, 24. iv.2025, coll: S. Khanra.

Diagnostic characters: Body length 14.4-15.2 mm in male, 14.7-16 mm in female. Body dark brown in colour. Head bears a distinct black diamond-shaped longitudinal marking, and a conspicuous black fascia is present on the lateral sides of the head at the inner margin of the eye. Pronotum with a median brown line and its adjacent region yellowish. In both sexes, the middle femur possesses a short black dorsal line near the basal region. Middle femur and tibia fringed with cilia. Connexival spines extend beyond the tip of the abdomen; sternum VIII of the male is sublaterally with a V-shaped process.

Distribution: India: As per Figure 1E

Elsewhere: Thailand, Malaysia, Indonesia, Philippines.

Remarks: During collection, the species was observed moving swiftly across the surface of slow-moving canal water, where it was found co-occurring with *Limnometra fluviorum*. *Limnometra ciliata* Mayr, 1865 was previously known from the island region of India and was subsequently

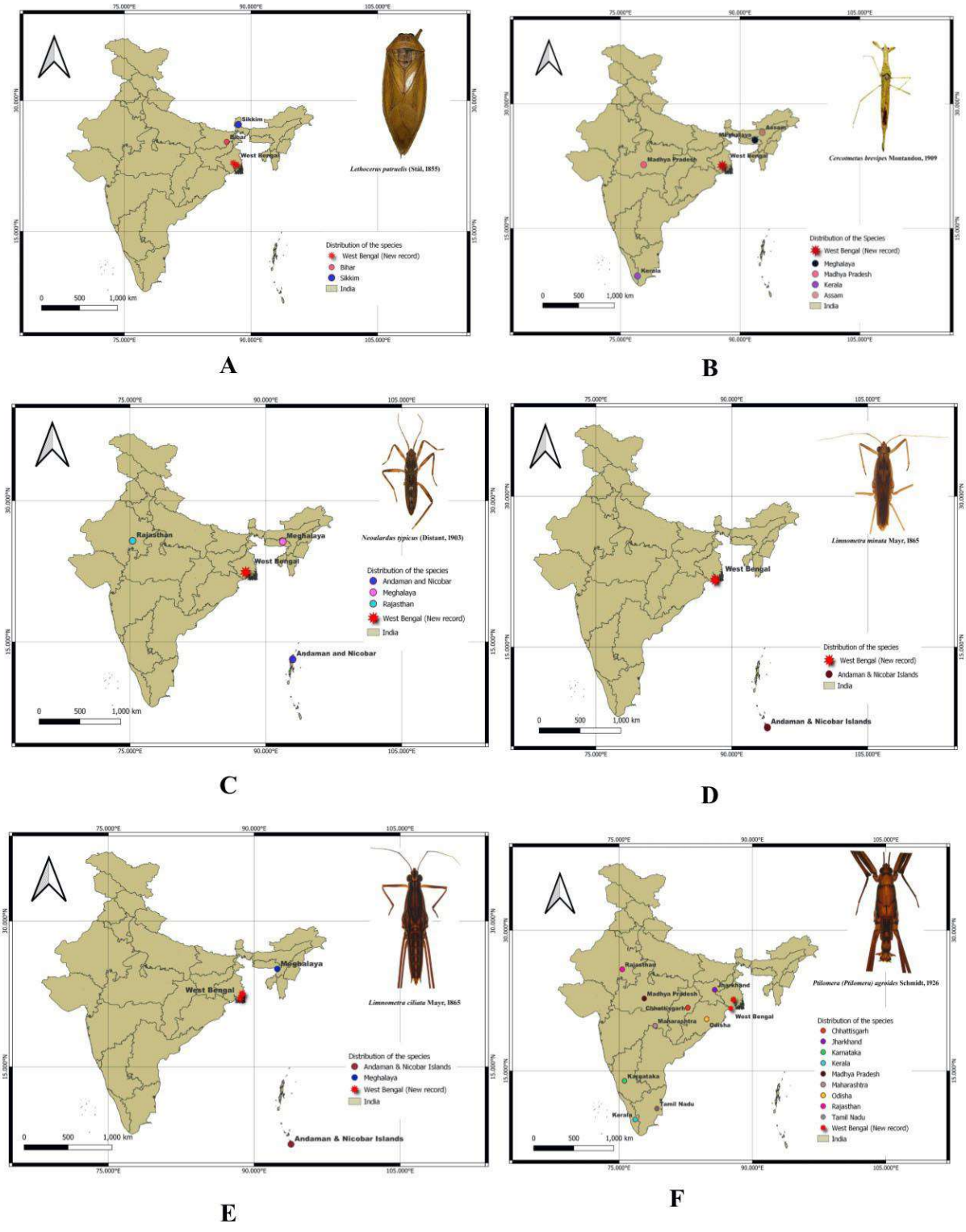


Figure 1. Distribution maps of six aquatic Hemiptera species newly recorded from West Bengal, based on previously published records and records obtained during the present study. A. *Lethocerus patruelis* (Stål, 1855), B. *Cercotmetus brevipes* Montandon, 1909, C. *Neoalardus typicus* (Distant, 1903), D. *Limnometra minuta* Mayr, 1865, E. *Limnometra ciliata* Mayr, 1865, F. *Ptilomera (Ptilomera) agroides* Schmidt, 1926

reported from Meghalaya, northeastern India, by Jehamalar and Chandra (2020), constituting its first record from mainland India. The present record from North and South 24 Parganas, West Bengal, therefore represents the second record of the species from mainland India and the first confirmed record from the Gangetic Plain biogeographic zone.

Genus: *Ptilometra* Amyot & Serville, 1843

3.1.6. *Ptilomera (Ptilomera) agroides* Schmidt, 1926

Material examined: 2♂, 1♀, Champa river, Nilpur, East Midnapore, 21.647037°N, 87.566392°E, 8.v.2025, Coll: S. Khanra.; 1♂, 3♀, canal connected to Rupnarayan river, Marokhana, Khanakul, Hooghly, 22.607255°N, 87.854666 °E, 12.v.2025, Coll: S. Khanra.

Diagnostic characters: Body length of male 17.3-18.5 mm, in female 15.7-16.3 mm. Body yellowish brown in colour. Apex of frontoclypes trilobed, with the antennae arising from the lateral lobes and the rostrum arising from the central lobe. Vertex convex, bearing a distinct oblique brown band extending to the upper inner margin of the eye. First antennal segment longer than combined length of 2nd to 4th antennal segments. Lateral margin of mesonotum and metanotum with silvery white fascia. Hind coxa provided with a distinct lateral spine. In males, the distal half of hind femur fringed with dense black setae, which gradually become sparse towards the proximal region; such setae absent in females. Male proctiger with a bisinuate posteromedian margin. A pair of stout, spinelike lateral processes of the pygophore extends distinctly beyond the lateral processes of the proctiger. In females, the connexival process is short and slightly surpasses the abdominal apex.

Distribution: India: As per Figure 1F

Remarks: Individuals were observed actively skimming the surface of running water, often accompanied by nymphs. Although this species is widespread and relatively common, no previous records are available from West Bengal. Its known distribution in India was previously restricted to the Deccan Plateau, Western Ghats, and Semi-arid biogeographic zones (Jehamalar et al*.,* 2018). The present record constitutes the first report from the Gangetic Plain biogeographic province and significantly extends its known range.

3.2. Key Insights

The present study significantly improves the understanding of the aquatic Hemiptera fauna of West Bengal by documenting six species as new records to the state, including four new records to the Gangetic Plain Biogeographic Zone. Additionally, *Limnometra minuta* (Mayr, 1865) recorded for the first time from mainland India, as it was previously reported only from Andaman and Nicobar Islands. These new records significantly expand the known distributional ranges and highlight the importance of riverine and floodplain systems of Southern West Bengal in supporting a diverse aquatic Hemiptera fauna. The updated distributional data for all the species reported along with their diagnostic features, coloured photographs and distributional maps provided herein constitute an important reference for future taxonomic and ecological studies.

4. CONCLUSION

The present study documented 6 previously unrecorded species of aquatic Hemiptera from West Bengal, thereby extending their known distribution range. Furthermore, one species previously known only from Andaman and Nicobar Islands represents new records for mainland India, suggesting a wider distribution than previously recognized. As most of the specimens were collected from canals connected to rivers, signifies the ecological importance of interconnected freshwater habitats.

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CRediT authorship contribution statement

Susmita Khanra: Conceptualisation, Data collection, Writing, original draft, Formal analysis; **Swetapadma Dash:** Supervision, Reviewing and editing, Visualization.

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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.

Data availability statement

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

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Diversity and Indigenous Usage Pattern of Socio-economically Important Plants in the *Pinus gerardiana* Forests in Kinnaur District of Himachal Pradesh, Northwestern Himalaya, India

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ABSTRACT: In the Indian Himalayan region, the tribal inhabitants largely depend on wild plants to meet their livelihood needs. As wild plants are part of their sustenance, they have rich knowledge of their indigenous uses but require proper documentation for their long-term conservation. *Pinus gerardiana* Wall. ex D. Don is an important ecological, economic, and near-threatened species with a restricted distribution in the dry temperate region of the North-Western Himalaya, mainly in the district of Kinnaur, Himachal Pradesh. The Kinnaura tribal communities of the area are highly dependent on Chilgoza pine forests for various daily livelihood needs, including fodder, fuel, timber, medicines, and spices. Hence, the present study was conducted to document the diversity and indigenous usage patterns of socio-economically important plants occurring in the *P. gerardiana* forests in Kinnaur district, and recorded 148 species, of which 62 were native to the Himalayan region and 20 were native to the Himalayan region and other biogeographical regions. *Angelica glauca*, *Dactylorhiza hatagirea, Ephedra gerardiana, Arnebia euchroma*, *Berberis aristata*, *Dioscorea deltoidea*, *Betula utilis, Hyoscyamus niger, Hyssopus officinalis, Bunium persicum*, *Juniperus communis* and *Rheum australe* are threatened. However, species such as *Prunus mira, P. armeniaca, Bunium persicum, Carum carvi, Hippophae* spp., *Elaeagnus parviflora, Juglans regia,* and *Morchella esculenta* showed considerable economic potential. Therefore, the adoption of integrated conservation measures, sustainable resource utilization practices, and community-based awareness programmes is crucial for safeguarding the biodiversity, ecological integrity, and long-term availability of these valuable Himalayan plant resources.

Keywords: Diversity, Forests, Indigenous use, Kinnaura tribes, *Pinus gerardiana*, Socio-economically important plants.

1. INTRODUCTION

The Indian Himalayan region (IHR) is one of the major repositories of biodiversity. The distinct topography of the IHR contributes to its unique ecosystems and forest types (Singh & Singh 1992). Tropical, sub-tropical, temperate, dry temperate, subalpine, and alpine are the major vegetation types (Samant et al., 1998). This rich floristic diversity of IHR has been utilised by the local inhabitants in many different ways. Currently, in the IHR, anthropogenic pressures and natural calamities have led to the degradation of natural habitats, placing many species on the verge of extinction (Pandit et al., 2007). Thus, the proper documentation and conservation of biodiversity is crucial for the proper functioning of ecosystems, and for the maintenance of environmental services they provide (Parthipan et al., 2016).

Pines dominate vast natural forests of the world (Richardson et al., 2007). The distribution of *P. gerardiana* (Chilgoza Pine) is quite sparse in the world, and it prefers to grow in the dry temperate forests in Tibet, India, Afghanistan, Baluchistan and Pakistan, at altitudes ranging

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from 1800-3300 m (Kumar et al., 2013). In India, natural populations of the Chilgoza pine are restricted to certain areas (the Kinnaur and Chamba districts of Himachal Pradesh and the Kishtwar district of Jammu & Kashmir) of the North-Western Himalaya. Among the different forest types available in the district of Kinnaur, Chilgoza pine forests are a major forest subtype (Dogra, 1964; Chib, 1978). Chilgoza pine forests harbour rich plant diversity, being utilized by tribal communities in a variety of ways. Currently, due to the conversion of Chilgoza pine forest to agricultural land, developmental activities, excessive seed cone harvesting, overgrazing, forest fires, and over-exploitation for firewood and unsustainable collection of needles, not only the population of the Chilgoza pine but also associated flora is quickly declining (Lata et al., 2020).

The review of available literature indicates that only a limited number of studies have been conducted on the ecology, vegetation structure and dynamics, regeneration constraints, ecological niche modelling, genetic diversity, socioeconomics, and marketing efficiency of Chilgoza pine (Ahmed et al., 1991; Kumar et al., 2016; Vipasha et al., 2018; Kumar et al., 2022; Paul et al., 2023; Kumar et al., 2025; Ahmadzai et al., 2026; Kumar et al., 2026). However, no comprehensive study has yet been conducted on the diversity and indigenous usage patterns of socio-economically important plant species in Chilgoza pine forests.

Despite the recognized ecological significance of *P. gerardiana* and its importance for sustaining local livelihoods, the associated plant diversity and its utilization by indigenous communities have not yet been systematically documented. Therefore, this study aims to enhance understanding of the dependence of local communities on the plant resources associated with *P. gerardiana* forests by generating baseline information on the diversity, indigenous knowledge, and utilization patterns of socio-economically important plant species occurring within these ecosystems.

2. MATERIALS AND METHODS

2.1. Study Sites

Extensive field surveys conducted in 56 natural populations of *P. gerardiana* in Moorang Forest Range (Moorang, Rarang-1, Rarang-II, Rarang-III, Ribba-1, Ribba-II, Ribba-III, Rispa-1, Rispa-II, Skiba, Akpa, Thangi, Lippa-1, Lippa-II, Lamber, Jhangi-1, Jhangi-II), Kalpa Forest Range (Powari-I, Powari-II, Powari-III, Shudarang, Telangi, Barang-I, Barang-II, Barang-III, Kalpa-I, Kalpa-II, Pangi, Tangling-I, Tangling-II, Purbani, Meeru-I, Meeru-II, Yulla, Urni, Chagaon, Janakpuri, Rarang), Pooh Forest Range (Dubling-I, Dubling-II, Ropa, Gyabung, Karla, Labrang, Kannam), Kilba Forest Range (Kilba-I, Kilba-II, Sapni, Ralli, Brua, Lingni, Kuppa-I, Kuppa-II, Karcham, Punang) and Bhabanagar Forest Range (Jani) of Kinnaur Forest Division of Himachal Pradesh (Figure 1).

2.2. Data Collection

For information on the diversity and indigenous usage patterns of socio-economically important plants occurring in the *P. gerardiana* forests, oral interviews, personal observations, and conversations with locals were used. A total of 514 respondents from 39 villages were interviewed during the years 2021 to 2025 (Table1). Prior informed consent was sought from the inhabitants of the area where the research was to be conducted by explaining the study's purpose to them. The locals verbally consented to the transmission of their traditional wisdom (Martin 1995; Collins et al., 2006). In each village, knowledgeable individuals were interviewed using semi-structured questionnaires. The knowledgeable tribal people were those who had been using wild plant resources for the last 30-40 years. The documented ethnobotanical information was further validated through group discussions in different villages and by comparing responses across different villages. In addition, the recorded ethnobotanical information was corroborated with relevant published literature wherever applicable.

2.3. Data Analysis

Samples of the species were also collected from each site and identified with the help of available floras (Chowdhery & Wadhwa, 1984; Polunin & Stainton 1984; Dhaliwal & Sharma 1999; Singh & Rawat 2000), the herbarium in ICFRE-Himalayan Forest Research Institute, Shimla, and other published literature. All identified species were listed and analyzed for diversity following Samant et al. (1998). Index Kewensis and The World Flora Online were followed for nomenclature and nativity (Anonymous 1885). Native species were those that originated or were first reported in the Himalayan region.

3. RESULTS AND DISCUSSION

In the present study, a total of 148 socio-economically important species belonging to 120 genera and 55 families have been recorded from the Chilgoza forests of the district

Table 1. Details of surveyed households and villages in Himachal Pradesh

Forest division	Forest range	Villages (48)	Household (622)	Elevation (m)	Latitude (N)	Longitude (E)
Kinnaur	Kalpa	Barang	21	2500	31°30'18.80"	78° 16' 3.90"
		Chagoan	12	1750	31°30'56.19"	78° 6'35.03"
		Janakpuri	12	2104	31°31'19.84"	78° 6'8.76"
		Kalpa	18	2235	31°32'15.21"	78°16'30.67"
		Meeru	11	2500	31°31'58.81"	78° 8'31.68"
		Pangi	16	2700	31°35'21.10"	78°16'39.04"
		Powari	10	2050	31°31'16.06"	78°16'18.41"
		Purbani	10	2400	31°35'18.62"	78°18'1.04"
		Runang	13	2230	31°31'16.55"	78° 9'7.80"
		Shudarang	12	2550	31°31'21.27"	78°15'27.24"
		Tangling	13	2240	31°31'47.59"	78°17'25.16"
		Telangi	16	2340	31°33'23.25"	78°16'23.67"
		Urni	10	2690	31°31'52.98"	78° 7'30.29"
		Yulla	14	2445	31°32'53.80"	78° 8'21.91"
	Moorang	Akpa	15	2370	31°35'7.32"	78°23'18.53"
		Jangi	14	2690	31°36'34.01"	78°25'44.37"
		Lamber	15	3017	31°30'55.24"	78°30'2.73"
		Lippa	13	2690	31°39'31.44"	78°23'0.61"
		Moorang	10	2550	31°36'2.85"	78°26'58.62"
		Rarang	20	2715	31°36'7.81"	78°21'11.83"
		Ribba	12	2400	31°35'9.88"	78°21'56.63"
		Rispa	18	2400	31°34'34.34"	78°25'36.76"
		Skibba	10	2800	31°34'21.09"	78°23'13.39"
		Thangi	10	2840	31°33'21.42"	78°28'50.46"
	Kilba	Brua	20	2255	31°27'52.28"	78°10'39.99"
		Karcham	10	1830	31°30'3.96"	78°10'33.05"
		Kilba	10	1950	31°30'53.49"	78° 8'37.00"
		Kuppa	10	2650	31°26'8.09"	78°14'41.17"
		Lingni	10	1935	31° 30"38.9'	78° 07"16.8'
		Punang	12	1975	31°30'44.55"	78° 6'8.17"
		Rali	10	1950	31°29'45.16"	78°12'43.25"
		Sapni	21	2470	31°29'6.01"	78°10'14.51"
	Pooh	Dubling	15	2800	31°44'38.97	78°37'51.62"
		Giabong	11	2890	31°46'23.98"	78°26'43.26"
		Kanam	15	2750	31°40'31.72"	78°27'3.71"
		Karla	10	2800	31°39'56.81"	78°26'4.49"
		Labrang	14	2900	31°40'41.10"	78°26'25.55
		Ropa	13	3090	31°47'49.77"	78°25'18.84"
	Bhawanagar	Jani	8	2200	31°30'57.06"	78° 4'46.05"

of Kinnaur. Out of 148 socio-economically important species (Food= 56; Medicinal= 107; Fodder=42; Fuel=33; Timber=05; Spice=05; Tannin=02; Religious=23; Agricultural tools=03; Household items=09; Manure=04), 138 species belong to Angiosperms, 09 species belong to Gymnosperms and 01 species belongs to Fungus (Figure 2). These species represented Trees (16 spp.), Shrubs (38 spp.), Herbs (85 spp.) Climbers (8 spp.) and Fungus (01). Among families, Rosaceae, Lamiaceae & Asteraceae and Apiaceae were found to be dominant families, represented by a maximum species (15 spp.), (12 spp. each), and (07 spp.), respectively, followed by Brassicaceae and Ericaceae (06 spp., each) and Cupressaceae, Pinaceae, Urticaceae, Elaeagnaceae, Caprifoliaceae, and Boraginaceae (03 spp., each).

Among the genera, *Rumex, Cotoneaster, Rosa, Rubus, Pinus,* and *Juniperus* (04 spp. each), and *Viola, Ephedra, Bergenia, Urtica, Prunus, Clematis, Artemisia, Impatiens, Lonicera, Chenopodium, Hippophae, Geranium,* and *Malva* (2 spp. each) were dominant genera. Of these, 62 species were native to the Himalayan region; 20 were native to the Himalayan region and other biogeographical regions collectively; and the remaining species were not native to the Himalayan region.

Allium stracheyi, Bupleurum falcatum, Carum carvi, *Chaerophyllum villosum, Echinops cornigerus, Tragopogon gracilis, Capparis spinosa, Nepeta longibracteata, N. eriostachya, Physochlaina praealta, Bergenia stracheyi, P.gerardiana* and *Myricaria elegans* (13 spp.) were endemic and *Heracleum candicans, Arisaema jacquemontii, Cirsium wallichii* var. *glabratum, Impatiens sulcata, Berberis aristata, B. lycium, Alnus nitida, Codonopsis ovata, Hippophae salicifolia, Ribes alpestre, Juglans regia, Reinwardtia indica, Dactylorhiza hatagirea, Rheum australe, Cotoneaster bacillaris, Rosa macrophylla, R. webbiana, Viburnum cotinifolium, Cedrus deodara, Pinus roxburghii, P. wallichiana, Ephedra gerardiana* (22 spp.) were near-endemic to the Himalaya (Table 2). Representative photographs of socioeconomically important plant species found in the P. gerardiana forests of the Kinnaur district are presented in Figure 3.

As per the IUCN Red List of Threatened Species, *Angelica glauca* and *Dactylorhiza hatagirea* are Endangered (Ved et al., 2015; Chauhan 2022); *Ephedra gerardiana* is Vulnerable (Roland 2020); *Pinus gerardiana* is Near Threatened (Farjon 2013); *Arisaema jacquemontii, Berberis aristata, B. lycium, Alnus nitida, Betula utilis, Capparis spinosa, Silene vulgaris, Desmodium elegans,*

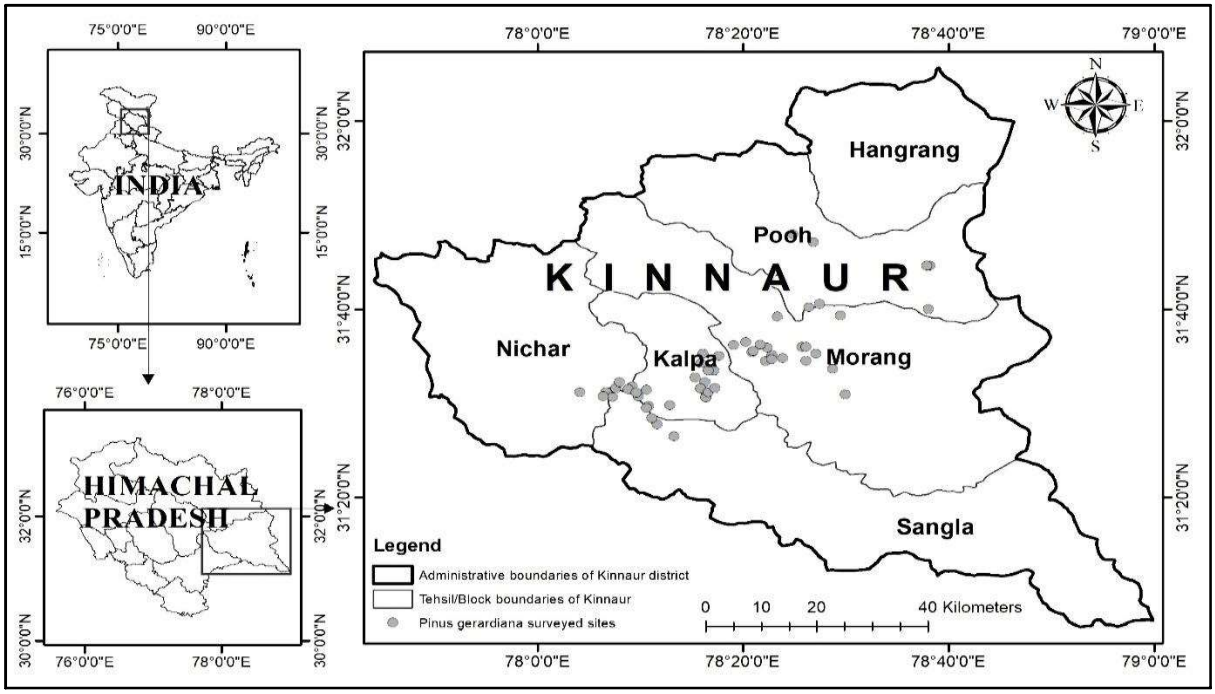


Figure 1. Surveyed sites of *Pinus gerardiana* in Kinnaur district. The graph represents surveyed villages in various tehsils/blocks of Kinnaur district of Himachal Pradesh

Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
ANGIOSPERMS							
Acanthaceae							
Dicliptera bupleuroides Nees	-	H	1800-2500	Ind Or	Leaves	Medicinal, Fodder	Leaf powder used to cure leucorrhoea and cough. Also used as fodder.
Amaryllidaceae							
Allium stracheyi Baker**	Kuthae, Pyaze	H	1900-2800	Ind Or	Leaves	Food, Spice	Leaves used as a vegetable, and dried leaves used as a spice.
Amaranthaceae							
Amaranthus spinosus L.	Jungli dankar	H	1800-2500	Reg Trop	Leaves	Food, Medicinal, Fodder	Leaves eaten as a vegetable to cure leucorrhoea and chicken pox. Also used as fodder.
Cyathula tomentosa Moq.	--	H	1800-2000	Reg Himal	Leaves	Medicinal, Fodder	Leaves used to cure eye problems. Also used as fodder.
Anacardiaceae							
Pistacia chinensis Bunge subsp. *integerrima* (J. L. Stewart ex Brandis) Rech. f.	Kakad-singhi	T	1800-2000	Egypt Persia Reg Himal	Galls	Medicinal	Powder of galls used to cure cough, vomiting, fever, skin diseases, leucorrhoea, asthma and loss of appetite.
Apiaceae							
Angelica glauca Edgew.	Chora, Sapal	H	1800-2600	Reg Himal	Roots	Medicinal, Spice	Roots used as spice and to cure stomach problems, fever, asthma and indigestion.
Bunium persicum B.Fedtsch	Kala-jeera	H	1800-2900	Persia	Seeds, Tubers, Leaves	Spice, Food, Medicinal	Decoction of the seeds used to cure stomachache, swelling, leucorrhea and urinary problems. Seeds and leaves used as spice. Tubers eaten raw or roasted.
Bupleurum falcatum L.**	Jungli jeera	H	2400-3300	Reg Himal	Seeds	Medicinal	Decoction of seeds used to cure stomach problems.
Carum carvi Linn**	Mako zeera	H	2600-3300	Europe As Bor	Seeds, Tubers, Leaves	Spice, Food, Medicinal	Decoction of the seeds used to cure stomachache, vomiting, leg pain, swelling, leucorrhea and urinary problems. Seeds and leaves used as spice. Tubers eaten raw or roasted.
Chaerophyllum villosum Wall. & DC**	--	H	1800-2700	Reg Himal	Roots	Medicinal, Fodder	Roots eaten to cures indigestion problem.
Ferula jaeschkeana Vatke	Kedmo, Sapal	H	1900-2600	Himal Bor Occ Turkestan	Roots, Twigs	Medicinal, Religious, Spice	Roots powder used as spice and to cure stomach ache, joint pain, backache, dysentery and vomiting. Twigs used for worshipping deities.
Heracleum candicans Wall. ex DC*	Poryal	H	2400-2800	Reg Himal Ind Or As Trop	Shoots, Roots	Food, Medicinal, Fodder	Tender shoots eaten with curd. Shoots chopped in small pieces and given to cows to enhance milk production. Root paste used to cure skin problems, arthritis and bleeding wounds.
Araceae							
Arisaema jacquemontii Blume*	Jamashang	H	1800-2000	Reg Himal	Tubers	Medicinal	Tubers used to cure fever, stomachache, swelling, pneumonia and chest congestion.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Araliaceae							
Hedera nepalensis K. Koch	Lacha	C	1800-2700	Reg Himal	Leaves	Medicinal, Religious	Leaves used in Shivratri festival and in purification ceremonies. Powder of leaves used to cure urinary problems.
Asteraceae							
Anaphalis triplinervis Sims ex C.B.Clarke	Kagzi uh	H	2900-3300	Reg Himal	Leaves	Medicinal	Used to cure chronic disease.
Artemisia maritima L.	Byur	S	2000-3000	Europe Reg Caucas Sibir	Leaves, Seeds, Roots, Twigs	Medicinal, Fodder, Fuel	Used to cure toothache, Jaundice and fever. Brooms of the plant used as to kill flea. Also used as fodder and fuel.
A. vestita Wall. ex Besser	Rang byur	S	2000-3000	Reg Himal China	Leaves	Medicinal, Fodder, Fuel	Leaves used to cure bad odour of under arms.
Bidens pilosa L.	--	H	1800-2800	Ind Occ Am Austr	Leaves	Medicinal, Fodder	Used to cure ear and eye problems. Used as fodder.
Cirsium wallichii var. *glabratum* (Hook. f.) Wendelbo*	Tee cho	H	1800-2900	Reg Himal	Roots	Food	Roots edible.
Cousinia thomsonii C.B.Clarke*	Tee Cho		2200-2400	Reg Himal	Roots	Food	Roots edible.
Echinops cornigerus DC. **	Cho	H	2200-2900	Reg Himal	Roots	Medicinal	Roots used to cure cough, cold and urinary troubles.
Lactuca dolichophylla Kitam.	--	H	2000-2700	Reg Himal	Leaves	Food, Fodder	Leaves eaten as vegetable. Also used as fodder.
Himalaiella heteromalla (D.Don) Raab-Straube	--	H	2200-2900	Reg Himal	Leaves	Medicinal	Leaf juice used to cure wounds.
Senecio laetus Edgew.	--	H	1800-2800	Reg Himal	Whole plant	Medicinal	Decoction of the plant used to cure fever and abdominal pain
Taraxacum officinale F.H.Wigg.	Kan	H	1800-3300	Reg Temp Bor et Austr	Leaves	Food, Medicinal, Fodder	Leaves eaten as vegetable. Decoction of root used as tonic and to cure kidney and blood related problems. Also used as fodder.
Tragopogon gracilis D.Don**	--	H	2400-3300	Reg Himal	Leaves	Medicinal	Leaf paste used for wound healing.
Balsaminaceae							
Impatiens micranthemum Edgew.	Tiktok	H	1800-2500	Reg Himal	Seeds	Fodder	Seeds edible. Also used as fodder.
I. sulcata Wall. *	Tiktok	H	1800-2600	Reg Himal	Seeds	Fodder	Seeds edible. Also used as fodder.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Berberidaceae							
Berberis aristata DC. *	Chutrum	S	1800-2800	Reg Himal	Fruits, Roots, Branches	Medicinal, Fodder, Fuel, Food	Fruits edible and decoction of roots used to cure eye problem. Branches used as fodder and fuel.
B. lycium Royle*	Chutrum	S	1800-2800	Reg Himal	Fruits, Roots, Branches	Medicinal, Fodder, Fuel, Food	Fruits edible and decoction of roots used to cure eye problems. Branches used as fodder and fuel.
Betulaceae							
Alnus nitida (Spach) Endl. *	Nyu	T	1800-2900	Reg Himal	Bark, Leaves, Twigs	Fodder, Fuel, Tannins	Tannins obtained from bark of the tree and used as writing ink. Wood used as fuel and leaves and twigs used as fodder.
Betula utilis D. Don	Bojpater, Pdh, Shag	T	3000-3300	Reg Himal Japan	Bark, Branches	Religious, Medicinal, Fuel, Fodder	Branches used for worshipping deities in festivals. Bark used in cremation ceremonies, roofing wooden traditional houses and in writing Buddhist scriptures. Decoction of bark used to cure urino-genital diseases, hysteria and earache. Also used as fuel and fodder.
Boraginaceae							
Arnebia euchroma (Royle) I.M.Johnst.	Rattanjot, Khome	H	2900-3300	Reg Himal Turkestan	Roots	Religious, Medicinal, Tannins	Roots used in Buddhist birth and death rituals as colouring agent known as 'Khome'. Roots mixed with mustard oil is used to cure hair fall.
Cynoglossum lanceolatum Forssk.	--	H	2400-2900	Arabia	Whole plant	Medicinal	Used as aphrodisiac.
Myostis sylvatica Ehrh. ex Hoffm.	--	H	2400-2900	Reg Bor Temp Oriens	Whole plant	Medicinal	Used to cure dysentery.
Brassicaceae							
Alliaria petiolata (M. Bieb.) Cavara & Grande	Shob, Sutral	H	1800-3300	Eurasia	Leaves	Food, Fodder	Leaves eaten as vegetable. Paste of leaves used to cure gangrene. Also used as fodder.
Arabidopsis thaliana (L.) Heynh.	Teskan	H	1800-2700	North America	Leaves	Food, Medicinal	Leaves eaten as vegetable and to cure stomach pain.
Capsella bursa-pastoris (L.) Medik.	Tiskan	H	1800-2600	Reg Temp	Leaves	Food, Fodder	Leaves eaten as vegetable.
Cardamine impatiens L.	--	H	1800-2400	Europe As	Leaves	Food, Fodder	Leaves eaten as vegetable.
Lepidium latifolium L.	--	H	1800-2400	Europe As Bor Oriens	Leaves	Food, Fodder	Leaves eaten as vegetable.
Nasturtium officinale R. Br	Bolku	H	1800-2900	Ind Or China	Leaves	Food	Leaves eaten as vegetable to cures cold and loss of appetite.
Campanulaceae							
Codonopsis ovata Benth. *	--	C	2400-2900	Reg Himal	Roots	Medicinal, Fodder	Roots edible. Decoction of roots used as aphrodisiac and to cure swollen joints and wound infections. Also used as fodder.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Cannabaceae							
Cannabis sativa L.	Sulfa, Bang	H	1800-2900	As Centr Reg Himal Bor Occ	Leaves	Medicinal	Leaves eaten as pakoras in Shivratri festival. Leaves also used for preparation of narcotic substance 'Charas'.
Humulus lupulus L.	Hopsa, Hung shuli	C	2400-2900	Europe Am Bor	Whole plant	Medicinal, Food	Used to cure hysteria. Also used in wine preparation.
Capparaceae							
Capparis spinosa L. **	Kakri	S	2600-3000	Reg Himal	Flowers, Fruits	Food, Medicinal	The flower buds and fruits edible and used as flavouring agent. Also used to cure earache, toothache, snake bite and to kill worms.
Caprifoliaceae							
Lonicera hypoleuca Decne.	--	S	1800-3000	Reg Himal	Branches	Fuel, Fodder	Used as fodder and fuel.
Lonicera quinquelocularis Hardw.	--	S	1800-3000	Reg Himal	Branches	Fuel, Fodder	Used as fodder and fuel.
Zabelia triflora (R.Br.) Makino	Bang	S	2400-2900	Reg Himal	Branches	Fuel, Agricultural tools, Religious	Branches used as fuel and for making walking sticks and handles of agricultural tools. In the Dakhrain festival, it is offered to deities.
Caryophyllaceae							
Cerastium cerastioides (L.) Britton	--	H	1800-2600	Reg Himal	Whole plant	Medicinal	Used to cure backache and body ache.
Silene vulgaris (Moench) Garcke	Shoshorch	H	1800-2900	Europe, Temp Asia and Northern Africa	Leaves	Food, Fodder	Leaves eaten as vegetable. Also used as fodder.
Chenopodiaceae							
Chenopodium album L.	Bithu	H	1800-3300	Reg Temp et Trop	Leaves, Flowers	Food, Medicinal	Leaves eaten as vegetable and flowers used to cure stomach problems.
C. foliosum (Moench) Aschers	Bithu	H	1800-3300	Reg Bor et Austr Iran	Leaves	Medicinal	Leaves eaten to cure stomach ache.
Convolvulaceae							
Cuscuta reflexa Roxb.	Amar bel, Aakash bel	C	1800-2900	Ind Or	Whole plant	Medicinal	Decoction of the plant used to cure jaundice, pains in muscles, joints and paralysis.
Dioscorea deltoidea Wall.ex Griseb.	Singli-Mingli	C	1800-2700	As Trop	Tubers	Medicinal, Fodder	Tubers used to kill lice in hairs. Also used as fodder.
Dipsacaceae							
Dipsacus inermis Wall.	Tis kan	H	1800-2700	Reg Himal	Leaves	Food	Leaves eaten as vegetable. Also used as fodder.

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Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Elaeagnaceae							
Elaeagnus parvifolia Wall. ex. Royle	Ral	S	2000-2800	Japan	Fruits	Food	Fruits edible used to cure throat problem.
Hippophae salicifolia D.Don*	Surch, Surchu	S	2400-2800	Reg Himal	Fruits	Food, Medicinal, Fuel	Fruits edible and juice used to cure throat infection and cough. Dried fruits and leaves also used in preparation of tea. Also used as fuel, and as live fence of orchards.
H. rhamnoides L. spp. *turkestanica* Rousi	Surch, Surchu	S	2700-3300	Europe As Temp	Fruits	Food, Medicinal, Fuel	Fruits edible and juice used to cure throat infection, skin problems and cough. Dried fruits and leaves also used for preparation of tea. Also used as fuel, and as live fence of orchards.
Ericaceae							
Cicer microphyllum Royle ex Benth.	Jungli matar	H	2000-2800	Soongar Reg Himal Bor Occ	Seeds	Food	Seeds edible.
Colutea nepalensis Sims.	--	S	2100-3000	Reg Himal	Leaves, Twigs	Medicinal, Fuel, Fodder, Household items	Twigs used as fodder and for preparation of traditional baskets 'Kilta' used for translocation fruits and manure. Leaves used to cure fever. Also used as fuel.
Desmodium elegans DC.	Chamyar	S	2000-2900	China	Roots, Twigs	Medicinal, Fuel, Agricultural tools, Fodder, Household items	Twigs used as fodder and for preparation of traditional baskets 'Kilta' used for translocation fruits and manure. Branches used as fuel and for making handles of agricultural tools. Roots used to cure toothache, piles and scorpion-sting.
Indigofera heterantha Wall. ex Brandis	Kasting	S	2000-2900	Reg Himal	Flowers, Twigs	Food, Fodder, Household items	Flowers eaten as vegetable. Twigs used as fodder and for preparation of traditional baskets 'Kilta' used for translocation fruits and manure.
Lotus corniculatus L.	--	H	2200-2800	Europe	Flowers	Religious	Women wear it on caps in the Aasharang Ramdus festival.
Medicago falcata L.	--	H	1800-2700	Geront BorTemp	Leaves	Food, Fodder	Leaves eaten as vegetable. Also used as fodder.
Fagaceae							
Quercus ilex L.	Brey	T	1800-2800	Reg Mediterr Oriens	Sap, Bark, Branches, Leaves	Medicinal, Fodder, Fuel, Religious	Sap of stem used to cure stomach problem. Bark powder used to cure toothache. Branches used to keep away evil spirits. Also used as fodder and fuel.
Gentianaceae							
Gentiana leucomelaena Maxim.	--	H	2200-2900	Mongolia Tibet	Flowers	Medicinal	Flowers used in jaundice.
Geraniaceae							
Geranium pratense L.	--	H	2800-3300	Europe Asia	Flowers	Religious	Women wear it on caps in Aasharang Ramdus festival.
G. wallichianum D. Don ex Sw.	--	H	2400-2900	Reg Himal	Roots, Flowers	Medicinal	Roots used to cure gonorrhoea and eye problems. Women wear it on caps in Aasharang Ramdus festival.

Cont...

Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Grossulariaceae							
Ribes alpestre Wall. ex Decne.*	Cho soch	S	2800-3300	Europe Afr Bor Reg Himal China	Fruits	Food, Medicinal	Fruits edible and used to cure gastric trouble, vomiting and dysentery.
R. orientale Desf.	Cho soch	S	2800-3300	Reg Himal	Fruits	Food, Medicinal	Fruits are edible and used to cure gastric trouble, vomiting and dysentery.
Juglandaceae							
Juglans regia L. *	Ka	T	1800-2700	As Occ Reg Himal	Wood, Leaves, Bark	Medicinal, Religious, Timber, Fuel	Garlands made from kernels used in marriage, birth and death ceremonies. Wood used as timber and for preparation of palanquins of deities. Roots, bark and leaves used for brushing teeth. Also used as fuel.
Lamiaceae							
Clinopodium vulgare L.	--	H	2200-2800	Reg Bor Temp	Leaves	Medicinal	Decoction of leaves used to cure flatulence.
Hyssopus officinalis L.	Tyngu	H	2900-3300	Europe As Temp	Leaves and Flowers	Medicinal	Used in hysteria, asthma, earache, cough, cold and throat infection.
Isodon rugosus (Wall. ex Benth.) Codd	--	S	1800-2800	Reg Himal	Leaves	Medicinal	Used to cure stomachache.
Leucas lanata Benth.	--	H	2400-2900	Ind Or China	Leaves	Medicinal	Leaves are used to cure piles.
Mentha longifolia (L.) L.	Van tulsi	H	2000-2900	Reg Bor Temp	Leaves	Food, Medicinal	Leaves used for preparation of tea and to cure fever.
Micromeria biflora (Buch.-Ham. ex D.Don) Benth.	--	H	1800-3000	Ind or Arab Afr trop	Leaves	Medicinal	Leaves used to cure stomach pain.
Nepeta longibracteata Benth. **		H	2800-3300	Reg Himal	Leaves	Medicinal	Leaves use to cure stomachache.
N. eriostachya Benth. **	--	H	2000-3300	Reg Himal	Leaves	Medicinal	Leaves use to cure eye problems.
Origanum vulgare L.	Van ajwain	H	2000-3000	Europe As et Afr	Leaves	Food	Leaves used for preparation of tea and chutney.
Prunella vulgaris L.	--	H	2100-3000	Reg temp	Whole plant	Medicinal	Used in healing wound.
Salvia moorcroftiana Wall. ex Benth.	--	H	2200-2800	Reg Himal	Whole plant	Medicinal	Used in colic, dysentery and skin infections.
Thymus linearis Benth.	Van ajwain	H	1800-3300	Europe As et Afr Bor Hispan	Leaves	Food, Medicinal	Used for preparation of tea and chutney. Decoction used as tonic and to cure headache, asthma, cough, stomachache, hair fall and urine problem.

Cont...

Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Linaceae							
Reinwardtia indica Dumort.*	Basanti	S	1800-2200	Ind Or	Flowers	Food, Medicinal	Flowers eaten as vegetable and to cure cold and jaundice.
Malvaceae							
Malva neglecta Wallr	--	H	1800-2700	Europe As et Afr	Leaves	Edible, Fodder	Leaves eaten as a vegetable.
Malva verticillata L.	--	H	1800-2700	Europe As et Afr Bor	Leaves	Edible, Fodder	Leaves eaten as a vegetable.
Moraceae							
Ficus palmata Forssk.	Dudhi, Fegura	T	1800-2200	Ind or	Fruits, Twigs	Food, Medicinal, Fodder, Fuel	Young fruits and twigs are eaten as vegetables. Fruits used to cure indigestion, lung and bladder related problems. Also used as fodder and fuel.
Oleaceae							
Fraxinus xanthoxyloides (G.Don) Wall. ex A.DC.	Thum	T	2200-2700	Reg Himal Bor Occ	Leaves, Bark, Branches	Fodder, Fuel, Medicinal	Leaves and twigs used as fodder and branches used as fuel. Paste of leaves and bark used to cure bone fractures and skin problems.
Dactylorhiza hatagirea (D.Don.) Soo*	Salampanja, Hathpanja	H	2400-2900	Europe Afr Bor Oriens Reg Himal	Roots	Medicinal	Roots used as tonic, aphrodisiac, and to cure fever and weakness.
Oxalidaceae							
Oxalis corniculata L.	Khatti mithi	H	1800-3300	Amphig Temp et Trop	Leaves	Medicinal, Food	Leaves eaten raw and used for preparation of chutney.
Poaceae							
Arundo donax L.	Rajal	S	1800-2900	Reg Mediterr Oriens	Branches	Household items	Twigs used as fodder and for preparation of traditional baskets 'Kilta' used for translocation fruits and manure. Small twigs used for making writing pen for small kid.
Polygonaceae							
Fagopyrum acutatum Mansf. ex K.Hammer	Jungli phapra	H	2400-2800	Reg Himal China	Leaves	Fodder, Food, Medicinal	Leaves eaten as vegetable to cure diabetes, jaundice, obesity, kidney, urinary and intestinal problems.
Oxyria digyna (L.) Hill	Shupchu	H	2200-3300	Reg Bor Alp et Arct	Leaves	Food	Leaves eaten as vegetable.
Polygonum tortuosum D. Don	--	H	2800-3300	Reg Himal	Leaves	Medicinal	Leaves used to cure dysentery.
Rheum australe D.Don*	Archa, Arch	H	2800-3300	Reg Himal	Leaves, Roots	Medicinal	Leaves eaten as vegetable. Roots used to cure swelling and joint pain.
Rumex acetosa L.	--	H	2600-2800	Europe As Bor	Leaves	Medicinal	Leaves used to cure stomachache.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
R. hastatus D. Don	--	H	1800-3300	Reg Himal	Leaves	Fodder, Medicinal	Decoction of the leaves used to cure cuts and wounds and to stop bleeding and to relieve pain from nettle sting. It is also used as fodder.
R. nepalensis Spreng	Jungli Palak	H	1800-3000	As Occ Ind Or Malaya Afr Austr	Leaves	Food	Leaves eaten as vegetable.
Primulaceae							
Anagallis arvensis L.	--	H	1800-2900	Europe	Roots	Medicinal	Paste of the roots used for wound healing.
Androsace sarmentosa Wall.	--	H	2400-2800	Reg Himal	Whole Plant	Medicinal	Decoction of roots used as tonic.
Primula denticulata Sm.	Chukl uh, Phanting Uh		1800-3300	Reg Himal	Roots	Medicinal, Religious	Roots used to cure liver problem.
Plantaginaceae							
Plantago depressa Willd.	--	H	1800-2600	Sibir	Leaves	Food	Leaves eaten as a vegetable.
Ranunculaceae							
Caltha palustris L.	--	H	2700-3300	Reg Bor Temp et Arct	Leaves	Medicinal, Religious	Leaves used for washing hands and utensils. Women wear it on caps in Aasharang Ramdus festival.
Clematis grata Wall.	Hondag	C	1800-2800	Reg Himal China Afr trop	Flowers, Branches	Medicinal, Household items	Paste of the flowers used to cure itching. Branches used for preparation of traditional pan support known as 'Kila' for utensils.
Clematis montana Buch.-Ham. ex DC.	Hondag	C	2500-2900	Reg Himal	Leaves, Branches	Medicinal, Household items	Leaves used to cure skin problems. Branches used for preparation of traditional pan support known as 'Kila' for utensils.
Thalictrum foliolosum DC.	--	H	1800-2800	Reg Himal	Roots	Medicinal	Paste of roots used in jaundice.
Rosaceae							
Cerasus prostata (Labill.)	--	S	2800-3300	Reg Mediterr Oriens	Branches, Fruits	Food, Fuel	Fruits edible and branches used as fuel.
Cotoneaster bacillaris Wall. ex Lind*	--	S	2000-2800	Reg Himal	Fruits	Food	Fruits edible.
C. microphyllus Wall. ex Lindl	--	S	1800-2600	Reg Himal	Fruits	Food	Fruits edible.
C.nummularius Fisch. & C.A.Mey.	--	S	2400-2800	Afr Bor Oriens	Fruits	Food	Fruits edible.
Fragaria nubicola (Lindl. ex Hook.f.) Lacaita	Babashoch	H	1800-3000	Ind Or Sikkim	Fruits	Food	Fruits edible.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Prinsepia utilis Royle	Behkhal	S	1800-2400	Reg Himal	Fruits, Roots, Branches	Food, Medicinal, Religious, Fuel	Fruits edible and roots used to cure diarrhoea. Branches used in various traditional rituals. Also used as fuel.
Prunus armeniaca L.	Chul	T	1800-3000	Reg Caucas	Fruits, Seeds	Food, Medicinal, Fodder, Fuel	Fruits edible and used for preparation of traditional dish 'Chul phanting and Remo thukpa' and also for preparation of alcoholic beverage known as 'Chul rak' which is used to worshipping deities in festivals, marriage and birth ceremonies. Seeds used for extraction of oil known as 'Chul telang' which is used to cure joint pain, skin problems, eye and ear problems, body weakness, indigestion, loss of appetite, constipation, flatulence and swelling. Dried leaves used as winter fodder and wood used as fuel.
P. mira Koehne	Reg	T	2000-3000	China	Fruits, Seeds, Branches	Food, Medicinal, Fodder, Fuel, Religious	Fruits edible and used for preparation of traditional dish 'Remo thukpa' and also for preparation of alcoholic beverage known as 'Regu rak' which is used to worshipping deities in festivals, marriage and birth ceremonies. Seeds used for extraction of oil known as 'Reg telang' which is used to cure joint pain, skin problems, eye and ear problems, body weakness, indigestion, loss of appetite, constipation, flatulence and swelling. Branches used in Khepa festival for the preparation of traditional food 'Dhu'. Dried leaves used as fodder and wood used as fuel.
Pyrus pashia Buch.-Ham. ex D.Don	Kainth	T	1800-2600	Reg Himal	Fruits	Food, Fodder, Fuel	Fruits edible. Also used as fodder and fuel.
Rosa brunonii Lindl.	Yal uh	S	1800-2800	Reg Himal	Flowers, Twigs, Leaves	Religious, Medicinal	In Khepa festival locals hang the twigs on main door to keep away evil spirits. Locals also wear flowers on their caps. Dried flower powder used to cure diarrhea. Leaf and flower juice used to cure wounds and eye problems.
R. macrophylla Lindl. *	Jungli gulab	S	2000-2700	Reg Himal, China	Fruits	Food	Fruits edible.
R. webbiana Wall. ex Royle*	Lama uh	S	2600-3300	Reg Himal	Fruits, Branches, Flowers	Religious, Medicinal, Fuel	Fruits edible. Branches used as fuel and in performing various Buddhist traditional rituals and for fencing the orchards and agricultural fields. Flowers offered to deities and worn on caps by women and used to cure stomach pain. Also used as fuel.
Rubus ellipticus Sm.	Cho soch	S	1800-2200	Reg Himal	Fruits	Food, Medicinal	Fruits edible. Root extract used to cure cough.
R. fruticosus Hegetschw.	Cho soch	S	1900-2900	Europe	Fruits	Food, Medicinal	Fruits edible. Infusion of leaf used to cure diarrhea.
R. niveus Thunb.	Cho soch	S	1800-3300	Ind Or	Fruits	Food, Medicinal	Fruits edible. Extract of fruits used to cure menstrual cramps.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Rubiaceae							
Rubia cordifolia L.	--	C	1800-2800	As Trop Temp Afr Tro	Whole plant	Medicinal	Decoction of the plant used as vermifuge.
Scrophulariaceae							
Verbascum thapsus L.	Phochu Kanang, Ban Tambaku	H	1800-3300	Reg Himal	Leaves	Medicinal	Leaves given to cows to cure diarrhoea and expulsion of placenta. Paste of leaf used to cure insect bite.
Simaroubaceae							
Ailanthus altissima (Mill.) Sw.	--	T	1800-2600	China	Leaves, Branches	Fodder, Fuel	Used as fodder and fuel.
Solanaceae							
Datura stramonium L.	Datura	H	1800-2500	America	Flowers	Medicinal, Religious	Flowers used to cure tonsils, dandruff and hair fall. Also used in religious ceremonies especially to keep away evil spirits.
Hyoscyamus niger L.	Khursani ajwain	H	2500-2900	Europe As Occ Reg Himal	Seeds	Medicinal	Seeds used to cure toothache, asthma and urinary problems.
Physochlaina praealta (Decne.) Miers. **	Jungli-tombaku			Reg Himal	Leaves, Seeds	Medicinal	Powder of leaves and seeds used to cure swellings and toothache.
Solanum nigrum L.	Makoi	H	1800-3300	Trop Geront	Fruits	Medicinal	Fruits edible.
Saxifragaceae							
Bergenia ciliata (Haw.) Sternb.	Patter phot	H	1800-2800	Reg Himal	Roots	Medicinal	Roots used to cure kidney problems.
Bergenia stracheyi (Hk. f. & Th.) Engl.**	Rachu kanang	H	3000-3300	Reg Himal	Roots	Medicinal, Religious	Roots used as aphrodisiac and to cure kidney stone. Flowers are used in Phulaich festival.
Tamaricaceae							
Myricaria elegans Royle**	Hombu	S	2700-3000	Reg Himal	Leaves, Branches	Medicinal, Religious	Leaf paste used as antiseptic. Branches used in religious ceremonies, especially in hawan.
Thymelaeaceae							
Daphne oleoides Schreb.	Aagru, Jiko, Jikog	S	2000-2800	Europ As Min	Leaves, Branches	Medicinal, Religious	Leaves or its infusion used to cure gonorrhoea. Branches used in religious ceremonies, especially in hawan.
Urticaceae							
Debregeasia saeneb (Forssk.) Hepper & J.R.I.Wood	Pincho	S	1800-2200	Ind Or	Fruits	Medicinal, Fodder, Fuel	Fruits used to cure Kidney stones. Also used as fodder and fuel.
Urtica dioca L.	Choya	H	1800-3300	Reg Bor Temp	Leaves	Food, Medicinal	Leaves cooked as vegetable known as 'Choya kan' used to cure body weakness, diabetes, skin problems jaundice and kidney problems.

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
U. hyperborea Jacq. ex Wedd	Choya	H	2500-3300	Reg Himal	Leaves	Food, Medicinal, Fodder	Leaves cooked as vegetable known as 'Choya kan' used to cure body weakness, diabetic, skin problems, jaundice and kidney problems. Also used as fodder and decoction of leaves used to cure skin problems.
Viburnaceae							
Viburnum cotinifolium D. Don*	Tustus	S	2500-3200	Reg Himal	Fruits	Food, Medicinal, Fuel, Agricultural tools	Fruits edible. The decoction of the bark is used in urino-genital problems. Branches used as handles of agricultural tools and as fuel.
Violaceae							
Viola biflora L.	Banaksha	H	1800-2900	Reg Bor Temp	Flowers	Medicinal	Decoction of the flowers is used to cure fever.
V. serpens Wall. ex Ging.	Banaksha	H	1800-2700	Reg Himal	Flowers	Medicinal	Decoction of the flowers is used to cure fever.
GYMNOSPERMS							
Pinaceae							
Cedrus deodara (Roxb. ex D. Don) G.Don*	Kyalbang	T	1700-3300	Reg Himal	Resins, Needles, Wood, Branches	Medicinal, Manure, Timber, Fuel, Religious, Household items	Resins with mustard oil are used to cure arthritis and are chewed for stronger gums. Needles used as manure for orchards and fields and used for bedding in cowsheds. Wood used for construction purposes and for making household items. Branches also used in various sacred rituals.
Pinus gerardiana Wall. ex D.Don	Ree	T	1800-3600	Reg Himal	Resin, Wood, Seeds, Leaves	Medicinal, Edible, Manure, Fuel, Household items	Resin with mustard oil used to cure joint pain. Seeds edible and used to preparation of garlands for marriage and other traditional ceremonies. Needles used as manure for orchards and fields and used for bedding in cowsheds.
Pinus roxburghii Sarg.*	Cheel	T	1600-1800	Reg Himal	Resins, Wood, Leaves	Medicinal, Manure, Timber, Fuel	Resin used to treat cracks on feet and chewed for stronger gums. Wood used for construction purposes and for making household items. Needles used as manure for orchards and fields and used for bedding in cowsheds.
P. wallichiana (Wall. ex D. Don) A.B. Jacks.*	Lim	T	1800-3800	Reg Himal	Resins, Wood, Branches, Leaves	Medicinal, Manure, Timber, Fuel	Resin used to treat cracks on feet and chewed for stronger gums. Wood used for construction purposes and for making household items. Needles used as manure for orchards and fields and used for bedding in cowsheds.
Cupressaceae							
Juniperus excelsa subsp. *polycarpos* (K. Koch) Takht.	Shur, Shukpa	T	2400-3300	Persia Reg Himal	Leaves, Wood	Timber, Fuel, Religious, Household items	Leaves used as incense in houses, temples and monasteries. Wood used for construction of houses, monasteries and temples and preparation of multipurpose wooden boxes for storage of grains, apricots and wine.
J. indica Bertol.	Shur, Shukpa	S	2800-3300	Reg Himal	Leaves	Fuel, Religious	Leaves used as incense in houses, temples and monasteries
J. squamata Buch.-Ham. ex D. Don	Shur, Shukpa	S	2600-3300	Reg Himal China	Leaves	Fuel, Religious	Leaves used as incense in houses, temples and monasteries

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Table 2. Diversity, nativity, life forms, indigenous uses, and other ethnobotanical attributes of socio-economically important plants in the *Pinus gerardiana* forests of Kinnaur district, Himachal Pradesh

Family/Taxa	Local name	Life form	Altitude range (m)	Nativity	Part used	Utilization pattern	Indigenous uses
Ephederaceae							
Ephedra gerardiana Wall. ex Stapf*	Som lata	S	2800-3300	As Centr Himal China	Branches	Fuel, Medicinal	Used to cure asthma and rheumatism.
E. intermedia Schrenk & C.A.Mey.	Som lata	S	2000-2400	As Centr Himal China	Roots	Medicinal	Used to cure blood pressure.
Fungus							
Morchellaceae							
Morchella esculenta Fr.	Rangmoch, Zangmoch	F	1800-2900	--	Fruiting body	Medicinal, Edible	Fruiting bodies edible and soup used to cure body weakness and loss of appetite.

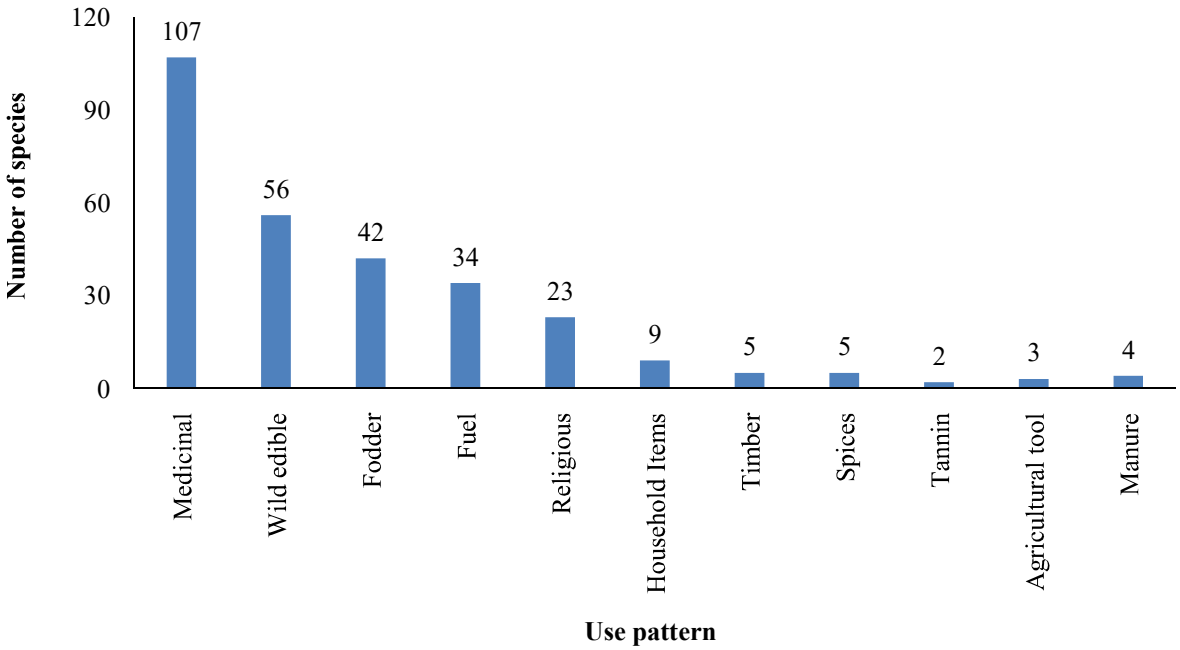


Figure 2. Use pattern of socio-economically important plants documented in Chilgoza forests. The graph represents the use pattern of species reported from Chilgoza forest in the district Kinnaur (H.P.) More than two uses of a single species have been recorded from the studied sites

Indigofera heterantha, Juglans regia, Fraxinus xanthoxyloides, Myricaria elegans, Cedrus deodara, P. wallichiana and *Juniperus excelsa* subsp. *polycarpos, J. squamata* are Least Concern, and *Rheum australe* is Data Deficient. However, as per the Conservation Assessment and Management (CAMP) report, *Dactylorhiza hatagirea* is Critically Endangered; *Angelica glauca*, *Arnebia euchroma*, *Berberis aristata*, *Dioscorea deltoidea* and *Betula utilis* are Endangered; *Hyoscyamus niger* and *Hyssopus officinalis* are Near Threatened; *Bunium persicum*, *Ephedra gerardiana, Juniperus communis* and *Rheum australe* are Vulnerable (Goraya et al., 2013). Thus, *in situ* habitat protection, sustainable harvesting, and improving natural regeneration through community-based forest management along with long-term monitoring are suggested for conservation of these threatened species.

Allium stracheyi, Angelica glauca, Betula utilis, Bunium persicum, Dioscorea deltoidea, Hippophae spp., *Juglans*



Figure 3. Socio-economically important plants found in the *P. gerardiana* forests of Kinnaur district of Himachal Pradesh, Northwestern Himalaya, India. Herbs constituted the majority (57%) of the recorded species, followed by shrubs (25.67%) and trees (10.81%). The families Rosaceae, Lamiaceae, Asteraceae, and Apiaceae were found dominant, collectively accounting for 22.97% of the recorded species

regia, Pinus gerardiana, Prunus armeniaca, P. mira and *Podophyllum hexandrum* are highly exploited species in district Kinnaur, Himachal Pradesh (Negi et al., 2015). *Pinus gerardiana, Prunus mira, Prunus armeniaca, Bhunium persicum, Carum carvi, Berberis aristata, Elaeagnus umbellata, Chenopodium album,* and *Morchellla esculenta* are the major species having huge market potential (Singh et al., 2016; Lata et al., 2021). The local inhabitants also collect *Bhunium persicum* (Kala jeera) and *Carum carvi* (Mako-jeera) from the forest and sell them in the local market at prices of Rs. 2000/kg and Rs. 500/kg, respectively (Paul, 2021; Klate et al., 2023). *Hippophae rhamnoides* ssp. *turkestanica* (sea buckthorn) is a valuable multipurpose shrub recognized for its ecological, nutritional, and medicinal importance in the cold desert ecosystems. (Singh et al., 2012). Due to its high medicinal properties, the Defence Institute of High-Altitude Research, Leh, has developed a wide range of antioxidant-rich products using the fruits and leaves of sea buckthorn along with other trans-Himalayan medicinal plants (Saxena et al., 2018). Despite their immense economic and pharmaceutical value, these wild plant resources also remain largely underutilized due to limited public awareness. Hence, there is a need to conserve these species while enhancing awareness of their medicinal, nutritional, and commercial significance.

4. CONCLUSION

P. gerardiana forests of Kinnaur district in the northwestern Himalaya are biologically rich and socio-culturally significant ecosystems. In addition to maintaining ecological balance and supporting biodiversity, these forests provide a wide range of ecosystem services and sustain the livelihoods of the tribal communities of Kinnaur, Himachal Pradesh. The communities' long-standing dependence on these forest resources has also fostered a deep-rooted traditional ecological knowledge system. However, increasing anthropogenic pressures, unsustainable resource extraction, habitat degradation, and the impacts of climate change pose serious threats to the long-term sustainability of these forests and the ecosystem services they provide.

Therefore, the development of comprehensive conservation and management strategies for Chilgoza pine forests is urgently needed. Priority actions should include population assessment and habitat monitoring using standardized ecological methods, development of a species recovery plan, development of propagation protocols, and the establishment of both *in situ* and *ex situ* conservation programmes. These measures would not only contribute to the conservation and restoration of *P. gerardiana* forests but also help sustain the socio-economic well-being of the Kinnaura tribal communities that depend on them. Furthermore, awareness and education programmes promoting sustainable harvesting and utilization of forest resources should be strengthened through collaborative efforts involving local communities, research institutions, non-governmental organizations (NGOs), Panchayati Raj Institutions, and the Forest Department.

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Swaran Lata: Conceptualization, Methodology, Investigation, Data curation, Data analysis, writing - original draft, writing - review & editing. The author has read and approved the final manuscript.

Data availability statement

The results described and data used henceforth was collected during the field visits performed during the execution of the ICFRE-PLAN funded project.

Conflict of Interests

The author declares no competing interests.

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

The author declares that no artificial intelligence tools were used in the writing of this manuscript.

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Indirect Bee Vectoring in a Non-Entomophilous Crop: A Proof-of-Concept

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ABSTRACT: Bee-mediated biocontrol (apivectoring) uses foraging bees to disseminate biological control agents within crop systems. Although this approach has been successfully applied in several entomophilous crops, its feasibility in non-entomophilous systems remains poorly documented. We evaluated an indirect apivectoring strategy in which bees foraging on interplanted strawberries (*Fragaria × ananassa*) acquired and transported viable propagules of *Trichoderma asperellum* from a dispenser to support the biological control of gray mold *Botrytis cinerea* in adjacent greenhouse roses (*Rosa hybrida*). A 3D-printed PLA dispenser was attached to a Langstroth hive containing *Apis mellifera carnica* and deployed in a rose greenhouse in Cotopaxi, Ecuador. Under laboratory conditions, *T. asperellum* inhibited the mycelial growth of *B. cinerea* by 61.7% and maintained a viability of 2.31×10^4 CFU/g. In the greenhouse, foraging bees carried 590.6 ± 244.3 CFU per individual. Disease severity was 26.8% lower in the apivectoring treatment than in the control, while strawberry fruit weight increased by 30%. Farmer perception surveys identified the perceived potential to reduce fungicide use and provide additional pollination services, while also highlighting operational constraints related to hive management and technical training. These findings provide initial evidence supporting the feasibility of indirect apivectoring in a non-entomophilous crop, although further studies are needed to confirm fungal deposition on rose tissues.

Keywords: Bee vectoring, Pollination, Bee-crop interactions, Sustainable pest control.

1. INTRODUCTION

The development of sustainable strategies for integrated pest and disease management is a priority in contemporary agriculture, particularly the resistance to excessive pesticide use (Deguigne et al., 2023). Innovation in integrated pest and pollinator management is key to reduce agrochemical use and providing sustainable alternatives to farmers (Sharma & Sharma, 2024). Among the most promising alternatives is vector-mediated biological control, particularly bee-mediated biocontrol (apivectoring), a technique implemented since the 1990s that uses bees to disperse

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beneficial microorganisms directly onto crops (Peng et al., 1992; Hokkanen et al., 2015). The success of apivectoring relies on efficient interactions among bees, the biocontrol agent, and the crop, as well as on suitable formulations and well-designed hive dispensers (Hokkanen et al., 2015). This technique exploits the natural foraging behavior of pollinators such as *Apis mellifera*, *Bombus* spp., and *Osmia* spp. to transport agents like *Trichoderma* spp., *Beauveria bassiana*, or *Bacillus subtilis* to flowers, facilitating targeted and continuous application of the biocontrol agent (Kevan et al., 2008; Sharma & Sharma, 2024). Numerous studies have demonstrated the efficacy of apivectoring in disease control, pollination enhancement, and increased agricultural productivity across fruit crops, including strawberry, apple, raspberry, and blueberry, both in greenhouses and in open-field conditions (see Supp. Table S1). Significant reductions in pathogen incidence, such as gray mold *Botrytis cinerea*, have been reported, with control rates of up to 45% in Swedish greenhouses and 77% in Colombian production systems (Iqbal et al., 2022; Espinosa et al., 2020; Coates et al., 2023). Likewise, notable yield increases have been observed, ranging from 10% to 18% in Estonian fruit production (Hokkanen et al., 2015) and from 40% to 100% in fruit weight in New York production systems (Kovach et al., 2000). Additional benefits include improvements in fruit quality and shelf life, reflected in higher sugar content and firmness in Italian crops (Albano et al., 2009), as well as extended postharvest longevity observed in trials in Sweden (Iqbal et al., 2022). Under controlled conditions, delivery precision of biocontrol agents such as *Bacillus amyloliquefaciens* has reached 96%-98 % efficiency in South Korea (Choi et al., 2024). According to the reviewed literature (Supp. Table S1), approximately 90% of apivectoring studies have focused on fruit crops such as strawberries and berries, whereas evidence for other globally relevant crops remains limited. This limitation arises because many commercially important crops (e.g., flowers, herbs, and several vegetables) are rarely visited by bees, thereby restricting the direct application of this technique. To overcome this obstacle, an indirect apivectoring approach has been proposed in which bees forage on companion flowering plants after acquiring a biological control agent from a hive-mounted dispenser, potentially facilitating its transfer within non-entomophilous cropping systems. To our knowledge, the use of an attractive companion crop to facilitate bee-mediated biological control has not been experimentally evaluated. Additionally, relatively few studies have considered the social factors that may influence the adoption of apivectoring technologies (Shafik, 2025). In this study, we implemented an exploratory proof-of-concept of an indirect apivectoring system in which honeybees foraged on interplanted strawberries (*Fragaria* × *ananassa*) after acquiring *T. asperellum* propagules from a hive-mounted dispenser, while disease suppression was evaluated in adjacent greenhouse roses (*Rosa hybrida*) naturally affected by *B. cinerea*. If deposited on susceptible rose tissues, *T. asperellum* could potentially interact with *B. cinerea* through antagonistic mechanisms such as competition and mycoparasitism, while the host plant mediates both pathogen infection and the establishment of the beneficial microorganism. Together, these interactions are expected to influence the effectiveness of indirect apivectoring under agricultural conditions (Kovach et al., 2000; Kevan et al., 2008). Additionally, although apivectoring has been extensively evaluated under experimental conditions, relatively few studies have incorporated farmers' perspectives or considered social factors influencing technology adoption (Otte et al., 2018; Shafik, 2025). Additionally, although apivectoring has been extensively evaluated under experimental conditions, relatively few studies have incorporated farmers' perspectives or considered social factors influencing technology adoption (Otte et al., 2018). Therefore, this study was conducted in collaboration with farmers from the parish of Aláquez (Cotopaxi, Ecuador), who face recurrent fungal diseases in commercial rose production. Our specific objectives were to: i) evaluate the viability and antagonistic activity of *T. asperellum* against *B. cinerea*; ii) design and validate a 3D-printed PLA dispenser coupled to Langstroth hives; iii) analyze bee traffic behavior; iv) quantify the *T. asperellum* propagules carried by foraging bees and evaluate their association with gray mold severity in roses and strawberry fruit weight; and v) assess farmers' perceptions of the technique, identifying its perceived benefits, limitations, and willingness to adopt it in their cropping systems.

2. MATERIALS AND METHODS

2.1. Laboratory Experiments

2.1.1. Preparation of fungal cultures

To evaluate the antagonistic activity of *T. asperellum* against *B. cinerea*, both fungal isolates were obtained and cultured. *B. cinerea* was isolated from symptomatic floral buds and petals collected in an organic rose greenhouse

located in Aláquez, Cotopaxi, Ecuador. The plant material was incubated in a moist chamber at 25-27 °C for 72 h to promote sporulation, after which the fungus was transferred to potato dextrose agar (PDA) to obtain pure cultures. A commercial soluble powder formulation of *Trichoderma asperellum* (Microbiolab, Quito, Ecuador) was used due to its local availability and formulation stability. For viability assessment, 1 g of the commercial formulation was suspended in 9 mL of sterile peptone water (initial 10^{-1} dilution), followed by serial decimal dilutions up to 10^{-2}. Aliquots of 100 µL from the selected dilution were plated on PDA supplemented with 1200 µL L^{-1} lactic acid to reduce bacterial contamination. Ten replicates were prepared and incubated at 27 °C for 48 h in darkness. Fungal growth was monitored to confirm propagule viability prior to downstream assays. Viability was calculated as colony-forming units per gram (CFU g^{-1}) based on colony counts, dilution factors, and plated volume, and values are reported as mean ± standard deviation.

2.1.2. Strain identification

Fungal DNA was extracted from seven-day-old cultures grown on PDA using the Wizard® Genomic DNA Purification Kit (Promega, 2019). Species identification was performed by amplifying the ITS region (ITS1-5.8S-ITS2) and the EF-1α gene (elongation factor 1-alpha) via PCR. Each PCR reaction was carried out in a final volume of 25 µL. The thermal cycling program included an initial denaturation at 95 °C for 1 min, followed by 30 cycles of 95 °C for 1 min, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Amplified products were visualized on a 1.5% agarose gel (95 V, 60 min), purified, and sequenced using Sanger sequencing (Macrogen Inc., Seoul, South Korea). Sequences were compared against the GenBank database (NCBI) using BLAST (Chinnasamy et al., 2023), confirming the identity of the isolates as *Trichoderma asperellum* (Samuels, Lieckf. & Nirenberg, 1999) and *Botrytis cinerea* (Pers., 1794).

2.1.3. In vitro antagonism assay

The antagonistic interaction between *T. asperellum* and *B. cinerea* was evaluated using a dual-culture assay on potato dextrose agar (PDA) in Petri dishes. Mycelial plugs (5 mm diameter) of both fungi were placed on opposite sides of the same plate, separated by approximately 4 cm. Two experimental conditions were established: (i) co-culture plates containing both *T. asperellum* and *B. cinerea*, and (ii) control plates in which *B. cinerea* was grown alone under identical conditions. Each treatment consisted of 10 replicates. Plates were incubated at 27 °C for seven days. Fungal growth was monitored daily, and the radial growth of *B. cinerea* was measured at the end of the incubation period. The percentage of radial inhibition (PRI) was calculated according to Skidmore and Dickinson (1976) (Eq. 1):

$$PRI = \frac{R_1 - R_2}{R_1} \times 100 \ldots\ldots\ldots\ldots (1)$$

where R_1 represents the radial growth of *B. cinerea* in the absence of *T. asperellum*, and R_2 represents its growth in dual-culture conditions.

2.2. Apivectoring System and Dispenser Design

2.2.1. Experimental hive

A commercial Langstroth hive containing approximately 20.000 workers of *Apis mellifera carnica* (Apiario Los Cerros, Quito, Ecuador) was used. This subspecies is characterized by a proboscis length of 6-6.6 mm and wingspan of 9.6-13.2 mm, traits associated with efficient foraging and pollen transport capacity (Taha & Al-Kahtani, 2017). The hive followed a standard Langstroth configuration, including a brood chamber, supers, inner cover, and outer cover (Magem, 2017). Maintaining a standardized structure helped ensure stable colony behavior during the apivectoring trials.

2.2.2. Construction and evaluation of the dispenser

Successful apivectoring depends on the ability of foragers to consistently acquire propagules of the biological control agent during hive exit. Based on previous designs and recommendations (Bilu et al., 2004; Kevan et al., 2008; Shelley et al., 2024), a custom dispenser was developed to ensure contact between bees and the powdered formulation without interfering with normal foraging activity (Mommaerts & Smagghe, 2011). The design process integrated feedback from beekeepers, a bee management specialist (co-author), and materials experts. This iterative input focused mainly on improving hive integration, handling during routine inspections, and reducing disturbance to colony activity under field conditions. Prototypes were fabricated using a Prusa MK3S+ 3D printer (Prusa Research, Prague) with polylactic acid (PLA) filament (Supplementary Figure S1). Each version was first tested for fit at the hive entrance and for bee passage flow, ensuring that traffic was not obstructed while still promoting contact with the biological control agent.

2.2.3. Bee traffic monitoring

Bee traffic at the dispenser was recorded using a Sony Cybershot video camera placed at the hive entrance in Quito,

Ecuador. Inbound and outbound bees were counted in two-minute intervals. Observations were conducted between 10:00 and 14:00 on two consecutive days under two conditions: dispenser loaded with *T. asperellum* and empty dispenser. A 24 h acclimation period was allowed before measurements to reduce behavioral disturbance caused by installation.

2.2.4. Conidia carried per bee

A total of 32 foraging bees were collected over two days at the dispenser exit. Individuals were immobilized at 4 °C and then washed in sterile water to detach externally carried *T. asperellum* conidia. The resulting suspension was plated on PDA and incubated at 27 °C for 48 h. Colony-forming units (CFU) per bee were then quantified following Iqbal et al. (2022).

2.3. Field-Condition Experiment

The field experiment was conducted in Aláquez (Cotopaxi Province, central Ecuador), an Andean agricultural region characterized by diverse cropping systems and environmental conditions suitable for floriculture. The site is located at 2.950 m a.s.l., with mean annual temperatures of 10-20 °C and precipitation between 500-1.250 mm (GAD Parroquial de Aláquez, 2020). The study was carried out in a 60 m² greenhouse (−0.8589002, −78.589811) containing 100 *Rosa* × *hybrida* plants divided into two equal blocks (n = 50 per block): (i) an experimental block with bee-mediated *T. asperellum* dispersal, and (ii) a control block without apivectoring. A plastic barrier separated both sections to prevent bee movement between them. A single Langstroth hive equipped with the 3D-printed dispenser was placed within the experimental block. This positioning restricted bee activity, including both pollination and fungal dispersal, to the treated area, while the control block remained isolated from visitation. Each block included the rose varieties Sweetness, Super Sun, and Orange Crush. The treatment and control greenhouse blocks were considered the experimental units, whereas individual rose plants were treated as observational subsamples for repeated disease assessments. The experiment consisted of one treatment block and one control block supplied by a single honey bee colony. Consequently, no independent replication of the treatment was possible, and the study was designed as an exploratory proof-of-concept under commercial greenhouse conditions. Because roses provide limited floral resources for honey bees, 50 flowering *Fragaria* × *ananassa* plants were intercropped within each block to attract foraging bees and promote their activity within the greenhouse. The hive was placed near the center of the experimental block to encourage homogeneous foraging distribution (Figure 1). For disease assessment, roses were artificially inoculated with 5-mm plugs of *Botrytis cinerea*. Disease severity on flower buds was evaluated weekly over six weeks using a 0-5 visual scale (Silva et al., 2016; Figure S2), generating repeated observations over time. As a proxy for pollination effects, 50 strawberry fruits per block were randomly sampled at harvest, and mean fruit weight (g) was compared between treatments.

2.3.1. Farmer perception assessment of apivectoring

A farmer perception workshop was conducted with 27 farmers from the Asociación Magna Unión y Progreso de Aláquez to explore perceptions of apivectoring, identify potential constraints to adoption, and relate the experimental results to local agricultural contexts. The workshop was designed to facilitate dialogue between researchers and farmers and to gather feedback on the potential applicability of the technique under local production conditions (Berthet et al., 2015). The session began with an open discussion on local knowledge of bees, pollination, and plant diseases in key crops, which helped situate the topic within the farmers' own experiences. Emphasis was placed on *B. cinerea* in roses and strawberries, as well as on the availability of floral resources for *A. mellifera carnica*. To facilitate understanding of the apivectoring mechanism, a simplified demonstration was conducted using floral structures and corn flour to simulate the transfer of *Trichoderma* propagules during bee foraging. The research team then presented the experimental setup and main results, including symptom images, a heatmap of disease reduction in roses, and estimates of strawberry-associated effects. A hive model equipped with the modified dispenser was also shown, and a local farmer-beekeeper contributed practical observations on bee behavior and dispenser performance. The discussion was guided around three main topics: perceived usefulness of the technique, crops that could benefit from apivectoring, and perceived limitations. Participants subsequently completed a structured questionnaire assessing perceived benefits, perceived barriers to adoption, and willingness to implement the approach. Responses were summarized using descriptive statistics.

2.3.2. Data analysis

All statistical analyses were performed in R (R Core Team, 2025) using RStudio (RStudio Team, 2025).

Graphical outputs were generated with ggplot2 within the tidyverse ecosystem (Wickham et al., 2019). Radial growth of *Botrytis cinerea* in dual cultures with. *T. asperellum* and in control plates (n = 10 per treatment) was compared using a Wilcoxon rank-sum test, selected due to non-normal distribution of the data. Bee traffic at the hive entrance was analyzed using a three-way ANOVA, with condition (presence/absence of *T. asperellum*), direction (entry/exit), and day as fixed factors, including interaction terms. In addition, Wilcoxon tests were applied to compare frequencies of correct entry and exit movements between treatments. Fungal carriage in a subset of 32 bees was summarized descriptively (mean, minimum, and maximum CFU per individual). Disease severity over time was analyzed using linear mixed-effects models (lme4 package), with treatment, week, and their interaction as fixed effects, and plant identity as a random intercept. Significance was assessed using Type III ANOVA with Satterthwaite's approximation. Fruit weight was compared between treatments using Welch's t-test, and results were expressed as mean ± SD and percentage differences. Questionnaire responses on perceived benefits, limitations, and willingness to adopt apivectoring were summarized using frequency distributions and percentages.

3. RESULTS AND DISCUSSION

3.1. Viability and In Vitro Antagonism

The commercial formulation of *Trichoderma asperellum* showed consistent viability on PDA medium, with an average of 2.31×10^4 CFU g^{-1} (n = 10), indicating that the

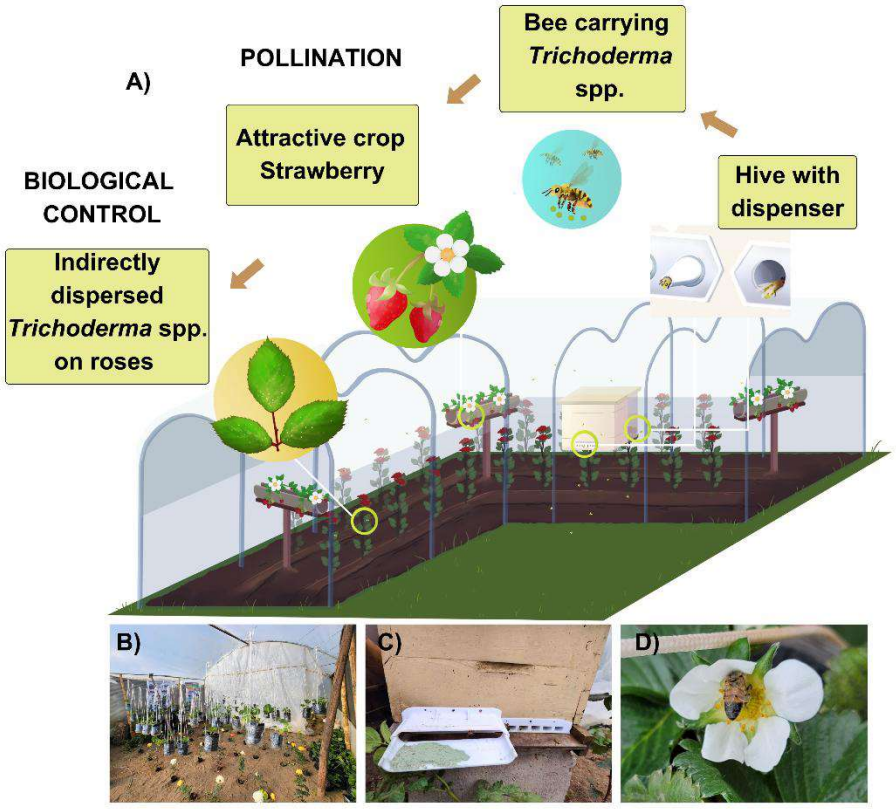


Figure 1. Experimental Setup of the apivectoring system for the biological control of *B. cinerea* in rose cultivation. **(A)** Schematic overview of the apivectoring process: forager bees acquire *T. asperellum* spores when exiting the hive through the dispenser and subsequently deposit them while visiting strawberry flowers and moving within the crop system. **(B)** Greenhouse layout showing the spatial arrangement of strawberry and rose plants used in the experiment. **(C)** Langstroth hive fitted with the 3D-printed *T. asperellum* dispenser at the hive entrance. **(D)** *A mellifera carnica* forager on a strawberry flower, illustrating concurrent pollination and dispersal of the biological control agent

formulation remained active prior to experimental application. In the dual-culture assay, *T. asperellum* reduced the radial growth of *Botrytis cinerea*, reaching a mean inhibition of 61.7 ± 3% (n = 10; Wilcoxon test, $p < 0.001$). At the interaction zone between both fungi, *B. cinerea* showed reduced mycelial density compared with control plates (Figure 2), confirming antagonistic activity under in vitro conditions. The antagonistic response observed is consistent with previously described mechanisms of *Trichoderma*, including mycoparasitism, competition for nutrients, and production of antifungal metabolites (Benítez et al., 2004; Sood et al., 2020). These results support the suitability of the formulation as a biological control agent for integration into bee-mediated delivery systems.

3.2. Dispenser Performance, Bee Traffic Behavior and Fungal Carriage

Bee traffic at the hive entrance showed no significant differences between treatments with and without *T. asperellum* in the dispenser. Frequencies of correct entries (Wilcoxon test, $p = 0.985$) and exits ($p = 0.651$) were similar across conditions. A three-way ANOVA including condition (presence/absence of *T. asperellum*), direction (entry/exit), and day indicated that the presence of the biological control agent did not significantly affect bee traffic (entries: $F_{1,56} = 0.159$, $p = 0.692$). However, variation was observed between days and between entry and exit directions (Direction × Day interaction: $F_{1,56} = 5.811$, $p = 0.019$). Overall, bee movement patterns remained consistent during dispenser operation.

Bees exiting through the dispenser carrying *T. asperellum* showed an average load of 590.6 ± 244.3 CFU per individual, with values ranging from 100 to 1100 CFU, indicating variability among foragers in propagule acquisition (Figure 3).

Although greenhouse conditions may affect bee orientation due to altered light environments (Dag, 2008),

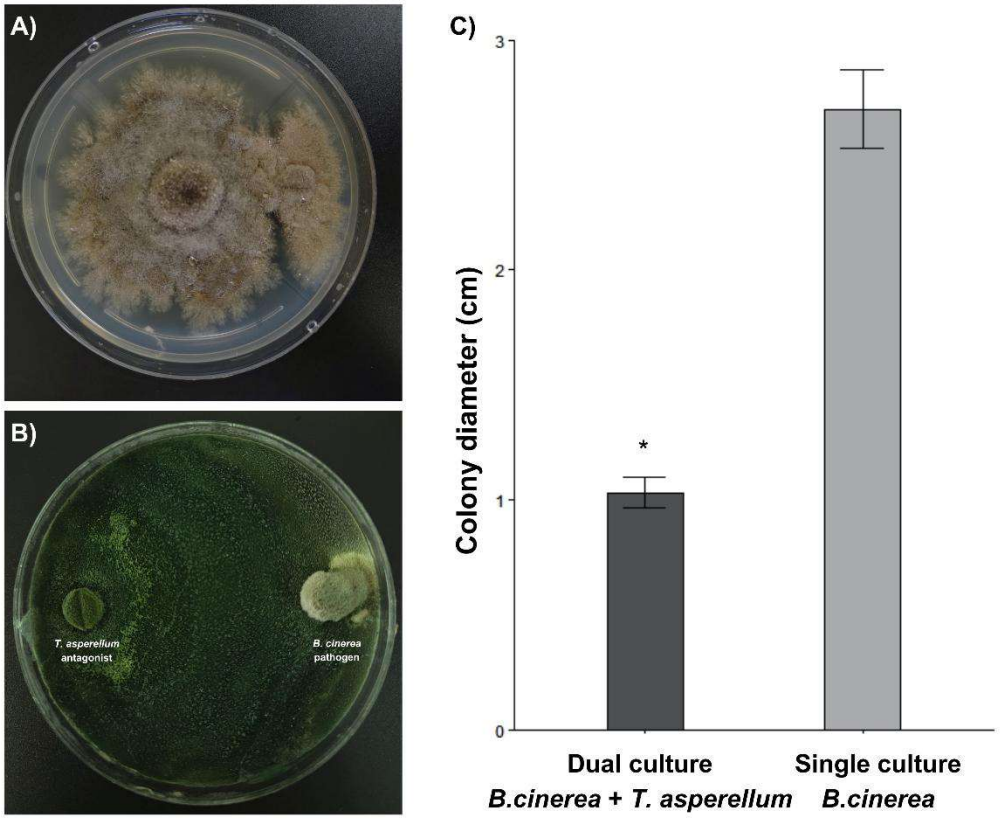


Figure 2. Radial growth suppression of *Botrytis cinerea* by *Trichoderma asperellum* in dual-culture assays. **(A)** Radial growth of *B. cinerea* in pure culture on PDA. **(B)** Dual-culture assay showing partial inhibition of *B. cinerea* mycelial expansion in the presence of *Trichoderma* spp. **(C)** Comparison of *B. cinerea* radial growth (cm) between pure culture and dual culture; bars represent mean ± standard deviation (n = 10)

the dispenser did not produce detectable changes in traffic behavior. Similar observations have been reported in other entomovectoring approaches, suggesting that guidance systems can be incorporated into hive management without substantially interfering with bee activity (Shelley et al., 2024). The variability in fungal load among individuals likely reflects differences in contact with the formulation, bee behavior, and foraging patterns. Compared with direct-dispensation systems (Kovach et al., 2000; Shafir et al., 2006), the lower propagule loads observed here may represent a trade-off between delivery efficiency and the design requirements of indirect vectoring systems. Further evaluation of microbial deposition on floral tissues will be necessary to determine how transported propagules translate into effective delivery within the crop.

3.3. Disease Severity Assessment

Disease severity caused by *Botrytis cinerea* in roses varied between treatments and across weeks (Figure 4; Figure S3). Control plants showed a progressive increase in severity, with values reaching the upper end of the scoring scale by week 6. In contrast, roses exposed to bee-mediated *T. asperellum* treatment showed lower severity values and a more stable progression over time. The linear mixed-effects model indicated a significant effect of treatment ($F_{1,96}$ = 12.47, $p < 0.01$) and a significant treatment × week interaction ($F_{5,96}$ = 9.62, $p < 0.05$), indicating different disease trajectories between treatments. By week 6, disease severity was reduced by 26.8% in the bee-mediated treatment compared with the control. No differences were detected among rose varieties ($F_{2,96}$ = 1.12, $p = 0.33$). The observed disease suppression is consistent with the potential contribution of bee-mediated dissemination of *T. asperellum* to biological control within the greenhouse system. Similar effects have been reported for bee-vectored *Trichoderma* applications (Espinosa et al., 2020) and for direct applications of *Trichoderma* against *B. cinerea* under greenhouse conditions (Elad & Shtienberg, 1995; Freeman et al., 2004). The response may result from a combination of direct antagonistic activity and possible induction of plant defense mechanisms (Contreras-Cornejo et al., 2017; Wang et al., 2024), although the relative contribution of each mechanism was not evaluated in this study. Compared with direct-dispensation systems, the lower level of disease

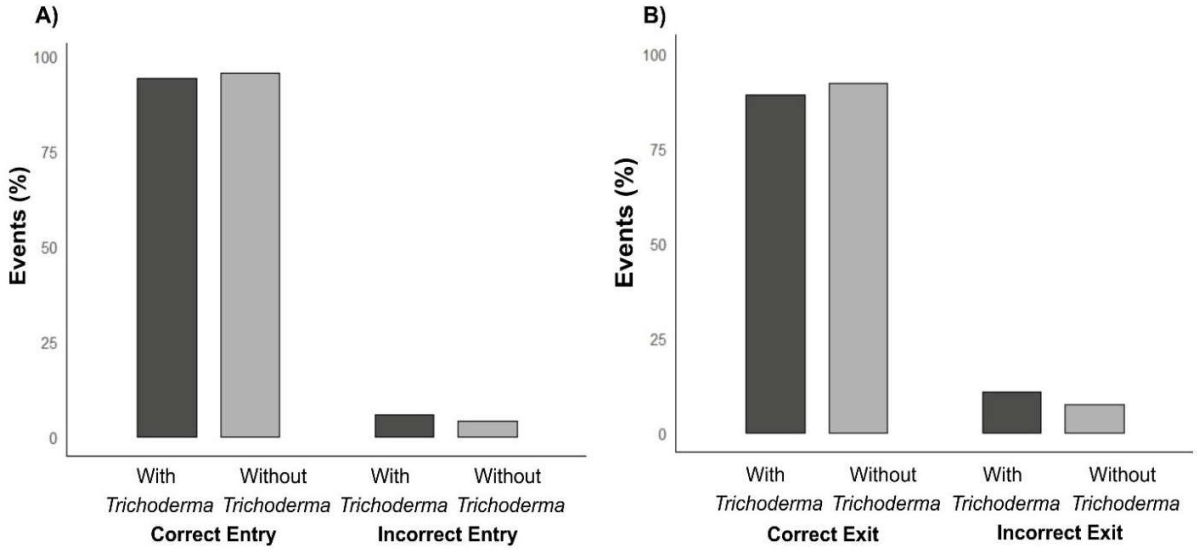


Figure 3. Orientation accuracy of *A. mellifera carnica* during hive entry and exit under treatments with and without *T. asperellum.* Percentage of correct and incorrect entry **(A)** and exit **(B)** events performed by *A. mellifera carnica* during pollination assays in strawberry crops under two conditions: hives supplemented with .*T. asperellum* and hives without *T. asperellum*. Bars represent the proportion of observed events for each behavioral category. Correct entries and exits correspond to the use of the designated openings in the 3D-printed dispenser, whereas incorrect events include attempts through non-designated or lateral openings. This comparison assesses whether the dispenser or the presence of *T. asperellum* influences bees' orientation accuracy during departure and return flights

suppression observed here may reflect the indirect nature of the delivery pathway, which introduces variability associated with bee behavior, inoculum acquisition, and deposition dynamics. Environmental factors, including temperature, humidity, and floral resource availability, may further influence bee activity and fungal performance (Mommaerts & Smagghe, 2011; Kevan et al., 2008). Future studies incorporating microbial traceability approaches will be necessary to confirm deposition and persistence of *T. asperellum* on plant tissues.

The observed reduction in disease severity is consistent with a potential contribution of bee-mediated dissemination of *T. asperellum* to biological control within the greenhouse system. Similar disease suppression has been reported in previous studies using bee-vectored *Trichoderma* (Espinosa et al., 2020) and in direct applications of *Trichoderma* against *B. cinerea* under greenhouse conditions (Elad & Shtienberg, 1995; Freeman et al., 2004). The observed response may involve a combination of direct antagonistic activity and possible induction of plant defense mechanisms (Contreras-Cornejo et al., 2017; Wang et al., 2024), although the contribution of each mechanism was not evaluated in this study. Disease suppression was lower than that reported in some direct-dispensation systems, which may reflect the indirect nature of the delivery pathway and the variability associated with bee behavior, inoculum acquisition, and deposition processes. Environmental factors, including temperature, humidity, and floral resource availability, may further influence bee activity and fungal performance, affecting overall dissemination efficiency (Mommaerts &

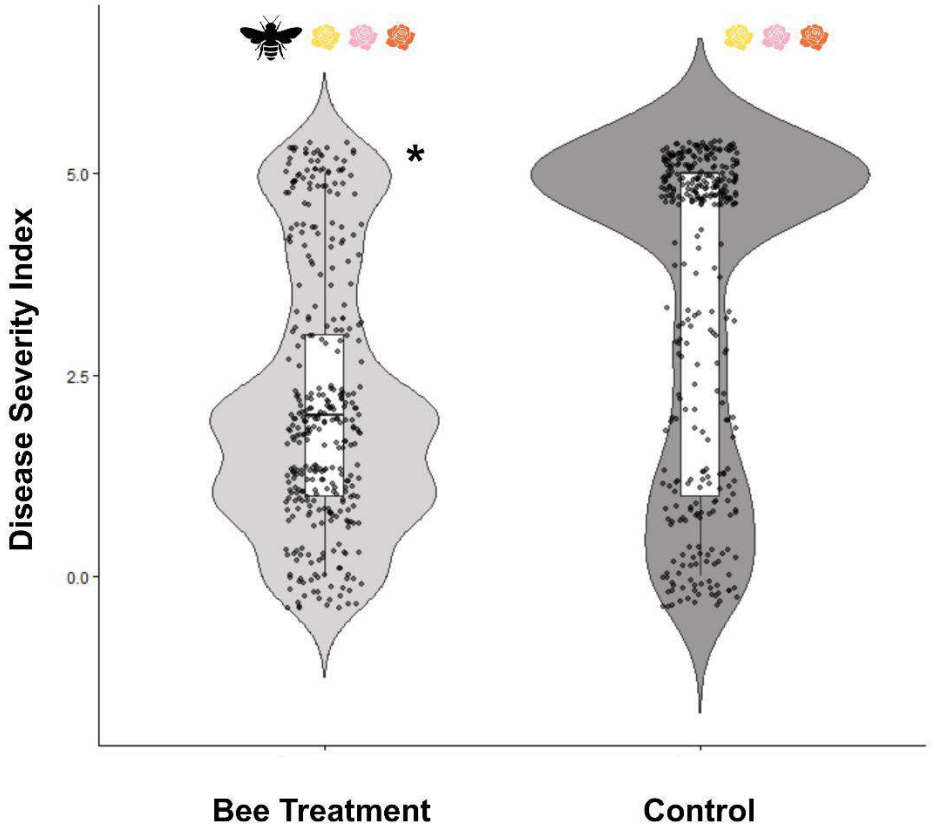


Figure 4. Disease severity in rose plants across treatments and varieties after six weeks. Violin plots illustrate the distribution of *B. cinerea* severity scores for three rose varieties under (A) the experimental bee-mediated *T. asperellum.* treatment and (B) the control treatment. Individual observations are shown as points jittered horizontally and vertically for clarity. Note that the violin plots extend slightly beyond the actual scoring range (0-5) for visualization purposes only.

Smagghe, 2011; Kevan et al., 2008). Future studies incorporating microbial traceability approaches will be necessary to confirm deposition and persistence of *T. asperellum* on plant tissues.

3.4. Effect of Pollination on Strawberry Fructification

Strawberries in the experimental treatment (hive + *T. asperellum* dispenser) produced heavier fruits with greater uniformity compared with the control treatment (Figure 5). Fruits from the control treatment showed a higher frequency of visible deformities, consistent with incomplete or uneven pollination. Fruit weight was significantly higher in the experimental group than in the control (n=50, Welch t-test, $t_{96} = 3.32$, $p = 0.001$), corresponding to an increase of approximately 30%. The observed improvement in fruit development is consistent with the recognized role of managed pollinators in enhancing pollen transfer and fruit set in strawberry crops. However, because the experimental treatment combined bee activity and microbial delivery, the individual contribution of pollination and *T. asperellum* dispersal cannot be separated in the current design. Future experiments, including treatments with bees without T. asperellum and with T. asperellum without bees, would help clarify their respective effects on fruit development and disease management.

3.5. Farmers' Perception of Benefits and Constraints of Bee-Vectoring

Survey responses indicated that enhanced crop pollination was the most frequently perceived benefit of bee-vectoring, mentioned by 74.1% of participants. Disease management support (66.7%) and increased productivity (55.6%) were also commonly reported. Other perceived advantages included potential additional income from beekeeping (22.2%), reduced reliance on synthetic chemicals (22.2%), improved bee nutrition (11.1%), and access to technical assistance (3.7%). Regarding constraints, the absence of managed bee colonies on farms was the most commonly cited limitation (68.3%), followed by limited knowledge of bee management practices (61.9%). Concerns about bee stings affecting workers or family members were reported by 40.7% of respondents, while 29.6% mentioned potential risks to livestock. Other less frequent concerns included neighbor acceptance, lack of technical support, pesticide-related bee mortality, and colony maintenance requirements (Figure 6). The strong association between apivectoring and pollination services suggests that farmers primarily perceive this technology in terms of its potential contribution to crop productivity. This perception aligns with previous studies highlighting the importance of pollinators in agricultural systems (Breeze et al., 2019;

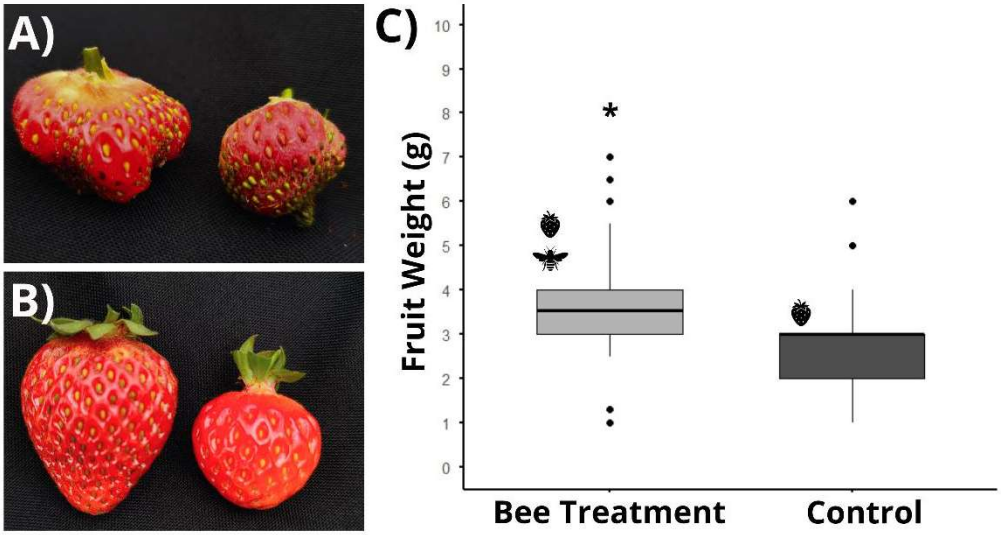


Figure 5. Effect of hive-mediated pollination on strawberry fruit size and uniformity **(A-B)** Representative fruit, from the control (**A**; small and deformed) and experimental (**B**; large and regular) treatments. **(C)** Boxplot comparing fruit weight between control (no hive) and experimental (hive + *T. asperellum*) treatments. Lines indicate the median. Hive presence enhanced fruit size and uniformity

Osterman et al., 2021). The lower frequency of responses related to beekeeping income suggests that apiculture may be considered a complementary activity rather than a primary economic strategy (Grimaldi & Ladio, 2025). The reported constraints, particularly limited technical knowledge and concerns regarding bee management, are consistent with barriers described in other smallholder farming contexts (Hecklé et al., 2018; Ricketts & Shackleton, 2019). These findings highlight the importance of training initiatives, risk management strategies, and technical support to facilitate adoption. This study should be interpreted as a proof of concept because it was conducted in

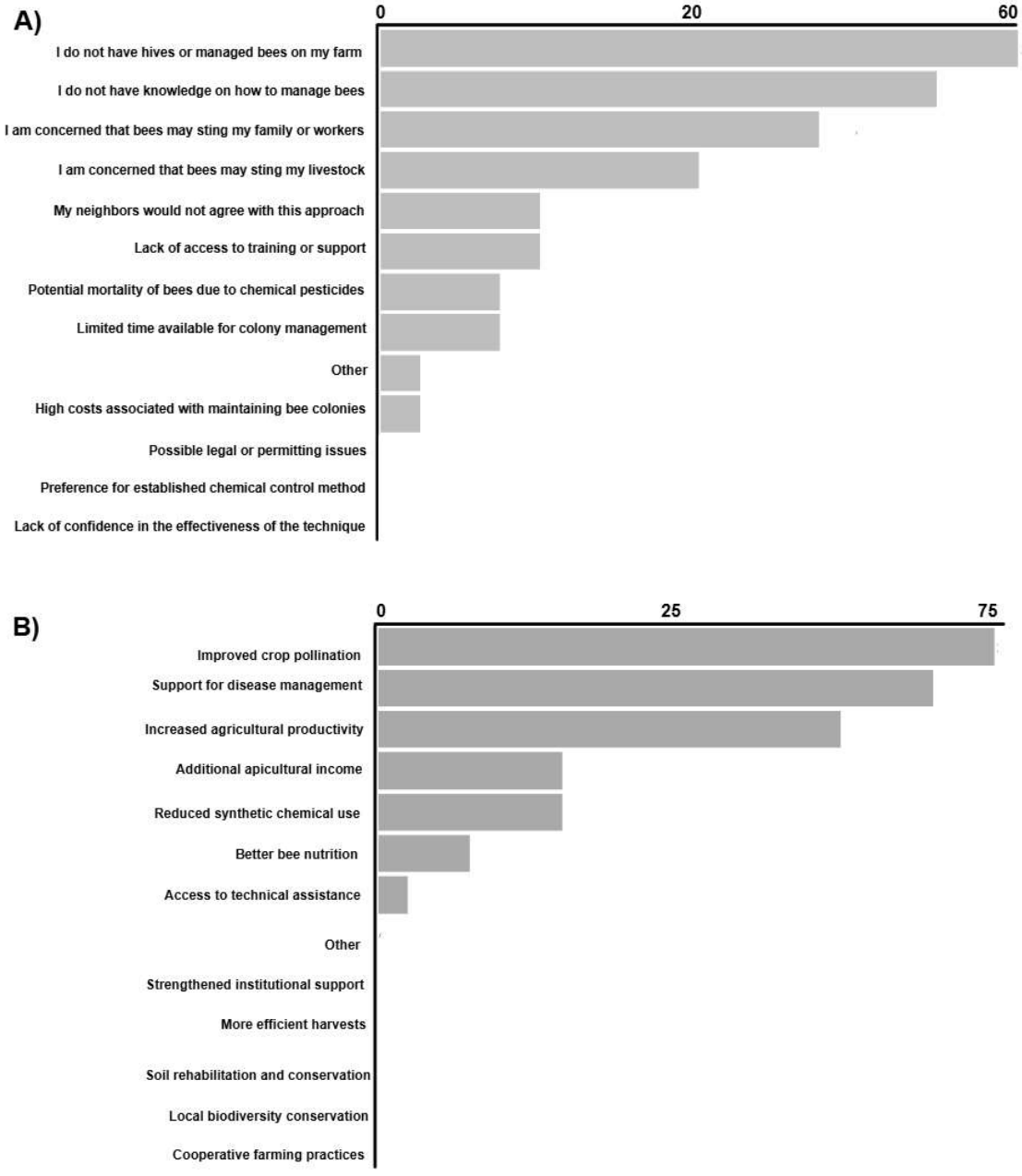


Figure 6. Farmers' perception of apivectoring **(A)** Limitations of apivectoring as reported by farmers. **(B)** Perceived benefits of apivectoring. Horizontal bars represent the percentage of farmers mentioning each option

a single greenhouse, with a single colony, and over a single flowering cycle. Additionally, the experimental design did not separate the effects of pollination from those of microbial inoculation, limiting interpretation of their individual contributions. Future studies should incorporate replicated experimental designs, microbial traceability tools, and evaluations under commercial production conditions. Ecological considerations, including potential interactions with wild pollinators and pathogen spillover, should also be assessed before broader implementation of apivectoring systems.

4. CONCLUSION

This study shows that indirect bee vectoring using *A. mellifera carnica* can contribute to both biological control and pollination services under greenhouse conditions. *T. asperellum* showed antagonistic activity against *Botrytis cinerea* and was transported by foraging bees without detectable effects on hive traffic or orientation through the dispenser system. Bee-mediated delivery of *T. asperellum* was associated with reduced disease severity in roses, while pollination activity corresponded with increased strawberry fruit weight. Overall, the results suggest that combining managed pollinators with microbial biocontrol agents may offer a complementary approach for greenhouse production systems. Farmers generally viewed apivectoring positively, particularly regarding productivity and pollination benefits, although limitations related to technical knowledge and access to managed colonies remain relevant. Given the limited experimental scale, these findings should be considered exploratory. Further studies under larger and replicated conditions are needed to assess the robustness, scalability, and ecological implications of the approach.

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CRediT authorship contribution statement

Michelle Cañizares Huilca: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing; **Diego Mina:** Conceptualization, Investigation, Methodology, Project administration, Supervision; **Jeniffer Yánez**: Investigation, Methodology, Supervision, Writing - original draft; **Andrés Basantes:** Investigation, Methodology, Validation, Writing - original draft; **Alden Yépez:** Methodology, Supervision, Writing - original draft; **Olivier Dangles:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - original draft, Writing - review & editing.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The surveys and methodology for this study were approved by the Ethics Committee of the Pontificia Universidad Católica del Ecuador (PUCE; approval number CEI-OE-07-2024).

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

The authors used generative artificial intelligence tools solely to improve language, grammar, readability, and formatting of the manuscript. All scientific content, analyses, interpretations, and conclusions were developed and verified by the authors, who take full responsibility for the final version of the manuscript.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Floristic Composition and Spatio-temporal Dynamics of Land Use of Two Contiguous Forest Massifs in the Sudano-Sahelian Zone of Cameroon

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ABSTRACT: Land use is essential for implementing numerous developments and to fulfill the increasing demands of basic human needs. The study examined the tree vegetation and land use of two contiguous forest massifs of high conservation value in the sudano-sahelian zone of Cameroon. However, these forest massifs have been experiencing high levels of anthropogenic pressure over the past few decades, leading to biodiversity loss and changes in land use. The diversity inventory design was a split-plot of 2 x 3 x 9, and tree species were inventoried in quadrats of 200 m x 50 m. We used diachronic analysis of three Landsat satellite images of 1978, 1998 and 2023 on the USGS Earth Explorer platform. Statistical analyses were performed with XLSTAT software, and the satellite images were processed using ArcGIS software. In total, 9807 tree stems were inventoried in Bougaye and Lokoro forest massifs and distributed in 55 species, 38 genera and 19 families. The Shannon-Weaver diversity index values varied from 2.26 to 2.66 and from 2.35 to 2.47 in Bougaye and Lokoro forest massifs, respectively. The Pielou's evenness index values oscillated between 0.35 and 0.42 and between 0.29 and 0.45 in Bougaye and Lokoro forest massifs, respectively. There was a significant decline of 3153.551 ha in woody vegetation and an increase of 1075.207 ha in bare soil and built-up area. Agricultural expansion and bare soil/built-up areas would have led to the disappearance of woody vegetation. Implementation of an appropriate land-use policy is needed in this area, including the delimitation of agricultural, grazing, and protected forest zones.

Keywords: Cameroon, Forest massif, Land use, Vegetation.

INTRODUCTION

Tropical forests are extraordinary reservoirs of biodiversity, and within a few decades, they have become a centre of attention in the scope of international challenges in climate change and biodiversity conservation for the countries they cover (Marquant et al., 2015; FAO, 2020). The Congo Basin, the second largest tropical forest in one

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piece after the Amazon, provides subsistence for 60 million people who live either within or in the vicinity of the forests (Nasi et al., 2012). They also fulfil social and cultural functions essential to local and indigenous populations and contribute to feeding 40 million people living in urban centres near these forestry areas (De Wasseige et al., 2014; Dalimier et al., 2021). The importance of tropical forests in the Congo Basin has gradually given these ecosystems the status of a global common asset, and many multilateral agreements now address their management and conservation of these ecosystems in partnership with the States of the region (Marquant et al., 2015). Central Africa, a region that kept its forests unspoiled for a long time, is now increasingly subject to deforestation (FAO, 2020). Cameroon has about twenty-two million hectares of forests, representing 46% of the country's total area (De Wasseige et al., 2015). These forest ecosystems provide more than eight million forest-dwellers with important nutritional supplements, traditional medicines, fuelwood, construction materials, raw materials for handicrafts, and other products such as resins and honey (Topa et al., 2009). However, the population is growing rapidly, and among the corollaries of sprawling cities, there's an increasing demand for agricultural and forest products, such as fuelwood, and for new road networks. Forest massifs face considerable threats and are diminishing rapidly due of human activities and global climate change (Laurance et al., 2015).

The sudano-sahelian zone of Cameroon consists of wooded savannahs, shrub savannahs and grass savannahs, which cover important areas and show a degradation trend towards the Saharan desert (Hansen et al., 2013). Assessments of sub-regional forest cover in the Congo Basin have been developed over several years, and the general trend in the deforestation rate shows an increase from 1990 to 2010 (Desclée et al., 2014). In Cameroon and more particularly in the sudano-sahelian zone, despite the intervention of the Government and some Non-governmental Organizations in the conservation of biodiversity, the pressure on land and species is increasing as urban centres grow and needs become more pressing. Land use change, which is the transformation of natural land into agricultural and urban areas, and grazing pressures, is among the most important human impacts on biodiversity and ecosystem functioning (Pereira et al., 2010). It also represents one of the most important components of global environmental changes (Grau et al., 2003). However, the two contiguous forest massifs, which have strong conservation value because of their ecological connectivity, have not been the subject of scientific studies, particularly regarding the integration of floristic diversity and remote sensing. Furthermore, anthropogenic activities in riparian villages threaten the maintenance of biological corridors. Based on insights from previous research, we hypothesised that the Bougaye and Lokoro forest massifs are experiencing degradation and loss of plant biodiversity due to anthropogenic disturbances. The survey was conducted specifically to:

i) Assess the floristic composition and tree species diversity in the study site;

ii) Estimate the spatio-temporal changes (1978, 1998, 2023) in the area.

2. MATERIALS AND METHODS

2.1. Study site

Investigations were carried out in two forest massifs, Lokoro and Bougaye forests, in the Sudano-Sahelian zone of Cameroon. The site lies between 10°0'0"N and 10'24'0"N of latitude and 15°0'0"E and 15°12'0"E of longitude (Figure 1). The zone is characterised by fragile ecosystems, a high population density and strong pressure on land (Bakoulou et al., 2021). The geological substratum consists of four types of soil (Sieffermann & Vallerie, 1963): clayey soils known locally under the name of Karals; ferruginous soils subdivided into two groups, namely brown-yellow sandy soils and red soils; halomorphic soils which are generally uncultivated soils and are known locally under the name of hardé soils; hydromorphic soils divided into two subclasses, namely organic hydromorphic soils and mineral hydromorphic soils. The mean annual rainfall is 726.2 mm/year, with mean monthly temperatures ranging from 26 to 34°C, a long dry season (8 to 9 months), and a short rainy season (3 months) (Fotsing, 2009). The main vegetation types consist of gallery forests and savannas (Letouzey, 1985), dominated by Combretum glutinosum, C. aculeatum, Guiera senegalensis, Anogeissus leiocarpa, Acacia senegal, A. seyal, A. nilotica, and *Faidherbia albida*. Most of the people living adjacent to the forest massifs rely on agriculture, livestock and forest resources to meet their basic needs.

2.2. Data Collection

2.2.1. Assessment of the tree species diversity and floristic composition

Study design: The inventory design is a split-plot with two

factors (Table 1): the two forest massifs, fixed by nature and thus cannot be randomised, constitute the main factors; the collection units, the secondary factors; and replications, 2 x 3 x 9. Three temporary collection units (CU_1, CU_2, CU_3) were randomly installed and GPS coordinates recorded in each forest massif. These collection units were situated at least 1 km from the villages to avoid anthropogenic edge effects, following the long-term Sahelian rangeland monitoring protocol described by Hiernaux et al. (2009). In each CU, nine quadrats (200 m x 50 m) were established. The space between two quadrats was 200 m. Woody plants with a diameter at breast height (1.30 m) (dbh ≥ 5 cm) were inventoried and identified by their scientific names according to Angiosperm Phylogeny Group IV (APG IV) with the help of the different volumes of Flora of Cameroon (Letouzey, 1985). Actually, the inventory was conducted in a single passage during the dry season. Sudano-sahelian savannah structure, particularly regeneration and

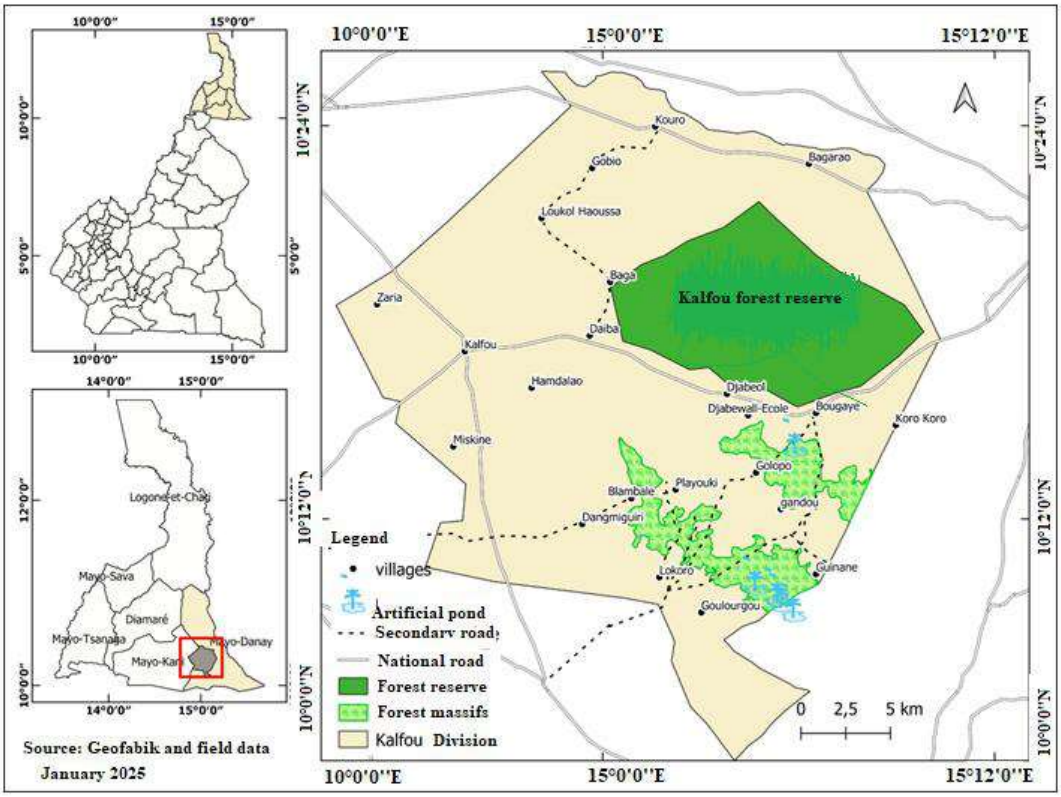


Figure 1. The figure shows the location map of the study site, situated in the Sudano-Sahelian zone of Cameroon

Table 1. Experimental design

Main factors	Secondary factors	Replications									Total
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	
Bougaye forest massif	BCU_1, BCU_2, BCU_3	R_1BCU_1, R_1BCU_2, R_1BCU_3	R_2BCU_1, R_2BCU_2, R_2BCU_3	R_3BCU_1, R_3BCU_2, R_3BCU_3	R_4BCU_1, R_4BCU_2, R_4BCU_3	R_5BCU_1, R_5BCU_2, R_5BCU_3	R_6BCU_1, R_6BCU_2, R_6BCU_3	R_7BCU_1, R_7BCU_2, R_7BCU_3	R8BCU1, R_8BCU_2, R_8BCU_3	R_9BCU_1, R_9BCU_2, R_9BCU_3	27
Lokoro forest massif	LCU_1, LCU_2, LCU_3	R_1LCU_1, R_1LCU_2, R_1LCU_3	R_2LCU_1, R_2LCU_2, R_2LCU_3	R_3LCU_1, R_3LCU_2, R_3LCU_3	R_4LCU_1, R_4LCU_2, R_4LCU_3	R_5LCU_1, R_5LCU_2, R_5LCU_3	R_6LCU_1, R_6LCU_2, R_6LCU_3	R_7LCU_1, R_7LCU_2, R_7LCU_3	R_8LCU_1, R_8LCU_2, R_8LCU_3	R_9LCU_1, R_9LCU_2, R_9LCU_3	27
Total											54

B : Bougaye forest massif L : Lokoro forest massif CU : Collection unit R : Replication

herbaceous cover, is highly seasonal.

2.2.2. Estimation of the Spatio-temporal changes (1978, 1998, 2023) in the area

Acquisition and processing of satellite images: The images were acquired from the Landsat MSS (1978), Landsat TM (1998) and Landsat OLI (2023) satellites on the USGS Earth Explorer platform. All satellite images were acquired during the dry season (December-March), when cloud cover was lower, vegetation discrimination was better, and farming and grazing pressure was lower. Two corrections were made in order to obtain usable images:

Radiometric correction: at the time of acquisition of the image, a certain amount of "radiometric noise" was present in the image, due either to sensor deficiencies, or to data transmission problems, or to interpretation (coding/decoding). This correction made it possible to modify and adjust the levels of radiance levels by applying a calibration (Shlien, 2014). In other words, it involved changing radiance values and brightness to obtain the geophysical parameters of the environment;

Atmospheric correction: this correction has enabled us to extract information linked to the atmosphere (clouds, humidity, shadows), variable in time and space, and to extract information independent of these effects (kergomard, 2000). Once pre-processed, satellite images were used to conduct spatial analyses over time to extract essential information on the extent of plant cover. These images, downloaded from the Earth Explorer platform, were analysed using ENVI 5.6 software. The images were selected based of spatial, temporal, and spectral resolution. Processing with ENVI 5.6 produced a spatialization of land use by supervised classification and vegetation cover. The classes were distinguished spectrally by combining dry season Normalised Difference Vegetation Index (NDVI), Soil Adjusted Vegetation Index (SAVI) to minimise soil brightness, and woody vegetation was separated from shrubby vegetation using a late dry season (February) NDVI threshold of 0.35 combined with SAVI > 0.30 (Brandt et al., 2016).

The various maps were finalised and formatted using QGIS 3.28 mapping/SIG software. This operation focused first on geometric correction, then on categorisation of the various classes resulting from ENVI processing, followed by statistical production of surface areas, and finally on the layout of the map. The quality of the classification obtained was assessed using the parameters calculated from the confusion matrix: overall accuracy and the Kappa coefficient (Girard & Girard, 1999).

2.3. Data Processing and Analysis

The tree species diversity was assessed with the Shannon-Weaver diversity index (H') (Eq.1) (Magurran, 2004), Pielou's Evenness index (EQ) (Eq.2) and the Margalef diversity index (Mg) (Eq.3) (Margalef, 1958).

$H' = -\Sigma Ni/N \log 2Ni/N$…….(1)

where H' = index of species diversity, Ni = number of individuals of a given species i, N = total number of individuals, log2 = logarithm in basis 2.

$EQ = H'/\log 2N$……..(2)

where H' is Shannon-Wiener diversity, and N is the total number of species in a sample. This index varies from 0 to 1.

$Mg = S-1/\ln N$……(3)

where Mg is the Margalef's Richness Index value, S is the total number of species found, N is the total number of individuals in the sample, and ln(N) is the natural logarithm of N. This index varies from 0 to 6.

To describe the ecological importance of species and families within each collection unit as well as for the total flora within the forest massifs, the species importance value index (IVI) (Eq.4) (Mori et al., 1983) and family importance value index (FIV) (Eq.5) were also calculated:

IVI = relative density + relative frequency + relative dominance…….(4)

Relative density = (Number of individuals of a species (Ni)/total number of individuals of all species (N)) × 100

Relative frequency = (Frequency of a species/sum of all frequencies) × 100

Relative dominance = (basal area of a species/basal area of all the species) x 100.

FIV = Family relative diversity + relative density + relative dominance…….(5)

The heterogeneity of the forest massifs was assessed using Sörensen's Coefficient Similarity Index (SCSI) with the following formula (Eq.6) (Sörensen, 1948):

$$SCSI = \frac{2c}{(a+b)} \qquad (6)$$

where SCSI is Sörensen's Coefficient Similarity Index, a the number of species present in survey a, b the number of species present in survey b, and c the number of common species.

Mean comparisons of basal area between the collection units in each forest massif, and between the two forest massifs were performed with One-way ANOVA test.

All the statistical analyses were performed with Statgraphic 5.0 and XLSTAT softwares and the graphs with Microsoft Office Excel 2007 software.

3. RESULTS AND DISCUSSION

3.1. Floristic Composition and Tree Species Diversity of the Forest Massifs

In total, 9807 tree stems of dbh ≥ 5 cm were inventoried in Bougaye and Lokoro forest massifs. They were distributed within 55 species, 38 genera and 19 families (Table 2). The number of species per collection unit ranged from 26 to 38. The number of species was lower in CU_1 (27) and CU_3 (26) in the Bougaye and Lokoro forest massifs, respectively, due to agricultural activities and pastures in these collection units. Actually, the two collection units were situated near the riparian villages, and as such are under strong anthropogenic pressure. These results were different from those obtained by Froumsia et al. (2012) in Kalfou forest reserve in Cameroon, who identified 86 species distributed in 42 genera and 25 families. This difference could be explained by the fact that our study site was accessible to local people for the extraction of wood and non-timber forest products. However, these results were similar to those obtained by Gonsi et al. (2025) in the Masgaya forest massif in the Sudano-Sahelian zone of Cameroon, with 64 species distributed in 46 genera and 19 families.

The Shannon- Weaver diversity index (H') values varied from 2.26 to 2.66 in Bougaye forest massif and from 2.35 to 2.47 in Lokoro forest massif (Table 3). Following the index values of Shannon-Weaver (H'), the X^2 test of homogeneity suggests that the null hypothesis is accepted ($p = 0.170$, $\alpha = 0.05$). Kent and Coker (1992) stated that forest communities considered rich are characterised by a Shannon diversity value (H') of about 3.5 or higher. Shannon diversity index values were weak because of anthropogenic activities in the forest massifs. Nevertheless, according to Todou et al. (2016), for any Shannon diversity value between 3 and 4, the diversity is moderate and could be considered good regeneration of species in the Sahelian ecoregion. The Pielou's evenness index (EQ) values oscillated between 0.35 and 0.42 in Bougaye forest massif, and between 0.29 and 0.45 in Lokoro forest massif. In fact, according to Dajoz (1982), ecosystems which are subject to permanent anthropogenic disturbances have low Pielou's evenness index values. These results are in accordance with those of Souare et al. (2020), Bakoulou et al. (2020), and Gonsi et al. (2025), who obtained respectively 0.47 in Laf forest reserve; 0.45 in Bangana forest massif; and 0.41 in Masgaya forest massif. The similarities of the results should be explained by the fact that our study site was in the same ecological conditions. Gonsi et al. (2025) stated that ecological and climatic conditions influence the distribution of plants and plant growth. The low values could be attributed to anthropogenic activities such as logging for the needs of firewood, overgrazing, urbanization and agricultural

Table 2. Floristic composition of the forest massifs

Floristic composition	Bougaye forest massif				Lokoro forest massif				Global value
	CU_1	CU_2	CU_3	TBFM	CU_1	CU_2	CU_3	TLFM	
Species	27	38	36	45	38	35	26	46	55
Genera	22	28	30	32	28	28	21	33	38
Families	12	14	14	16	15	14	12	17	19
Number of stems	1726	1232	1780	4738	2507	1943	619	5069	9807

CU : Collection unit ; TBFM : Total Bougaye forest massif; TLFM: Total Lokoro forest massif

Table 3. Diversity indices of the forest massifs

Collection units	Bougaye forest massif			Lokoro forest massif			Global indices
	CU_1	CU_2	CU_3	CU_1	CU_2	CU_3	
H'	2.26	2.79	2.54	2.41	2.35	2.47	2.70
EQ	0.35	0.42	0.35	0.29	0.30	0.45	0.27
Mg	3.48	5.19	4.67	4.72	4.49	3.88	5.87
SCSI				0.79			

CU: collection unit; H': Shannon- Weaver diversity index; EQ: Pielou's evenness index; Mg: Margalef index; SCSI: Sörensen's Coefficient Similarity Index

development. The Margalef index (Mg) values varied from 3.48 to 5.19 in Bougaye forest massif and from 3.88 to 4.72 in Lokoro forest massif. The Sörensen's Coefficient Similarity Index value was 0.79, indicating a clear similarity in species composition between the two forest massifs. The Sörensen's Coefficient Similarity Index value was 0.79, indicating a clear similarity in species composition between the two contiguous forest massifs.

3.2. Structural Parameters: Basal Area and Density

The basal area varied from 238.26±98.03 m²/ha to 462.46±75.70 m²/ha (Table 4). These values were found in Lokoro forest massif in CU_2 and CU_1, respectively. The values were moderate in Bougaye forest massif, ranging from 249.25±60.02 m²/ha to 397.32±55.40 m²/ha. At the 0.05 level, the two-way ANOVA reveals no significant effect of massif ($F = 3.17$, $p = 0.08$), but a highly significant effect of collection unit ($F = 31.59$, $p < 0.0001$) and a highly significant effect of the massif x collection unit interaction ($F = 31.50$, $p < 0.0001$). This interaction indicates that the collection unit effect depends on the massif. Within Bougaye forest massif, one-way ANOVA is significant ($F = 29.05$, $p < 0.0001$), and within Lokoro forest massif, one-way ANOVA is also significant ($F = 32.83$, $p < 0.0001$). Tukey HSD post-hoc test (HSD 0.05 = 78.2 m^2/ha) grouped LCU_1 (462.46 m^2/ha) as group "a" and BCU_3 (397.32 m^2/ha) as group "b", both significantly higher than the other units. BCU_1 (318.15 m^2/ha) grouped with LCU_3 (275.88 m^2/ha) and LCU_2 (238.26 m^2/ha) in group "c" while BCU_2 (249.25 m^2/ha) was lowest in group d overlapping with LCU_2/CU_3. This confirms the interaction massif x CU. Assumptions for two-way ANOVA were verified. Normality of residuals was confirmed by the Shapiro-Wilk test ($W = 0.96$, $p = 0.13$). Homogeneity of variances was confirmed by Levene's test ($F = 0.914$, $p = 0.48$). At the 0.05 level, two-way ANOVA: all 3 effects are highly significant (massif, $F = 36.12$, $p < 0.00001$; CU, $F = 8.38$, $p < 0.0007$; massif x CU, $F = 9.54$, $p < 0.0003$), unlike basal area massif, which was $p = 0.08$. Tukey HSD is 58.1 stems/ha. After transformation, Levene's test ($p = 0.38$) and Shapiro residuals ($p = 0.21$) validate ANOVA. The low values of density were due to anthropogenic disturbances caused by the locals who depend on the natural resources in the forest massifs. According to Azaria et al. (2024), demographic growth, accompanied by certain modes of exploitation, leads to land degradation, resulting of a disruption of environmental balances.

3.3. Species Importance Value Index (IVI) and Family Importance Value Index (FIV)

The thirteen most important species (those of importance value index (IVI)≥10) represented 23.63 % in the two forest massifs. In Bougaye forest massif, the twelve most important species were found (Table 5), representing 78.48 % of the total IVI in that forest massif, especially *Balanites aegyptiaca* (L.) Del. (IVI = 40.02), *Guiera senegalensis* J.F. Gmel. (31.05), *Anogeissus leiocarpa* (DC.) Guill. & Perr. (IVI = 25.07), *Combretum glutinosum* Perr. ex DC. (IVI = 24.21), *Hexalobus monopetalus* (A. Rich.) Engl. & Diels (IVI = 21.06) with an IVI ≥ 20. In the Lokoro forest massif, 10 species were the most dominant, representing 75.43% of the total IVI. Amongst them were found *Guiera senegalensis* J.F. Gmel. (IVI = 35.35), *Anogeissus leiocarpa* (DC.) Guill. & Perr. (IVI = 33.30), *Combretum molle* R. Br. ex G. Don (IVI = 28.73), *Combretum glutinosum* Perr. ex DC. (IVI = 23.93) and *Prosopis africana* (Guill. Et Perr.) Taub. (IVI = 20.34) with an IVI≥ 20. The majority of the species, 67.39 % in the Lokoro forest massif and 73.33 % in the Bougaye forest massif, had IVI values less than 10, and 13 (28.26 %) species had IVI values less than 1 in the Lokoro forest massif and 16 (35.55 %) in the Bougaye forest massif. They were *Senegalia ataxacantha* (DC.) Kyal. & Boatwr., *Vachellia gerrardii* P.J.H. Hurter, *V. sieberiana* (DC.) Kyal. & Boatwr.,

Table 4. Basal area and density in the forest massifs

Structural parameters	Forest massifs	Collection units		
		CU_1	CU_2	CU_3
Basal area (m²/ha)	BFM	318.15±43.40^{c}	249.25±47.60^{d}	397.32±53.80^{b}
	LFM	462.46±75.70^{a}	238.26±98.03^{cd}	275.88±82.70^{cd}
Density (Stems/ha)	BFM	191.77±25.20b^{c}	136.88±27.80^{c}	197.77±20.78^{bc}
	LFM	278.55±86.60^{a}	215.88±87.90^{ab}	168.77±52.50^{bc}

BFM: Bougaye forest massif; LFM: Lokoro forest massif; CU: collection unit; values shown are means ± SD; within lines, means followed by different letters are significantly different ($p < 0.05$)

Table 5. Importance value index (IVI) of the 10 most important species (in bold) in the forest massifs

Species	Bougaye forest massif IVI				Lokoro forest massif IVI			
	CU_1	CU_2	CU_3	Global IVI	CU_1	CU_2	CU_3	Global IVI
Senegalia ataxacantha (DC.) Kyal. & Boatwr.	3.14	5.08	7.37	5.35	1.86			0.81
Vachellia gerrardii P.J.H. Hurter			0.75	0.29	0.75			0.33
Senegalia senegal (L.) Britton		0.69		0.24				
Vachellia seyal (Delile) P.J.H. Hurter					0.62	1.56	1.32	1.09
Vachellia sieberiana (DC.) Kyal. & Boatwr.			0.66	0.25	0.68			0.30
Annona senegalensis Pers.		0.77	0.67	0.51				
Anogeissus leiocarpa (DC.) Guill. & Perr.	31.52	22.92	21.23	25.07	33.18	30.57	40.60	33.30
Balanites aegyptiaca L.	29.33	35.19	52.08	40.02	16.17	16.40	27.42	18.37
Bombax costatum Pellegr. & Vuillet	3.76	2.31	2.31	2.71	1.48	1.76		1.29
Cadaba farinosa Forssk.		0.79	1.90	0.98	2.78		1.70	1.55
Capparis sepiaria L.	1.89	4.33	3.36	3.26	8.64	2.11		4.72
Combretum aculeatum Vent.	2.24	1.30		1.05		0.75	1.32	0.54
Combretum collinum Fresen.	28.87	13.47	4.98	15.53	4.86	20.16	29.76	14.62
Combretum fragrans F. Hoffm.		2.00		0.58	0.65	0.76	7.88	2.21
Combretum glutinosum Perr. & DC.	30.23	21.39	21.01	24.21	23.47	24.41	27.19	23.93
Combretum micranthum G. Don	9.42	12.50	9.60	10.30	24.48	17.31		17.68
Combretum molle R. Br.	6.59	12.77	22.19	14.33	43.56	25.16		28.73
Commiphora africana (A. Rich.) Engl.					2.22		1.33	0.98
Commiphora pedunculata (Kotschy & Peyr.) Engl.		2.96	2.57	1.97	5.54	2.67		3.65
Detarium microcarpum Guill.	2.79	14.04	6.54	7.34	3.91	9.70	12.41	7.10
Dichrostachys cinerea (L) Wight & Arn.		3.44	4.26	2.68	3.87	2.06	1.84	2.74
Entada africana Guill. & Perr.	3.51	3.19	1.42	2.51	5.68	4.72		4.09
Feretia apodanthera Del.	4.19	6.84	4.62	5.08	5.57	3.46		3.76
Ficus thonningii Blume							1.54	0.33
Ficus vogelii (Miq.) Miq.			1.46	0.58			2.86	0.70
Flueggea virosa (Roxb. Ex Willd.) Voigt					0.62	2.83		1.29
Guiera senegalensis J.F. Gmel.	38.01	23.61	30.89	31.05	28.96	52.13	19.67	35.35
Hexalobus monopetalus (A. Rich.) Engl.& Diels	10.21	24.58	28.54	21.06	4.95	7.45	21.81	9.10
Hymenocardia acida Tul.		2.32	1.91	1.49		2.89		1.01
Lannea fruticosa (A. Rich.) Engl.	2.11	2.71	1.66	2.12	4.35	2.97		2.89
Lannea microcarpa Engl. & Krause	1.87	4.30	6.39	4.39	9.85	3.98	6.49	7.21
Lonchocarpus laxiflorus Guill. & Perr.		0.99	3.75	1.76		0.83	2.15	0.75
Maerua angolensis DC.			1.32	0.50	1.85	0.88		1.11
Maytenus senegalensis (Lam.) Exell	0.86			0.24				
Pachystela brevipes (Baker) Baill.					0.72	1.85	1.77	1.32
Piliostigma reticulatum (DC.) Hochst.	0.90	5.08	4.01	3.39	0.66	3.47	3.03	2.11
Piliostigma thonningii (Schum.) Milne-Redh.		0.79		0.26				
Prosopis africana (Guill. & Perr.) Thaub.	21.69	23.30	13.78	18.76	15.73	22.53	28.51	20.34
Sclerocarya birrea (A. Rich.) Hochst.	12.92	15.97	7.48	11.39	2.96	3.43	35.41	10.98
Securidaca longipedunculata Fresen.		2.24		0.75				

Cont...

Table 5. Importance value index (IVI) of the 10 most important species (in bold) in the forest massifs

Species	Bougaye forest massif IVI				Lokoro forest massif IVI			
	CU_1	**CU_2**	**CU_3**	**Global IVI**	**CU_1**	**CU_2**	**CU_3**	**Global IVI**
Senna singueana Del.			0.62	0.24				
Sterculia setigera Del.	11.65	10.66	13.90	12.36	17.42	5.36	11.35	12.45
Stereospermum kunthianum Cham.						1.36		0.40
Strychnos innocua Del.		0.69		0.24				
Strychnos spinosa Lam.	26.93	2.79	3.37	11.27	3.27			1.42
Swartzia madagascariensis Desv.		2.08	0.63	0.89				
Tamarindus indica L.	1.75			0.48		0.76		0.27
Terminalia avicennioides Guill. & Perr.	5.11	8.00	6.62	6.40	13.25	8.01	7.97	10.48
Vitellaria paradoxa C.F. Gaertn.					0.70			0.31
Vitex doniana Sweet					0.62			0.27
Vitex simplicifolia Oliv.							2.02	0.36
Ximenia americana L.	1.79	1.47	2.74	2.01	2.50	5.67		3.00
Ziziphus abyssinica Hochst. ex A. Rich.		0.85		0.28				
Ziziphus mauritiana Lam.					0.69	0.95	1.33	0.90
Ziziphus mucronata Willd.	6.72	1.62	3.41	3.70	0.89	9.07	1.32	3.76

IVI: Importance value index ; CU: Collection unit

Annona senegalensis Pers.*, Cadaba farinosa* Forssk*, Ficus vogelii* (Miq.) Miq.*, Maytenus senegalensis* (Lam.) Exell, *Strychnos innocua* Del.*, Stereospermum kunthianum* Cham.*, Swartzia madagascariensis* Desv.*, Tamarindus indica* L.*, Vitellaria paradoxa* C.F. Gaertn.*, Vitex doniana* Sweet*, Ziziphus abyssinica* Hochst. ex A. Rich.*, Z. mauritiana* Lam.

The five most important families (those of the highest values of FIV index) (Table 6) were Combretaceae, Fabaceae, Zygophyllaceae, Anacardiaceae and Annonaceae in Bougaye forest massif, representing 84.16% of the total family importance value index, and Combretaceae, Fabaceae, Anacardiaceae, Zygophyllaceae and Malvaceae in Lokoro forest massif, representing 86.64 % of the entire forest massif. Considering the two forest massifs, Combretaceae, Fabaceae and Zygophyllaceae were the most dominant families, as shown by the principal component analysis (Figure 2). They were strongly and positively correlated to the F1 axis with a contribution of 97.66 % (Pearson r = 0.87). The least important families with FIV ≤ 1 were Celastraceae, Moraceae and Polygalaceae in Bougaye forest massif, representing 0.52%, and Bignoniaceae and Verbenaceae, which accounted for 0.36% in Lokoro forest massif. Species belonging to families with high values of FIV index were Combretaceae, Fabaceae, and Zygophyllaceae (Figure 2) and were the most dominant families in the study site due to their strong regeneration capacity. These results corroborate those of Kemeuze et al. (2015) and Souare et al. (2020), who stated that in the semi-arid zone of Cameroon, Combretaceae and Fabaceae families are recognized as the most dominant families. Really, the drought of the Sahel drought has allowed the natural selection of the most robust species, such as those in these families.

3.4. Spatio-temporal Changes from 1978 to 2023

Landsat images from 1978 to 2023 revealed four classes of land cover (Figure 3), notably woody vegetation, shrubby vegetation, herbaceous vegetation, and bare soil and built-up areas. The overall accuracies obtained were 99.16%, 97.81% and 97.19% with Kappa coefficients of 0.96, 0.85 and 0.87, respectively, for the images of 1978, 1998 and 2023.

The evolution of the areas of land use classes and the annual rate of change from the period from 1978 to 2023 in the two forest massifs showed that the cumulative area covered by the different formations accounted for 17036.13 ha (Table 7). The space covered by the different formations within it, namely woody vegetation, shrubby vegetation, herbaceous vegetation, bare soil, and built-up lands, varied

from one period to another and are unevenly distributed. The period from 1978 to 1998 was characterised by a slight variation in the space occupied by the formations. We noted the dominance of woody vegetation covering almost half of the area with an average of 45%, followed by shrubby vegetation with a little cover of 33%. The other formations followed the same trend, with a slight regression of around 0.21 ha of bare soil and built-up land. The trend is quite different from 1998 to 2023, with a significant regression of woody vegetation of 3153.551 ha and an increase in the remaining classes, respectively 942.027 ha, 1136.318 ha and 1075.207 ha for shrubby vegetation, herbaceous vegetation and bare soil and built-up land. Across the area, woody vegetation decreased with an annual rate of -0.69% (Figure 4). The other formations recorded an increase of approximately 0.2%.

Table 6. Family importance value (FIV) of the five most important families (in bold) of each forest massif censused within the collection units

Family	Bougaye forest massif				Lokoro forest massif			
	CU_1	FIV CU_2	CU_3	Global FIV	CU_1	FIV CU_2	CU_3	Global FIV
Anacardiaceae	16.91	22.97	15.53	17.90	17.17	10.38	41.90	21.08
Annonaceae	10.21	25.35	29.21	21.57	4.95	7.45	21.81	9.10
Bignoniaceae						1.36		0.45
Burseraceae		2.96	2.57	1.97	7.76	2.67	1.33	4.63
Capparaceae	1.89	5.12	6.57	4.74	13.28	2.99	1.70	7.39
Celastraceae	0.86			0.24				
Combretaceae	151.98	117.95	116.51	128.56	172.41	179.27	134.40	166.81
Fabaceae	33.79	58.68	43.80	44.43	33.77	45.63	49.25	39.93
Loganiaceae	26.93	3.48	3.37	11.50	3.27			1.43
Malvaceae	15.41	12.97	16.21	15.07	18.90	7.13	11.35	13.74
Moraceae			1.46	0.58			4.41	1.04
Phyllanthaceae		2.32	1.91	1.49	0.62	5.71		2.30
Polygalaceae		2.24		0.75				
Rhamnaceae	6.72	2.47	3.41	4.07	1.58	10.02	2.65	4.67
Rubiaceae	4.19	6.84	4.62	5.08	5.57	3.46		3.76
Sapotaceae					1.42	1.85	1.77	1.63
Verbenaceae					0.62		2.02	0.64
Ximeniaceae	1.79	1.47	2.74	2.03	2.50	5.67		3.05
Zygophyllaceae	29.33	35.19	52.08	40.02	16.17	16.40	27.42	18.37

FIV: Family importance value; CU: Collection unit

Table 7. Evolution of the land cover from 1978 to 2023 in the forest massifs

Year/Land cover	1978		1998		2023		Evolution in 45 years		Annual rate of evolution (%)
	Area (ha)	%	Area (ha)	%	Area (ha)	%	Area (ha)	%	
Woody vegetation	7669.971	45.02	7702.028	45.21	4548.477	26.7	-3121.494	-18.32	-0.69
Shrubby vegetation	5692.219	33.41	5704.816	33.49	6646.843	39.02	954.624	5,61	0.21
Herbaceous vegetation	2946.944	17.3	2938.447	17.25	4074.765	23.92	1127.821	6.62	0.25
Bare soil and built-up land	726.996	4.27	690.835	4.06	1766.042	10.37	1039.046	6.1	0.23
Total	17036.13	100	17036.13	100	17036.13	100	/	/	/

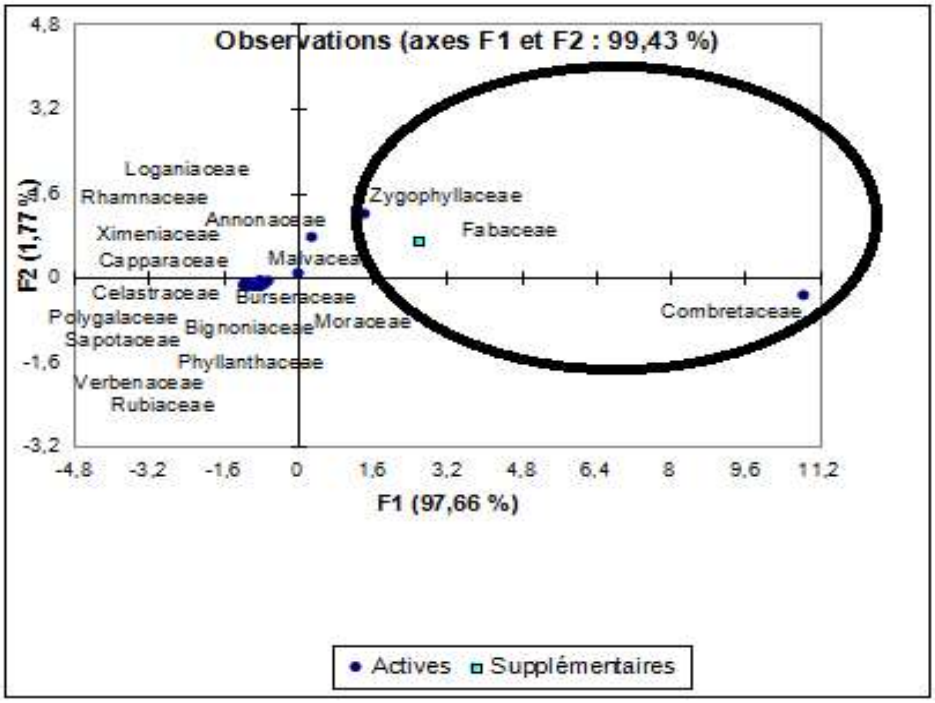


Figure 2. The figure indicates the ecological importance of the families in Bougaye and Lokoro forest massifs, highlighting the three most dominant families

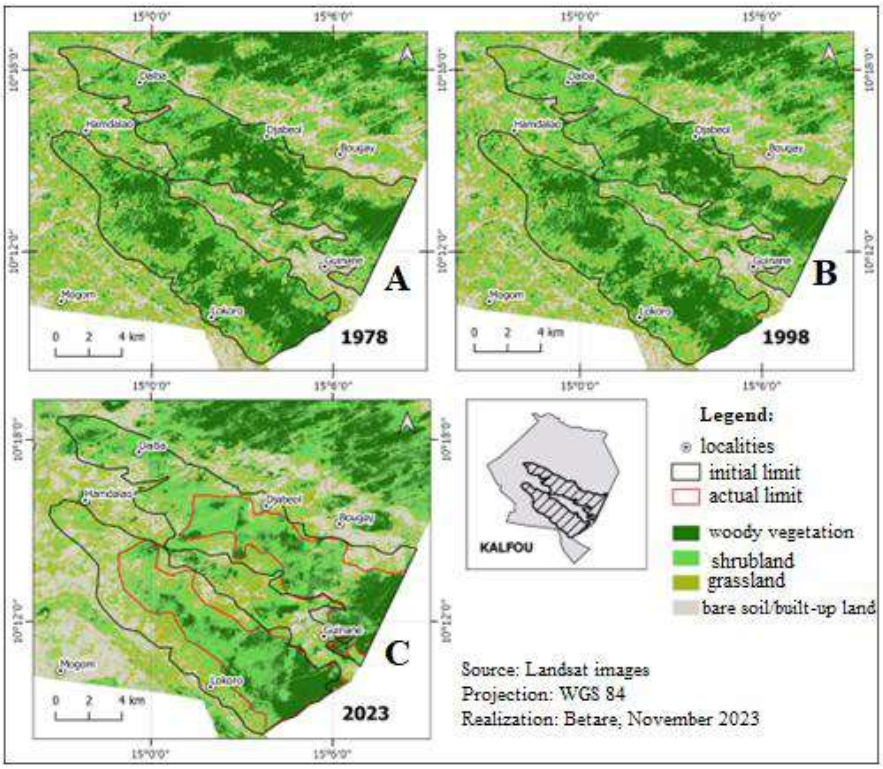


Figure 3. The figures show the change in land use and land cover from the year 1978 to 2023 in Bougaye and Lokoro forest massifs, in the sudano-sahelian zone of Cameroon (A = 1978; B= 1998; C= 2023)

The area of Bougaye and Lokoro forest massifs from 1978 to 2023 (about 45 years) showed variations across land-use classes. In-depth analysis (Figure 4) reveals a decline in woody vegetation with an annual rate of -1.2% between 1998 and 2023, and -0.6% between 1978 and 2023. This trend corresponds to a significant regression of 3153.551 ha of the woody vegetation and an increase in bare soil and built-up land of 1075.207 ha. The decrease in tree cover is explained by the increase population in this area, which has resulted in a cropland expansion and urbanisation. Agricultural development and urbanisation have led to the conversion of forest areas into anthropogenic occupation classes. All the same, grazing pressures, deforestation for roads, logging, fuelwood and charcoal production reduce total forest area, and fragmentation isolates populations, changes the local climate and water balance, limits gene flow and increases local extinctions. Diguera et al. (2024) noted a decrease in tree cover with a rate of annual expansion on the order of -1.22% in the Pette area situated in the same ecoregion of Cameroon. All the same, Azaria et al. (2024) in the eastern bank of Benoue river in sudanian zone of Cameroon showed a reduction of 25% of woody vegetation for about 30 years, and Dwivedi et al. (2025) who indicated that 58.32% of the total area of the Hindon river basin is used for agriculture purposes in India. Our results are also similar to those obtained by Sarfo et al. (2022) in Southwestern Ghana, who found evidence of expansion in farmlands and built-up areas over the given period. Arouna et al. (2010), Tente et al. (2010), and Temgoua et al. (2018) also showed that agricultural activities, house built-up, and logging are the major causes of vegetation degradation. Indeed, several authors have reported that the changes in landscape composition in the Sahelo-Sudanian, Sudanian, Sudano-Guinean and Guinean zones are the result of rapid and progressive anthropisation manifested by unsustainable agro-silvo-pastoral practices (Illiassou et al., 2015; Alohou et al., 2016).

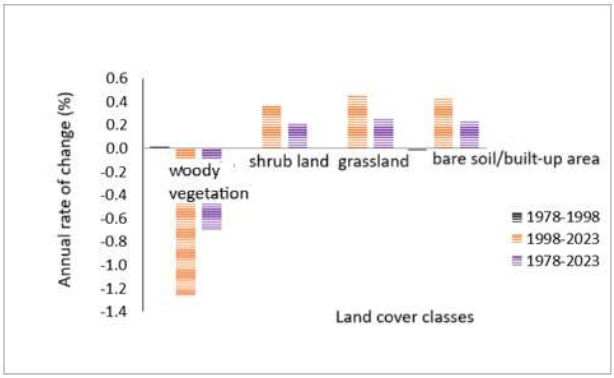


Figure 4. The figure shows the annual rate of changes of land cover classes from 1978 to 2023, in the area of Bougaye and Lokoro forest massifs in Cameroon

3.5. Study Limitations

In the context of this study, we had some constraints such as Landsat spatial resolution, the absence of socioeconomic surveys, the lack of regeneration assessment and the seasonal vegetation variability. A future study will be addressed specifically to these constraints which are important for conservation implications in this area. A complementary inventory with nested micro-plots of 5 m x 5 m will be necessary for regeneration assessment. Also, future work will integrate Sentinel-2 (10 m) and a diachronic analysis with at least 2 dates (early/late dry season), and a socioeconomic survey as complementary to the structural inventory to understand anthropogenic pressure drivers on the two massifs.

4. CONCLUSION

Anthropogenic activities, notably agricultural expansion, grazing, fuelwood and charcoal production in tropical forests, threaten biodiversity by removing forests, degrading them, and altering local climate, thereby affecting global ecosystem functions and services. The study aimed at assessing plant diversity and land use of two contiguous forest massifs in the Sudano-Sahelian zone of Cameroon. The floristic inventory used a split-plot design, and diachronic analysis was conducted of three Landsat satellite images: Landsat MSS (1978), Landsat TM (1998), and Landsat OLI (2023). The results revealed a moderate diversity of species within the two forest massifs, and the observed variations in index values and vegetation structure in different collection units indicate changes in vegetation vigour and cover. The findings also highlight significant variations in woody vegetation and bare soil/built-up area from 1978 to 2023. Agricultural development, grazing pressure, fuelwood and charcoal production would have led to forest loss and the depletion of plant diversity. An implementation of appropriate land use policy in this area should be a high priority, including the creation of buffer zones, the maintenance of connectivity corridors between the massifs, the promotion of assisted natural regeneration on overgrazed rangelands and the involvement of riparian communities in the participatory management of the forest massifs to ensure the sustainability of restoration actions.

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CRediT authorship contribution statement

Manga Tiga Job: Writing original draft, investigation, data analysis; **Bakoulou Ngamo Africa**: Investigation, data curation; **Djibrilla Mana**: Investigation, data curation; **Beidi Eugene**: Investigation; **Froumsia Moksia**: Investigation; **Todou Gilbert:** Investigation; **Souare Konsala**: Writing original draft, investigation, data analysis.

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Conflict of interest

The authors declare no competing interests that could influence the work reported in this paper.

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 or in the creation or alteration of any figures

Data availability statement

The data used and analysed in this study will be available from the corresponding author upon request.

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Contributions to the Life-history, Morphology, Chaetotaxy and Host-Plants of *Creatonotos transiens* (Walker, 1855) (Lepidoptera: Erebidae: Arctiinae) from Himachal Pradesh, India

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ABSTRACT: This study investigated the life-history characteristics, host-plant associations, immature-stage morphology, and larval chaetotaxy of the Clouded Tiger Moth, *Creatonotos transiens* (Walker, 1855), from different localities in the District of Kullu, Himachal Pradesh. Surveys were conducted regularly from April 2019 to October 2024, alongside laboratory rearing. The egg masses of this tiger moth were collected and reared under controlled conditions to document developmental stages, duration, feeding and pupation behaviour and morphological characteristics of its immature stages on Apple tree, *Malus domestica* (Suckow) Borkhausen, 1803 and Bitter Dock Weed, *Rumex obtusifolius* Linnaeus, 1753. The eggs were spherical and creamish-white, deposited in masses of 50-70, with an incubation period of 4-5 days. The larvae underwent six moults and seven instars, with a total larval period of 27-50 days. It completed its life cycle in 42-70 days, including an 11-15 day pupal period. Distinct morphological changes in colouration, body markings, tubercles and setal development were observed across successive larval instars. The species exhibited polyphagous feeding behaviour and successfully reared on apple and bitter dock under laboratory conditions. Detailed chaetotaxic examination of the final instar revealed characteristic arrangements of setal groups on the head, thoracic, and abdominal segments, together with uniordinal mesoseries of 20-25 crochets on the abdominal pro-legs. The findings provide baseline information on the immature morphology, developmental biology, host-plant relationships and chaetotaxy of *C. transiens* (Walker, 1855). Such information is valuable for accurate identification of immature stages, morpho-taxonomic studies, understanding host associations, and supporting future systematic and phylogenetic investigations of Lepidoptera.

Keywords: *Creatonotos transiens*, Clouded Tiger Moth, Life history, Immature stages, Host plants, Larval chaetotaxy, Kullu, Himachal Pradesh

1. INTRODUCTION

Insecta constitute the most diverse group of animals and occupy almost every terrestrial ecosystem. Widely distributed, they play an important role in global ecology because of their tremendous heterogeneity in form, function, and lifestyle. These creatures are major contributors to biodiversity in the majority of habitats and consequently play an array of hugely relevant ecological roles in many functions of an ecosystem. Moths and other Lepidoptera are particularly important components of these ecological networks because their larval stages are primarily herbivorous, while adults may contribute to pollination and serve as food for birds, bats and other insectivorous

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organisms. The ecological significance of Lepidoptera extends beyond their taxonomic diversity. Their larvae utilize a wide variety of plant resources and may influence plant growth, leaf biomass and vegetation dynamics through herbivory. At the same time, caterpillars constitute an important food resource for numerous predators and parasitoids, thereby contributing to energy transfer between trophic levels. The close association between lepidopteran larvae and their host plants makes host-plant availability an important determinant of their distribution, abundance, survival and reproductive success. Therefore, investigations of larval host plants, feeding behaviour and developmental biology provide valuable information for understanding species-plant interactions and the ecological niche occupied by individual moth species. Lepidoptera complete their life cycle in a particular season, and each species is host-dependent, feeding on a particular host plant. The availability of food plants directly affects their population. Knowledge about larval host-plants helps us to understand the host-plant relationships of different species. The identification of immature stages, particularly the larvae, remains an important aspect in the field of immature stage taxonomy. According to Hinton (1946), the setae and tubercles occur systematically across the whole body in even numbers in the moths. In India, only few scattered works of Issac and Venkatraman (1941), Mathur (1954, 1959), Singh (1956), Mathur and Singh (1963), Azam and Ali (1965), Goel and Kumar (1981), Singh and Goel (1987), Kaur (2004), Sri et al. (2010), Subramoniam and Chitra (2019), Chakravarty et al. (2021), Yusof et al. (2021), Divya et al. (2023) and Kaleka and Kapoor (2024) are there who investigated different aspects of life-history, morphology and larval chaetotaxy of moth species.

2. MATERIALS AND METHODS

2.1. Study Area

Field surveys were carried out in seven localities, i.e., Bhuntar, Dhaugi, Kanaun, Kullu, Larji, Manali and Naggar, of District Kullu in Himachal Pradesh from April 2019 to October 2024.

2.2. Sampling and Data Collection

The egg masses of *Creatonotos transiens* (Walker, 1855) were collected from Apple tree, *Malus domestica* (Suckow) Borkhausen, 1803 and Bitter Dock Weed, *Rumex obtusifolius* Linnaeus, 1753. We collected the adult moths, pinned them, spread their wings, and preserved them in well-fumigated wooden entomological boxes. Photographs of eggs, larvae, their feeding patterns, damaged host plants, pupae, and adults were taken with a Canon 300D digital camera in the field and in the laboratory.

2.3. Observations

The collected egg masses of *C. transiens* were transferred

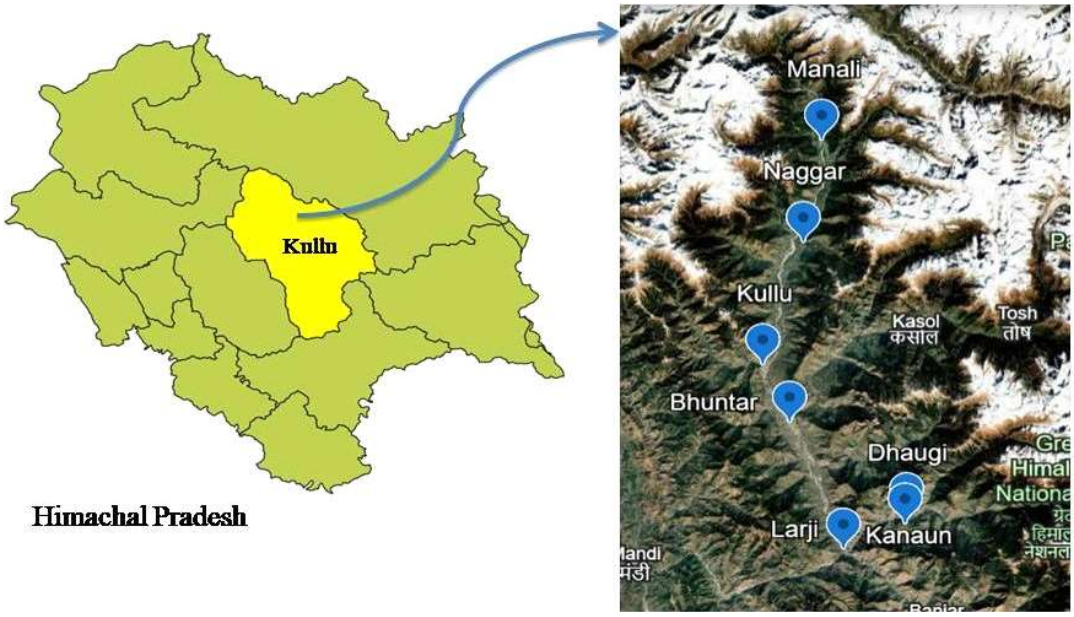


Figure 1. Surveyed localities of District Kullu, Himachal Pradesh

to Petri dishes for rearing. Fresh leaves of host-plants were kept in Petri dishes along with moist filter paper to keep the leaves fresh. This served as an immediate source of food for the first-instar larvae. After hatching, the larvae were placed in insect breeding cages (8″ x 8″ x 8″) with appropriate food, i.e., fresh leaves of the host plant, for further rearing. The final larval instars were transferred to separate cages for proper pupation. The pupae were sorted, collected and kept in small plastic jars covered with muslin cloth. The eggs, different larval instars and pupae were preserved in 70% alcohol for further studies. For larval chaetotaxy, we followed the methodology adopted by Kaleka and Kapoor (2024).

3. RESULTS AND DISCUSSION

3.1. Life-history Stages, Developmental Time and Host-plants of *Creatonotos transiens* (Walker, 1855): (Figures 2-13)

Eggs (Figures 2-4): Eggs were spherical in shape, creamish-white in colouration; approximately 0.35- 0.48 mm in size; laid in masses of 50-70 eggs on the lower surface of the leaves and not covered with hair. After 3-4 days of oviposition, the eggs turned dark yellow, then dark grey just prior to hatching. Hatching occurred in the morning, i.e., between 7:00 am and 11:00 am. The incubation period was of 4-5 days.

Larva: Larvae of *C. transiens* moulted six times and passed through seven instars. The total larval period lasted for 27-50 days.

1st Instar (Figure 5): The newly hatched larvae were light grey in colour with a black head. After 8-9 hours of hatching or on the next day of hatching, the colour of the larva became pale brown. 1st abdominal segment was brown. A pair of brown sub-dorsal longitudinal lines were present on either side of the body. There was a pair of supra-spiracular tubercles, and each tubercle carried a single grey hair. The length of the first instar was 1.26±0.58 mm, and its duration lasted for 5-7 days.

2nd Instar (Figure 6): After moulting, the larval body became yellowish-orange without any change in head colouration. The brown band on the 1st abdominal segment and brown sub-dorsal longitudinal lines remained same as in the first instar. No change in the pattern of tubercles, colour and number of hairs, it only increased its size. The length of the second instar was 2.78±0.45 mm, and its duration varied from 4 to 7 days.

3rd Instar (Figure 7): After moulting, size increased, and the larval body turned orange-brown without any change in head colouration. A pair of brown sub-dorsal lines were present as in the 2nd instar, but reduced to sub-dorsal brown spots on the thoracic and last three abdominal segments. A series of 5 mid-dorsal white T-shaped spots were present on the 2nd to 6th abdominal segments. 1st and 7th abdominal segments were blackish-brown in colour. Two pairs of tubercles, i.e., sub-dorsal and supra-spiracular, were present. Each tubercle carried 1-2 long greyish-white hair. The length of the third instar was 5.15±0.67 mm, and its duration varied from 3-9 days.



Figure 2. Freshly laid eggs of *Creatonotos transiens* (Walker, 1855)

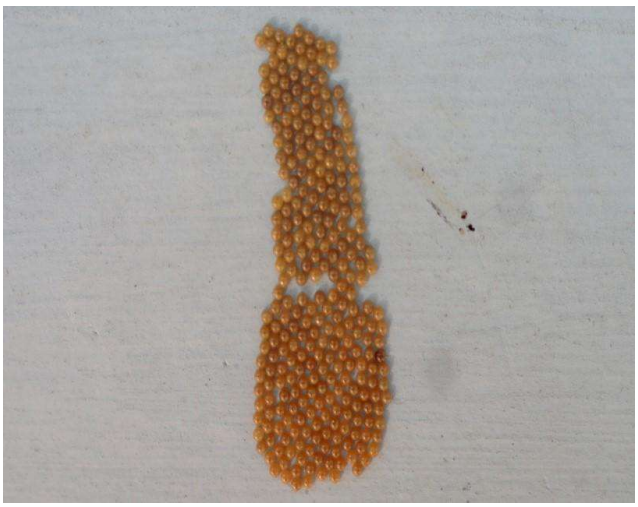


Figure 3. Eggs of *Creatonotos transiens* (Walker, 1855) after the 3rd Day



Figure 4. Eggs of *Creatonotos transiens* (Walker, 1855) prior to hatching



Figure 5. First instar of *Creatonotos transiens* (Walker, 1855)



Figure 6. Second instar of *Creatonotos transiens* (Walker, 1855)

4th Instar (Figure 8): After the third moult, it increased in size, and the larval body became darker. The sub-dorsal brown spots present on thoracic segments and the last three abdominal segments became black. 1st and 7th abdominal segments turned black. The sub-dorsal line was reduced to sub-dorsal black spots, edged with a brown line. The mid-dorsal T-shaped spots remained the same as in the previous instar. Both sub-dorsal and mid-dorsal spots numbered 5. Each sub-dorsal tubercle carried 2-3 long black hairs, and the supra-spiracular tubercle carried 3-5 long greyish-white hairs. The length of the fourth instar was 6.78±0.12 mm, and its duration of 3-8 days.

5th Instar (Figure 9): The fifth instar increased in size, and the larval body turned blackish-brown. The thoracic segments and the last three abdominal segments became dark brown, and sub-dorsal spots turned black. 1st and 7th abdominal segments remained the same as in the previous instar. The mid-dorsal line turned reddish-brown and sub-dorsal black spots present on the 2nd to 6th abdominal segments edged with reddish-brown lines. Each sub-dorsal tubercle carried 3-5 long black hairs, and the sub-spiracular tubercle carried 5-7 long greyish-white hairs. The length of the fifth instar was 8.65±1.34 mm, and its duration varied from 4-7 days.

6thInstar (Figure 10): After moulting, the larva increased its size without any change in colouration and pattern. There were five pairs of tubercles: mid-dorsal, sub-dorsal, supra-

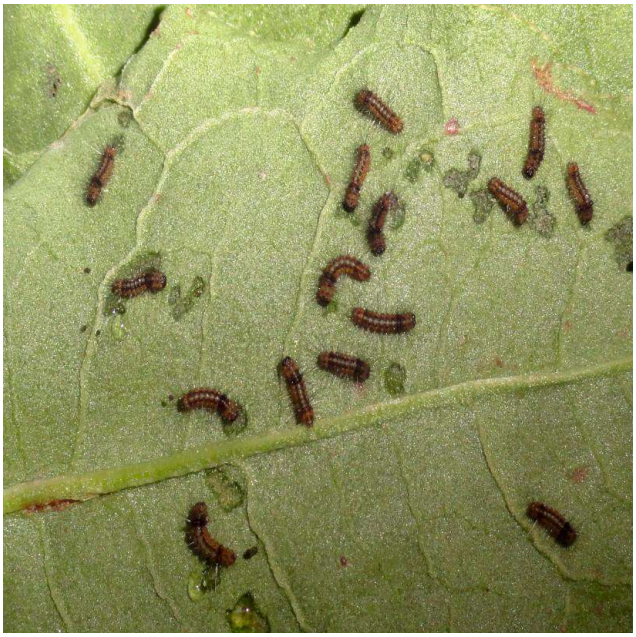


Figure 7. Third instar of *Creatonotos transiens* (Walker, 1855)

spiracular, infra-spiracular and basal tubercles and were brown in colour. Each mid-dorsal, sub-dorsal and supra-spiracular tubercle carried 3-5, 10-15, 5-7 long black hairs, respectively. Each infra-spiracular and basal tubercle had tufts of 15-20 long golden-brown hairs. The length of the sixth instar was 11.54±0.89 mm, and its duration varied from 4-7 days.



Figure 8. Fourth instar of *Creatonotos transiens* (Walker, 1855)



Figure 9. Fifth instar of *Creatonotos transiens* (Walker, 1855)

7th Instar (Figure 11): The final instar was totally distinct from the sixth instar. The larva increased in size, and the body became black with an orange head. The mid-dorsal lines and sub-dorsal spots became obsolete. The bean-shaped, pale-brown spiracles became visible. The tubercles became orange. Each tubercle carried a tuft of 20-25 long



Figure 10. Sixth instar of *Creatonotos transiens* (Walker, 1855)



Figure 11. Seventh instar of *Creatonotos transiens* (Walker, 1855)

orange hairs, which gave them a fuzzy appearance. The length of the seventh instar was 18.21 ±1.04 mm, and its duration varied from 3- 5 days.

Pre-pupation: Prior to pupation, the final instar stopped feeding, shed its hair and shrank in size. The body became motionless and turned brown. The pre-pupal period lasted for 1-2 days.

Pupation: It occurs with or without proper cocoon formation. The cocoon was brown in colour, and the pupa was 12.6±1.34mm in length. The male pupae were having X-shaped genital aperture on the 9th abdominal segment and female pupae had an inverted V-shaped genital aperture on the 8th abdominal segment on their ventral sides. The pupal duration lasted for 11-15 days.

Pupa (Figure 12): Adecticous and obtect; creamish-yellow immediately after pupation, turning reddish-brown after 2-3 days. It hardened and became brown after 4-5 days. Body became elongated, head rounded, with vertex extending towards the posterior region along the prothorax and antenna. Eyes were not so prominent. Thorax with partly visible legs. Abdomen tapering; edges of all segments well marked; dark brown patches were present on the dorsal side of abdominal segments. Cremaster well developed in both sexes with 7-9 hooked setae.

The entire life cycle of *C. transiens* completed in 42-70 days.

3.2. Larval Food Plant

Larvae are polyphagous, and the plant species recorded as host plants in the present study, as well as by Holloway (1988), are summarised in Table 3.



Figure 12. Pupae of *Creatonotos transiens* (Walker, 1855)

3.3. Observation and Behaviour

In the present study, *C. transiens* was reared on two host plants, i.e., apple tree, *M. domestica* and bitter dock weed, *R.*

Table 1. Stage-wise duration and size of immature stages of *Creatonotos transiens* (Walker) on *Malus domestica* (Suckow) Borkhausen

Stage of insect	Days	Size
Egg		0.35- 0.48 mm
Incubation Period	4 days	
Larval Instars		
I Instar	5 days	1.26±0.58mm
II Instar	4 days	2.78±0.45mm
III Instar	3 days	5.15±0.67mm
IV Instar	3 days	6.78±0.12mm
V Instar	4 days	8.65±1.34mm
VI Instar	5 days	11.54±0.89mm
VII Instar	3 days	18.21±1.04mm
Total larval period	27 days	
Pupa		12.6±1.34 mm
Pupal period	11 days	
Total life cycle	42 days	

Table 2. Stage-wise duration and size of immature stages of *Creatonotos transiens* (Walker) on *Rumex obtusifolius* Linnaeus

Stage of insect	Days	Size
Egg		0.35-0.48mm
Incubation Period	5 days	
Larval Instars		
I Instar	7 days	1.26±0.58mm
II Instar	7 days	2.78±0.45mm
III Instar	9 days	5.15±0.67mm
IV Instar	8 days	6.78±0.12mm
V Instar	7 days	8.65±1.34mm
VI Instar	7 days	11.54±0.89mm
VII Instar	5 days	18.21±1.04mm
Total Larval Period	50 days	
Pupa		12.6±1.34 mm
Pupal Period	15 days	
Total Life Cycle	70 days	

obtusifolius, at 25±3°C, 65-75% relative humidity and a photoperiod of 16:8 light-dark hours. Its immature stages were recorded in the field in the months of April to September. This species is observed to be multivoltine, and thrives in tropical and sub-tropical regions where food plants, particularly those belonging to the family Poaceae, are readily available throughout the year. The favourable environmental conditions coupled with abundant food sources allowed it to complete its life cycle multiple times annually. Observations were made to study immature stages and various aspects of its life history across its three generations.

Larval behaviour: The first instar larva emerged from the egg by chewing the eggshell and making a hole at the micropylar region. After hatching, it began feeding on the eggshell and left the remaining eggshell on the leaf. After that, the larvae crawled towards tender leaves of the host-plant for feeding. The first three instars fed only on the epidermal tissue of the leaf. During feeding, the larvae scraped the leaves and left small circular patches. The feeding patterns of progressive instars were distinct from earlier ones, and they consumed the whole leaf except the mid-rib region. Upon disturbance, the larvae showed typical dropping behaviour as they curled their bodies when touched with a brush or needle, even in laboratory conditions. They usually rested on the underside of the leaves.

Table 3. Larval food plants of *Creatonotos transiens* (Walker)

Food plant	Common name	Family
Beta sp.	Beet	Amaranthaceae
Cayratia sp.	Fox Grape	Vitaceae
Cissus sp.	Veldt Grape	Vitaceae
Dioscorea sp.	Wild Yam	Dioscoreaceae
Malus domestica (Suckow) Borkhausen	Apple Tree	Rosaceae
Musa sp.	Banana	Musaceae
Paspalum sp.	Kodo Millet	Poaceae
Pithecellobium sp.	Manila Tamarind	Fabaceae
Rumex obtusifolius Linnaeus	Bitter Dock Weed	Polygonaceae
Salix sp.	Willow	Salicaceae
Toona sp.	Red Cedar	Meliaceae
Vigna sp.	Cowpea	Fabaceae
Wisteria sp.	Chinese Wisteria	Fabaceae
Zea sp.	Maize	Poaceae

Moulting behaviour: Before moulting, the larvae stopped feeding and became motionless. The moulting took place during afternoon hours, i.e., between 13:00 and 16:00. It took 6-7 hours to moult into the next instar.

Pupation: Before pupation, the larvae stopped feeding and remained in a quiescent stage. Pupation was observed on the leaves of the host plant. The pupation took place with or without proper cocoon formation. It has been observed that pupation mainly took place during morning hours, i.e., between 6:00 and 10:00. It took about 7-8 hours to undergo pupation. The pupal period lasted for 11-15 days.

Eclosion: At the time of emergence, the adult secreted a liquid (meconium) from the tip of its abdomen. Meconium was reddish-brown in colour. The eclosion took place during the daytime, i.e., between 11:00 am and 02:00 pm .

Adult behaviour: The adults showed nocturnal behaviour and were attracted to light. It has been observed that the adults were usually seen resting on the leaves of the host-plants. After mating, the female started laying eggs on the lower surface of the leaves of its host-plant.

3.4. Chaetotaxy of Last Instar (Figures 14-18)

Cephalic Chaetotaxy (Figure 14): The cranium was sclerotized, orange, rounded; the epicranial notch was not as deep as in other Arctiin species, forming two lateral rounded hemispheres. The median epicranial suture was 1/5th length of the lateral adfrontal suture. The epicranial suture and



Figure 13. Copulating pair of *Creatonotos transiens* (Walker, 1855)

lateral adfrontal areas were not well marked. The frontoclypeus was long and broad. The stemmatal area was well differentiated from the rest of the cranium and had six stemmata, arranged in a semicircle on the lateral sides of the head capsule. The integument was smooth. Mandibles were serrated, and each mandible had two teeth. Each half of the head had 18 pairs of setae and 8 pairs of pores. Among them, there was one pair of adfrontal pores (AFa); five pairs of anterior setae (An1-An5); two pairs of clypeal setae (C1 and C2); one pair of frontal setae (F1); three pairs of frontal pores (Fa, Fb and Fc); three pairs of lateral setae (L1-L3); three pairs of micro-dorsal setae (MD1-MD3); three pairs of posterio-dorsal setae (P1-P3); one pair of posterio-dorsal pores (Pa); sub-ocellar group (SO1) and three pairs of subocellar pores (SOa, SOb and SOc).

Adfrontal group (AF): AFa positioned at the junction of the adfrontal suture.

Anterior group (An): These setae were filiform. An1 was close to the inferior margin of the epicranium; An2 was latero-dorsal to An1; An3 was anteriodorsal to An2; An4 was latero-dorsal to An3, and An5 was postero-ventral to An4. An3 was longer than An1, An2, An4 and An5, whereas An1 and An5 were of the same length, and An2 and An4 were of the same length. An1 and An5 were shorter in length, i.e., An3>An2 and An4>An1 and An5.

Clypeal group (C): C1 and C2 were filiform. C1 was close to the latero-ventral margin of the clypeus. C2 was vertically aligned and posterior to C1. C1 was shorter than C2.

Frontal group (F): F1 was filiform and anterior to Fa. Fa, Fb and Fc were in a straight line and parallel to the frontal suture.

Lateral group (L): All were filiform. L1 was located in the middle of the head capsule and close to the lateral margin of the epicranium. L2 was anterior to L1 and latero-dorsal to P1. L3 was latero-dorsal to L2 and ventral to MD3. L2 and L3 were of the same length, and L3 was longer than L1 and L2.

Micro-dorsal group (MD): All were filiform. MD1 was close to the epicranial suture. MD2 was posterior to MD1. MD3 was latero-ventral to MD2 and latero-dorsal to L3. MD2 and MD3 were of the same length, whereas MD1 was shorter in comparison to MD2 and MD3.

Posterio-dorsal group (P): All were filiform. P1 was latero-dorsal to An5; P2 anterio-dorsal to P1; P3 latero-dorsal to P2. Pa was anterior to P3 and close to the epicranial suture. P2 and P3 were of the same length, whereas P1 was longer than P2 and P3.

Sub-ocellar group (SO): All were filiform. SO1 was at the level of stemma 4. SOa, SOb and SOc were present. SOa was close to stemma 1, SOb close to stemma 2 and SOc close to stemma 3.

Chaetotaxy of Thoracic Segments (Figure 15)

Prothorax (T1): D, SD, L1 and L2 groups of setae were present. All were filiform. D and SD groups fused, i.e., not separately positioned. The fused warts aligned near the mid-dorsal line. L1-group was anterior to the spiracle and L2-group anterio-ventral to the spiracle. All were multi-setosed, arising from the wart and of the same length.

Mesothorax and metathorax (T2 and T3): Both segments had five groups of setae, i.e., D, SD, L1, L2 and SV. All were filiform. D-group of setae were located near mid-dorsal line. SD group was ventral to D-group. The L1 group was anteroventrally positioned to the SD group, whereas the L2 group was aligned near the coxal surface of the meso- and metathoracic legs. All were multi-setosed, arising from wart and of same length.

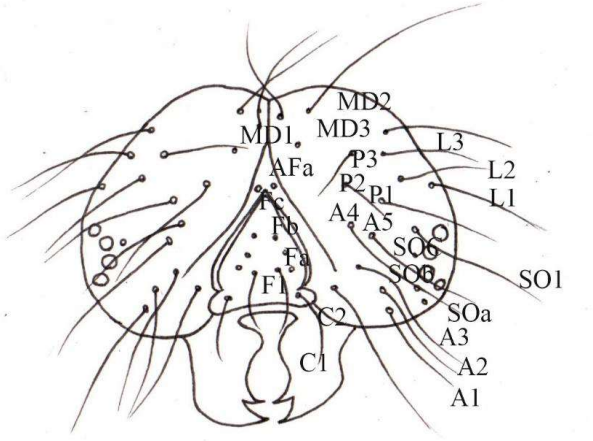


Figure 14. Cephalic capsule of final instar of *Creatonotos transiens* (Walker, 1855)

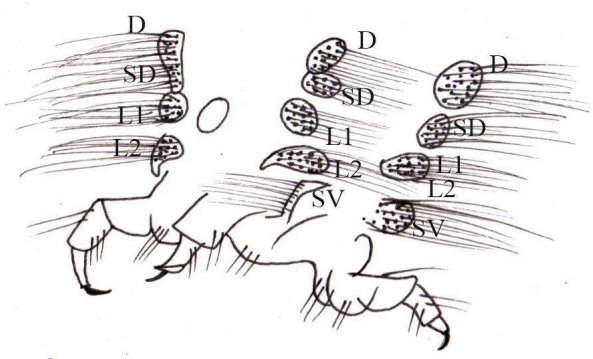


Figure 15. Thoracic segments of final instar of *Creatonotos transiens* (Walker, 1855)

True Legs: Coxa was with 2 pairs of setae, i.e., Cx1 and Cx2, on its middle part. Trochanter had 1 pair of setae, i.e., Tr1 on its distal end. The femur with 3 pairs of setae, i.e., Fe1, Fe2, and Fe3 on its middle part. The tibia was with 4 pairs of setae, i.e., Tb1, Tb2, Tb3 and Tb4 on its middle part. The tarsus was with 1 pair of setae, namely Ts1 near the claws. All setae were of equal length.

Chaetotaxy of Abdominal Segments (Figures 16-18):

Abdominal segment I and II (A1 and A2): Eight groups of setae, i.e., D, SD, L1, L2, SV1, SV2, MV1 and V1 observed on each segment. All were filiform. D-group of setae aligned near mid-dorsal surface. SD-group was located posterio-ventral to the D-group. L1-group was typically above the spiracle and L2-group below the spiracle. SV1-group was ventral to the L2-group of setae. SV2-group of setae aligned ventrally to SV1-group. MV1-group anterio-ventrally positioned to SV2-group. V1-group was ventral to MV1-group and close to the mid-ventral line. All were multi-setosed, arising from wart and of the same length.

Abdominal segments III to VI (A3 to A6): Each segment possessed six groups of setae, i.e., D, SD, L1, L2, SV1 and SV2. All were filiform. D-group was close to the mid-dorsal surface. The SD group was located posterior-ventral to the D group. L1-group was typically above the spiracle and L2-group below the spiracle. SV1-group anterio-ventrally aligned to L2-group of setae. SV2-group was ventral to SV1-group and near the upper surface of the pro-leg base. All were multi-setosed, arising from a wart and of the same length.

Abdominal segments VII and VIII (A7 and A8): D, SD, L1, L2, SV1, SV2 and V-groups of setae were present in both segments. All were filiform. D-group was close to the mid-dorsal surface. SD-group posterio-ventrally aligned with D-group. Below the SD-group, the L1-group of setae was positioned, which is typically above the spiracle, and the L2-group was below the spiracle. SV1-group was located ventral to the L2-group, and the SV2-group positioned just below the SV1-group. V-group of setae was ventral to SV2 and close to the mid-ventral line. All were multi-setosed, arising from the wart and of the same length.

Abdominal segment IX (A9): Five groups of setae, i.e., D, SD, L1, L2 and V were present. All were filiform. D and SD groups were fused, i.e., not separately positioned and close to the mid-dorsal surface. L1-group of setae was located ventrally to the fused setae. L2-group was ventral to L1-group. V-group of setae was close to the mid-ventral line. SV1 and SV2-groups were absent. All were multi-setosed, arising from a wart and of the same length.

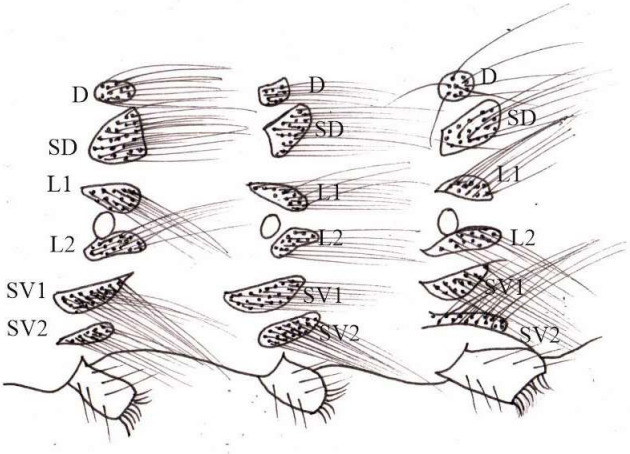


Figure 17. Abdominal segments (A3-A6) of final instar of *Creatonotos transiens* (Walker, 1855)

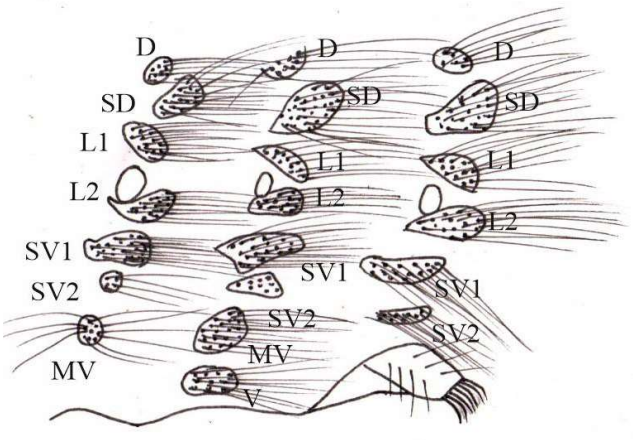


Figure 16. Abdominal segments (A1-A2) of final instar of *Creatonotos transiens* (Walker, 1855)

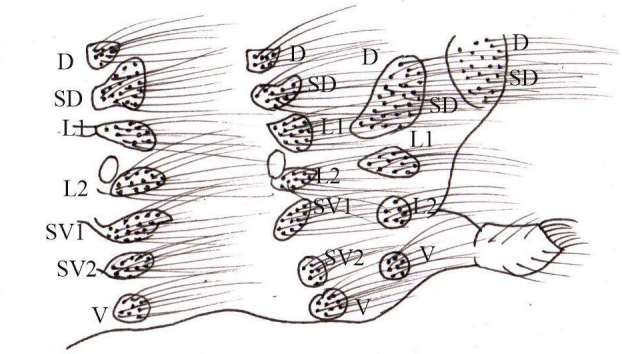


Figure 18. Abdominal segments (A7-A8) of final instar of *Creatonotos transiens* (Walker, 1855)

Abdominal segment X (A10): The sub-triangular anal shield had multi-setosed setae, i.e., D and SD-groups. V-group was present near the anal pro-leg. D and SD-groups of setae were longer than V-group. L-group was absent. All were filiform.

Pro-legs: Each fleshy pro-leg had 6 pairs of setae, i.e., L1, L2, L3, SV1, SV2 and V1. All were of the same length.

Crochets: Uniordinal mesoseries of 20-25 long crochets on aliform appendages. D and SD-groups were fused, i.e., not separately positioned and close to the mid-dorsal surface. L1-group of setae was located ventrally to the fused setae. L2-group was ventral to L1-group. V-group of setae was close to mid-ventral line. SV1 and SV2-groups were absent. All were multi-setose, arising from a wart and of the same length.

3.5. Discussion

In the present investigation, regular surveys were conducted during 2019-2024 to document eggs, successive larval instars, pupa, developmental duration, larval behaviour, host plants, and chaetotaxy of the last-instar larva. In total, 13 plant species were recorded as host plants during the study. In Lepidoptera, the immature larval stage can cause significant damage, and studying it is very important in applied entomology. The scrutiny of relevant literature showed that moths mostly lay their eggs either singly or in small groups/clusters on or near the host (Warren & Huddleston, 1962; Deseo, 1976; Common & Edward, 1991; Sardana, 1996; Cordaro et al., 2000; Silhacek et al., 2003; Mohandas et al., 2007; Calvo & Molina, 2008; Straten & Muus, 2010; Solovyev et al., 2012; Finale & Barro, 2014). For this, the female takes a considerable amount of time to select the food plant and a suitable substratum to deposit its eggs with the help of the abdominal tip (Chew & Robbins, 1984; Papaj & Probopy, 1989). While choosing the food plant, the females mostly prefer the fresh leaves of the host-plant for larval growth and sunlight (Singer et al., 1988; Schurian & Fiedler, 1996). In *C. transiens*, females lay eggs in clusters of 50-70 on the ventral surface of the leaves to protect them from predators or harsh climatic conditions. Before hatching, the eggs become darker, often black. The egg chorion serves as the first meal for freshly emerged larvae. Hinton (1981) also revealed that the young larvae eat partial or complete eggshells during hatching in most lepidopterans. In this species, the first instar larvae also eat partial eggshell. The information about the number of instars is essential for both basic and applied research, particularly for biological studies, construction of life tables and development of control strategies (Logan et al., 1998; Zawadneak et al., 2017). In this species, the larvae moult six times and has seven instars. The pupation, considered as a critical stage in an insect's development, becomes complicated under confinement and limit adult emergence (Gillott, 2005). The duration and behavioural aspects vary from species to species and these aspects have been noticed for this species.

The taxonomic value of setae and sensillar structures has long been recognised in order Lepidoptera by workers such as Heinrich (1916), Hinton (1946) and Stehr (1987). Dyar (1894) established a classification for lepidopterous larvae based on setae. In the present study, eight groups of setae, i.e., D, SD, L1, L2, SV1, SV2, MV1 and V1, are present on both the A1 and A2 segments. Each A3 to A6 segment bears six groups of setae, i.e., D, SD, L1, L2, SV1 and SV2. D, SD, L1, L2, SV1, SV2 and V-groups of setae are present in both A7 and A8 segments. Five groups of setae i.e., D, SD, L1, L2 and V are present in A9. A10 has a sub-triangular anal shield bearing multi-setosed setae, i.e., D and SD groups. V-group is present near the anal pro-leg. Each fleshy pro-leg has 6 pairs of setae, i.e., L1, L2, L3, SV1, SV2 and V1. The uniordinal mesoseries of 20-25 long crochets are present on abdominal pro-legs.

Understanding immature phases is crucial, as it compiles data on morphological, biological and ecological features and is helpful for systematics and taxonomy (Beebe et al., 1960; Fleming, 1960; Kaminski et al., 2002; Bizarro et al., 2003; Braga et al., 2014). The purpose of carrying out chaetotaxic studies is to identify the arrangement and distribution pattern of setae and punctures on the larval head and body segments, which further helps in differentiation of species. In India, few workers such as Issac and Venkatraman (1941), Mathur (1954, 1959), Singh (1956), Mathur and Singh (1963), Azam and Ali (1965), Goel and Kumar (1981), Singh and Goel (1987), Kaur (2004), Sri et al. (2010), Subramoniam and Chitra (2019), Chakravarty et al. (2021), Yusof et al. (2021), Divya et al. (2023) and Kaleka and Kapoor (2024) have made scattered contributions in this regard. The immature stages of Lepidoptera can help us to gain a broader view of morphological, biological and ecological aspects. The associations of the immature stages with their host-plants will certainly be of utmost significance to applied and fundamental sciences. The life-history aspects and host-plant relationships of the Clouded Tiger Moth shed important light on how it interacts with its surroundings and adapts to Himalayan habitats. The

association of its larvae with both cultivated apple (*Malus domestica* (Suckow) Borkhausen, 1803) of the family Rosaceae and the wild herb (*Rumex obtusifolius* Linnaeus, 1753) of the family Polygonaceae indicate a broad ecological niche that may enhance its survival under varying environmental conditions. In a broader sense, comprehensive knowledge of the breeding biology of moths helps us to understand how ecosystems function. These findings provide important baseline information for understanding species diversity, tracking ecological shifts, protecting Himalayan biodiversity, and developing ecologically sustainable pest control techniques in apple-growing regions.

6. CONCLUSION

The present study aimed to provide a comprehensive morphological characterisation of the immature stages of a ditrysian moth to identify diagnostic characters of taxonomic significance. The larval stage represents the principal feeding phase of the life cycle and plays a pivotal role in plant-insect interactions and ecosystem functioning, serving as herbivores, decomposers, predators, or an important food resource for higher trophic levels, depending on the species. In addition to their ecological importance, larval morphological characters, particularly chaetotaxy, provide reliable diagnostic features for distinguishing closely related taxa and contribute to resolving phylogenetic relationships and inferring evolutionary patterns within Lepidoptera. The findings strengthen the morphological framework for taxonomy and provide a robust foundation for future phylogenetic and systematic studies.

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Data availability statement

The data can be obtained from the corresponding author upon reasonable request.

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Traits in Traditional Rice Genotypes Influence Spinning and Folding Behaviour of Rice Leaffolder

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ABSTRACT: Traditional rice genotypes (TRGs) exhibit rich genetic and phenotypic diversity, which confers resilience against various insect pests. The present study explores the resistance traits in TRGs against the devastating foliage feeder, namely, leaffolder (*Cnaphalocrocis medinalis* Guen.), which spins, folds the leaf and causes significant damage. TRGs are season-specific, photosensitive and locally adapted. In the deltaic zone of Tamil Nadu, a southern state of India, TRGs are cultivated predominantly during two seasons, namely "*Kuruvai*" (June to August, suited for short-duration genotypes) and *Samba* (September to January, suited for long-duration genotypes). These seasons are almost analogous to the *Kharif* and *Rabi* seasons of North India, respectively. Considering this, a detailed behavioural study was conducted at the Department of Entomology, Annamalai University, Tamil Nadu, India, to observe the process from spinning to complete folding of leaf margins in selected TRGs suited for these two seasons. Observations on time taken for leaf selection, number of binds for making the primary fold, number of head swings from one end to the other end to make a bind, time consumed to construct primary fold, length of primary fold and extension of primary fold after 24 hours revealed that these parameters were significantly influenced by biophysical and biochemical traits of the selected TRGs. Subsequently, correlation analysis showed that *C. medinalis* larval feeding was positively correlated with the number of binds to form the primary fold, the length of the primary fold, and the number of binds in 24 hours. The time taken to construct the primary fold evinced a significant negative correlation. The resistant genotypes *Karunkuruvai* and *Kothamalli Samba* had narrow leaves with dense trichomes. Phenol content exerted a significant negative correlation with spinning. Moisture content showed a significant negative correlation with time taken for primary fold. The present investigation showed that leaf traits of selected TRGs, such as width and length, trichome density, phenol, and moisture content, determine the folding and feeding behaviour of *C. medinalis*.

Keywords: Folding, Indigenous rice, Leaffolder, Leaf traits, Spinning.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food, especially in India and other Asian countries. In India, 168 million hectares were under rice cultivation with a production of over 532.87 million metric tonnes of milled rice during 2024-2025 (Statista, 2025). India is home to many traditional rice genotypes (TRGs) known for their unique colour, plant height, architecture, grain size, shape, pigmentation, and nutrient content. Rice plants are generally considered highly photosensitive, and these traditional genotypes, which vary in duration and adaptation to different environmental conditions, possess valuable traits against biotic and abiotic stresses (Amudha et al*.,* 2023). In Tamil Nadu, these genotypes are specifically adapted to various rice-growing seasons, *viz*., *Navarai* (December-January)*, Sornavari* (April- May)*, Kar* (May-June)*, Kuruvai* (June-July)*, Samba* (August)*, Thaladi* (September- October) and *Late Thaladi* (October-November). Among these, *Kuruvai* (<120 days) and *Samba* (> 145 days) seasons are highly suited for growing TRGs in the Cauvery delta regions of Tamil Nadu (TNAU, 2026), and these two seasons broadly correspond to the *Kharif* (June- September) and *Rabi* (October- March) cropping periods. Among the insect pests, rice leaffolder, *Cnaphalocrocis medinalis* (Guen.) is an important foliage feeder that results in 30-80 per cent yield loss in rice-producing areas (Gangwar, 2015). The infestation is active across all phenological stages of the crop (Sharma et al., 2024). Previous studies have shown that morphological traits such as leaf trichomes and narrow leaves, along with biochemical traits such as increased phenol content, reduced sugar and amino acid, and increased deposition of silica, significantly influenced the feeding and oviposition behaviour of rice leaffolder (Ashrith et al*.,* 2020; Javvaji et al*.,* 2021). Spinning is an essential behaviour of the rice leaffolder, wherein the larva folds the leaf into a roll and feeds within it. This folded structure serves as a shelter to protect the insect life stages from the external environment (Scott & Yack, 2012). The duration and success of leaf folding are indirectly influenced by the external environment (Giri & Mohapatra, 2024). Furthermore, variations in morphological and biochemical traits of rice genotypes also directly influence the process of spinning (Punithavalli et al., 2013). Islam and Karim (1997) reported that larvae faced difficulties in making folds on narrower leaves, whereas longer and tougher leaf blades contribute to resistance.

Understanding these behavioural aspects helps in identifying the plant traits in selected TRGs that are either favourable or unfavourable for leaffolder to spinning and feeding on rice leaves. Although a few studies have examined the spinning behaviour of rice leaffolder in popular cultivars, information on the influence of specific traits in TRGs on the spinning and folder behaviour of rice leaffolder is limited. However, the present study was undertaken to evaluate such traits in selected TRGs that may confer resistance to rice leaffolder.

2. MATERIALS AND METHODS

Six TRGs suited for *Kuruvai* season (short-duration crop from June to August) and fifty TRGs suited for *Samba* season (long-duration crop from September to January) were field evaluated against rice leaffolder during the respective season from 2022 to 2024 at the Experimental Farm, Faculty of Agriculture, Annamalai University, India. Based on the per cent infestation under field conditions, the damage score for each genotype was calculated, and it was further converted to a 0-9 scale as per the standard evaluation system (SES) for *C. medinalis* suggested by IRRI (2002) (Table 1).

Based on this preliminary evaluation, the following eight genotypes were selected to study the spinning and folding behaviour of larvae of leaffolder in the Host Plant Resistance Research Laboratory, Department of Entomology, Annamalai University, Tamil Nadu, India. Among these, one resistant, one susceptible and two moderately resistant genotypes were selected based on their reactions to leaffolder infestation (Table 2).

2.1. Raising of TRGs

The seeds of selected genotypes harvested from field evaluation were sown in a potting mixture comprising two parts of clay soil + one part of farmyard manure inside

Table 1. Standard evaluation system (SES) for *C. medinalis* (IRRI, 2002)

Score	Adjusted damaged area rating (%ADAR)	Category
0	0	Highly resistant (HR)
1	1-20	Resistant (R)
3	21-40	Moderately resistant (MR)
5	41-60	Moderately susceptible (MS)
7	61-80	Susceptible (S)
9	81-100	Highly susceptible (HS)

cement pots of 45×30×30 cm (l×b×h) dimensions. These cement pots were placed inside an iron cage measuring 3.05×1.83×4.57 m, which was enclosed with cloth mesh. Twenty-five-day-old seedlings of each genotype were transplanted and maintained in small plastic pots of 12 cm diameter and 12 cm height for easy transport. Forty-day-old plants were used for conducting the experiment.

2.2. Mass culturing of rice leaffolder (*C. medinalis)*

The leaffolder was mass-cultured by adopting the method described by Waldbauer and Marciano (1979) with certain modifications. Field-collected adults were released into an iron oviposition cage (45×45×45 cm) containing 30-day-old plants of the susceptible variety TN1 at the rate of four pots per cage. The moths were supplied with a 10% sucrose solution through cotton balls. The female moths, after mating, were allowed to lay eggs on the plants. At the black head stage, eggs were collected and maintained in petri plates (14 cm diameter) up to the first instar to avoid natural mortality of neonates. The second-instar larvae were transferred singly onto freshly potted rice plants (40 days old) placed inside an iron cage. Larvae were allowed to feed, grow, and full-grown larvae were allowed to pupate within the leaf fold inside the cage without any disturbance. After pupation, pupae were collected and kept inside the oviposition cage containing one fresh potted rice plant for adult emergence. The process was continued, and thus, the culture of *C. medinalis* was maintained throughout the period of study.

2.3. Spinning and Folding Behaviour of *C. medinalis*

The spinning and folding behaviour of *C. medinalis* in response to different resistance levels of selected genotypes was analysed. For the study, forty-day-old potted plants of the selected TRGs, each maintained with a single tiller bearing four fully expanded vegetative leaves for uniformity were used. Five hours pre-starved single early third-instar larvae were transferred to a leaf of each selected genotype and maintained in the laboratory (25–28°C with 60% RH). A single larva released on a potted plant was considered an experimental setup, and five such independent experimental setups were conducted for each TRG, constituting five replications. For each setup, to avoid carry-over effects and influence of prior host preference on subsequent behavioural observations, fresh larvae were used. The behaviour of each larva was intensely observed from spinning to the complete folding of leaf margins. To avoid manual error, spinning was recorded in full using a mobile camera (Samsung M30S with 2.0 x magnification) attached to a stand (equal to the height of the plant) throughout the experimental period (Figure 1) with five replications. Observations on the time taken for leaf selection were recorded from larval release to the initiation of spinning; additionally, the time taken for construction of the primary fold was recorded using a stopwatch. The number of thickened binds forming the

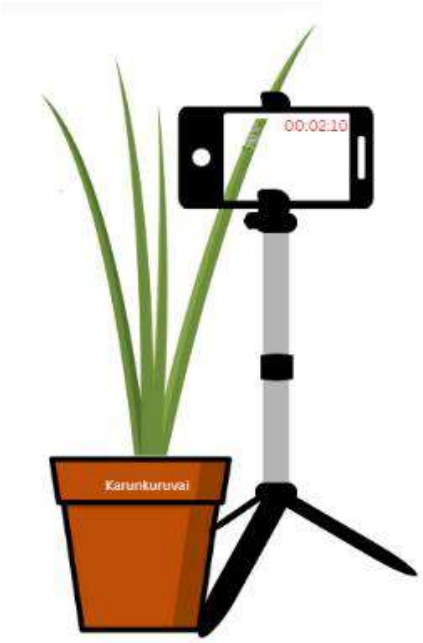


Figure 1. Experimental setup for continuous recording of larval spinning behaviour on a plant using a mobile camera mounted on a height-adjustable stand

Table 2. List of selected genotypes

Season	Selected genotypes			
	Resistant (R)	**Moderately resistant (MR)**		**Highly susceptible (HS)**
Kuruvai	*Karunkuruvai*	*Kullakar*	*Aruvatham Kuruvai*	TN 1
Samba	*Kothamalli Samba*	*Sivappu Kuruvikar*	*Mappillai Samba*	*Adanchilipa*

primary fold and the number of head swings between the leaf margins required to construct each bind were recorded. The length (cm) of the primary fold was also measured using a ruler (Figure 2a-2e) (Islam & Karim, 1997). After recording the initial measurements, the leaf was enclosed within a tubular plastic cover (35 cm in height and 3 cm in diameter) to confine the larva and allow the leaf to fold under undisturbed conditions. The extension of the primary fold and the number of binds made by the larva after 24 hours were measured (Islam & Karim, 1997), while the area of leaf damage was measured using ImageJ software and converted to per cent damage.

2.4. Studies on Factors of Resistance in Selected TRGs

2.4.1. Biophysical factors

The biophysical traits of leaves, namely, leaf length (cm), leaf breadth (cm) and trichomes (mm^2) in the selected TRGs, were recorded on forty-day-old plants from five potted plants in each replication. Five replications were maintained.

2.4.2. Biochemical factors

The total phenol content of leaves of selected TRGs was determined using the Folin-Ciocalteu assay method (Ainsworth & Gillespie, 2007). The leaf epicuticular wax was extracted and weighed by adopting the method described by Thomas (2015). Moisture content of leaves of selected TRGs was estimated using the weight method given by AOAC (2005).

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed using OPSTAT software (Sheoran et al., 1998) to differentiate the treatment means. Critical difference values were computed at 5% probability level and Duncan's Multiple Range Test (Duncan, 1951) was used to compare the treatment means. Pearson's correlation analysis was conducted using the R statistical package to examine the relationships between larval spinning and leaf-folding behaviour and the morphological and biochemical parameters of the leaves.

3. RESULTS AND DISCUSSION

In Tamil Nadu, *Kuruvai* and *Samba* represent short- and long-duration rice seasons, respectively. TRGs are season-specific and express good yield potential only in their respective seasons. Therefore, four genotypes suited to each season were separately selected and evaluated for pest resistance and yield performance in their respective seasons, which is a pertinent approach for selecting promising genotypes suited to that particular season.

C. medinalis is well known for its characteristic leaf-folding behaviour, which significantly reduces the photosynthetic efficiency of rice plants, eventually resulting in yield loss. The larva folds the leaves carefully by pulling the two longitudinal edges into a tubular shape by stretching its silk and placing many binds as desired, which slowly pull the two edges into the tubular position according to the larval body size (Greeney et al., 2010; Padmavathi et al., 2013).

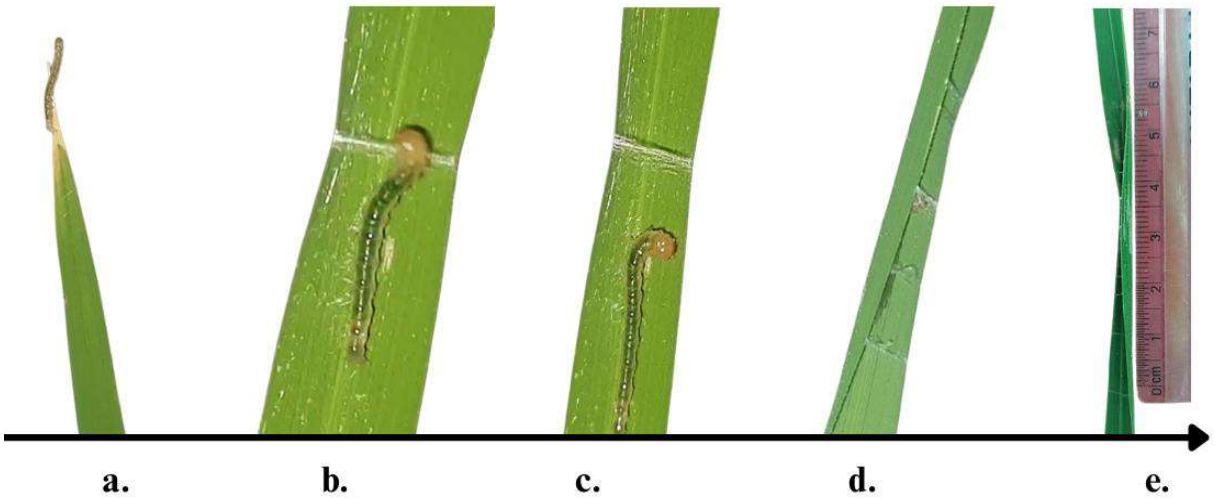


Figure 2. Sequential process of spinning by leaffolder larva 2a. Leaf selection; 2b. Head swings while silk binds are drawn from one margin to another; 2c. Primary bind- a thickened silken bind; 2d. Primary fold- first-formed fold at the binding region; 2e. Measurement of the primary fold using a ruler

Knowing the pattern and mechanism of spinning across various genotypes is important to understand the traits influencing spinning. This experiment was conducted using 40-day-old plants, as the larvae usually prefer tender leaves, which are soft and flexible and can be easily folded as desired, whereas mature leaves exhibit greater stiffness (Singh & Sarkar, 2021; Modert et al., 2024).

3.1. Spinning and Folding Behaviour of *C. medinalis* in *Kuruvai* Season Suited Genotypes

Upon releasing the larvae on genotypes suited for the *Kuruvai* season, the larvae took a prolonged time for leaf selection in the moderately resistant *Aruvatham Kuruvai* and *Kullakar*. After selection, they started to produce thick binds to fold the leaves to produce a primary fold. No significant variation was found between genotypes with regard to the number of binds to make a primary fold. A higher number of binds was produced in the susceptible genotype TN1, which subsequently resulted in a lengthier primary fold.

Alternatively, the head swings required to make a primary fold were higher in *Karunkuruvai*, and they were lower in *Kullakar*. Likewise, the time taken for primary folding was prolonged in moderately resistant genotypes *Aruvatham Kuruvai* and *Kullakar* compared with the susceptible genotype TN1. However, after 24 hours of spinning, the number of binds and length of the primary fold increased, and maximum binds with an elongated primary fold were observed in the resistant genotype *Karunkuruvai*. Shortened folding was found in moderately resistant genotypes. Feeding damage was higher in susceptible genotype TN1 than other genotypes (Table 3).

3.2. Spinning and Folding Behaviour of *C. medinalis* in *Samba* Season Suited Genotypes

Among the *Samba* season suited genotypes, the larva took the maximum time for leaf selection on resistant genotype *Kothamalli Samba*, followed by moderately resistant genotype *Mappillai Samba*. Maximum binds were produced in *Adanchilipa* and *Mappillai Samba* than *Kothamalli Samba* and *Sivappu Kuruvikar*. Resistant genotype *Kothamalli Samba* recorded the shortest primary fold. The susceptible genotype *Adanchilipa* registered the highest number of head swings per primary fold, whereas the least number of head swings was noticed in *Sivappu Kuruvikar*. Unlike *Kuruvai* suited genotypes, there was no variation after 24 hours of spinning and folding. A similar trend was followed in the number of binds made by the larva and reflected in the extension of the primary fold even after 24 hours. *Kothamalli Samba* was least preferred followed by other genotypes *viz*., *Mappillai Samba, Sivappu Kuruvikar* and *Adanchilipa* (Table 3).

The observations revealed that larvae preferred the resistant genotype *Karunkuruvai* suited for the *Kuruvai* season, started to spin and construct a longer leaf fold, but the leaf damage was less than other genotypes. Whereas, the resistant genotype *Kothamalli Samba* suited for *Samba* was least preferred and the larvae constructed a shorter primary fold. However, no significant differences were observed among the other genotypes. Interestingly, after releasing the larvae on *Kothamalli Samba*, many attempts to fold led to failure, wherein the larvae climbed to the top of the leaves, tried to escape and dispersed through bungee jumping from the whole plant in search of another host. This may be due to the release of any unpleasant volatile substance, which would have deterred the larvae. The potential role of volatile compounds as repellents in rice-mediated defence against insect pests has been discussed by Pattanaik et al. (2025). Meanwhile, the presence of a high number of trichomes delayed the time required for primary folding, even when forced to feed within, compared with the susceptible genotype. In an earlier study, Punithavalli et al. (2011) observed significantly shorter fold lengths in resistant genotypes TKM 6, Ptb33, *Oryza rhizomatis*, *O. minuta*.

Correlation studies showed that *C. medinalis* larval feeding exhibited a non-significant positive correlation with the number of binds to form the primary fold, the length of the primary fold, and the number of binds in 24 hours. Nevertheless, the time taken for constructing the primary fold showed a significant negative correlation (r= -0.84) with feeding (Figure 3). This shows that larvae had difficulty spinning the leaves of genotypes containing resistant traits, which might have resulted in spending more time folding than feeding. Similar observations were made by Liao and Chen (2017).

3.3. Factors of Resistance in Selected TRGs

With regard to the biophysical factors of resistance, the resistant genotypes *Karunkuruvai* and *Kothamalli Samba* recorded denser trichomes compared to other genotypes, whereas the least was noticed in susceptible genotypes TN1 and *Adanchilipa*. The moderately resistant genotype *Mappillai Samba* recorded the maximum leaf length. The susceptible genotype TN1 had wider leaves, whereas both the resistant genotypes *Karunkuruvai* and *Kothamalli Samba*

had narrow leaves (Table 4). A high negative correlation was observed between leaf length (r= -0.87) and the number of binds and extension of the primary fold after 24 h (r= -0.81), whereas leaf breadth showed a high positive correlation with feeding activity (Figure 3). Length of the primary fold was directly proportional to leaf breadth, as observed earlier by Punithavalli et al. (2013). A longer primary fold was observed in susceptible genotypes, which had broader leaves. In the resistant genotype *Karunkuruvai,* the extension of the primary fold was maximum, but the folding was incomplete due to reduced leaf breadth. The reduced leaf breadth may not provide enough space for the third instar larva, which could result in an increased number of binds and extension of the primary fold only after 24 hours. Hence, the larvae may be more susceptible to the external environment and more silk binds were needed to seal the opening of folding. Even after successful folding, it was less preferred for feeding, due to the interruption of trichomes on the leaves.

Among the biochemical factors, phenol content showed a highly significant negative correlation with the number of binds (r = -0.85) and the extension of the primary fold after 24 hours (r = -0.89) (Figure 3). This difference explains the reason behind the immediate preference towards *Karunkuruvai* for spinning and folding over *Kothamalli Samba,* which contained a higher amount of phenol (Table 4). Thus, it was inferred that phenol content reduces the performance of larvae, as reported earlier by Zhuang et al. (2022) and Nayak et al. (2025).

A significant negative correlation (r= -0.78) was observed between wax content and the time taken for leaf selection and in contrast, wax content exhibited a significant positive correlation (r= 0.80) with extension of the primary fold after 24 hours, suggesting greater fold extension with higher wax content.

Moisture content in leaves had an impact on larval spinning, and their effectiveness partly depended on leaf

Table 3. Leaf spinning and folding behaviour of *C. medinalis* on selected traditional rice genotypes suited for *Kuruvai* and *Samba* seasons

Category	Genotype	Resistant scoring	Time taken for leaf selection (min)	Number of binds to make primary fold	Length of primary fold (cm)	Number of binds in 24 hrs	Extension of primary fold in 24 hrs (cm)	Number of head swings per primary fold	Time taken for primary fold (min)	% Leaf damage#
Suited for *Kuruvai* season										
Resistant	*Karunkuruvai*	1	4.39 (2.09)^{c}	3.60 (1.87)	0.68 (0.79)^{b}	15.80 (3.94)^{a}	9.02 (2.99)^{a}	925.80 (30.42)^{a}	26.03 (5.06)^{ab}	13.15 (21.25)^{b}
Moderately resistant	*Kullakar*	3	20.71 (4.53)^{a}	2.80 (1.66)	1.32 (1.11)^{ab}	4.80 (2.17)^{b}	4.06 (2.00)^{b}	254.80 (15.90)^{d}	35.47 (5.94)^{a}	10.20 (18.62)^{c}
Moderately resistant	*Aruvatham Kuruvai*	3	11.68 (3.24)^{b}	4.20 (2.01)	1.72 (1.29)^{a}	6.80 (2.58)^{b}	2.34 (1.52)^{c}	721.00 (26.83)^{b}	37.16 (6.07)^{a}	7.50 (15.89)^{d}
Susceptible	*TN1*	9	7.28 (2.62)^{bc}	4.40 (2.09)	2.06 (1.40)^{a}	11.80 (3.40)^{a}	4.52 (2.12)^{b}	452.60 (21.13)^{c}	18.37 (4.15)^{b}	32.15 (34.53)^{a}
S. Ed.			0.30	NS	0.17	0.28	0.12	1.07	0.48	0.30
C.D. (p=0.05)			0.65		0.36	0.59	0.27	2.27	1.03	0.67
Suited for *Samba* season										
Resistant	*Kothamalli Samba*	1	36.84 (6.00)^{a}	2.60 (1.59)^{b}	0.30 (0.54)	4.20 (2.04)^{b}	1.40 (1.14)^{b}	740.00 (27.17)^{a}	29.88 (5.46)	9.33 (17.78)^{d}
Moderately resistant	*Mappillai Samba*	3	33.87 (5.65)^{a}	4.40 (2.08)^{a}	0.90 (0.88)	5.80 (2.39)^{b}	1.46 (1.16)^{b}	413.00 (20.24)^{b}	23.80 (4.86)	17.00 (24.34)^{c}
Moderately resistant	*Sivappu Kuruvikar*	3	13.49 (3.57)^{b}	2.80 (1.65)^{b}	0.90 (0.89)	4.40 (2.08)^{b}	1.42 (1.18)^{b}	346.80 (18.56)^{b}	24.27 (4.90)	26.49 (30.97)^{b}
Susceptible	*Adanchilipa*	9	11.48 (3.25)^{b}	4.80 (2.16)^{a}	0.92 (0.87)	10.60 (3.19)^{a}	4.70 (2.14)^{a}	851.40 (29.07)^{a}	25.92 (5.06)	30.76 (33.66)^{a}
S. Ed.			0.72	0.17	NS	0.25	0.18	1.26	NS	0.35
C.D. (p=0.05)			1.54	0.37		0.55	0.39	2.69		0.76

Each value is the mean of five replications

Values in parentheses are square root transformed and #arc sine transformed

Values followed by different alphabets are significantly different at P> 0.05 as per Duncan's Multiple Range Test

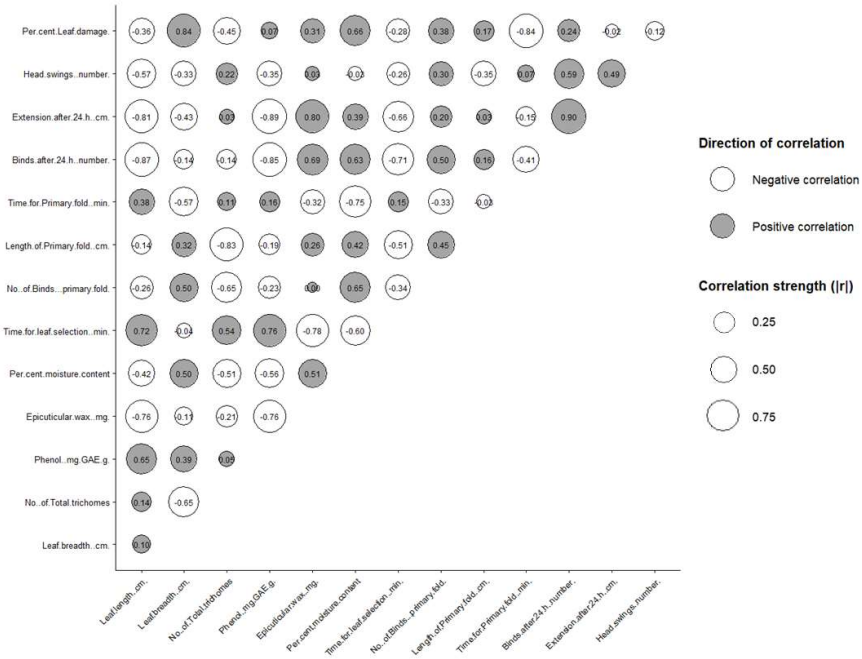


Figure 3. Graphical representation of the correlation between the folding behaviour and physico-chemical traits of selected traditional rice genotypes suited for *Kuruvai* and *Samba* seasons. Size and shading of the circles indicate the strength and direction (positive/ negative) of the correlation respectively

Table 4. Plant physicochemical parameters of selected traditional rice genotypes suited for *Kuruvai* and *Samba* seasons

Genotype	Leaf length (cm)	Leaf breadth (cm)	Number of trichomes	Epicuticular wax (mg)	Moisture content (%)	Phenol content (mg of GAE/g)
Suited for *Kuruvai* season						
Karunkuruvai	26.30 (5.12)^{b}	0.47 (0.68)^{c}	133.40 (11.54)^{a}	12.60 (3.55)^{a}	50.22 (45.12)^{b}	182.00 (13.49)^{d}
Kullakar	38.80 (6.17)^{a}	0.58 (0.79)^{b}	93.00 (9.64)^{b}	8.90 (2.98)^{c}	37.63 (37.83)^{d}	239.00 (15.46)^{a}
Aruvatham Kuruvai	38.19 (6.22)^{a}	0.63 (0.76)^{b}	63.40 (7.95)^{c}	2.50 (1.58)^{d}	41.89 (40.33)^{c}	234.20 (15.30)^{b}
TN1	27.98 (5.28)^{b}	0.83 (0.91)^{a}	53.20 (7.28)^{d}	10.70 (3.27)^{b}	55.33 (48.06)^{a}	227.00 (15.06)^{c}
S. Ed.	0.17	0.02	0.19	0.02	0.57	0.03
C.D. (p=0.05)	0.37	0.04	0.42	0.03	1.21	0.06
Suited for *Samba* season						
Kothamalli Samba	36.97 (6.07)^{b}	0.58 (0.76)^{b}	154.90 (12.43)^{a}	0.90 (0.94)^{c}	31.18 (33.94)^{c}	270.20 (16.43)^{a}
Mappillai Samba	44.48 (6.66)^{a}	0.77 (0.88)^{a}	104.30 (10.20)^{b}	0.76 (0.87)^{d}	49.60 (44.77)^{a}	248.00 (15.74)^{b}
Sivappu Kuruvikar	38.35 (6.18)^{b}	0.80 (0.89)^{a}	93.80 (9.68)^{c}	7.70 (2.77)^{a}	48.01 (43.86)^{b}	243.80 (15.61)^{bc}
Adanchilipa	30.08 (5.47)^{c}	0.84 (0.92)^{a}	60.30 (7.75)^{d}	7.26 (2.69)^{b}	48.03 (43.87)^{b}	241.40 (15.53)^{c}
S. Ed.	0.16	0.02	0.23	0.03	0.33	0.07
C.D. (p=0.05)	0.33	0.04	0.49	0.06	0.71	0.14

Values in parentheses are square root transformed
Values followed by different alphabets are significantly different at P> 0.05 as per Duncan's Multiple Range Test
Each value is the mean of five replications

turgidity. The susceptible genotype TN1 and the resistant genotype *Karunkuruvai* suited for the *Kuruvai* season had the highest moisture content, while it was minimum in the *Samba* season-suited resistant genotype *Kothamalli Samba* (Table 4). Moisture content had a significant negative correlation (r= -0.75) with the time taken for primary fold (Figure 3). Experiments of Mulwa et al. (2023) in green gram, Prabhakar et al. (2013) in mulberry, and Shi et al. (2023) in lily showed that lower moisture content does not favour pest activity as leaves become tougher and are not suitable for feeding. In addition, scraping leads to moisture loss, quick drying of leaves, and reduced larval development and spinning efficiency.

4. CONCLUSION

Leaf folding by larvae extends beyond physical damage by disrupting physiological processes essential for plant growth. The folding behaviour creates a microenvironment for the larva, protecting it from harsh external conditions and contact insecticides, thereby complicating leaffolder management efforts.

Based on the results of the study, we conclude that *Karunkuruvai* and *Kothamalli Samba,* which possess narrower leaves with abundant trichomes, can confer resistance against *C. medinalis,* and such traits could be useful in developing cultivars against rice leaffolder.

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Credit authorship contribution statement

Amirtha P: Conceptualization, Data collection, Writing –original draft; **Navin Kumar R:** Data collection, Data curation, Visualization; **Selvanarayanan V:** Conceptualization, Supervision; **Abishekkumar S:** Resources, Data collection

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 are available from the corresponding author upon reasonable request.

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

We declare that we did not use AI tools in writing the manuscript.

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