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Bird Diversity and Anthropogenic Impacts in the Southern Lake Chad Wetlands, Cameroon

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***Abstract*:** This study, conducted in the southern part of Lake Chad (Far North Region, Cameroon), assessed bird diversity and conservation status using data collection methods along linear transects and Japanese net captures. A total of 213 species belonging to 24 orders and 67 families were recorded. Species richness was higher in the dry season (210 species) than in the rainy season (193 species), and higher around water points (98 species). The Shannon index was higher in the rainy season (H' = 2.1283 bits) than in the dry season (H' = 1.8149 bits), indicating greater diversity during the rainy season. Nine types of human activities were identified, with pollution being highest in the dry season (44.72 signs/km) and grazing predominant in the rainy season (24.63 signs/km). These activities negatively affected avian diversity in the dry season and rainy season). Conservation measures are needed to preserve this rich but threatened birdlife.

***Keywords*:** Birds, Lake Chad, Cameroon, Diversity, Anthropogenic.

1. INTRODUCTION

Wetlands are highly diverse ecological systems that support a wide range of biological diversity and provide numerous services to human societies (Garba et al., 2025 ; Walakira et al., 2024 ; Aissaoui, 2012). The Cameroonian part of Lake Chad, located in the Far North region, was designated a Ramsar site in 2008 (Gbetkom, 2020). Of all the components of wetland biodiversity, birdlife is the most important element that gives meaning to aquatic environments. Indeed, wetlands are not only biodiversity hotspots, but also provide a large number of ecosystem services to society by contributing to the maintenance and improvement of water quality, the regulation of hydrological regimes, and the regulation of the local and global climate (Robinson et al., 2025). These ecosystems are a privileged habitat for many species of water birds (Green and Elmberg, 2014).

However, birds are considered to be good ecological indicators for these wetlands because they respond quickly to environmental changes on several spatial scales (Parchizadeh and Williams, 2018)., birds are a zoological group that can be easily used for ecological diagnosis, leading to conservation strategies that benefit other species of flora and fauna (BirdLife International. 2018). Despite well-known biodiversity conservation functions, wetlands are subject to intense land use changes that alternately alter the composition, structure, and diversity of bird communities (Thapa and Saund, 2012). This globally important wetland is seriously threatened by heavy population pressure and climate change. In addition, the Lake Chad area faces serious eutrophication problems that are reducing the lake's surface area (Ranger, 2016). Throughout Lake Chad, several factors contribute to its degradation and thus to the loss of avian biodiversity, namely anthropogenic factors (extensive agriculture, logging, crafts, excessive harvesting of medicinal plants and wild honey, vegetation fires, overgrazing, urbanization) resulting mainly from population growth on the one hand, and natural factors such as the drying up of the lake (climate change) on the other (Trolliet, 2014). The available knowledge on birdlife in this area is still fragmentary and is based on localized inventories and often unsystematic data. There is therefore a lack of quantitative and spatially extensive research capable of establishing causal links between anthropogenic factors and the structure of bird communities in this semi-arid region of Cameroon (BirdLife International, 2018). The study will contribute to a fundamental approach for assessing the consequences of different uses of ecosystems in this part of the country on bird communities and, more broadly, on biodiversity.

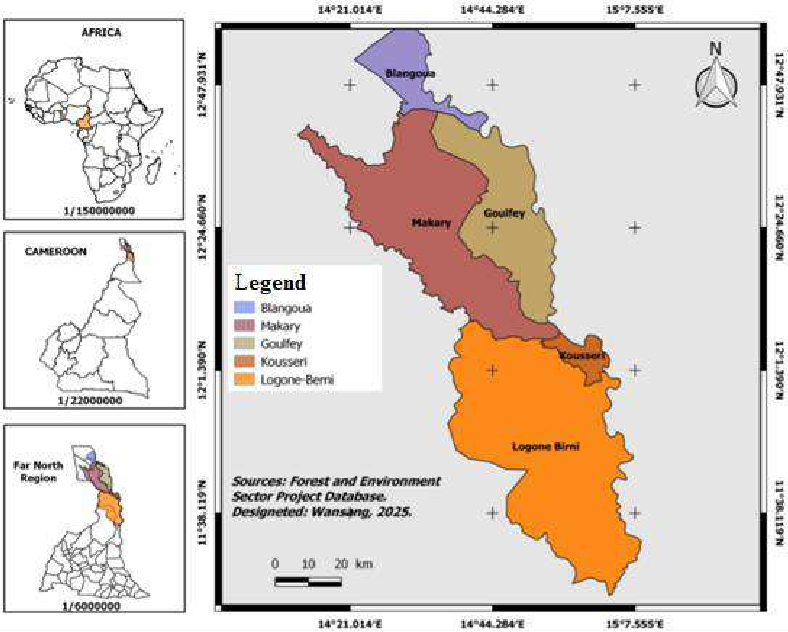
2. MATERIALS AND METHODS

2.1. Study site

This study was conducted in the southern part of Lake Chad, in Cameroon, located in the Far North region between approximately 10°30′ and 13°30′ N and 12°30′ and 16°00′ E (Figure 1), within the Sudanian-Sahelian ecological zone characterized by a semi-arid climate with a short rainy season (July–September) and a long dry season (United Nations Educational, Scientific and Cultural Organization (UNESCO), 2023). Annual rainfall is generally low and variable, while temperatures remain high throughout the year, often averaging above 30 °C, with higher relative humidity near the water body, especially during the rainy season (Britannica, 2024). Lake Chad is an extremely shallow wetland, with average depths generally less than 4 m and strong seasonal and interannual fluctuations linked to inflows from the Chari–Logone river system and regional climate variability (Gbetkom, 2022). Recent analyses based on remote sensing show that, despite a marked reduction in its surface area since the 1960s, the lake exhibits a complex hydrological dynamic with phases of contraction and expansion of surface water over the past decade, including temporary increases observed after wetter years (Pham-Duc et al., 2022). The vegetation in the Cameroonian part of the lake consists of a mosaic of aquatic and terrestrial formations, including floating and emergent macrophytes such as Cyperaceae, Typha, Phragmites, and Pistia in flooded areas, as well as wooded and shrub savannas dominated by *Acacia spp*., *Balanites aegyptiaca, Ziziphus mauritiana, and Hyphaene tebaica*(doum palm) formations, whose spatial distribution varies according to water gradients and the recent dynamics of water levels (Gbetkom, 2020; UNESCO, 2023).

2.2. Data collection using the transect method

The data was collected from January 8 to March 21, 2024, for the dry season and from June 13 to October 16, 2024, for the rainy season. The geo-referenced satellite images obtained showed the different variations in land use, namely plantation areas, wooded areas, dwellings, water points, and grassy areas. This was done in order to compare the diversity between the two (02) seasons and between habitats. A total of 80 linear transects, each 1 km long, were defined. The transects were oriented horizontally, with a spacing of at least approximately 1.5 km between them (Figure 2). The starting points of each transect and their coordinates were recorded using QGIS version 12.3 software to facilitate their location and orientation in the field. As part of this study, the 1-km transects were established and evenly distributed according to habitat type, with 16 transects per habitat. A total of five (transects corresponding to each habitat type were randomly selected for the placement of Japanese nets.



Sources: Forest and Environment Sector Project Database. Designeted: Wansang, 2025.

Figure 1: Study area and land use in the study area

At water points, were placed at the edge of an area considered likely to be used by birds. The inventory team arrives early in the morning (around 6 a.m.) at or near the sampling point and/or in the evening from 3 :30 p.m. onwards, to increase the chances of encountering birds, as most wild birds are most active during these periods (Kougoum et al., 2024). Each of these transects was surveyed during each season.

2.3. Data collection using the Japanese net capture method

In order to confirm or correct certain identifications, Japanese net captures were also carried out during the same period, on the same transects, to capture passerines (Figure 3). Twenty-five (25) transects out of the 80 used, at a rate of 5 per habitat type, were selected for the installation of nets. The Japanese nets, which were 3 m high, with a mesh size of 2 cm and a length of 12 m, were set up vertically very early in the morning between 5 :30 and 6 :00 a.m. and in the evening between 4 :00 and 5 :30 p.m. by attaching their rings to two stakes at least 3.5 m high and fixed to the ground. These nets were initially placed by observing the probable direction of the birds' movement in order to increase the chances of capture. The various measurements are recorded on a form, including the length of the beak, tarsus, tibia, wing, wingspan, total length of the bird, weight, and other morphological characteristics (color of plumage, shape of legs, toes, beak, etc.).

2.4. Identification of bird species in the southern part of Lake Chad, Cameroon

Bird identification equipment consists of a pair of binoculars for remote bird observation, a telescope, a bird identification guide for perfect bird recognition. the species through its morphological characteristics and scientific name, a camera for taking images of birds without doubt of identification in the field (Figure 4). The bird nomenclature is that used in the work of Borrow and Demey (2014).

2.5. Data analysis

2.5.1. Species richness estimate : Species richness was calculated as described by Blondel (1975).

Species richness estimate (E) = (species observed) / (species estimated) x 100

2.5.2. Shannon-Wiener diversity index (H')

This index was calculated according to each type of habitat and according to the seasons and expressed in Binary Digit Units (Bits). $H = \sum p_i \log_2(p_i)$

$\log_2$= logarithm to base 2; N_i = number of observations of a species i; N = total number of observations; p_i is the probability that a species i is present in a survey. In Practice, $p_i = N_i N^{-1}$

-If H' ° (H < 3) then H' is weak, which implies that the environment is not very diverse (case of specialized

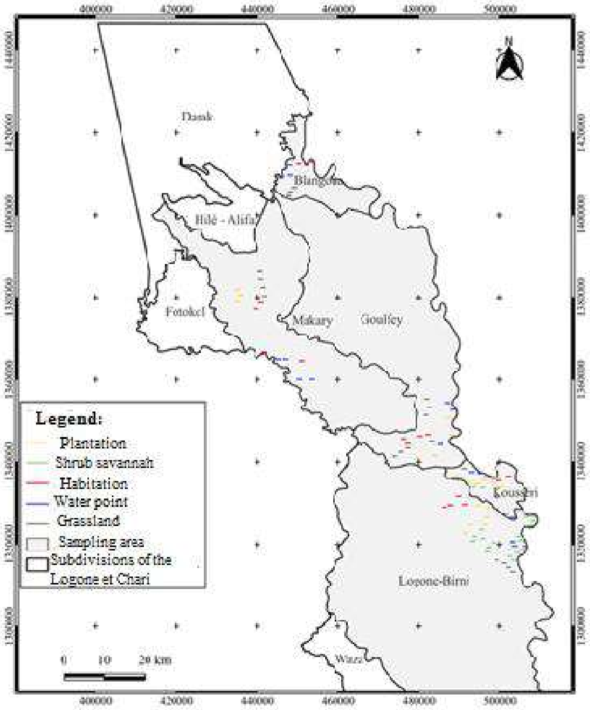


Figure 2: Lay out of transects in the sampling area

Figure 3: Taking measurements of a bird captured with a mist net

Figure 4: Group of birds in the study area

environments where we generally note the dominance of a single species or a small number of species species across all species in the community.

- If H' ° ($3 \leq H < 4$) then H' can be assumed to be average, which implies that the environment is relatively rich in species.
- If H' ° ($H \geq 4$) then H' can be assumed to be high. Here the study station or the environment presents favorable conditions for the installation of many species, but the number of individuals per species is low

To determine the differences in diversity between bird communities according to habitat type and season, the Jaccard similarity index was used.

2.6.3. Anthropogenic factors

This was based primarily on determining the anthropogenic disturbance index. The team reported the anthropogenic pressure indices encountered at each sampling point. These indices were grouped into categories : transhumance (any presence of domestic livestock), camping, poaching (any physical presence of poachers, poaching camps.), fishing, agricultural occupation (fields or human occupation), logging (harvesting of dead wood/fuel, logging sites), etc. To compare the intra-annual variability of species richness and diversity, we used the chi-square ($\chi2$) test and the diversity t-test, respectively. The nonparametric Kruskall-Wallis test was used to compare species richness between habitat types. General linear model was also used to determine the effect of human activities on the avifauna of the study area based on kilometer abundance indices. The probability threshold used for statistical analyses corresponding to a confidence level of 95.0%. Statistical tests were performed using PAST 4.03 and XLstat software.

3. RESULTS AND DISCUSSION

3.1. Overall species richness

The 80 transects carried out in the study area recorded 213 species. These birds are divided into 24 orders and 67 families. Among the orders, Passeriformes is the order with the largest number of families (27 families), representing 39.70%, followed by Bucerotiformes (11 families) and Pelicaniformes (4 families). Orders such as Strigiformes, Psittaciformes, Podicipediformes, Musophagiformes, Falconiformes, Cuculiformes, Columbiformes, Coliiformes, Ciconiiformes, Caprimulgiformes, Apodiformes, and Accipitriformes are the least represented, with only one family each. Most diverse families in terms of number of species are Accipitripidae with 19 species and Ardeidae with 16 species. Of the 68 families recorded, 29 each represent 0.79% (one species) (Table 1). According to the IUCN, three (03) species have been classified as vulnerable (*Aquila rapax ; Circaetus beaudouini* ; *Balearica pavonina)*, two (02) as near threatened (*Circus macrourus ; Ciconia episcopus*) and One (01) Critically Endangered (*Necrosyrtes monachus*).

3.2. Sampling success

The cumulative richness of bird species based on 80 surveys was carried out (Figure 5). Taking into account the species richness in the different habitats of the study area, the estimators Chao2 (261.335), Jackknife1 (221.464), Jackknife2 (237.202), and Bootstrap (231.906) give us an average of 238 species. As the number of species observed is 213, the estimators show that 25 species were not encountered in this study area. Considering the number of estimated species and those sampled, the sampling effort for study is 88.26%.

The results of counts carried out in the southern part of Lake Chad highlight a particularly high level of avian diversity. The abundance of individuals observed confirms the ecological importance of this ecosystem for both resident and migratory birds. The species richness represents approximately 22.3% of all known bird species in Cameroon (954 species) and 56.2% of Far North region (379 species) (Lepage, 2019; Languy, 2019). These proportions demonstrate the central role of this wetland in maintaining national and regional avian diversity.

The values observed are similar to those obtained in other wetlands in West Africa. Odoukpe et al. (2014) recorded 216 species in two seasons in a wetland in Côte d'Ivoire, confirming the high ecological productivity of these environments. In contrast, Kougoum et al. (2024), in the Kalfou Forest Reserve and surroundings, recorded a much lower species richness (124 species). This difference could be explained by the ecological contrasts between the two sites : the Lake Chad area is a vast wetland recognized as a Ramsar site, characterized by the presence of permanent water bodies favorable to waterfowl and Palearctic migratory birds (Gbetkom, 2020). Conversely, the Kalfou Reserve does not have such aquatic habitats, which limits its capacity to support water-dependent species.

Wetlands play a crucial role in the life cycle of birds, as breeding, wintering, and migratory stopover sites. Chokri (2024) emphasized that these environments are home to a wide variety of waterbirds, providing critical habitats for many migratory species. The probabilistic estimators applied in this study suggest a total species richness of around 237 species, which is higher than the estimate by Kougoum et al. (2024) in Kalfou (184 species) using the

Table 1: Distribution of specific richness by order and by family

Orders	Families	Species	IUCN status
Accipitriformes	Accipitridae	*Accipiter badius*	LC
		Accipiter minullus	LC
		Aquila rapax	VU
		Aquila wahbergi	LC
		Butastur rufipennis	LC
		Buteo auguralis	LC
		Chelictinia riocourii	VU
		Circaetus beaudouini	VU
		Circaetus cinereus	LC
		Circus macrourus	NT
		Circus pygargus	LC
		Elanus caeruleus	LC
		Hieraaetus spilogaster	LC
		Lophaetus occipitalis	LC
		Melierax metabates	LC
		Micronisus gabar	LC
		Milvus migrans	LC
		Necrosyrtes monachus	CR
		Polyboroides typus	LC
	Pandionidae	*Pandion haliaetus*	LC
Anseriformes	Anatidae	*Alopochen aegyptiaca*	LC
		Anas hottentota	LC
		Dendrocygna bicolor	LC
		Dendrocygna viduata	LC
		Plectropterus gambensis	LC
		Sarkidiornis melanotos	LC
		Thalassornis leuconotus	LC
Apodiformes	Apodidae	*Apus affinis*	LC
		Cypsiurus parvus	LC
Bucerotiformes	Bucerotidae	*Tockus erythrorhynchus*	LC
		Tockus nasutus	LC
	Upupidae	*Upupa epops*	LC
Caprimulgiformes	Caprimulgidae	*Caprimulgus inornatus*	LC
		Macrodipteryx longipennis	LC
Charadriiformes	Burhinidae	*Burhinus capensis*	LC
		Burhinus senegalensis	LC
	Charadriidae	*Charadrius hiaticula*	LC
		Charadrius marginatus	LC
		Vanellus crassirostris	LC
		Vanellus senegallus	LC
		Vanellus spinosus	LC
		Vanellus superciliosus	LC
		Vanellus tectus	LC

Cont...

Table 1: Distribution of specific richness by order and by family

Orders	Families	Species	IUCN status
	Glareolidae	Glareola cinerea	LC
	Jacanidae	Actophilornis africana	LC
		Microparra capensis	LC
	Laridae	Larus cirrocephalus	LC
		Rynchops flavirostris	NT
		Sterna albifrons	LC
	Pluvianidae	Pluvianus aegyptius	LC
	Recurvirostridae	Himantopus himantopus	LC
	Rostratulidae	Rostratula benghalensis	LC
	Scolopacidae	Calidris alpina	LC
		Calidris minuta	LC
		Gallinago gallinago	LC
		Lymnocryptes minimus	LC
		Philomachus pugnax	LC
		Tringa nebularia	LC
		Tringa totanus	LC
	Sternidae	Sterna caspia	LC
	Turnicidae	Ortyxelos meiffrenii	LC
		Turnix sylvaticus	LC
Ciconiiformes	Ciconiidae	Anastomus lamelligerus	LC
Ciconiiformes	Ciconiidae	*Anastomus lamelligerus*	LC
		Ciconia episcopus	LC
		Leptoptilos crumeniferus	LC
		Leptoptilos senegalensis	LC
		Mycteria ibis	LC
Coliiformes	Coliidae	*Urocolius macrourus*	LC
Columbiformes	Columbidae	*Columba guinea*	LC
		Oena capensis	LC
		Streptopelia abyssinicus	LC
		Streptopelia decipiens	LC
		Streptopelia roseogrisea	LC
		Streptopelia senegalensis	LC
		Streptopelia vinacea	LC
		Turtur abyssinicus	LC
Coraciiformes	Alcedinidae	*Ceryle rudis*	LC
		Ceyx pictus	LC
		Halcyon chelicuti	LC
		Halcyon leucocephala	LC
		Halcyon senegalensis	LC
		Megaceryle chelicuti	LC
		Megaceryle maxima	LC
	Coraciidae	*Coracias abyssinicus*	LC
		Coracias naevius	LC
		Eurystomus glaucurus	LC

Cont...

Table 1: Distribution of specific richness by order and by family

Orders	Families	Species	IUCN status
	Meropidae	*Merops albicollis*	LC
		Merops apiaster	LC
		Merops nubicus	LC
		Merops orientalis	LC
		Merops persicus	LC
		Merops pusillus	LC
Cuculiformes	Cuculidae	*Centropus senegalensis*	LC
		Chrysococcyx caprius	LC
		Cuculus gularis	LC
		Oxylophus levaillanti	LC
Falconiformes	Falconidae	*Falco ardosiaceus*	LC
		Falco biarmicus	LC
Galliformes	Numidae	*Numida meleagris*	LC
	Phasianidae	*Francolinus clappertoni*	LC
Gruiformes	Gruidae	*Balearica pavonina*	VU
	Rallidae	*Amaurornis flavirostra*	LC
		Crex egregia	LC
		Gallinula angula	LC
Musophagiformes	Musophagidae	*Crinifer piscator*	LC
Otidiformes	Otididae	*Eupodotis senegalensis*	LC
		Lissotis melanogaster	LC
Passeriformes	Acrocephalidae	*Acrocephalus arundinaceus*	LC
		Acrocephalus rufescens	LC
		Acrocephalus schoenobaenus	LC
		Acrocephalus scirpaeus	LC
	Alaudidae	*Eremopterix leucotis*	LC
		Galerida cristata	LC
	Buphagidae	*Buphagus africanus*	LC
	Cisticolidae	*Camaroptera brachyura*	LC
		Cisticola galactotes	LC
		Cisticola juncidis	LC
		Cisticola ruficeps	LC
		Cisticola marginatus	LC
		Prinia subflava	LC
	Corvidae	*Corvus albus*	LC
		Ptilostomus afer	LC
	Dicruridae	*Dicrurus adsimilis*	LC
	Estrildidae	*Estrilda melpoda*	LC
		Euodice cantans	LC
		Lagonosticta senegala	LC
		Pytilia melba	LC
		Spermestes cucullatus	LC
		Uraeginthus bengalus	LC

Cont...

Table 1: Distribution of specific richness by order and by family

Orders	Families	Species	IUCN status
	Fringillidae	*Serinus leucopygius*	LC
	Hirundinidae	*Delichon urbicum*	LC
		Hirundo aethiopica	LC
		Hirundo daurica	LC
		Hirundo rustica	LC
		Petrochelidon preussi	LC
		Hirundo senegalensis	LC
		Riparia cincta	LC
		Riparia paludicola	LC
	Indicatoridae	*Indicator indicator*	LC
	Laniidae	*Lanius collurio*	LC
	Malaconotidae	*Nilaus afer*	LC
		Tchagra senegalus	LC
	Motacillidae	*Anthus cervinus*	LC
		Macronyx croceus	LC
		Motacilla flava	LC
	Muscicapidae	*Muscicapa aquatica*	LC
		Saxicola torquatus	LC
	Nectariniidae	*Chalcomitra senegalensis*	LC
		Cinnyris pulchellus	LC
		Hedydipna platura	LC
	Passeridae	*Passer domesticus*	LC
		Passer griseus	LC
		Passer luteus	LC
		Petronia dentata	LC
		Plocepasser superciliosus	LC
		Sporopipes frontalis	LC
	Phylloscopidae	*Phylloscopus bonelli*	LC
	Ploceidae	*Bubalornis albirostris*	LC
		Euplectes franciscanus	LC
		Euplectes hordeaceus	LC
		Ploceus cucullatus	LC
		Ploceus heuglini	LC
		Ploceus luteolus	LC
		Ploceus vitellinus	LC
		Quelea quelea	LC
	Pycnonotidae	*Pycnonotus barbatus*	LC
	Sturnidae	*Lamprotornis caudatus*	LC
		Lamprotornis pulcher	LC
		Lamprotornis purpureus	LC
	Sylviidae	*Hippolais pallida*	LC
		Hippolais (pallida) opaca	LC
		Hippolais icterina	LC

Cont...

Table 1: Distribution of specific richness by order and by family

Orders	Families	Species	IUCN status
	Timaliidae	*Cercotrichas leucophrys*	LC
		Cercotrichas podobe	LC
		Turdoides plebejus	LC
	Turdidae	*Oenanthe deserti*	LC
		Oenanthe isabellina	LC
		Oenanthe oenanthe	LC
		Phoenicurus phoenicurus	LC
		Turdus pelios	LC
	Viduidae	*Vidua chalybeata*	LC
		Vidua macroura	LC
Pelicaniformes	Ardeidae	*Aigretta ardesiaca*	LC
		Ardea alba	LC
		Ardea cinerea	LC
		Ardea melanocephala	LC
		Ardea purpurea	LC
		Ardeola ralloides	LC
		Bubulcus ibis	LC
		Butorides striada	LC
		Egretta garzetta	LC
		Egretta intermedia	LC
		Ixobrychus sturmii	LC
		Lxobrychus minutus	LC
		Nycticorax nycticorax	LC
	Scopidae	*Scopus umbretta*	LC
	Threskiornithidae	*Bostrychia hagedash*	LC
		Platalea leucorodia	LC
		Plegadis falcinellus	LC
		Threskiornis aethiopica	LC
Piciformes	Capitonidae	*Lybius dubius*	LC
		Lybius minor	LC
	Picidae	*Dendropicos obsoletus*	LC
Podicipediformes	Podicipedidae	*Tachybaptus ruficollis*	LC
Psittaciformes	Psittacidae	*Psittacula krameri*	LC
Pterocliformes	Pteroclidae	*Pterocles exustus*	LC
		Pterocles quadricinctus	LC
Strigiformes	Strigidae	*Bubo(africanus) cinerascens*	LC
		Glaucidium perlatum	LC
		Ptilopsis leucotis	LC
Suliformes	Anhingidae	*Anhinga rufa*	LC
	Phalacrocoracidae	*Microcarbo africanus*	LC
		Phalacrocorax africanus	LC

Legend: LC=Least Concern ; NT=Near Threatened ; VU=Vulnerable ; CR=Critically Endangered ; IUCN=International Union for Conservation of Nature

same estimation tools. This difference corroborates the hypothesis of greater habitat heterogeneity in the Lake Chad area, favoring increased species diversity. Although the accumulation curve obtained indicates a satisfactory estimate of species richness, additional sampling would be necessary to identify rare or undetected species. In the same ecological gradient, the nearby Waza National Park (WNP) is home to around 370 bird species, including more than 71 species of water birds, of which around 20 are migratory (BirdLife International, 2018 ; MINFOF, 2020). This park, which enjoys protected status, preserves an exceptional wealth of fauna and flora (Gbetkom, 2022). A comparison between the WNP and the southern part of Lake Chad illustrates the impact of the level of protection on the diversity observed.

Among the species recorded in the study area, three endangered species, all birds of prey, and two near-threatened species were identified. This finding is consistent with the observations made by Kougoum et al. (2024) in the Kalfou Reserve, as well as by Adhikari et al. (2019) in Chitwan National Park in Nepal. The presence of these globally threatened species gives the southern Lake Chad area high biological value and makes it a priority site for conservation.

3.3. Spatio-temporal diversity of avifauna

Of the 213 species encountered in the area, shows that 98.59% (210 species) are in the dry season, compared to 193 species in the rainy season, or 91.07% of the total. Surveys conducted in the five habitat types enabled to encounter 98 species at water points, 88 species in the shrub savanna, 87 species in plantations, 77 species in grassy savanna, and 65 species in residential areas. The Krukall-Wallis test shows no significant difference between these different habitats. The cumulative species richness curves are logarithmic in nature, showing a quasi-asymptotic plateau after a certain number of surveys, beyond which the probability of encountering a new species becomes very low (Figure 6).

The avian diversity observed within habitats (Table 2) is relatively high (3.821 bits) at water points and lower in plantations (0.985 bits) according to the Shannon index. This result shows that water point habitats are more diverse than other types of habitats. Comparing the Shannon indices of the different habitats in pairs, indicate that there is a highly significant difference between shrub savanna and plantation followd by plantations and grassy savannah, grassy savannah and water points, water points and residential areas, and grassy savannah and shrub savannah. Comparison of the Jaccard similarity index shows that there are differences between habitat types. The greatest similarity is

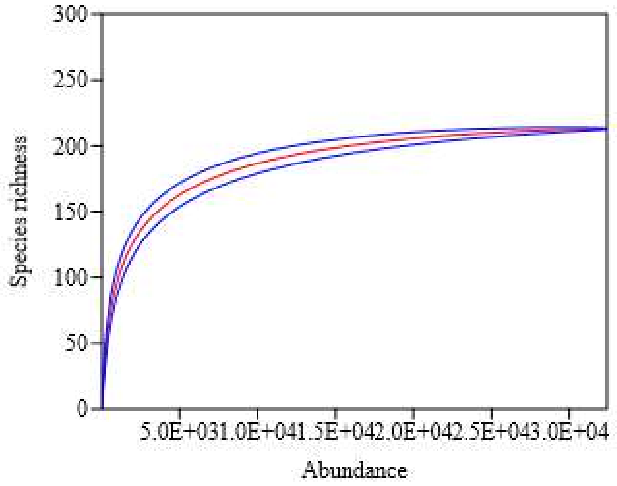


Figure 5: Global rarefaction curve

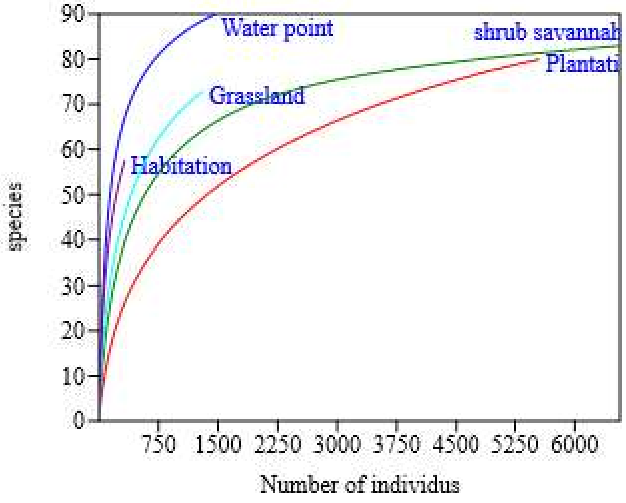


Figure 6: Rarefaction curves depending on habitats

Table 2: Diversity indices

	Shannon index				Simpson index			
	H'	**T**	**Dl**	**P**	**D**	**T**	**Dl**	**P**
Rainy season	2.1283	11.432	32052	3.3101E-30	0.43261	-12.246	32309	2.0951E-34
Dry season	1.8149				0.5179			

Legend: H'= Shannon index; T=diversity indices T; df = degree of freedom; P = probability; D = Simpson index

between shrub savanna and plantation (78.66%). In contrast, there is little similarity between the residential area and the plantation (6.8%).

Although diversity is low during both seasons, the Shannon index in the rainy season (H' = 2.1283) is higher than in the dry season (H' = 1.8149). Comparison of the Shannon index between the two seasons showed a highly significant difference. The probability that two randomly selected individuals belonging to the same species is higher in the rainy season (D=0.43261) than in the dry season (D=0.5179) according to the Simpson index. This means that certain species are more dominant in the rainy season compared to the dry season.

3.4. Specific diversity of birds according to the seasons

The results indicate that bird species richness is higher during the dry season than during the rainy season. This trend has also been highlighted in several previous studies (Kougoum et al., 2024 ; Sadip et al., 2022 ; Nepali et al., 2021). Kougoum et al. (2024), mentiond that seasonal variation could be explained by increased availability and better access to food resources during the dry season, a period also marked by the arrival of many migratory species between October and March, which temporarily increases species richness. Conversely, heavy rainfall and flooding during the rainy season can disrupt aquatic habitats, leading to a decrease in the number of species present.

However, the rainy season has a higher Shannon diversity index than the dry season. This result could be explained by a more balanced distribution of abundance among species, particularly due to the scarcity of migratory species during this period. The high species diversity during the rainy season would thus be linked to the lesser dominance of certain abundant species observed in the dry season. Habitat structure also plays a decisive role in the composition and organization of avian communities (Earnst and Holmes, 2012, Krizler et al., 2022). In this study, a comparison of species richness and diversity in five habitat types (shrub savanna, residential areas, plantations, water areas, and grassy savanna) south of Lake Chad highlights a significant influence of habitat type on bird distribution. Previous studies confirm that avian diversity is closely linked to habitat structure and complexity (Kumar et al., 2017, Adhikari et al., 2019). The differences observed between habitats could therefore be due to variability in resource availability and the degree of anthropogenic disturbance. Water areas have the highest species diversity, followed by grassy savannahs, with which they share a strong similarity. This ecological proximity is probably explained by comparable vegetation composition and environmental conditions, as the grassy savannahs south of Lake Chad are periodically flooded and offer food resources similar to those found at water points. The shrub savanna shows a notable similarity to plantation areas, a result consistent with Krizler (2015) and can be attributed to analogous plant structures—trees and shrubs scattered across herbaceous cover—which promote similar ecological assemblages. Nevertheless, differences in management practices and the level of anthropization introduce significant variations between these two environments (Bett et al., 2016, Thakur et al., 2022).

The lowest similarity was observed between plantation areas and residential areas which is consistent with the observations of Gatesire et al. (2014) and SpringerLink. (2023), where reduced bird diversity in urban environments. Human infrastructure (roads, buildings, schools, and health facilities) in residential areas significantly alter natural habitats and limit the niches available to birdlife. These transformations explain the low diversity and dissimilarity observed in the inhabited areas of southern Lake Chad.

3.5. Human activities and impact on birdlife in the southern part of Lake Chad, Cameroon, according to the seasons

Analysis of data on indicators of human presence revealed nine (09) types of human pressure in the southern part of Lake Chad, Cameroon, including poaching, bush fires, grazing, deforestation, pollution, poisoning, agricultural activities, and fishing. The HIs for the various activities range from $0.04<HI<24.7$ in the dry season to $0.04<HI<44.8$ in the rainy season. Grazing has the highest IKA (24.63 signs/km) in the rainy season compared to other activities., pollution indicate highest IKA (44.72 signs/km) o in the dry season (Table 3). Comparing the IKAs of these different activities observed in the two seasons, there are significant differences between grazing (and fishing and very significant differences between activities related to pollution

3.6. Correlation and level of influence of human activities and bird presence

Depending on the distribution of bird species and human activities, overall, the species appear to be influenced by anthropogenic activities. To assess the impact of human activities on avifauna, Pearson correlation and determination coefficients were evaluated between the IKA of activities and the IKA of birds. It therefore appears that birds are strongly influenced and are negatively, highly, and significantly affected by human activities (r= -0.38 and p=0.00012446) in the dry season (Figure 7a) and

Table 3: Kilometer indices of abundance of human activities in the southern part of Lake Chad, Cameroon

Type of threats	Activities	Frequency (n) rainy season	PCKI (n/80km) rainy season	Frequency (n) dry season	PCKI (n/80km) dry season
Poaching	Bird catch nets	39	0,487	44	0,55
	Hunter seen	23	0,287	8	0,1
	Gunshot	26	0,325	0	0
	Holes dug by hunters	42	0,525	18	0,225
	Pitfalls	37	0,462	21	0,262
	Torch/Battery/bulb	7	0,087	_	-
	Cartridges	6	0,075	-	-
	Birds injured by the weapon	27	0,337	-	-
	Total Poaching activities	**206**	**2,575**	**91**	**1,1375**
Bushfires	Landscape consumed /ha	6	0,075	13	0,16
	Total bushfire activity	**36**	**0,45**	**6**	**0,075**
Deforestation	Pruning	41	0,512	11	0,137
	Trees felled	256	3,2	256	3,2
	Wood cutting	63	0,78	36	0,45
	Coal mining	22	0,275	3	0,0375
	Total deforestation activities	**306**	**3,825**	**457**	**5,712**
Pasture	Shepherds seen	27	0,337	14	0,175
	Sheep or goats seen	629	7,862	370	4,625
	Cattle seen	882	11,025	328	4,1
	Cattle tracks	433	5,412	612	7,65
	Total pasture activities	**1971**	**24,63**	**1234**	**15,425**
Pollution	Engine noise	8	0,1	18	0,22
	Fuel leaks into water	8	0,1	18	0,22
	Plastic waste	1224	15,3	3435	42,9375
	Chemical fertilizers	27	0,337	11	0,13
	Scarecrows	15	0,187	89	1,11
	Slaughterhouse	1	0,012	1	0,012
	Pesticides	21	0,262	6	0,075
	Total pollution activities	**1304**	**16,3**	**3554**	**44,425**
Poisoning	Poisoned water	30	0,375	22	0,275
	Potentially poisoned birds	93	0,92	52	0,65
	Total poisoning activities	**123**	**1,537**	**74**	**0,925**
Electrocution	Post or wires involved	4	0,05	1	0,0125
	Electrocuted birds	-	-	3	0,0375
	Total electrocution activities	**4**	**0,05**	**4**	**0,0375**
Agricultural activities	Fallow	66	0,825	90	1,125
	Cultivated fields	73	0,912	250	3,125
	New fields	13	0,162	12	0,15
	Farmers' Tracks	2	0,025	8	0,1
	Total agricultural activities	**154**	**1,925**	**360**	**4,5**
Peaches	Fishing nets	67	0,837	128	1,6
	Traps	18	0,225	54	0,675
	Fishermen	47	0,587	162	2,025
	Total fishing activities	**132**	**1,65**	**344**	**4,3**

Legend: PCKI (Pressure Contact Kilometer Index)

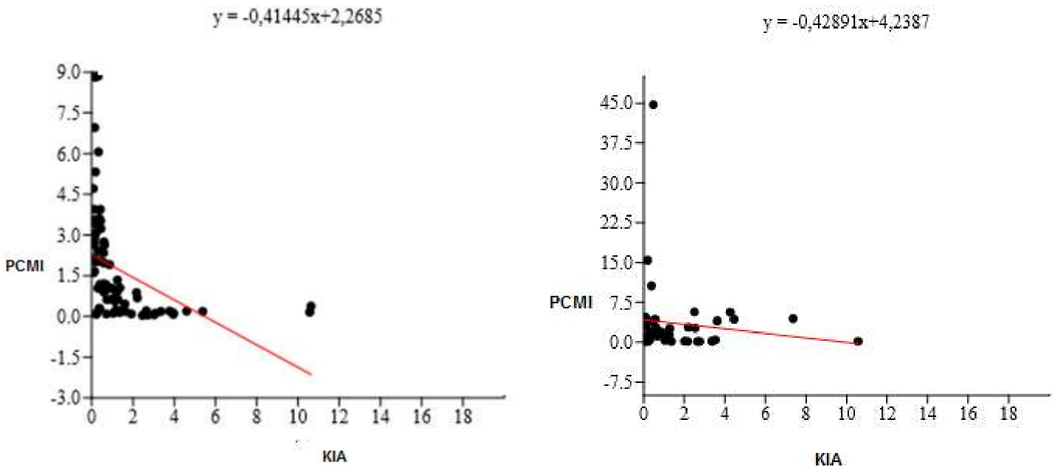


Figure 7: Correlation between the IKA values of anthropogenic activities and birds in the southern part of Lake Chad, Cameroon in the dry season (a) and in the rainy season (b)

significantly in the rainy season (r= -0.167 and p=0.42911) (Figure 7b).

The bird communities respond strongly to various pressures from human activities such as poaching, fishing, pastoralism, pollution, poisoning, deforestation, bush fires, electrocution, and agriculture. The values of the kilometer-based abundance indices (IKA) associated with these pressures vary according to the seasons, reflecting both the temporal and spatial influence of anthropogenic disturbances on avifauna.During the rainy season, pastoralism was most significant pressure. This situation can be explained by the influx of transhumant herders to the southern area of Lake Chad at this time of year, due to the increased availability of pasture and water sources (Rangé, 2016). In the dry season, these groups move and concentrate around the few remaining functional wetlands (Gbetkom, 2020), altering the distribution of pastoral pressure on habitats. In contrast, pollution was higher during the dry season, probably due to the intensification of economic and agricultural activities such as market gardening and off-season crops, as well as the increased use of motorized equipment and chemical inputs. These factors contribute to the degradation of environmental quality and the disruption of ecosystems frequented by birds. Similar trends were reported by Kougoum et al. (2023) in the Sena Oura and Zakouma national parks, where pastoralism was also the most frequent activity according to IKA surveys. However, contrary to their conclusions suggesting a limited impact of human activities on birdlife, the analyses from this study highlight a significant negative correlation between the intensity of anthropogenic pressures and bird abundance, both in the rainy and dry seasons. This difference could be linked to the protection status of the sites : in unprotected areas, pressures are generally stronger and less regulated, which amplifies the impacts on wildlife. Chavez-Lugo et al. (2024) also observed same trend where bird communities in unprotected environments are significantly influenced by human activities. The weaker correlation recorded during the rainy season could be explained by the greater availability of food resources and nesting habitats, which temporarily mitigates the effects of human disturbance. However, this buffering effect remains limited, as most of the pressures observed persist throughout the year. Overall, these results highlight the need for integrated management of the Lake Chad basin ecosystems.

4. CONCLUSION

The study conducted in the southern part of Lake Chad highlights remarkable avian diversity, with 213 species recorded, representing more than half of the region's total. Water areas have the greatest diversity, while anthropized environments are the poorest. The dry season is richer in species, particularly migratory species, while the rainy season has greater species diversity. Human activities (grazing, poaching, deforestation, fires, pollution) negatively affect bird communities. The presence of endangered species confirms the ecological importance of the site. Integrated habitat management, combining conservation, regulation of uses, and community participation, is essential to ensure the sustainability of biodiversity in southern Lake Chad.

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Authors' Contributions

Wansang Daksala: led the research, conducted the fieldwork, and prepared the manuscript. Kougoum Piebeng Ghislain Noé: conducted the fieldwork, performed the literature review. Kondasso Taiga Lea: interpreted and analyzed the data, reviewed the manuscript. Bakwo Fils Eric Moise: conducted the fieldwork, reviewed the manuscript.

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

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

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Multi-Dimensional Yield Decline in Kerala's Kole Wetlands: Climate, Agronomy, and Governance Challenges

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Abstract: The Kole wetlands of Kerala constitute a vital agro-ecological system that supports a large share of the state's rice production but has experienced a persistent decline in productivity over the past decade. This study examined the multiple drivers of yield reduction through an integrated approach combining secondary datasets (2019–2024), structured farmer surveys, and field-level diagnostics. Annual rice yield, area sown, and rainfall records were compiled from official agricultural and meteorological agencies, while primary data documented farmers' perspectives on production constraints and management practices. Key limiting factors were erratic climatic patterns, delayed or inadequate drainage maintenance, sub-optimal agronomic practices, and gaps in the institutional delivery of inputs and technical support. The combined quantitative and qualitative evidence demonstrates that climate stressors, infrastructural limitations, and governance bottlenecks act synergistically to depress yields in this seasonally inundated landscape. The findings underscore the urgency of coordinated interventions focused on water-management governance, timely input supply, and locally responsive policy measures to enhance resilience and restore the long-term sustainability of rice cultivation in Kerala Kole wetlands.

Keywords: Agronomic constraints, Climate variability, Drainage infrastructure, Kole lands, Institutional governance, Paddy productivity, Wetland ecosystems.

1. INTRODUCTION

The Kole wetlands in Kerala, encompassing approximately 13,632 ha across Thrissur and Malappuram districts, form a crucial agro-ecological landscape that contributes significantly to the state's *Puncha* (dry-season) rice production. Their hydrology, characterized by an intricate system of bunds, sluices, and canals, supports both agriculture and key ecosystem services, including flood control and groundwater recharge (Sujana & Sivaperuman, 2008; Krishnankutty et al., 2013; Sunil et al., 2024). Despite these functions and a history of high productivity, paddy yields in this region have exhibited a sustained decline over the past decade (Prasad & Kuruvila, 2024).

This yield downturn reflects the interplay of climatic, agronomic, and institutional stressors. Climate projections for Kerala indicate rising temperatures and increasing rainfall variability during critical crop-growth stages, phenomena that disrupt flowering, induce heat stress, and prolong waterlogging—each of which adversely affects yield and crop duration (Aswathi et al., 2022; Riya & Ajithkumar, 2023). Erratic rainfall patterns have been shown to delay transplanting and impede fertilizer application, while high-intensity storms can damage bunds and drainage structures, exacerbating the effects of flooding and submergence. Agronomic constraints further reduce productivity. Soil salinity, continued use of obsolete rice varieties, and deterioration of field infrastructure contribute to declining yields (Krishnankutty et al., 2013; The Hindu, 2023). Limited seed replacement and overuse of nitrogenous fertilizers encourage pest outbreaks and nutrient imbalances, compounding the effects of climate stress. In several *padasekharams*, maintenance of drainage canals and sluice gates has lagged behind needs, decreasing the efficiency of water removal and increasing susceptibility to both flood and drought conditions. Institutional factors also play a decisive role. Weak coordination among local agencies, delays in input delivery, and non-compliance with bunding deadlines undermine field management and the collective action required to maintain the delicate hydrology of the wetlands. Such governance gaps reduce the capacity of farmers to respond to changing climatic conditions, creating a cycle of risk that further depresses yields (Prasad & Kuruvila, 2024).

Given the strategic importance of the Kole wetlands for Kerala's food security and the livelihoods of farming communities, there is an urgent need for an empirically grounded assessment of the drivers of yield decline. While previous studies have documented aspects of climate variability, agronomic stress, and institutional constraints, a comprehensive, multi-dimensional analysis integrating these factors remains limited.The present study addresses this gap by combining time-series data from 2019–2024 with structured farmer surveys, field diagnostics, and robust statistical modeling, including regression analysis. This approach aims to isolate the principal yield-limiting factors and provide actionable recommendations for scalable, context-specific interventions to restore productivity and enhance the sustainability of rice cultivation in this ecologically and economically vital wetland system.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling Design

The investigation was conducted in the Thrissur–Ponnani Kole wetlands, India, situated between 10°20' and 10°40'N latitudes and 75°58' and 76°11'E longitudes. The Kole region covers about 13,632 ha of seasonally inundated lowlands distributed across Thrissur and Malappuram districts. The wetlands are characterized by clayey alluvial soils, prolonged monsoon flooding, and rich aquatic biodiversity that supports *Puncha* (dry-season) rice cultivation. The wetlands also perform crucial ecological functions such as flood attenuation, groundwater recharge, and habitat support for migratory birds and aquatic fauna. The rice fields are organized into cooperative farming units known locally as *padasekharams*, each administered by farmer committees (*Padasekharam samithis*) that coordinate activities under the jurisdiction of the grama and block panchayats (Vivek & Bonny, 2024).

For field investigation, twelve padasekharams (>50 ha each) were selected using a stratified sampling approach to capture variation in hydrology, drainage condition, and management intensity. These representative sites provided a cross-section of the region's agro-ecological and institutional diversity for subsequent data collection and analysis.

2.2. Data Sources and Collection

Both secondary and primary data streams were integrated to provide a comprehensive dataset. Secondary data covering the period 2019–2024 on annual paddy yield (kg ha^{-1}) and total sown area (ha) were compiled from the *Atlas of Kole Lands of Kerala, Volume 2* (Sunil et al., 2024). Temperature data (°C) for Thrissur and Malappuram were retrieved from the India Meteorological Department's Hydromet Web Portal (IMD, 2025) and cross-validated with satellite-derived gridded dataset available through NASA's POWER Data Access Viewer (NASA Langley Research Center, 2025).

Primary data were collected between December 2023 and March 2024 through structured interviews with 120 farmers proportionally distributed across the 12 sampled padasekharams. Respondents were identified using a snowball sampling technique to ensure adequate representation of experienced rice farmers within each farming unit. The semi-structured questionnaire elicited detailed information on drainage performance, bund and sluice conditions, fertilizer application rates and timing, seed replacement and varietal choice, pest and disease incidence, labour availability and cost, and awareness and uptake of government agricultural support schemes. To complement self-reported information, field-level observations were simultaneously undertaken using a standardized checklist to document canal siltation, weed burden, crop establishment success, and the physical integrity of bunds and sluice gates. This mixed-method approach ensured that both farmer perceptions and objective field diagnostics informed the analysis.

2.3. Analytical Variables

The dependent variable was paddy yield (kg ha^{-1}), obtained from official production records for the study period. Explanatory variables included maximum temperature during the flowering stage (°C), drainage status, sluice and bund maintenance, fertilizer application rate (kg ha^{-1}), seed type, visible salinity indicators, labour cost (₹ ha^{-1}), and participation in government support schemes.

Maximum temperature (°C) was treated as a continuous variable. Drainage status was assessed through field observations using a three-point ordinal scale (Good = 3, Moderate = 2, Poor = 1) based on channel openness, water stagnation, and drainage efficiency. Sluice and bund maintenance were recorded as a binary variable (Maintained = 1, Not maintained = 0) depending on structural condition and timely operation.

Fertilizer application rate (kg ha^{-1}) was estimated from farmer-reported nutrient inputs per hectare. Seed type was categorised as high-yielding variety (HYV = 1) or local landrace (0). Visible salinity indicators were recorded as a binary variable (Present = 1, Absent = 0) based on field signs such as salt crust formation and poor crop establishment. Labour cost (₹ ha^{-1}) was calculated as the total expenditure on hired and family labour per hectare for the cropping season. Participation in government support schemes was measured as a binary variable (Participated = 1, Did not participate = 0).

All economic figures were standardised to 2024 price levels using the Kerala Consumer Price Index to ensure comparability across years (Kerala State Planning Board, 2024b). Farmer-reported production constraints were prioritised using the Garrett ranking technique to identify the most critical challenges affecting paddy cultivation.

2.4. Statistical Analysis

Data were analysed using IBM SPSS version 28.0. Descriptive statistics were used to summarise temporal trends in yield, maximum temperature and cultivated area. Pearson's correlation coefficients were calculated to measure the strength and direction of relationships between maximum temperature and yield. An empirical simple linear regression model was employed to estimate the influence of maximum temperature during the flowering stage on yield variability. The model was specified as (Eq.1):

$$Y_i = \beta_0 + \beta_1 T_i + \varepsilon_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where Y_i represents paddy yield (kg ha^{-1}), T_i denotes maximum temperature (°C), β_0 is the intercept, β_1 is the regression coefficient, and ε_i is the random error term. Model adequacy was evaluated using the coefficient of determination (R^2), and statistical significance was tested at the 5% probability level.

To evaluate productivity differences between well-managed and poorly managed *padasekharams*, a one-way analysis of variance was conducted. In addition, Chi-square tests were used to assess the association between farmers' awareness of government agricultural schemes and actual scheme uptake. Prior to analysis, assumptions of normality, linearity, and homogeneity of variance were verified to ensure the validity of statistical inferences.

3. RESULTS AND DISCUSSION

3.1. Yield trends and climatic variation

Padasekharam-level data for 2019–2023 show a coordinated decline in mean paddy yields concurrent with a rise in average maximum temperature (Tmax) during the reproductive (flowering) window. Specifically, reported values indicate mean Tmax at flowering increasing from 33.8 °C (2019) to 35.5 °C (2023), while mean yields dropped from 6,026 kg ha^{-1} to 4,112 kg ha^{-1} (Figure 1). Simple linear regression fitted to the five annual observations estimated a slope of aproximately –230 kg ha^{-1} $°C^{-1}$ (Yield = 12,800 – 230 × Tmax). Pearson's correlation analysis revealed a strong negative relationship between maximum temperature during the flowering stage and paddy yield ($r = -0.90$, $p < 0.05$). Simple linear regression further identified temperature as a major determinant of yield variability, explaining approximately 81% of the observed variation ($R^2 = 0.81$). The fitted model indicated that each 1°C increase in temperature was associated with an average yield decline of about 230 kg ha^{-1}. However, given the limited number of annual observations, the relationship should be interpreted as indicative rather than predictive.

The observed temperature–yield relationship is consistent

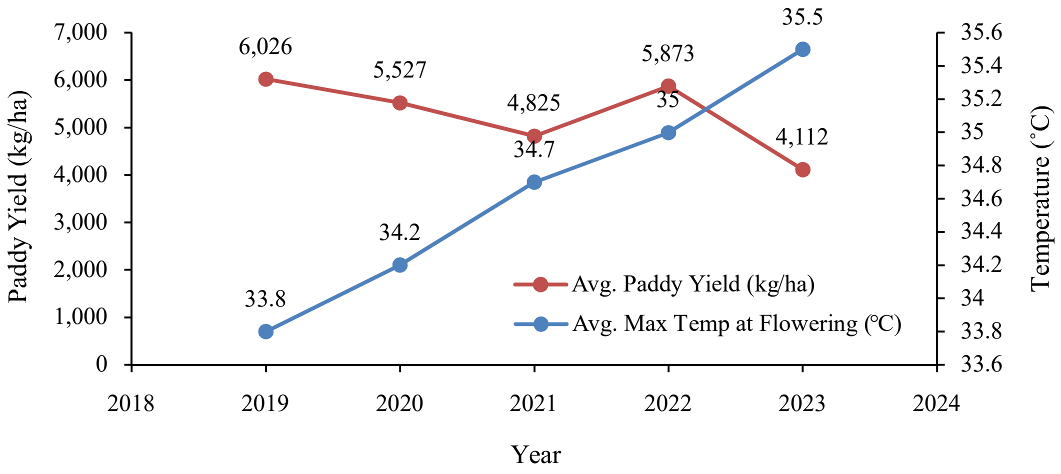

Figure 1. Average maximum temperature during flowering and corresponding paddy yield

with physiological and field evidence that reproductive-stage heat increases spikelet sterility and reduces grain set, thus sharply lowering yield (Peng et al., 2004; Jagadish et al., 2015; Krishnan et al., 2011). Peng et al. (2004) and Jagadish et al. (2015) document that both daytime maxima and elevated night temperatures at anthesis markedly reduce grain number and grain weight, while Krishnan et al. (2011) further highlight interactions among Tmax, vapor pressure deficit and water status that modulate realized damage in the field. Recent agronomic syntheses also demonstrate that modest reductions in spikelet fertility under high Tmax translate to large yield penalties at farm scale (Zhang et al., 2023). Given projected warming and increases in heat-extreme frequency in the region (state climate assessments and national projections), the observed sensitivity points to high exposure of Kole Puncha systems. Short-term adaptive measures include synchronizing planting dates to avoid peak anthesis heat, actively maintaining field water levels during flowering, and promoting heat-tolerant varieties (Jagadish et al., 2015; Zhang et al., 2023). Medium-term measures include strengthening the seed system for rapid deployment of tolerant cultivars and breeding programs targeting spikelet fertility under high T (Krishnan et al., 2011; Zhang et al., 2023).

3.2. Agronomic Drivers: Pests, Seed Systems and Soil Health

Garrett ranking of farmer responses places insect pest and disease outbreaks as the top agronomic constraint (Garrett mean = 79.6), followed by seed quality/varietal fatigue (74.3) and nutrient imbalance (69.8) (Table 1). Field diagnostics corroborate the survey: stem borer (*Scirpophaga incertulas*) and leaf folder (*Cnaphalocrocis medinalis*) dominated insect pressure in low, poorly drained fields, while bacterial leaf blight and sheath blight were more frequent in waterlogged and dense canopy conditions. These patterns reflect well-known pest–water–crop interactions: high humidity and delayed drainage favour fungal and bacterial pathogens and sustain vector populations (Savary et al., 2019).

Seed quality and varietal fatigue emerged as the second major constraint based on Garrett ranking (mean score = 74.3). Farmers reported the predominant and repeated use of the same local variety, indicating limited seed replacement and degeneration over time. This pattern may be due to dependence on farm-saved seed and inadequate access to certified seed systems, which can lead to reduced vigour and accumulation of seed-borne pathogens, as observed in similar smallholder systems (Jagadish et al., 2015; Vanlauwe et al., 2015).

Soil health and nutrient imbalance were ranked third to fourth. Field assessments and farmer responses indicated low surface soil organic carbon (often < 0.5%) and an over-reliance on nitrogenous fertilizers with limited use of phosphatic and organic amendments. Such nutrient imbalance may be responsible for declining soil fertility and reduced yield stability, consistent with findings reported by Vanlauwe et al. (2015).

Overall, these agronomic constraints—poor seed renewal and nutrient imbalance—may collectively increase vulnerability to climatic stress by lowering crop resilience and potential yield (Savary et al., 2019).

3.3. Infrastructure and Water-Management Constraints

Physical drainage infrastructure and operational synchronization emerged as key limiting factors influencing yield performance across years. Field inspections conducted between December 2023 and February 2024 revealed that a majority of padasekharams had partially blocked secondary drains (58%) due to siltation and weed growth, while 42% of sites had non-functional or poorly timed sluice operations (Table 2). Outer bund breaches were limited to one location (8.3%), but water-release and sowing mismatches occurred in nearly 67% of sites, causing transplanting delays of more than 10 days.

These drainage and scheduling deficiencies had a clear impact on productivity during 2019–2023. Padasekharams with blocked channels and delayed sowing consistently recorded lower mean yields (3,800 kg ha^{-1}) compared with well-maintained sites (6,750 kg ha^{-1}) (Table 4), indicating that prolonged submergence and late transplanting may be responsible for the observed year-to-year decline in yield. Similar associations between poor water control, disease incidence, and yield reduction in irrigated rice systems have been reported by Bouman et al. (2007) and Arouna et al. (2023).

Table 1. Ranking of agronomic constraints in Kole Padasekharams

Constraint	Garrett mean score	Rank
Pest and disease outbreaks	79.6	1
Poor seed quality and varietal fatigue	74.3	2
Nutrient imbalance	69.8	3
Decline in soil organic carbon	63.5	4
Lack of crop rotation	58.4	5
Inadequate extension services	52.7	6

Water-management literature further supports that timely desilting, synchronized sluice operation, and controlled water release are critical for maintaining crop health and uniform growth stages. The observed mismatches between canal-release schedules and farmer field readiness amplified inter-annual variation in yield by creating asynchronous planting and flowering periods across the padasekharam network. Operational lapses such as late bund closure or unsynchronized pumping schedules therefore translate directly into agronomic losses. Regular maintenance regimes, clear standard operating procedures (SOPs) for water release, and performance-linked maintenance funds may help reduce such variability, as evidenced in successful irrigation governance models elsewhere (Bouman et al., 2007; Meinzen-Dick, 2007).

3.4. Socio-economic Constraints: Labour, Land Fragmentation and Mechanization

The surveyed population shows an ageing farmer cohort (mean age ≈ 58 years) and high off-farm dependency (~53%) (Table 3). Labour shortages and rising wages (reported ~₹19,000 ha^{-1} $season^{-1}$) constrain time-sensitive farm operations (bunding, transplanting, weeding), while small, highly fragmented landholdings (~0.39 ha mean; 70% < 0.4 ha) limit mechanisation economies of scale and reduce uptake of custom-hire services. These demographic and structural patterns are consistent with regional studies documenting youth disengagement, rising agricultural wages, and area contraction for rice in Kerala (Kerala State Planning Board, 2024a).

Mechanisation can reduce labour bottlenecks if services are timely and appropriately localized; however, subsidy schemes and mechanisation programs often fail to reach smallholders without accompanying service-provision models (custom hiring centres) and capacity building (Regina et al., 2019). The net result in Kole is a reliance on contract labour and ad hoc machinery, which introduces timing uncertainty and inconsistent operational quality, further enlarging yield gaps between well-managed and poorly-managed padasekharams.

3.5. Yield Differentials by Management Intensity

The study indicates significant mean yield gap: 6,750 kg ha^{-1} in well-managed sites vs 3,800 kg ha^{-1} in poorly-managed sites (Table 4). The magnitude of this gap demonstrates large recoverable potential through improved management. One-way analysis of variance revealed a highly significant difference in paddy yield between well-managed and poorly managed padasekharams ($F(1,58)= 861.72$, $p < 0.001$), indicating that management quality is a major determinant of productivity. Empirical and experimental literature supports the idea that bundled management interventions (timely bunding, certified seed, synchronized transplanting and balanced fertilization) produce multiplicative benefits — i.e., the package effect often outperforms the sum of single interventions (Bouman et al., 2007; Vanlauwe et al., 2015). The implication for Kole is clear: targeted interventions that simultaneously address timing (water and operations), seed quality, and soil fertility are likely to yield the largest and most cost-effective recovery in productivity.

Table 2. Observed infrastructure and drainage constraints across Padasekharams

Parameter	Observed incidence (%)	Key observations
Blocked secondary channels	58	Channels obstructed by silt and vegetation; lack of systematic desilting
Non-operational sluices	42	Sluice malfunction or delayed closure; contributes to prolonged submergence
Outer bund breaches	8.3 (1 of 12)	Localized failure in structural integrity of outer bund in one location
Water release and sowing mismatch	67	Sowing delayed >10 days due to misaligned bund closure and irrigation

Table 3. Socio-economic characteristics of Kole Farmers (n = 120)

Parameter	Statistic	Observations
Average farmer age (years)	58.20	Majority over 55; youth disengagement evident
Average landholding size (ha)	0.39	70% hold < 0.4 ha; high fragmentation
Off-farm income dependency	53%	Reflects low return from monocrop rice
Labor cost (₹/ha/season)	19000	Increasing trend; impacts transplanting schedules
Machinery subsidy access	34%	Procedural hurdles and delayed disbursement

Table 4. Yield differentials between well-managed and poorly-managed Padasekharams

Management category	Mean yield (kg/ha)	Standard deviation	Remarks
Well-managed	6,750	412	Early bunding, timely sowing, certified inputs
Poorly-managed	3,800	365	Delayed transplanting, poor bund status, low input

3.6. Institutional and Policy Gaps

Despite multiple schemes (e.g., Kole Paddy Development Programme, mechanisation subsidies), effective uptake is low (38.3% of respondents accessed schemes successfully). Chi-square analysis revealed a highly significant association between farmer awareness and scheme uptake ($\chi^2(1) = 20.80$, $p < 0.001$), indicating that awareness plays a critical role in determining access to institutional support. Key institutional bottlenecks identified in interviews were limited outreach, complex procedures, delayed disbursements and weak field monitoring for compliance (bund construction, desilting timelines). Coordination among the Agriculture Department, Irrigation Department and Local Self-Government Institutions (LSGIs) was inconsistent; farmers in 7 of 12 padasekharams reported conflicting directives on sowing windows and bund timelines.

Institutional literature on irrigation and collective resource management emphasises that generic panaceas fall short; sustainable performance requires locally-fit arrangements, clear accountability, and farmer participation in monitoring and maintenance (Meinzen-Dick, 2007). In Kole, strengthening padasekharam-level institutions (formalizing padasekharam samithis, performance-linked maintenance funds, simple accountability mechanisms) and aligning departmental SOPs to the Puncha calendar would reduce operational delays and improve responsiveness.

3.7. Synthesis: Interacting Drivers and Priority Interventions

The evidence converges on a multi-causal and synergistic explanation for the observed yield decline. Rising Tmax at anthesis substantially increases biological vulnerability (spikelet sterility), but the degree of realized yield loss depends on the capacity of farmers and institutions to implement timely countermeasures — i.e., planting date shifting, precise water control and access to tolerant seed (Peng et al., 2004; Jagadish et al., 2015; Zhang et al., 2023). Agronomic deficits (seed, pests, soil fertility) amplify susceptibility; poor drainage converts episodic rainfall extremes into chronic waterlogging; and institutional/ operational failures prevent timely corrective actions.

Based on these empirical relationships, the study identifies four priority implications for enhancing productivity:

1. Climate-responsive crop management: The observed temperature–yield sensitivity suggests that aligning transplanting schedules to avoid peak anthesis heat and promoting access to regionally adapted, certified varieties may help mitigate yield loss under warming conditions (Jagadish et al., 2015; Zhang et al., 2023).

2. Drainage and operational synchronization: Field diagnostics confirmed that yield gaps were largest in padasekharams with poor drainage and delayed bund closure. Regular desilting, timely sluice operation, and better canal–field coordination may reduce inter-annual variability in yield (Bouman et al., 2007; Meinzen-Dick, 2007).

3. Soil fertility and pest balance: Soil organic carbon levels (<0.5%) and unbalanced fertilizer use were strongly associated with lower yields. Balanced nutrient management with organic amendments, combined with community pest monitoring, may enhance soil resilience (Vanlauwe et al., 2015; Savary et al., 2019).

4. Labour and mechanisation access: Reported labour shortages and low mechanisation access (34%) constrained timely transplanting. Expanding localized custom-hiring models could improve operational timeliness and reduce yield gaps (Regina et al., 2019).

Together, these findings suggest that improving timeliness of operations, drainage maintenance, and balanced input use at the padasekharam level offers the greatest potential to restore productivity in the Kole wetlands. Future interventions should therefore be sequenced to first strengthen water control and synchronization, followed by improvements in seed systems, soil fertility, and mechanisation support, consistent with the study's observed relationships.

4. CONCLUSION

The study analysed multi-year (2019–2024) data to identify the principal factors contributing to yield decline in Kerala's Kole wetlands. Each 1 °C rise in maximum temperature during flowering was associated with a measurable decline in paddy yield.. Padasekharams with efficient drainage, timely bund closure, and synchronized transplanting achieved maximum yields. Agronomic assessments revealed low seed replacement, nutrient imbalance, and frequent pest and disease incidence, supported by soil organic-carbon levels often below 0.5 %. Blocked drains and non-functional sluices were widespread, reducing the capacity for timely water control. Socio-economic observations showed high labour costs, small fragmented holdings and limited mechanisation access, all of which constrained timely operations. Institutional and policy analysis indicated low scheme uptake delayed disbursement of input subsidies, and weak coordination among agriculture, irrigation, and local-governance departments. These governance gaps limited the implementation of drainage maintenance and bunding schedules, thereby influencing field-level productivity. Overall, the findings indicate that yield performance in the Kole wetlands is influenced by multiple interacting factors including climatic stress, management quality, soil fertility, infrastructural condition, and institutional efficiency, with significant variability arising from the interaction of these factors across years.

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Credit authorship contribution statement

V.G. Sunil: Conceptualization, methodology, supervision, and manuscript review.

Asish Benny: Data collection, formal analysis, manuscript drafting, and editing.

V.S. Chinchu: Field investigation and data validation.

M.K. Jaliya: Statistical analysis and interpretation.

Suma Nair: Technical guidance and critical revision of the manuscript.

Amaljith V J: Literature review and data compilation.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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Biodiversity Citizen Science in Meghalaya: Case Study of Potentials, Public Perceptions and Attitudes in the Eastern Himalayas

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***Abstract*:** Biodiversity Citizen Science (BCS) has emerged as an effective participatory approach for biodiversity monitoring and conservation, particularly in regions with limited formal data. This pilot study evaluates the potential of BCS in Meghalaya, a biodiversity-rich state in Northeast India, by examining public perceptions, attitudes, and participation patterns. Using a mixed-methods approach, data were collected through structured questionnaires, semi-structured interviews, and two biodiversity photography contests conducted under varying motivational conditions. A total of 94 participants contributed 1,690 biodiversity records across taxonomic groups and regions. Quantitative analysis using analysis of variance revealed that age ($p = 0.030$), urban ecological orientation ($p = 0.035$), and mental connection with nature ($p = 0.020$) significantly influenced participation, whereas gender and education were not significant predictors. Qualitative findings highlighted emotional affinity, cultural ties, and curiosity as key drivers of engagement. Participation increased notably when incentives were introduced, although elevated engagement persisted beyond the incentive phase. The study demonstrates that BCS can effectively bridge traditional ecological knowledge and scientific documentation, fostering ecological awareness and inclusive conservation. These findings underscore the relevance of citizen science as a scalable tool for participatory biodiversity monitoring and policy-relevant conservation in the Eastern Himalayas.

***Keywords*:** Biodiversity citizen science, Meghalaya, Eastern Himalayas, Biodiversity.

1. INTRODUCTION

Global biodiversity loss has reached catastrophic levels, posing unprecedented challenges to conservation science and environmental governance (McKinley et al., 2017). In response, citizen science (CS), the active participation of non-professional volunteers in scientific research—has emerged as a powerful tool for biodiversity monitoring (Conrad and Hilchey, 2011; Sullivan et al., 2014; Theobald et al., 2015). CS initiatives generate reliable ecological data at scales often unattainable by professional science alone (Chandler et al., 2017; Danielsen et al., 2014; Callaghan et al., 2025) and simultaneously enhance environmental awareness, social capital, and stewardship (McKinley et al., 2015; Pretty and Smith, 2004; Voigt-Heucke et al., 2023).

Biodiversity Citizen Science (BCS) specifically empowers individuals to document species, monitor ecosystems, and engage directly with conservation efforts. With global biodiversity threatened by climate change, habitat loss, and unsustainable development, BCS offers a participatory framework that bridges data gaps while fostering ecological literacy and inclusive conservation (Kelly et al., 2020; Mason et al., 2025). Motivations for involvement often include learning, emotional connection to nature, and a desire to contribute to conservation goals (Tiago et al., 2017; Jansen et al., 2024). Digital platforms and mobile applications have expanded accessibility and retention (Mason et al., 2025), while integration into classrooms demonstrates educational benefits (Kelemen-Finan et al., 2018).

In India, citizen science platforms such as the Indian Bioresource Information Network (IBIN) and the India Biodiversity Portal have expanded biodiversity documentation, particularly in underrepresented regions (Singh et al., 2018; Malhotra, 2018; Barve et al., 2023). In the Northeast, initiatives such as the North-East India Biodiversity Portal have shown how participatory approaches can rapidly enrich datasets (ATREE, 2016)., despite Northeast India's exceptional biodiversity and cultural traditions of ecological stewardship (e.g., sacred groves, clan-managed forests), systematic studies of public perceptions toward BCS remain limited. We assessed the public attitudes toward BCS, identify factors influencing participation, and evaluate the role of intrinsic and extrinsic motivators in sustaining engagement.

2. MATERIALS AND METHODS

2.1. Study Area

Meghalaya, located in Northeast India, spans approximately 22,429 km² and shares borders with Assam to the north and Bangladesh to the south. Characterized by high annual rainfall, the state supports diverse ecosystems ranging from subtropical forests and sacred groves to limestone caves and high-altitude grasslands. More than 75% of its land is under forest cover, harboring rare and endangered species such as the clouded leopard (*Neofelis nebulosa*), hoolock gibbon (*Hoolock hoolock*), and numerous endemic orchids and carnivorous plants.

The population is predominantly composed of the Khasi, Jaintia, and Garo tribes, residing in Khasi, Jaintia and Garo Hills respectively, whose traditional practices emphasize sustainable resource management. Sacred groves exemplify indigenous ecological knowledge and community-based conservation. However, Meghalaya's biodiversity faces mounting threats from deforestation, unregulated coal mining, shifting agriculture, and infrastructure expansion, which contribute to habitat loss and ecosystem fragmentation. These pressures, coupled with shifting cultural values, highlight the urgency of participatory conservation strategies that integrate local knowledge with scientific approaches.

2.2. Methodology

This study employed an exploratory mixed-methods design to examine motivational drivers in a pilot biodiversity citizen science initiative. Quantitative indicators were combined with qualitative insights to capture participation patterns and underlying motivations.

2.3. Sampling Frame

The study included 94 voluntary participants recruited through open calls disseminated via local and online networks. Participation was self-selected, informed consent was obtained prior to data collection, and no exclusion criteria were applied.

2.4. Study Variables and Data Collection

Data were collected in two phases using biodiversity photography contests:

1. a contest conducted without prior disclosure of incentives, and.
2. a contest conducted with prizes and recognition. These phases were designed as motivational probes rather than controlled experiments.

A structured questionnaire captured demographic variables (age, gender, education, rural–urban background) and psychological variables (mental connection with nature, perception of ecological balance, urban ecological orientation), measured using Likert-type scale items. Biodiversity submissions were compiled and categorized taxonomically. Semi-structured interviews were conducted with selected participants to capture qualitative insights into motivations and perceptions. Data collection occurred during and immediately following each contest phase.

2.5. Data Analysis

Quantitative data were analyzed using R software (version 4.3.2). Descriptive statistics and analysis of variance (ANOVA) were applied to assess the influence of demographic and psychological variables on willingness to participate. Qualitative interview data were analyzed using thematic coding.

2.6. Likert-scale instrument and measurement

Attitudinal and perception-based variables were measured using Likert-type scale items following standard social science survey practice (Likert, 1932). Statements were developed based on themes commonly used in citizen science and environmental psychology literature, including perceived importance of biodiversity documentation, ecological balance, urban ecology, climate change concern, and mental connection with nature.

Participants indicated their level of agreement with each statement on a five-point Likert scale ranging from 1 (“Strongly disagree”) to 5 (“Strongly agree”). The instrument was designed for exploratory purposes rather than scale development or psychometric validation. Internal consistency and reliability testing were therefore not emphasized; instead, individual items were treated as independent indicators and analyzed descriptively and inferentially as appropriate for pilot studies.

3. RESULTS AND DISCUSSION

3.1. Participation and Biodiversity Records

A total of 94 respondents participated in the survey, aged 11–47 years (mean age ≈ 24 years), with the majority falling in the 18–30 year age group. The sample was male-dominated (52.64%), with largely urban (46.8%), rural (42.5%), followed by semi urban (10.6%) residents. Education is predominantly undergraduate-level education or higher (73.4%), representing diverse academic backgrounds across science, social science, and professional disciplines. A total of 1,690 biodiversity submissions were received from across Meghalaya. The Khasi Hills recorded the highest number of submissions (n = 675), followed by the Garo Hills (n = 229) and the Jaintia Hills (n = 24). Across taxonomic groups, Insecta dominated submissions (n = 824), followed by Aves (n = 134), with additional records from Arachnida, Reptilia, Mammalia, Gastropoda, Nemertea, and Diplopoda (Fig. 1).

3.2. Demographic and Psychological Predictors of Participation

Analysis of variance identified three significant predictors of participation in biodiversity documentation: age (p = 0.030), urban ecological orientation (p = 0.035), and mental connection with nature (p = 0.020). Gender, education level, prior conservation experience, and political views were not significant predictors of participation (Table 1).

Table 1. Demographic and psychological predictors of participation in biodiversity documentation

Variable	Pr(>F)
Age	0.030
Gender	0.161
Rural–Urban (RuUrb)	0.341
Education	0.361
Prior Experience (Exp)	0.414
Participation History (Parti)	0.314
Ecological Balance (EcoBal)	0.335
Political Views (Poli)	0.597
Urban Ecological Orientation (UrEco)	0.035
Climate Change Concern (Cchange)	0.693
Mental Connection with Nature (Mental)	0.020
Future Orientation (Future)	0.150
Residuals	—

Significant predictors are in bold (p < 0.05)

3.3. Participant Attitudes and Perceptions Towards Biodiversity Documentation

Survey responses revealed strong positive attitudes towards biodiversity documentation. More than 70% of participants agreed that biodiversity documentation is important for maintaining ecological balance, that urban ecology should be incorporated into conservation planning, and that climate change affects mental well-being. Nearly all participants expressed willingness to participate in future biodiversity citizen science initiatives.

Likert-type scale responses were strongly skewed towards agreement and strong agreement across most statements, indicating a generally positive orientation towards biodiversity documentation and conservation-related themes (Figure 2). Neutral and negative responses were comparatively limited, suggesting low resistance or disengagement among participants.

3.4. Engagement Patterns across Project Phases

Engagement levels were lowest during the initial pre-phase, increased during the photography contest conducted without prior disclosure of incentives, and peaked during the incentive-based phase. Although participation declined after incentives were removed, engagement levels remained higher than baseline, indicating persistence of participation beyond purely extrinsic motivation (Figure 3).

3.5. Participation Patterns and Taxonomic Bias

The dominance of insect and bird records reflects widely observed patterns in biodiversity citizen science, where visually conspicuous and easily observable taxa are more frequently documented (Chandler et al., 2017; Sullivan et al., 2014). The uneven geographic distribution of submissions, with higher participation from the Khasi Hills, likely reflects differences in population density, accessibility, and exposure to environmental networks, as noted in other regional citizen science studies (Tiago et al., 2017).

3.6. Psychological and Demographic Drivers of Participation

The significant influence of age on participation aligns with previous findings indicating greater conservation engagement among older individuals (West and Pateman, 2017). The role of urban ecological orientation highlights the increasing relevance of urban environmental awareness in shaping conservation behaviours, particularly in rapidly urbanising landscapes (Voigt-Heucke et al., 2023). The association between mental connection with nature and participation reinforces evidence that emotional and psychological bonds with nature are key motivators in citizen science engagement (Peter et al., 2019; Kelly et al., 2020).

3.7. Attitudes towards Biodiversity Documentation and Conservation

The overwhelmingly positive attitudes observed among participants suggest a strong latent interest in biodiversity documentation and conservation. Similar trends have been reported in citizen science initiatives globally, where participants demonstrate high levels of environmental concern and willingness to engage when accessible platforms are provided (McKinley et al., 2017; Jansen et al., 2024). This indicates that limited participation in biodiversity monitoring may stem more from lack of opportunity than lack of interest.

3.8. Role of Incentives and Sustained Engagement

The observed increase in participation during the incentive-based phase supports evidence that extrinsic motivators can effectively stimulate short-term engagement in citizen science projects (West and Pateman, 2017). Importantly, the persistence of participation beyond the incentive phase suggests the activation of intrinsic motivation, a phenomenon reported in other participatory conservation initiatives (Pretty and Smith, 2004; McKinley et al., 2015). This highlights the potential of short-term incentives to catalyse longer-term ecological engagement.

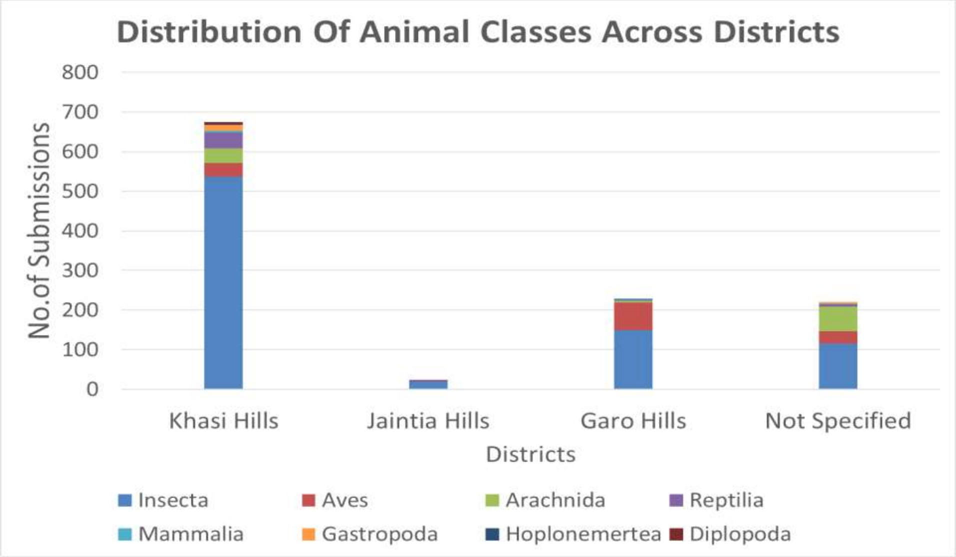

Figure 1. Distribution of animal classes across districts in Meghalaya

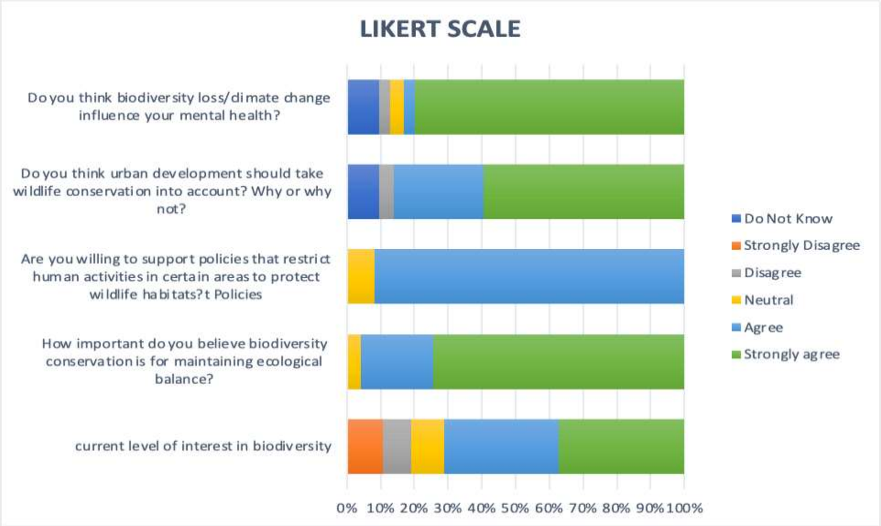

Figure 2. Distribution of participant attitudes and perceptions towards biodiversity documentation measured using Likert-type scale item

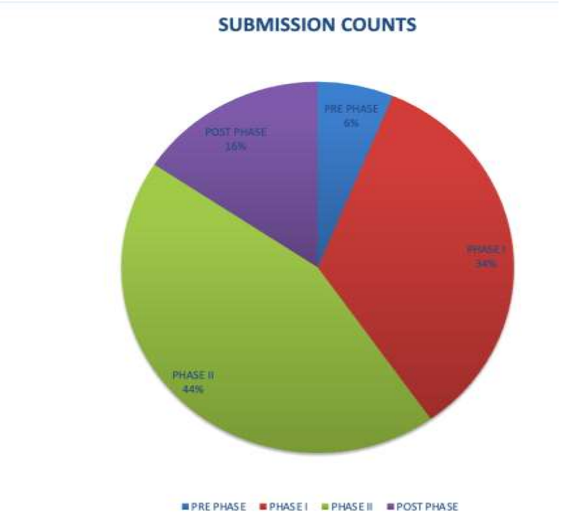

Figure 3. Engagement levels across project phases showing intrinsic and extrinsic motivators

4. CONCLUSION

This exploratory case study demonstrates that Biodiversity Citizen Science is a viable and transformative approach for participatory conservation in Meghalaya. Empirical findings reveal that age, urban ecological orientation, and psychological affinity with nature significantly influence willingness to participate, while factors such as gender and education are less predictive in this context. The emotional and cultural connections to nature may transcend formal education in shaping engagement with biodiversity documentation. The study highlights the capacity of BCS to bridge traditional ecological practices, such as sacred grove conservation with modern scientific documentation. By positioning communities not merely as beneficiaries but as co-creators of ecological knowledge, BCS strengthens stewardship, enriches biodiversity databases, and fosters inclusive governance. Importantly, this is one of the first independent, youth-led citizen science initiatives in Northeast India. It underscores the role of young leaders in re-grounding ecological awareness in a region where human–nature connections are at risk of dilution. Future research should expand longitudinal tracking of citizen engagement, integrate digital innovations, and institutionalize citizen science within local governance structures. By doing so, BCS can evolve into a cornerstone of participatory conservation in biodiversity-rich yet vulnerable landscapes.

Authors' Contributions

JDJ conceptualized the research, analyzed the data, and wrote the manuscript. GPF collected data and led data management and compilation. PR, BK, SS, LM, BL, and VF conducted interviews and collected data. SB co-wrote the manuscript, and NBD co-conceptualized and supervised the entire research, finalized the draft and helped in funding opportunities.

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Conflict of interest

The authors declare no conflict of interest related to the research, authorship, or publication of this article. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Species Drift Without Decline: Avian Responses to Climate and Habitat

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***Abstract*:** Birds are a faunal taxon that is highly sensitive to global climatic changes. Bird migration is one of the major bioindicators of habitat destruction and climate change. This study refers to bird species drift and migratory pattern changes due to habitat destruction and climate variation. The study was conducted at Jigani Lake Anekal Taluk, Bangalore, Karnataka, India (comparative analysis of 45 avian species was performed based on the available e-bird data and personal documentations (2020 to 2024). Out of 175 recorded species, considering the deviations that have occurred from the usual trend followed until 2020. Desertification, a decrease in water-holding capacity, rising temperatures, and altered precipitation patterns have affected migratory timing and vulnerability during long migrations, distribution ranges, egg-laying timing, clutch size, and the mismatch between behavior and the environment, causing a drift in avian species and populations. These changes have not caused a decrease in avian species numbers but have led to considerable species drift. These changes have led to an increased reproductive success in avian species like *Pavo cristatus, Vanellinae sp.* Etc. Conversely, many species have altered their migratory patterns. Many of the water birds, which were the regular migrants to the lake, have become passage migrants, whereas the migratory birds that prefer dry habitats like *Coracias garrulus* (European Roller) are observed . Introduction to invasive plantations, pollution, constructions, and other anthropogenic disturbances has caused degradation to the actual ecosystem. This can be considered as an epitome of climate change and ecosystem changes occurring in Bangalore. The paper emphasises the importance of wetland conservation, the need to restore and maintain biodiversity.

Keywords: Avian species drift, Migratory pattern shifts, Wetland conservation, Anthropogenic disturbance.

1. INTRODUCTION

Bird migration is one of the bioindicators of climate change (George et al., 2024)]. Wetlands (and lakes) are among the most productive ecosystems, serving as life support systems and habitats for rich and diverse biodiversity. Wetland bird diversity greatly depends on the quality, quantity, and local water cycle of the lake (Pant et al., 2024)]. The bird species diversity and population index reflect the relationship with seasons (Rai and Sharma, 2024). Reports suggest that large green patches always host larger bird diversity than small patches (Zhu et al., 2024). Urbanisation has led to loss of wetlands, floods, a decline in the groundwater table, urban heat island effects, and elevated carbon footprints (Ramachandra et al., 2015). Approximately 389 avian species have been found in urban Bangalore (https://ebird.org/region/IN-KA-BN/bird-list). Among these, around 50% of birds are migratory. Winter migration, which ranges from early October to March, is more prevalent in Bangalore compared to the summer migration. This lake hosts 190 species of birds (https://ebird.org/hotspot/L5174545/bird-list?rank=lrec), including key species such as *Buteo buteo* (Buzzards), *Scolopacidae sp.* (Sandpipers), *Alaudidae sp.* (Larks), and *Passeri sp.* (Warblers). Many avian species exhibit migration, the seasonal habitual movement, and these birds are sensitive to climate change and specific habitats. They can be considered as bioindicators of environmental change (Arya et al., 2019).

The city has an approximate of 1,350 lakes spread across Bengaluru (Kulranjan and Palur, 2022), with around 850 active lakes in Bangalore Urban County, out of which some of the lakes are hotspots of avian biodiversity in the Bangalore urban county. The 98% of the lakes are encroached, and 90% of the lakes are sewage-fed (Ramachandra et al., 2015). 167 lakes have been protected by the BBMP (Government of Karnataka), which has caused improvements as well as destruction for the well-being and biodiversity of the lakes. The current storage capacity of Bengaluru's lakes is about 5 TMC (5,000,000,000 cubic feet), but due to siltation and other polluting factors, the actual storage capacity is only about 1.2 TMC (thousand million cubic feet). Lake-filling as part of industrialization has led to great destruction of the lakes and the biodiversity around them. These lakes host more than 1000 species of biodiversity, which greatly depend on these lake ecosystems. These include numerous species of fish, amphibians, birds, insects, phytoplankton, and zooplankton. Lakes play a critical role in controlling soil erosion, floods, microclimate variations, and biogeological cycles (Naeem, 2024). E-bird is a platform hosted by the Cornell Lab that helps to collect and compile bird data. The study is based on the eBird data uploaded by 90 birdwatchers over the years 2020-2024. Every week for the past 2 years, the field work was done to record the biodiversity and to study the destruction and changes in and around the lake. The changes in habitat and climate conditions have caused a great change in migratory patterns of birds, and there is a hike in the number of bird species and population that prefer dry habitats in and around the lake (Scridel et al., 2024) indicating decline in water levels of the lake.

This study focuses on the anthropogenic destruction that has caused a change in the overall drift in the bird diversity and population without a decline in the total species count in Jigani Lake, Anekal Taluk, which is situated in the South of Urban Bengaluru (about 5 km from Bannerghatta National Park (air distance).

2. MATERIALS AND METHODS

2.1. Study Area

Bangalore - located at 12°59′ north latitude and 77°57′ east longitude, is nearly equidistant from both the eastern and western coasts of the South Indian peninsula. Situated at an altitude of 920 m above the mean sea level [Nölke (2021)]. The city experiences a tropical climate, with the monsoon extending from June to October and the dry season from November to April. In the first half of the monsoon (June-July), the rains are not abundant. The September and October bring peak rainfall, with monthly precipitation reaching up to 194 mm (Rainwater Harvesting. (n.d.). *Rainwater harvesting in India.* Retrieved November 03, 2025, from http://www.rainwaterharvesting.org). From mid-October, the north-east winds begin to result in reduced rainfall (Climates to Travel. (n.d.). *Bangalore climate: Weather by month, temperature and rainfall.* Retrieved November 06, 2025, from https://www.climatestotravel.com). In winter, from November to February, nights are cool, other for occasional falls in temperature to 10/12 °C (50/54 °F). In terms of precipitation, winter is the driest season. (Seasons Year. (n.d.). *Seasons in Bangalore: Weather and climate.* Retrieved November 12, 2025, from https://seasonsyear.com).

Jigani Kere (Jigani Lake) is a water body covering an area of 2 hectares in the urban county of Bangalore city. The lake is at the coordinates - 12.7944° N, 77.6346° E, Anekal taluk, Bangalore, Karnataka, India. The lake can be considered a wetland as it has a large area with shallow water-filled grassland. During the summer, these areas dry up, whereas the major water body of the lake retains the water. The lake is surrounded by agricultural sites, plantations, mango county, plant nurseries, residential areas, temples, industrial areas, automobile workshops, and granite industries. Agricultural sites include paddy, groundnut, mustard, and vegetables. Plantations include eucalyptus (*Eucalyptus globulus),* mango (*Mangifera indica*), and coconut palms (*Cocos nucifera*). The industrial area hosts many large-scale and small-scale industries, including Cipla Ltd. (https://www.cipla.com), Aragen Life Science (https://www.aragen.com), Yazaki India Pvt. Ltd. (https://www.yazaki-group.com), and SAAB Engineering Pvt. Ltd. (https://saabengg.com). The East side of the lake has a rubberised road. Bannerghatta Anekal road runs along the lake, whereas other parts of the lake are encircled with a walking track. The lake also has many water holes around the main water body, which become a part of the lake when the lake overflows. The main water body has an island in the middle housing nesting perches for cormorants, raptors, and other water birds. The lake is facilitated with a canal, which gets active when the main water body overflows.

2.2. Bird Sampling and Data Collection

Field visits were done for a minimum period of 3 hours at least once a month in person for the past 4 years, i.e., 2020, 2021, 2022, 2023, and the bird data were updated in eBird. The available data is divided into Spring Migration (March - May), Breeding Season (June - July), Fall Migration (August - November), and Winter migration (December - February). E-bird is an online platform that collects and compiles the bird data uploaded and updated by the bird watchers and ornithologists who visit the field (https://ebird.org/home). This platform was launched and is maintained by the Cornell Lab of Ornithology. Jigani Lake is recognised as a hot spot by eBird (https://ebird.org/hotspot/L5174545). The observations were made with Binoculars and photographed with cameras. 190 species of birds have been recorded by 90 birders from this location, out of which 120 species of birds are recorded in person (https://ebird.org/lifelist/L5174545). 45 species among the above-mentioned birds are selected depending on their impact on the study. These 45 species of birds are selected based on the changes in migratory patterns or because of the emergence of a new species in the location. Other geographical details and observations were recorded during the visit to the work field. E-bird events like the backyard bird count and big day counts were also conducted to record the species.

3. RESULTS AND DISCUSSION

Birds spotted for the 1st time during the observation periods includes avian species Cotton pygmy goose, Garganey, Northern pin tail, Baillon's crake (Figure 1.3), Osprey, Indian spotted eagle, Common buzzard (Figure 1.2), Large cuckoo shrike, Spot-breasted fantail (Figure 1.4), Thick-billed warbler, Paddy field warbler, Palla's grasshopper warbler, Lesser white throat, Asian brown flycatcher, Siberian stonechat, Oriental skylark and European roller (Figure 1.1). These 17 species of birds showed difference in population and migratory pattern in the years observed.

Many open ground-nesting birds lay and bury their eggs in sand or mud, which gives a long dry gestation period and provides camouflage to protect them from predators [Vishwa Jagati et al. (2023)]. Jigini lake is now occupied by *dry ground birds* as a breeding ground since it has dried up. The lake provides sand and dried grass, making it a suitable breeding ground (Figure 2).

Birds which prefer dry habitats, increased in population when compared to other places, indicating the rapid drying up of the Jigani lake. The above-mentioned reasons have also attracted Oriental skylark during the migratory track. These birds have started migrating here because the drying of the lake and flora has made it a suitable habitat (Dinda et al., 2020; Scridel et al., 2024; Barik et al., 2021; Nath et al., 2022).

3.1. Paddy-Field PIPIT

Raptors like bird Short-toed Snake Eagle (Cauli et al., 2022) and bird Common buzzard (Sidorovich et al., 2016), which prefer dry grasslands with a good population of rodents and herps for their diet, were spotted for the first time during the study period. This shows that the lake is drying up. The decrease in water level helps the raptors catch fish and has caused an increase in the prey population. Insectivorous birds like bird Spot-breasted fantail (Sharma et al., 2024) and Bay-backed shrike, prefer shrub patches along small water bodies or streams, find great diversity and population of arthropods suitable for their diet (Figure 3). Invasive plants like *Bougainvillea spectabilis* (Bougainvillea)and Lantana (*Lantana camara*) have made a suitable habitat for them. Bird Thick-billed warbler, Paddy field warbler, Palla's grasshopper warbler, Lesser white throat, Yellow-eyed babbler, Siberian stonechat (Figure. 3.2), *Muscicapa latirostris (*Asian brown flycatcher), and Taiga flycatcher prefer dried flora as their habitat. The dried grass, shrubs, herbs, and trees support their habitat. All these birds, except bird Yellow-eyed babbler, are migrants to the lake. These birds were spotted for the first time during the study period. The eucalyptus plantations have made their habitat prosperous.

Larger waders like bird Asian openbill (Greeshma and Jayson 2018) and Yellow bittern have increased in population. Bird 79 has increased in population because of the excess availability of food materials like molluscs and fish. As the average water levels have come down in the lake

Figure 1. New bird species that have visited the place during the study period; 1.1: European Roller, Figure 1.2: Common Buzzard and 1.3 : Ballion Crake and Figure 1.4: Spot-breasted Fantail

Figure 2. Birds that have increased population due to availability of better breeding grounds; **2.1**(R): Red wattled Lapwings, 2.1 (L): Yellow Wattled Lapwings; **2.2:** Jerdon's Bush Lark and **2.3** : Zitting Cisticola and Figure

through the years, these waders are able to feed more easily with their specialized bills. Other waders like bird Yellow bittern have increased their populations due to shallow water grounds to build their nest in the grass. Warblers like bird, Booted warbler, *Acrocephalus dumetorum* (Blyth's reed warbler), and Clamorous reed warbler have become more common during their migratory seasons. These birds have started spending more time in the lake compared to previous years. This could be due to the exposure of grass and herbs of the lake above the water levels (Varga et al., 2023). These birds are usually found on short, dense patches of herbs above the water. During the observations, was found that the grass and shrubs on the mainland of the lakes started to get exposed.

Birds like *Coracina macei* (Large Cuckoo shrikes), Common Cuckoo [Puswal et al. (2024)], Chestnut-headed bee-eater and Ashy drongo have become winter visitors to the lakes, as there is an increase in deciduous habitat, and bird species are more common in places with more deciduous and arid habitats. Bird 66 is a passage winter migrant to Bangalore's rural areas. However, the bird was spotted for the first time in the lake in 2024, which is on the urban side of Bangalore (Figure 1.1). The bird was seen in the fields and barren lands around the lake. According to the studies, bird 66 prefer warmer and arid habitats (Kiss et al., 2020).

Smaller waders like *Tringa stagnatilis* (Marsh sandpiper), Wood sandpiper were observed to change their migratory patterns (Rischette et al., 2024). Baillon's crake, a migratory bird seen in the same habitats, was spotted for the 1st time in the lake during the observation period. These changes in migratory patterns may be due to the filling of the lake. It is noted that the depth of the lake has decreased largely because of the waste dumping and silt sedimentation. These shallow waters are preferred by these small waders (Figure 4). Late winters have also caused these birds' migratory patterns.

The study uses the bird data as a scale for climate change (Copernicus Climate Change Service. 2021). *Bird migration and climate indicators*. ECMWF. Retrieved November 14, 2025, from https://climate.copernicus.eu). Industrialization has caused great destruction to the lake, which has affected the birds. Plantation of invasive species like eucalyptus has caused water levels to go down (Kabir et al., 2023). Dumping of construction waste or solid waste has caused silting and filling of lakes. Pollution and dumping of waste and chemicals have caused the water quality to decline.

3.3. NDVI (Normalized Difference Vegetation Index)

NDVI (Normalized Difference Vegetation Index) is an indicator of vegetative health, controlled by satellites, where darker green areas indicate healthy, dense vegetation, and white or pale areas represent less or no vegetation, such as bare soil, dry grassland, or buildings (Janani et al., 2017). NDVI maps are generated using the Google Earth Engine tool (https://earthengine.google.com/platform/) for the spring migration season (March–May) across five years (2020–2024) to understand the habitat transformation around Jigani Lake and its influence on migratory bird patterns (Zeng et al., 2022).

Moderate vegetation coverage was observed around Jigani Lake, indicating balanced wetland-grassland ecosystems that supported both wetland birds and seasonal migrants (Figure 1) (NASA Earth Observatory. (n.d.). *Measuring vegetation (NDVI & EVI)*. Retrieved November 20, 2025, from https://earthobservatory.nasa.gov /features/MeasuringVegetation].

Figure 3. Birds that have prospered due to the emergence of shrub habitat- **3.1:** Indian White -eye and **3.2:** Siberian Stone-chat

Figure 4. Birds that are reported throughout the year which were once visitors- Black Winged Stilt

Table 1. Presence of various birds in different seasons during 2020 to 2024

Common name	Scientific name	Spring	Breeding	Fall	Winter	Iucn status	Residential status
Cotton pygmy goose	*Nettapus coromandelianus*	-	-	-	+	LC	Resident
Garganey	*Spatula querquedula*	+	-	-	+	LC	Winter migrant
Northern pintail	*Anas acuta*	-	-	-	+	LC	Winter migrant
Eurasian collared dove	*Streptopelia decaocto*	+	-	-	-	LC	Resident & winter migrant
Laughing dove	*Spilopelia senegalensis*	+	+	+	+	LC	Resident
Baillon's crake	*Zapornia pusilla*	-	-	-	+	LC	Winter migrant
Watercock	*Gallicrex cinerea*	-	-	+	-	LC	Resident & Within-India Migrant
Rudy breasted crake	*Zapornia fusca*	+	+	+	2020-21 -	LC	Resident
		+	-	-	2023-24 +		
Striated heron	*Butorides striata*	+	-	+	+	LC	Resident
Spot-billed pelican	*Pelecanus philippensis*	-	-	+	2020-21 -	NT	Resident
		+	+	+	2023-24 +		
Yellow bittern	*Botaurus sinensis*	+	+	+	2020-21 -	LC	Resident & Local Migrant
		+	-	-	2023-24 +		
Eurasian spoonbill	*Platalea leucorodia*	-	-	-	+	LC	Resident & Local Migrant
Black crowned night heron	*Nycticorax nycticorax*	-	+	+	2020-21 +	LC	Resident
		+	+	+	2023-24 +		
Osprey	*Pandion haliaetus*	-	-	-	+	LC	Winter migrant
Indian spotted eagle	*Clanga hastata*	-	-	-	+	VU	Resident & Within-India Migrant
Common buzzard	*Buteo buteo*	+	-	-	+	LC	Winter migrant
Short-toed Snake Eagle	*Circaetus gallicus*	-	-	-	+	LC	Resident
Booted eagle	*Hieraaetus pennatus*	-	-	+	2020-21 +	LC	Winter migrant
		-	+	+	2023-24 +		
Oriental honey buzzard	*Pernis ptilorhynchus*	-	+	+	2020-21 -	LC	Resident & Winter Migrant
		+	+	+	2023-24 +		
Crested serpent eagle	*Spilornis cheela*	-	-	+	-	LC	Resident
Greater spotted eagle	*Clanga clanga*	-	-	+	2020-21 +	VU	Winter migrant
		+	-	+	2023-24 +		
Indian pitta	*Pitta brachyura*	-	-	-	+	LC	Within-India Migrant

Cont...

Table 1. Presence of various birds in different seasons during 2020 to 2024

Common name	Scientific name	Spring	Breeding	Fall	Winter	Iucn status	Residential status
Large cuckoo shrike	Coracina macei	+	-	-	+	LC	Resident
Spot-breasted fantail	*Rhipidura albogularis*	+	-	-	+	LC	Resident
Bay-backed shrike	*Lanius vittatus*	+	-	+	+	LC	Resident & Local Migrant
Thick-billed warbler	*Arundinax aedon*	+	-	-	-	LC	Winter migrant
Paddyfield warbler	*Acrocephalus agricola*	-	-	-	+	LC	Winter migrant
Palla's grasshopper warbler	*Helopsaltes certhiola*	-	-	-	+	LC	Winter migrant
Streak-throated swallow	*Petrochelidon fluvicola*	+	-	-	-	LC	Local Migrant
Lesser white throat	*Curruca curruca*	+	-	-	+	LC	Winter Migrant & Localized Summer Migrant
Yellow-eyed babbler	*Chrysomma sinense*	+	-	-	-	LC	Resident
Asian brown flycatcher	*Muscicapa dauurica*	+	-	-	+	LC	Within-India Migrant & Winter Migrant
Tickell's blue flycatcher	*Cyornis tickelliae*	+	-	-	+	LC	Resident
Taiga flycatcher	*Ficedula albicilla*	+	-	-	+	LC	Winter migrant
Siberian stonechat	*Saxicola maurus*	-	-	+	-	NR	Winter Migrant & Localized Summer Migrant
Streaked weaver	*Ploceus manyar*	+	-	-	-	LC	Resident
Black-headed cuckooshrike	*Lalage melanoptera*	-	-	+	2020-21	LC	Within-India Migrant
		+	-	-	2023-24 +		
Ashy drongo	*Dicrurus leucophaeus*	-	-	-	2020-21 +	LC	Within-India Migrant
		-	+	+	2023-24 +		
Oriental skylark	*Alauda gulgula*	-	+	-	2020-21	LC	Resident & Local Migrant
		+	-	-	2023-24 +		
Booted warbler	*Iduna caligata*	+	-	-	2020-21	LC	Winter migrant
		+	-	+	2023-24 +		
Clamorous reed warbler	*Acrocephalus stentoreus*	+	-	-	2020-21 +	LC	Resident & Winter Migrant
		+	+	+	2023-24 +		
Rosy starling	*Pastor roseus*	-	-	+	2020-21	LC	Winter migrant
		+	-		2023-24 +		
Chestnut-tailed starling	*Sturnia malabarica*	-	-	-	2020-21 +	LC	Resident & Within-India Migrant
		+	-	+	2023-24 +		
Indian cuckooshrike	*Coracina macei*	+	-	-	+	LC	Resident & Local Migrant

Cont...

Table 1. Presence of various birds in different seasons during 2020 to 2024

Common name	Scientific name	Spring	Breeding	Fall	Winter	Iucn status	Residential status
Black-headed oriole	*Oriolus larvatus*	-	-	-	+	LC	Resident & Local Migrant
White-bellied drongo	*Dicrurus caerulescens*	+	+	-	-	LC	Resident
Indian paradise-Flycatcher	*Terpsiphone paradisi*	-	-	+	2020-21 -	LC	Resident & Within-India Migrant
		+	-	-	2023-24 +		
Rufous treepie	*Dendrocitta vagabunda*	+	-	+	+	LC	Resident
Ashy-crowned sparrow-Lark	*Eremopterix griseus*	+	-	-	-	LC	Resident
Jungle prinia	*Prinia sylvatica*	+	-	+	-	LC	Resident
Sykes's warbler	*Iduna rama*	-	-	-	+	LC	Winter migrant
Green warbler	*Phylloscopus nitidus*	-	-	+	+	LC	Summer Migrant & Localized Winter Migrant
Greenish warbler	*Phylloscopus trochiloides*	+	-	+	+	LC	Winter Migrant & Localized Summer Migrant
Tawny-bellied babbler	*Dumetia hyperythra*	-	-	+	+	LC	Resident
Puff-throated babbler	*Pellorneum ruficeps*	+	-	-	+	LC	Resident
Loten's sunbird	*Cinnyris lotenius*	+	-	+	+	LC	Resident
Jerdon's leafbird	*Chloropsis jerdoni*	-	-	+	2020-21 -	LC	Resident
		-	-	-	2023-24 +		
Western yellow wagtail	*Motacilla flava*	-	-	+	+	LC	Winter migrant
Brown shrike	*Lanius cristatus*	+	-	+	2020-21 +	LC	Winter migrant
		-	-	+	2023-24 +		
Common Cuckoo	*Cuculus canorus*	-	-	+	2020-21 +	LC	Summer Migrant & Passage Migrant
		-	-	+	2023-24 -		
Grey-bellied cuckoo	*Cacomantis passerinus*	+	+	+	2020-21 +	LC	Resident & Local Migrant
		+	-	-	23-24 +		
Pied cuckoo	*Clamator jacobinus*	+	+	+	2020-21 +	LC	Resident & Summer Migrant
		+	-	+	2023-24 +		
Common hawk-Cuckoo	*Hierococcyx varius*	-	+	+	2020-21 +	LC	Resident & Local Migrant
		+	+	+	2023-24 +		
Indian scops-owl	*Otus bakkamoena*	-	-	+	2020-21 -	LC	Resident
Mottled wood owl	*Strix ocellata*	-	-	+	+	LC	Resident

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Table 1. Presence of various birds in different seasons during 2020 to 2024

Common name	Scientific name	Spring	Breeding	Fall	Winter	Iucn status	Residential status
European roller	*Coracias garrulus*	-	-	+	-	LC	Passage Migrant & Localised Summer Migrant
Chestnut-headed bee-eater	*Merops leschenaulti*	-	-	-	+	LC	Resident & Within-India Migrant
Green sandpiper	*Tringa ochropus*	- +	- -	- -	2020-21 + 2023-24 +	LC	Winter migrant
Wood sandpiper	*Tringa glareola*	- +	+ -	+ +	2020-21 - 2023-24 +	LC	Winter migrant
Yellow wattled lapwing	*Vanellus malabaricus*	-	-	-	+	LC	Resident
Black-winged slit	*Himantopus himantopus*	- +	- +	+ -	2020-21 - 2023-24 +	LC	Winter migrant
Little ringed plover	*Charadrius dubius*	-	-	-	+	LC	Resident & Winter Migrant
Common snipe	*Gallinago gallinago*	+	-	-	+	LC	Winter migrant
Common sandpiper	*Actitis hypoleucos*	+	-	+	+	LC	Winter migrant
Whiskered tern	*Chlidonias hybrida*	+ +	- -	+ +	2020-21 - 2023-24 +	LC	Winter Migrant & Localized Summer Migrant
River tern	*Sterna aurantia*	- +	- -	+ +	2020-21 + 2023-24 +	VU	Resident & Local Migrant
Greater painted snipe	*Rostratula benghalensis*	+ +	+ -	+ +	2020-21 - 2023-24 +	LC	Resident
Pheasant-tailed jacana	*Hydrophasianus chirurgus*	- +	+ -	+ +	2020-21 - 2023-24 +	LC	Resident
Asian openbill	*Anastomus oscitans*	- +	- -	+ +	2020-21 + 2023-24 +	LC	Resident
Painted stork	*Mycteria leucocephala*	- +	+ +	+ +	2020-21 - 2023-24 +	LC	Resident
Eurassian hoopoe	*Upupa epops*	- +	- -	+ -	2020-21 - 2023-24 +	LC	Resident

Cont...

Table 1. Presence of various birds in different seasons during 2020 to 2024

Common name	Scientific name	Spring	Breeding	Fall	Winter	Iucn status	Residential status
Indian peafowl	*Pavo cristatus*	-	-	+	2020-21 -	LC	Resident
		+	+	+	2023-24 +		
Grey junglefowl	*Gallus sonneratii*	-	-	+	+	LC	Resident
Jungle bush quail	*Perdicula asiatica*	+	-	+		LC	Resident
Indian nightjar	*Caprimulgus asiaticus*	-	-	-	+	LC	Resident
Little swift	*Apus affinis*	+	+	+	2020-21 -	LC	Resident
		+	+	+	2023-24 +		
Asian palm swift	*Cypsiurus balasiensis*	-	-	+	2020-21	LC	Resident
		+	-	+	2023-24 +		
Little grebe	*Tachybaptus ruficollis*	-	+	+	2020-21 +	LC	Resident
		+	+	+	2023-24 +		
White-naped woodpecker	*Chrysocolaptes festivus*	+	-	+	-	LC	Resident
Plum-headed parakeet	*Psittacula cyanocephala*	-	-	+	+	LC	Resident

Breeding Code - C- Carnivores, O-Omnivore, G-Granivore, N-Nectarivore, F Frugivore .[The SoIB Partnership. (2023). State of India's birds. Retrieved November 17, 2025, from https://stateofindiasbirds.in/. The table consists IUCN- International Union for Conservation of Nature (https://iucn.org/) status birds were classified as The IUCN Red listing is done based on the database collected and analysed by the SIS committee (https://www.iucnredlist.org/assessment/process). The 43 birds come under the classification of LC (Least concern) except for the Indian Spotted Eagle, which is VU(Vulnerable), and the Spot-billed Pelican, which is NT (near threatened) (https://www.iucnredlist.org/) The table also consists of the residential status in which some birds are R(residential) and WM (winter migrant). This classification is done based on the presence of the individual in the study area in the chosen period. This data is mentioned based on the observation and analysis of the data available in e-bird (https://ebird.org/barchart?r=L5174545). The bird is considered resident if the bird is present throughout the year. Winter migrants are the birds that visit the study area during their migratory tracts or as migrants during the winter season.

NDVI values slightly increased in central zones, due to favorable monsoon conditions in the previous year during Spring 2021 (Fig 5.2) This corresponds with sightings of migratory ducks such as *Spatula querquedula*(Garganey) and *Anas acuta (*Northern Pintail) as regular visitors during the spring (Lu et al., 2025). There was patchy reduction in vegetation, especially at the lake margins spring 2022, indicating the start of the drying phase (Figure 5.3). This aligned with the spotting of (yellow-wattled lapwing) *Vanellus malabaricus* and (Oriental Skylark) *Alauda gulgula* as new nesting species in the area (Saikia et al. 2025). The vegetation reduced further, especially in shallow and peripheral areas of the lake. This shift in habitat favored birds adapted to dry and semi-open landscapes, such as (Indian Pitta) *Pitta brachyura*, (Bay-backed Shrike) *Lanius vittatus*, and various flycatchers and warblers (Figure 5.4) (Ramachandra and Aithal 2016). NDVI maps showed extensive vegetation in the middle of the lake, while peripheral areas show less vegetation during spring 2024 (Figure 5.5). This indicates the growth of invasive plant species like Lantana camara and Eucalyptus spp., which support insectivorous and shrub-eating birds (Figure 5) (Liu et al. 2024) Change detection NDVI map (Figure 2) shows the difference in vegetation over five years. Red zones represent loss in vegetation, possibly due to drying, deforestation, and construction of buildings (Figure 5.6).

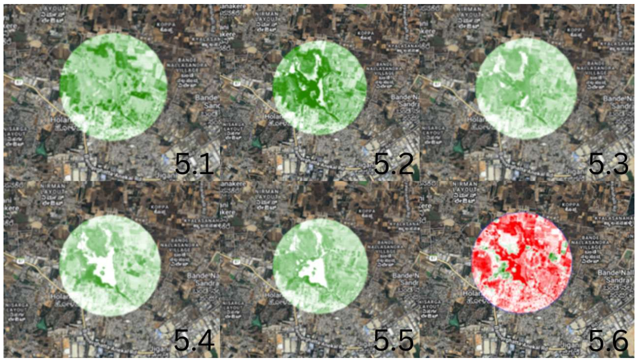

Figure 5.1. NDVI Map of Jigani Lake - Spring 2020

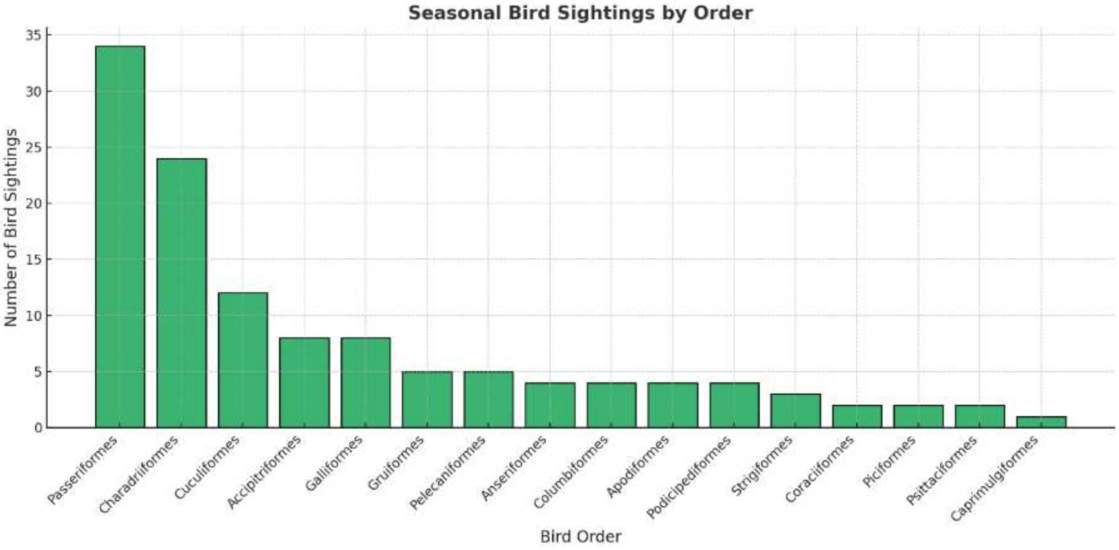

Figure 6. Number of birds in each family according to taxonomy

Green zones indicate an increase in vegetation, due to the growth of seasonal crops and invasive plant species and white zones indicate little or no change, due to permanently dry areas. This satellite evidence confirms the findings of the study, i.e, climate change and drying up of the lake, indicated by a change in the migratory pattern of bird species around Jigani Lake.

The Order Passeriformes has the highest number of avian species, (22), followed by the order Accipitriformes (5 species). Anseriformes and Charadriiformes, (3 bird species) (Figure 6). The study indicate that number of Charadriiformes (the shore birds), which are supposed to be high in numbers in a lake ecosystem, is being overtaken by the Passeriformes (the perching birds). This drift in population can be considered an indication of the habitat drift happening in the ecosystem.

The study has shown that habitat destruction and climatic changes have affected the migratory patterns and relative population density of several affected species. The study results demonstrate that the bird data can be used for climate monitoring and observations and as a measure of habitat drift (Buriro and Lund, 2025). This supports the fact that birds are effective climate indicators (Liang, 2021). Rise in temperature, shift in monsoon patterns, and habitat alterations have led to a shift in migration timing, species distribution, and breeding behaviour (Lal et al., 2025). The reduction in water level and the expansion in dry habitat have resulted in an increase in birds preferring dry habitat and a decrease in water birds (Deomurari et al., 2023). The observations of population and species drift of birds indicate the factors of depletion in the ecosystem and the affected habitat. Unregulated industrialization, pollution, and the introduction of invasive species have degraded the ecosystem, changing the natural balance of the wetland habitat (Buriro and Lund, 2025). Non-scientific and unethical developmental operations like concreting of lake banks, road construction around the lakes, waste dispersal and treatment plants around the lakes, non-scientific weeding in lakes, and introduction of Alien Invasive Species (fishes and plants) for commercial and aesthetic reasons by the government have also contributed a large percentage to the destruction of the wetland ecosystem (Prasanya et al., 2024). From the study area, it is also noted that the dumping of solid wastes, including construction wastes, cut wastes from granite industries, and plastic wastes, has played a major role in the depletion of the water holding capacity of the lake. (Buriro and Lund, 2025);

4. CONCLUSION

The study reveals that habitat destruction and climate change have caused changes in the bird population and visiting terns in the lake. The diversity of the bird species has shifted from wetland species to dry land species. Due to the shift, there is only a negligible decline in the total avian species considering the study duration. The observation shows that there is a slight positive deviation in the total number of species. The paper emphasizes the importance of wetland conservation, the need to restore and maintain biodiversity. Measures such as controlling pollution and restoring native vegetation can help lessen the harmful effects of habitat shift and climate change on the bird populations. Bird migration patterns are bio-indicators of environmental changes. Protecting Jigani Lake and similar habitats is crucial for preserving biodiversity and moderating the long-term effects of climate change on migratory bird species.

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Authors' Contributions

Data collection, data analysis and content design: Abhijith S. Scientific writing: Steven S. Guidance and content design: Jincy George.

Disclosure statement

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

Data availability statement

Data is available and can be made available on demand.

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Context-Dependent Effects of Monsoon Seasonality on Riverine Fish Assemblages: Case Study From Bharathapuzha River, India

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Abstract: In tropical monsoon rivers, understanding how powerful seasonal hydrology interacts with localized anthropogenic degradation to shape fish assemblages is a central ecological and management challenge. This study investigated fish community dynamics in the Bharathapuzha River, India, using a comprehensive spatio-temporal dataset from 108 sampling events across nine sites and three seasons. Environmental heterogeneity was characterized through hierarchical cluster analysis and principal component analysis, revealing distinct spatial groupings and dominant axes of variation, including flow dynamics (explaining 23.9% of environmental variation) and habitat integrity (explaining 22.8% of environmental variation). Fish community composition showed significant differences based on study site (explaining 33.7% of community variance) and season (explaining 16.4% of community variance), with a crucial and significant interaction between study site and season (explaining 17.6% of community variance). This interaction demonstrated that the monsoon's influence on fish assemblages in the Bharathapuzha River is profoundly context-dependent, varying significantly among sites. Redundancy analysis, explaining 11.6% of adjusted community variance, further identified physical habitat structure as a greater unique driver (5.7%) compared to water quality (3.5%). These findings establish that fish assemblages in the Bharathapuzha River are primarily structured by the intricate interplay between its natural flow regime and site-specific habitat conditions, providing a nuanced understanding essential for localized conservation.

Keywords: River ecology, Fish community, Variance Partitioning, Habitat heterogeneity, Bharathapuzha

1. INTRODUCTION

Riverine ecosystems are globally significant hotspots of biodiversity, yet they are increasingly threatened by a suite of anthropogenic stressors, including flow alteration, habitat degradation, and pollution (Vörösmarty et al., 2010; Reid et al., 2019; Tickner et al., 2020). In tropical regions, which support a disproportionately high share of global freshwater biodiversity, these pressures are often intensified by rapid development and land-use change, leading to profound ecological degradation (Arthington et al., 2018; Albert et al., 2021). This degradation is typically mediated through two primary pathways: the deterioration of water quality and the physical alteration of habitat structure, both acting as critical environmental filters on biological communities (Schippert et al., 2022). A central and ongoing debate in river ecology is to determine the relative influence of these filters in structuring biological communities. While the physical structure of a river—its channel morphology, substrate, and flow heterogeneity—is often considered the primary framework selecting for species with specific traits (Power et al., 2015), severe pollution can shift this dynamic, making physiological stressors such as hypoxia or chemical toxicity dominant filters shaping community assembly (Palmer et al., 2015; Landman et al., 2022).

This dynamic is further complicated in monsoon-driven rivers, where an annual cycle of extreme floods and droughts imposes a powerful, system-wide disturbance (Datry et al., 2016). This strong seasonality poses a fundamental ecological question: does the monsoon's influence homogenize biological communities across a riverscape, effectively masking the effects of localized degradation? or, conversely, does it interact with local conditions to amplify differences between sites? Resolving this is essential for predicting ecosystem resilience and for developing robust management strategies in these naturally variable systems (Poff, 2018; Tonkin et al., 2018).

The River Bharathapuzha in Kerala, India, serves as a pertinent case study for these complex challenges. As a vital water resource, it is heavily impacted by a mosaic of pressures, including flow regulation, sand mining, and pollution from urban and agricultural sources (Gopi and Sreepriya, 2018; Remya and Joseph, 2021). Therefore, the overarching objective of this study was to disentangle the complex interplay between powerful seasonal hydrology and localized anthropogenic degradation in shaping fish assemblages within this tropical monsoon river, providing nuanced insights essential for developing effective, localized conservation strategies.

2. MATERIALS AND METHODS

2.1. Study Area and Sampling Design

The study was conducted along the River Bharathapuzha, a 209 km long, monsoon-fed river in Kerala, India (Figure 1). A purposive stratified sampling procedure was used to select nine distinct study sites along the river's longitudinal gradient. This strategic selection aimed to represent the diverse spectrum of geomorphological settings—from high-energy mid-reaches to the low-energy estuarine mouth at Chamravattom—and varying degrees of anthropogenic influences, including agricultural runoff, urban waste, and ritualistic activities (Table 1). Sampling was conducted across two full hydrological years (2023 and 2024), with each site visited during three seasons: Pre-monsoon (February-May), Monsoon (June-September), and Post-monsoon (October-January). This design yielded a total of 108 independent sampling events (9 sites × 3 seasons × 2 years).

2.2. Environmental Data Collection

At each sampling site and event, key physicochemical water quality parameters were quantified, including dissolved oxygen (DO), pH, total suspended solids (TSS), and nitrate concentrations. Physical habitat was characterized using a semi-quantitative protocol (Barbour et al., 1999) where 20 parameters related to channel morphology, flow regime, riparian condition, and human activities were scored on an ordinal scale (0-5) along a representative 100-200m reach. For multivariate analysis, these 20 parameters were aggregated a priori into six functional strata reflecting distinct pressure types (direct pollution/waste, ritualistic activities, channel morphology). This approach was chosen to create predictor variables with direct relevance to specific management actions.

2.3. Fish Community Data Collection

Fish were sampled concurrently with environmental data collection along a standardized 100m reach using a multi-gear approach. The effort included active sampling with a cast net (4.0 m diameter, 10 mm mesh) and passive sampling with a fleet of three gill nets of varying mesh sizes (15, 30, and 50 mm). All captured fish were identified to the species level, counted, and released. For community analysis, raw species abundance data were Hellinger-transformed to down-weigh the influence of rare species (Legendre and Gallagher 2001). Species richness and Shannon diversity were calculated for each sample.

2.4. Statistical Analysis

All statistical procedures were performed in R (v. 4.4.3) using the 'vegan', 'cluster', and 'indicspecies' packages. Prior to analysis, fish abundance data were Hellinger-transformed to down-weigh the influence of rare species, and environmental variables were centered and scaled to ensure comparability.

2.4.1. Environmental heterogeneity: To identify spatial groupings of sites with similar physical characteristics, Hierarchical Cluster Analysis (HCA) was performed on site-averaged, scaled habitat data using Euclidean distance and Ward's linkage. To identify the primary axes of

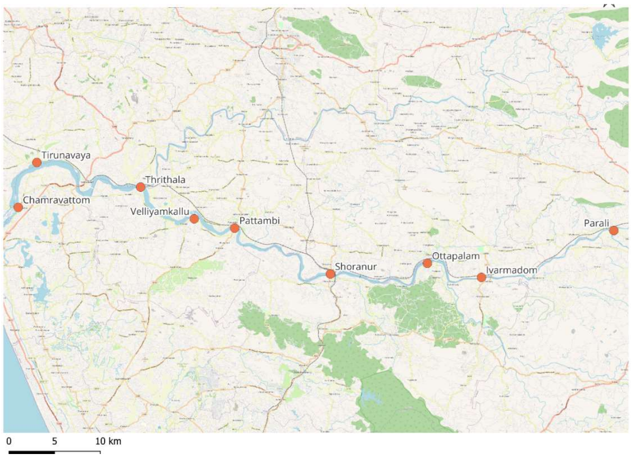

Figure 1. Study area showing the location of the nine sampling sites along the Bharathapuzha river, Kerala

Table 1. Geographic coordinates of the nine sampling sites along the Bharathapuzha river

Site ID	Site name	Latitude (°N)	Longitude (°E)
1	Parali	10.7968	76.5594
2	Ivarmadom	10.7507	76.427
3	Ottapalam	10.7646	76.373
4	Shoranur	10.7541	76.276
5	Pattambi	10.7992	76.1803
6	Velliyamkallu	10.8083	76.1398
7	Thrithala	10.83953	76.0862
8	Tirunavaya	10.8637	75.9825
9	Chamravattom	10.8196	75.963

environmental variation across all 108 sampling events, Principal Component Analysis (PCA) was conducted on the correlation matrix of the habitat variables.

2.4.2. Fish community structure and spatio-temporal variation: To test for differences in fish community composition across sites and seasons, a factorial Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using a Bray-Curtis dissimilarity matrix (Anderson 2001). The empirical model for PERMANOVA tested the marginal effects of Study Site, Season, and their Interaction as follows:

Distance Matrix ~ Study Site + Season + (Study Site * Season)

The statistical significance was determined using pseudo-F statistics calculated through 999 permutations. To identify specific species significantly associated with particular sites or seasons, Indicator Species Analysis (IndVal) was performed using the multipatt function (Dufrene and Legendre 1997). The Indicator Value (IndVal) was calculated based on the product of specificity (A) and fidelity (B) for each species.

2.4.3. Environment-biotic linkages: The relationship between the fish community and environmental predictors was modeled using Redundancy Analysis (RDA). The empirical model for RDA is defined as:

$$Y = XB + E$$

where Y represents the matrix of Hellinger-transformed species data, X is the matrix of environmental predictors (6 Habitat Strata and 6 Water Quality variables), B is the matrix of regression weights, and E is the residual error. Variance partitioning (Borcard et al., 1992) was subsequently used to decompose the community variance into unique fractions explained by Physical Habitat (fraction [a]) and Water Quality (fraction [b]).

2.4.4. Diversity indices and correlations: Alpha diversity was quantified using Species Richness, Shannon Diversity, Simpson Diversity, and Pielou's Evenness. Relationships between these indices and individual environmental predictors were assessed using Spearman rank correlation coefficients (Spearman 1904). To control for the false discovery rate, p-values were adjusted using the Benjamini-Hochberg (BH) procedure (Benjamini and Hochberg 1995).

3. RESULTS AND DISCUSSION

3.1. Spatio-Temporal Environmental Heterogeneity

All 20 physical habitat parameters showed significant variation among study sites confirming a strong spatial gradient of physical conditions. Seasonally, 14 of the 20 parameters varied significantly, driven primarily by the monsoon.

Hierarchical cluster analysis on site-averaged habitat data identified four distinct groups of sites (Figure 2). Group 1 -Chamravattom, Velliyamkallu was characterized by high scores for Flow Modification and Bank Modification. Group 2 -Ivarmadom, Shoranur) was distinguished by high scores for parameters related to ritualistic activities (cremation, religious use). Group 3 -Thrithala, Thirunavaya exhibited the lowest relative scores for most disturbance parameters. Group 4 -Ottapalam, Parali, Pattambi represented sites with mixed, moderate impacts.

Principal component analysis (PCA) of the 108 samples distilled the environmental variation into three primary axes explaining 62.5% of the total variance (Figure 3). PC1

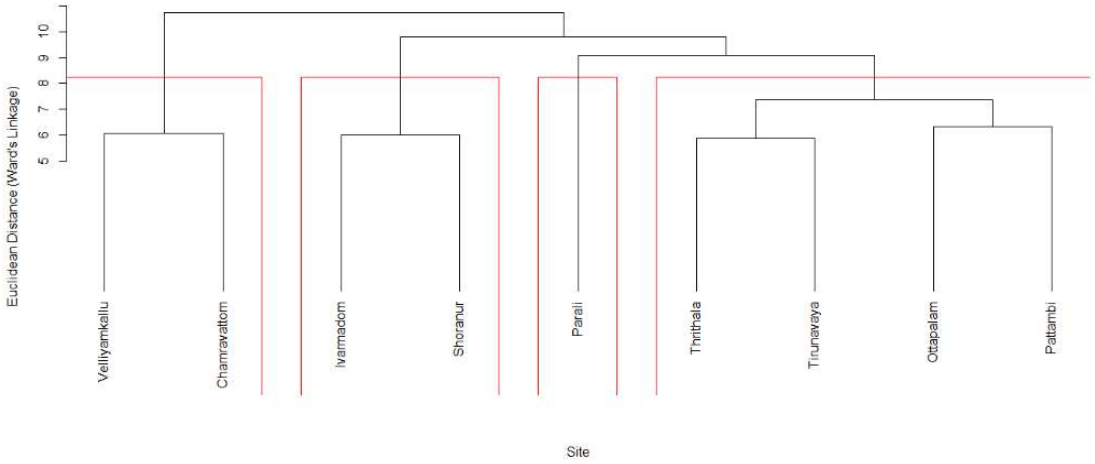

Figure 2. Dendrogram from the hierarchical cluster analysis (HCA) of the nine study sites based on their site-averaged habitat characteristics. The red lines delineate the cluster groupings discussed in the text

(23.9%) represented a 'Flow Dynamics' axis, contrasting high-flow monsoon conditions (high turbidity, flow, width) with low-flow conditions. PC2 (22.8%) represented habitat integrity'axis, separating sites with high tree cover and woody debris from those with high bank modification, trash, and flow obstructions. PC3 (15.9%) separated sites based on specific land uses, contrasting impacts from ritualistic activities with those from agriculture.

3.2. Fish Community Structure

The factorial PERMANOVA revealed significant effects for both study site (F=6.99, R^2=0.337) and season (F=8.53, R^2=0.164) on fish community composition. Crucially, the interaction study site × season was also highly significant (R^2=0.176), indicating that the seasonal trajectory of the fish community differed significantly among the study sites (Table 1).

The environmental analyses provide a mechanistic basis for this interaction. The HCA and PCA results clearly define the distinct physical characteristics of the study sites, grouping them based on dominant pressures such as flow regulation (Group 1) or specific land uses (Group 2). The significant PERMANOVA interaction term can thus be interpreted as evidence that the trajectory of community change—from the pre-monsoon drought to the monsoon flood and subsequent recovery-differs between these site groups. The community response in a highly regulated reach with artificial banks is unlikely to mirror the response in a less-impacted reach with intact riparian zones and natural channel features (Poff et al., 2010).

The study demonstrates that fish assemblages in the River Bharathapuzha are not structured by the monsoon cycle alone, but by a complex interplay between this powerful seasonal pulse and a spatially heterogeneous mosaic of local habitat conditions. The significant site × season interaction is the central finding, rejecting the notion of the monsoon as a uniform, system-wide driver. Instead, it reveals that the ecological response of the fish community to seasonal hydrological shifts is fundamentally context-dependent, filtered through the specific physical template of each river reach.

3.3. Environment- Community Linkages

The RDA model relating environmental predictors to fish community structure was significant with all predictors explaining 11.6% of the adjusted variance. Variance

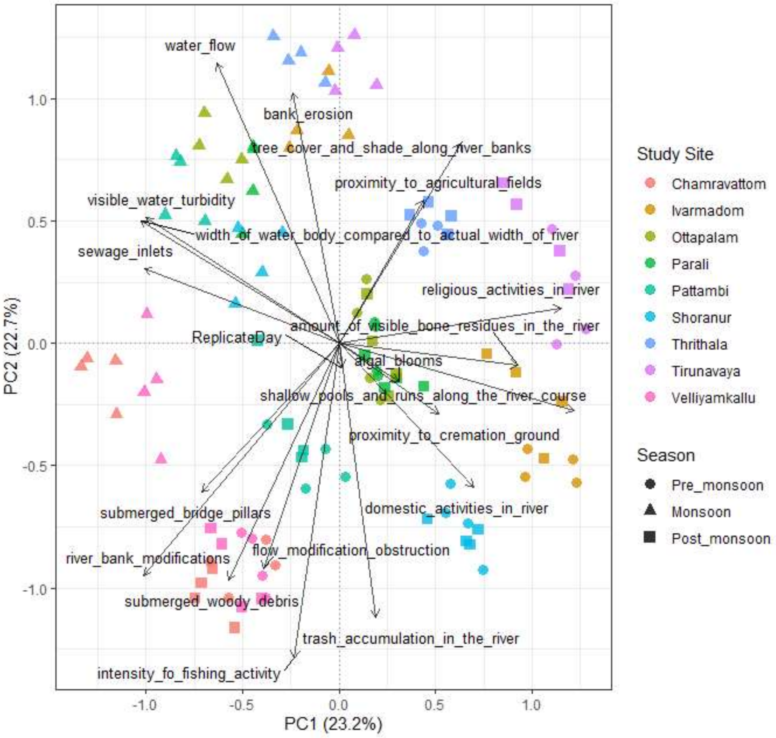

Figure 3. Principal component analysis (PCA) biplot of environmental habitat parameters, showing individual sampling events by site and season. The first two components (PC1: 23.9%, PC2: 22.8%) are displayed

partitioning revealed that the unique fraction of variance explained by the habitat strata (5.7%)was greater than the unique fraction explained by water quality variables (3.5%**).** The shared variance between the two sets of predictors was 2.5% (Figure 4).

The Spearman rank correlation analysis (Table 3) provided critical insights into the environmental drivers of fish alpha diversity. Statistically, the significant rho values indicate the strength and direction of monotonic relationships between predictors and diversity indices. Two results from Table 3 are particularly significant for the management of the Bharathapuzha River.

First, the significant positive correlation between 'Stratum 1: Pollution and Waste' and both species richness (rho = 0.43) and Shannon diversity (rho = 0.46) suggests a pattern of incidental structural enrichment. In river reaches where the natural habitat has been simplified by sand mining or channelization, the presence of solid waste or moderate nutrient inputs may provide artificial substrate or hydraulic refugia, thereby supporting a higher number of opportunistic or tolerant species. This aligns with the 'Intermediate Disturbance Hypothesis,' where moderate levels of anthropogenic pressure can temporarily increase local richness before reaching a threshold of collapse.

Second, the significant negative correlation between Dissolved Oxygen (DO) and diversity (rho = -0.36 for both indices) is an indicator of ecological instability. In tropical monsoon rivers like the Bharathapuzha, high daytime DO readings are often a symptom of advanced eutrophication, where nutrient-fueled algal blooms cause daytime supersaturation followed by severe nighttime hypoxia. Therefore, the lower diversity observed at higher DO levels likely reflects a community filtered by these extreme diurnal fluctuations, where only the most resilient species persist. These correlations highlight that simple water quality metrics must be interpreted through the lens of the river's specific ecological context.

Physical habitat structure emerged as a stronger unique predictor of fish community composition than water quality (Figure 4). The influence of physiological stressors like dissolved oxygen is evident, but the greater explanatory power of habitat variables underscores the foundational importance of the physical template in structuring these assemblages. The positive correlation between the stratum1_pollutionwaste metric and fish diversity supports our third hypothesis of non-linear responses. This counter-intuitive result does not imply that waste is beneficial; rather, it suggests a mechanism of incidental structural enrichment. In river reaches that may be otherwise simplified by factors like sand mining or channelization, the physical structure

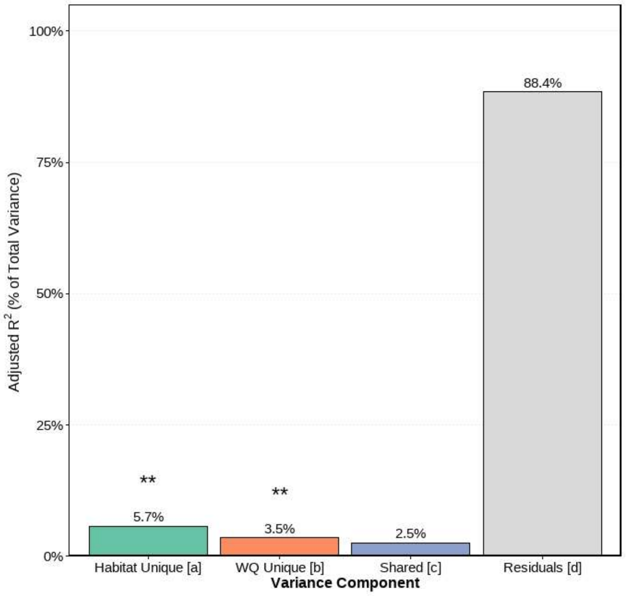

Fig. 4. Partitioning of variance in fish community composition by habitat strata and water quality predictors. Stars indicate statistical significance (*** $p < 0.001$)

Table 2. Factorial PERMANOVA results showing significant effects of Study Site, Season, and their interaction on fish community composition

Source	R^2	F-value	p-value
Study site	0.337	6.999	0.001
Season	0.164	8.531	0.001
Study site: Season	0.176	1.830	0.001
Residuals	0.442		
Total	1.000		

Table 3. Spearman rank correlations between key environmental predictors and fish diversity indices

Predictor	Diversity Index	Rho (ρ)
Stratum1_PollutionWaste	Richness	0.43
Stratum1_PollutionWaste	Shannon	0.46
Dissolved Oxygen (DO)	Richness	-0.36
Dissolved Oxygen (DO)	Shannon	-0.36

provided by solid waste could inadvertently create hydraulic refuges or substrate complexity, a phenomenon observed in other modified aquatic systems (Reis et al., 2017). This highlights that the loss of natural structural elements is a critical, and perhaps primary, component of degradation.

To investigate the mechanism behind the significant site × season interaction and the counter-intuitive negative correlation with DO, the relationship between dissolved oxygen and Shannon diversity was plotted for each site specifically during the pre-monsoon season (Figure 5). The analysis revealed that the nature of the relationship was highly context-dependent. A strong negative linear trend was observed in sites characterized by pollution and/or flow modification (Ivarmadom, Shoranur, Pattambi), whereas a neutral or weakly positive trend was observed in the least-disturbed sites (Thrithala, Thirunavaya).

Similarly, the significant negative correlation between dissolved oxygen and fish diversity is best explained not as a direct effect, but as a statistical signature of advanced eutrophication. As shown in our site-specific analysis for the pre-monsoon season (Figure 5). This negative trend is driven exclusively by the most polluted sites. It is hypothesized that in these reaches, high nutrient loads fuel intense daytime algal photosynthesis, leading to the supersaturated DO levels we recorded and is likely followed by severe nightly hypoxia due to high ecosystem respiration, creating an unstable environment that filters out sensitive species. The absence of this negative trend in cleaner sites like Thrithala (Figure 5) confirms that high daytime DO in this system is a symptom of instability, not health. This finding illustrates why ecological responses are context-dependent and why single-parameter assessments can be misleading.

RDA model explained a modest, though significant, portion of community variance (11.6% Adj. R^2). This low value should not be interpreted as a model deficiency but as a significant ecological finding in itself. The large proportion of unexplained variance is likely a signature of the substantial role of stochastic processes—such as chance colonization, extinction, and dispersal dynamics—which are known to be prominent in disturbance-driven systems like monsoon rivers (Chase 2010; Heino et al., 2015).

The study has direct implications for the conservation and management of the Bharathapuzha and similar tropical rivers. The demonstrated context-dependency of ecological responses means that uniform, basin-wide management policies (e.g., a single environmental flow standard) are likely to be inefficient or even ineffective. Restoration efforts must be tailored to the specific pressures impacting a given reach, as identified by our site groupings. For sites in Group 1, management should prioritize mitigating the effects of hydrological alteration, while for sites in Group 2, efforts might focus on local waste management and stakeholder engagement.

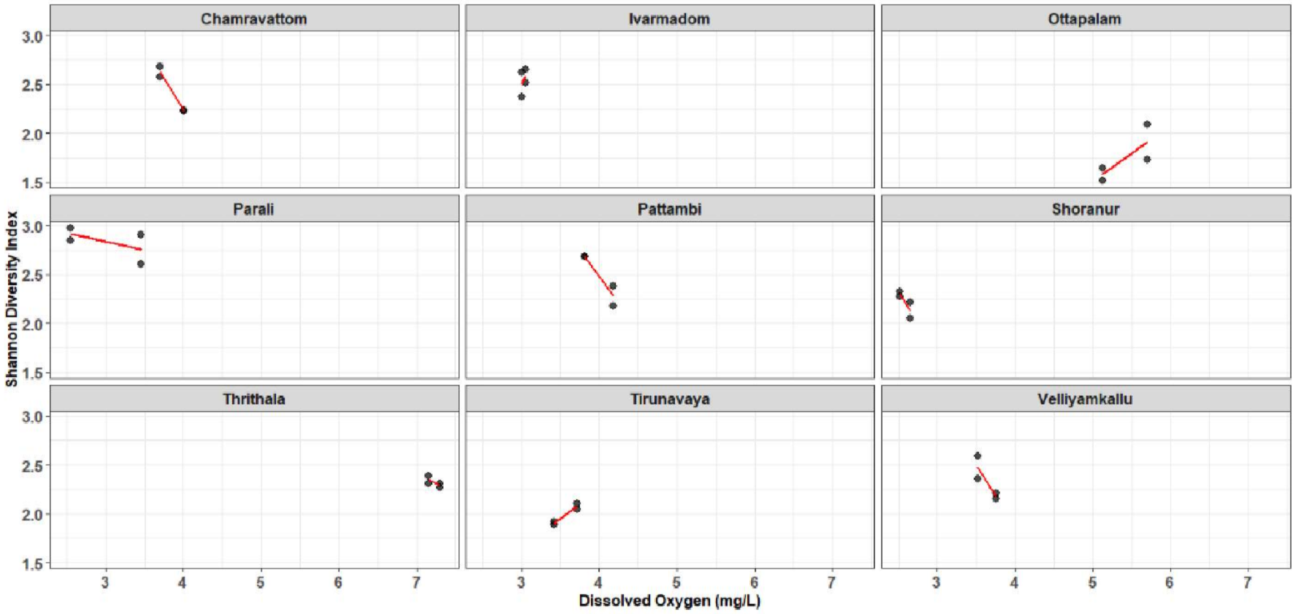

Fig. 5. Site-specific relationships between Dissolved Oxygen and Shannon Diversity Index during the pre-monsoon season

4. CONCLUSION

The structure of fish assemblages in the Bharathapuzha River is governed by a complex interplay of drivers where the regional monsoon cycle is fundamentally mediated by site-specific habitat conditions. Our findings lead to three specific recommendations for the management of this system:

First, because physical habitat structure explained significantly more unique variance (5.7%) in fish community composition than water quality (3.5%), conservation priority should be shifted toward physical habitat restoration—such as bank stabilization and the removal of flow obstructions—rather than focusing solely on chemical water quality monitoring.

Second, the highly significant interaction between study site and season demonstrates that "one-size-fits-all" management is inappropriate for this river. Management actions must be spatially explicit; for instance, reaches like Ivarmadom and Shoranur require targeted mitigation of ritualistic waste, while the Chamravattom and Velliyamkallu reaches require strategies to address the ecological impacts of flow regulation and bank modification.

Finally, our discovery that high daytime dissolved oxygen levels were negatively correlated with fish diversity suggests that standard water quality snapshots can be misleading in eutrophic tropical rivers. We recommend that local environmental agencies adopt the site-specific indicator species identified in this study (e.g., Silago sihama for estuarine reaches and Hypselobarbus dubius for mid-reaches) as more reliable longitudinal benchmarks for river health than chemical parameters alone.

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Conflicts of Interest

The authors declare they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the study design; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

Authors' Contributions

Gopika. S. G. Unni: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Visualization. Sreeparvathi. C. K: Conceptualization, Writing – Review & Editing. Remia. K. M: Supervision, Project administration, Writing – Review & Editing. All authors have read and agreed to the published version of the manuscript.

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Diversity of the Microbiome in Fish: Composition, Function and Implications for Aquatic Health & Aquaculture: A Review

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***Abstract*:** The fish microbiome represents a highly diverse and dynamic assemblage of microorganisms inhabiting key anatomical sites such as the gut, skin, and gills, where they play essential roles in host nutrition, immune regulation, physiological homeostasis, and protection against pathogens. Increasing evidence highlights that microbiome diversity and stability are critical determinants of fish health, disease resistance, and productivity, with significant implications for sustainable aquaculture and aquatic ecosystem management. The composition and function of fish-associated microbial communities are shaped by intrinsic host factors including species, developmental stage, genetics, and diet, as well as extrinsic environmental variables such as water quality, temperature, salinity, and aquaculture system design. Disruptions in these microbial communities can lead to dysbiosis, increasing susceptibility to infectious diseases and compromising mucosal barrier function. Advances in microbiome research have enabled the development of targeted strategies such as probiotic and prebiotic supplementation, environmental microbiome management, and microbiome-based disease control to enhance fish health and reduce reliance on antibiotics. This review synthesizes global literature on the anatomical and functional diversity of the fish microbiome, elucidates its role in host–microbe–environment interactions and disease processes, and highlights emerging microbiome-centered applications as key tools for improving aquatic health and sustainability in modern aquaculture.

***Keywords*:** Microbiome, Fish, Probiotics, Aquatic Health, Aquaculture.

1. INTRODUCTION

A microbiome includes all the microorganisms in a particular ecosystem. Occasionally it comprises the total of the genetic material of the microbes, symbiotic and pathogenic, living in and on all vertebrate animals. Microbiomes play important roles in maintaining aquatic animal health, nutrient cycling and climate change regulation (Lennon et al., 2023). The composition and function of gut, skin, and gill microbiomes in fish and shellfish, as well as aquatic ecological drivers behind tissue microbiome assembly. Microbes perform a number of important ecosystem services in water. Microbes also support ecosystems through their involvement in nutrient cycling, especially the nitrogen cycle (McKenney et al., 2018). Interestingly, a study of 140 sample of ocean water from around the world wide revealed that 73% of the prokaryotic gene abundance in all of the ocean samples can be attributed to the same functional core of the human gastrointestinal microbiome.

Aquatic animals are surrounded by a milieu of microbes and evidence suggests that the composition of environmental microbiomes influences the microbiomes of aquatic animals. For example, in zooplankton, alterations of the environmental microbiome by anthropogenically introduced antibiotics to aquaculture industry causes a shift in the host-associated microbiome, which in turn influences growth of plankton populations (Callens et al., 2018). A meta-analysis of the gut microbiomes of freshwater and saltwater fish and surrounding environmental samples indicated that the gut microbiome is not a reflection of the local habitat but rather the specific gut environment of the fish (Wong and Rawls, 2012).This review explains the role of the fish microbiota in regards to the host physiology, feeding habitat, digestion and metabolism, and also its control over stress responses, reproduction, development, and immune responses. In addition to future implications of fish microbiota handling and possible research guidelines for this growing aquaculture field.

2. Microbial Diversity across Anatomical Sites

The microbiome of fish is a complex and group of microorganisms inhabiting various anatomical sites including the gastrointestinal tract, skin and gills. These communities vary in structure, abundance and function depending on the specific micro environment they occupy and play crucial roles in host immunity, metabolism and disease resistance against pathogens. The niche-specific microbiota not only reflect the physiological role of each tissue but also respond distinctly to environmental, dietary and host genetic factors.

2.1. Gut Microbiome

The fish gut microbiome presents an invaluable window into host microbiota-environment interactions, offering insights with direct implications for aquaculture sector. The composition of fish gut microbiota is shaped by a combination of environmental, biological and behavioural parameters, which collectively influence microbial communities across diverse fish species (Nayfach et al., 2021; Bertoncin et al., 2022). The gut microbiota includes Proteobacteria, Firmicutes, Fusobacteria, Actinobacteria and Bacteroidetes. Their relative abundance varies not only between species but also within individuals depending on environmental exposure and rearing practices. For instance, Fusobacteria, such as *Cetobacterium*, are often abundant in freshwater fishes and are known for producing vitamin B12 and fatty acids that contribute to host metabolism (Ghanbari et al., 2015).

The gut environment is predominantly anaerobic or microaerophilic, favouring the proliferation of fermentative bacteria capable of producing the acetate, propionate and butyrate. These microbial metabolites play essential roles in maintaining intestinal epithelial health, regulating immune responses and inhibiting pathogen colonization (Ghanbari et al., 2015; Nayak, 2010). The gut microbiome is not only a reflection of the host's internal structure but also of external management factors. Microbiomes are leveraged to enhance fatty acid production in muscle tissue and improve fish health development. The gut microbiome helps protect the intestinal barrier, prevent the overgrowth of opportunistic pathogens and modulate the host immune system, all of which are crucial for maintaining fish health (Nohesara et al., 2023). Therefore, understanding and manipulating the fish gut microbiome has become an important strategy for developing sustainable and disease-resistant aquaculture practices. The gut microbiome is increasingly recognized as an important indicator of environmental health and the adaptability of fish populations, offering valuable insights for conservation efforts (Soh et al., 2024).

In addition, the gut microbiome also has implications for disease prevention and therapeutic intervention. A gut microbiome-characterized by reduced microbial diversity and increased abundance of opportunistic pathogens of aquatic animals such as *Aeromonas*, *Vibrio*, or *Pseudomonas* has been linked to disease susceptibility and inflammation (Ghanbari et al., 2015). Therefore, maintaining a stable and diverse gut microbiota is essential for sustainable fish farming and health management. Advancing sustainable aquaculture and implementing effective conservation strategies for vulnerable fish populations hinges on a comprehensive understanding of gut microbiome dynamics. Research has shown that gut microbiota plays a fundamental role in host resilience, immune function, and adaptation to environmental changes, all of which are critical for both farmed and wild fish (Fonseca and Fuentes, 2023; Zhu and Wang, 2023).

2.2. Skin Microbiome

Teleost are associated with diversified microbial communities in the mucosa of the skin, gill and gut. Healthy fish skin harbours an indigenous microbial community that facilitates the homeostasis of host immunity, removes waste products and outcompetes the colonization of pathogens (Hu et al., 2021). The epidermal mucus exerts an innate immune response secreted from goblet cells, which act as a primary barrier for pathogenic microbes. In mucus contains antimicrobial peptides, proteases, lysozymes and lectins that are known to support innate immunity (Dash et al., 2018). The mucus in fish skin is known to entrap and slough off microbes constantly; therefore, skin prevents the colonization of pathogenic microbes. Although fish skin constantly renews mucus, a healthy resident microbial community is maintained in the mucus layer. However, the community structure of the skin microbiome is shaped by multiple factors, such as environmental conditions, health status and host genetics (Van Cise et al., 2020; Xavier et al., 2020). As microbiome of fish plays significant role in their health and development through symbiotic relationship (Collin et al., 2021) though less abundance of community exists in skin mucus compared to gut (Hu et al., 2021).

The bacterial phyla of fish skin such as Proteobacteria, Firmicutes, Actinobacteria and Cyanobacteria. At the genus level, bacterial organisms include *Aeromonas*, *Pseudomonas*, *Vibrio*, and *Shewanella* are commonly reported. These microbes can exist as commensals under normal conditions but may act as opportunistic pathogens when the host is stressed or immunocompromised (Lowrey et al., 2015). A component of the fish skin is the mucus layer, which contains various innate immune molecules such as lysozymes, lectins, antimicrobial peptides (AMPs) and immunoglobulin-like proteins. This mucus not only acts as a physical barrier but also serves as a selective substrate for beneficial microbes, thus helping to shape and stabilize the skin microbiota (Salinas et al., 2011).

Environmental condition or husbandry-related factors such as stocking density, handling, and poor water quality and temperature shocks can destabilize the skin microbial community, a condition known as dysbiosis. It is often results in reduced microbial diversity and a shift toward potentially pathogenic taxa. Such imbalances have been associated with increased incidence of parasitic and bacterial infections, including diseases caused by *Ichthyophthirius multifiliis* (white spot disease) and *Flavobacterium columnare* (columnaris disease) (Boutin et al., 2014). Skin ulceration and muscular necrosis are dominated by *A. hydrophila;* the freshwater pathogen would cause infection-related mortality in catfish farming system (Siao et al., 2021). Infections caused the loss of 3 million pounds of farm-raised catfish in Alabama, United States, in 2017, making *A. hydrophila* the most prevalent pathogen in catfish (Siao et al., 2021). However, within the same species of fish, they found different mucosal microbiomes (Butt and Volkoff, 2019). Gaining a better understanding of the skin-mucus microbiota of farmed fish could help to manage the health and disease of high densities aquaculture species.

In aquaculture, maintaining beneficial skin microbiota is importance for disease prevention and improved aquatic animal health. Strategies such as probiotic bathing, water microbiome management, and stress minimization are increasingly being explored to support the natural skin microbiome and reduce dependency on antibiotics.

2.3. Gill Microbiome

Gill surfaces are the primary site of gas exchange in fish and are colonized by microbial communities. Like the skin and gut, the gills are coated in a layer of mucus that is constantly replaced, providing both a defence against and habitat for pathogenic or commensal prokaryotes. Antimicrobial peptides found in the skin and intestines of fish are also present in the gill mucus, highlighting the importance of the gills as a first line of defence against pathogenic infection. Gill microbiota can reflect internal and external diseases (Legrand et al., 2018).

The mucosal surface of the gills constitutes the most extensive biological interface between the fish and external environment. This surface is colonized by a complex highly diverse microbial community that promotes gill mucosal homeostasis and thereby contributes to overall fish health (Elsheshtawy et al., 2021). Recently, an increased prevalence of the gill pathogen *Candidatus Branchiomonas cysticola* and changes in mRNA abundance of cellular stress, inflammation, and repair and proliferation genes in gill tissue were reported after thermal delousing (Ostevik et al., 2022).

Gill microbiomes respond to changes in the environment. Following exposure to suspended sediments, clownfish (Amphiprion percula) larvae gill microbes had higher abundances of pathogenic taxa (Flavobacterium, Pasteurella, Edwardsiella, Chryseobacterium Pseudomonas, Corynebacterium) (Hess et al., 2015). Though the microbiome was not characterized, a study with Atlantic salmon found that gill mucus cell count increases

Table 1. Major microbial groups associated with fish microbiome, their functional roles, and implications for aquatic health and aquaculture

Microbial group	Dominant Taxa (Examples)	Primary functions	Implications for fish health	Relevance to aquaculture
Proteobacteria	*Vibrio, Pseudomonas, Aeromonas*	Nutrient metabolism, immune modulation	Includes both beneficial and opportunistic pathogens	Dysbiosis linked to disease outbreaks
Firmicutes	*Lactobacillus, Bacillus*	Enzyme production, pathogen inhibition	Enhances gut health and digestion	Widely used as probiotics
Bacteroidetes	*Flavobacterium, Bacteroides*	Carbohydrate degradation	Supports nutrient absorption	Influenced by diet formulation
Actinobacteria	*Microbacterium, Corynebacterium*	Vitamin synthesis, antimicrobial activity	Maintains microbial balance	Sensitive to water quality
Fusobacteria	*Cetobacterium*	Vitamin B_{12} production	Improves growth and feed efficiency	Common in freshwater species
Archaea (minor)	Methanogens	Fermentation processes	Limited but emerging roles	Potential biomarker for gut health

with salinity and fluctuations in ion regulation-additional research is needed to determine whether gill microbiomes play an active role in ion regulation (Hess et al., 2015).

The function of gill-associated bacteria may contribute to mucus degradation**,** ammonia detoxification and modulation of innate and non-specific immune responses, helping to maintain the integrity of gill tissues. Some commensal organisms may also produce antimicrobial compounds that inhibit colonization by pathogenic bacteria. However, due to the thin epithelium and rich vascularization of the gills, this site is particularly vulnerable to environmental insults and microbial dysbiosis (Dhanasiri et al., 2011). Stressful conditions, such as poor water quality (e.g., high ammonia or nitrite levels), elevated organic loads, hypoxia, or abrupt temperature changes, can destabilize the gill microbiota. This can lead to increased susceptibility to opportunistic pathogens, including *Flavobacterium*, *Vibrio*, and *Tenacibaculum* species. Such infections are commonly associated with bacterial gill disease (BGD)**,** a condition characterized by hyperplasia, necrosis, and impaired gas exchange (Dhanasiri et al., 2011; Rosado et al., 2019). From an aquaculture perspective, the health of the gill microbiome is critical for fish performance, survival, and health. Strategies such as maintaining high water quality, reducing stocking stress and administering water probiotics or immunostimulants are being explored to preserve gill microbial homeostasis and prevent gill-associated diseases.

3. Microbial Transmission and Colonization

The initial development of microbiome colonization may occur vertically (from parent to offspring) or horizontally from the surrounding environment, with the relative contribution of these routes varying across species and rearing conditions (Funkhouser & Bordenstein, 2013). In hatchery systems, egg disinfection and water sterilization practices can disrupt natural microbial transmission, potentially reducing microbial diversity during early developmental stages (Llewellyn et al., 2014; De Schryver & Vadstein, 2014). Such alterations in early microbial exposure may influence host health and immune development, thereby prompting growing interest in microbial inoculation strategies and probiotic interventions to support healthy microbiome establishment in the aquaculture industry (Ringo et al., 2018; Vadstein et al., 2018).

4. Microbiome and Disease in Fish

The composition and stability of the fish microbiome are closely related to disease susceptibility and overall health status. A microbiome helps protect fish from pathogens, whereas any disruption of this balances-known as dysbiosis. It is increasing their susceptibility to opportunistic infections and inflammatory disease. This relationship is essential for disease prevention and health management strategies in aquaculture systems.

4.1. Dysbiosis and Disease Susceptibility

4.1.1. Dysbiosis: Refers to an imbalance normal microbial community, often characterized by reduced microbial diversity and overgrowth of pathogens. It can be triggered by a variety of stressors including poor water quality, overcrowding, temperature fluctuations, dietary changes, and antibiotic use (Xiong et al., 2019).

For example, high levels of *Aeromonas hydrophila* and *Vibrio* spp. have been reported in diseased fish suffering from dysbiosis, such as catla and tilapia with hemorrhagic septicemia and fin rot (Huang et al., 2020; Bujan et al., 2022). These pathogens exploit weakened mucosal barriers and immune suppression, often resulting in systemic infections.

4.2. Mucosal Microbiomes as Barriers

The skin, gill and gut microbiomes serve as critical first-line defenses by occupying niche spaces on mucosal surfaces and secreting antimicrobial substances. In healthy fish, these microbial barriers competitively exclude invading pathogens by outcompeting them for nutrients level and adhesion sites. When these microbial communities are destabilized due to environmental stressors or antibiotic exposure of pathogen colonization increases, leading to epithelial cell damage and inflammation of acute or chronic level (Llewellyn et al., 2014). Recent studies have shown that fish with stable and diverse microbiota exhibit stronger resistance to infections such as *Edwardsiella tarda* and *Streptococcus iniae*, compared to those with low microbial richness (Lowrey et al., 2015). This suggests that microbial diversity itself may be a biomarker of fish health and resilience.

4.3. Role in Chronic Inflammation and Gut Pathology

Chronic dysbiosis has been related to gut pathologies such as enteritis and epithelial erosion in salmon and common carp. In Atlantic salmon, prolonged feeding of soybean meal-based diets induces an inflammatory gut response and microbial shifts that favor pro-inflammatory taxa, including *Mycoplasma* and *Desulfo vibrio* (Ulloa et al., 2021). Gut microbiota imbalances have also been associated with altered intestinal barrier integrity, leading to leaky gut syndrome and translocation of bacteria into the bloodstream-further worsening systemic infections.

4.4. Antibiotic Use and Microbiome Disruption

The use of antibiotics in aquaculture sector, especially in developing countries, has led to serious consequences for microbial health. Antibiotics not only eliminate pathogenic bacteria but also disrupt beneficial microbial communities in aquatic animals (Dehler et al., 2017). Post-antibiotic dysbiosis is frequently associated with secondary infections of parasites, reduced immunity and poor feed conversion efficiency. Moreover, the emergence of antimicrobial resistance (AMR) within fish microbiota has raised concerns about zoonotic transmission and environmental contamination. Multi-drug-resistant *Vibrio* and *Aeromonas* strains have been detected in both wild and farmed fish microbiomes, posing a threat to both aquaculture sustainability and public health concern by Santos and Ramos (2018).

5. Applications in Aquaculture

The fish microbiome has emerged as a promising way for improving aquaculture productivity, sustainability and fish health. The microbiome is increasingly being viewed as a targetable aquaculture industry that can be engineered or enhanced to promote disease resistance, feed efficiency, water quality, and stress tolerance. With the advent of omics technologies, microbiome-based interventions are becoming more precise and impactful.

5.1. Probiotics and Prebiotics

The use of probiotics-live microbial supplements that give health benefits to the host. In aquaculture, probiotics such as *Lactobacillus spp.*, *Bacillus spp.*, *Shewanella putrefaciens* and *Pediococcus acidilactici* are administered through feed or water to enhance gut microbial balance, inhibit pathogens and boost immunity (Nayak, 2010; Ringo et al., 2018). These beneficial microbes produce antimicrobial compounds (e.g., bacteriocins, organic acids), compete for adhesion sites and stimulate mucosal immunity.

Prebiotics are non-digestible feed ingredients (e.g., inulin and β-glucans) that selectively stimulate the growth of beneficial microbes. Their inclusion in diets enhances microbial richness and metabolic activity, improving nutrient utilization and gut integrity (Hoseinifar et al., 2016). When combined, synbiotics (probiotic + prebiotic) have result on synergistic effects, improving feed conversion ratio and survival in several fish species including tilapia, carp, and salmonids.

5.2. Microbiome-Targeted Disease Management

Microbiome modulation is a non-antibiotic strategy for disease prevention. It is promoting microbial communities that resist pathogen colonization; fish are better protected from infections like *Vibriosis*, *Aeromoniasis*, and Enteritis. Probiotic administration has been shown to reduce mortality in *Litopenaeus vannamei* challenged with *Vibrio harveyi* and to enhance resistance to *Aeromonas hydrophila* in common carp (Kim and Austin., 2006; Xiong et al., 2019). Furthermore, viable exclusion strategies are being developed, where selected beneficial strains are introduced early in larval stages to occupy ecological niches and prevent pathogenic colonization. Such approaches reduce the need for antibiotics and lower the risk of antimicrobial resistance (Dehler et al., 2017).

5.3. Environmental Microbiome Management

In Recirculating Aquaculture Systems (RAS) and biofloc systems, managing the environmental microbiome is as important as animal health. Biofilters and microbial additives are used to maintain nitrifying bacterial load that convert ammonia to nitrate, improving water quality and reducing stress-induced dysbiosis (Giatsis et al., 2015). In aquaculture systems incorporate bioremediation microbes such as *Nitrosomonas* and *Nitrobacter* to stabilize water chemistry and suppress pathogen loads. The practice of "green water" (algal-bacterial mixtures) in larviculture is a microbiome-centered strategy that improves larval survival and promotes the development of beneficial gut flora (Caruffo et al., 2021).

5.4. Next-Generation Technologies

Modern multi-omics tools (metagenomics, meta transcriptomics, and metabolomics) have enabled in-depth profiling of microbiomes in different aquaculture species and production systems. These tools facilitate real-time health monitoring by identifying shifts in microbial signatures that precede disease outbreaks or stress responses (Li et al., 2021). These approaches aim to directly manipulate microbial community structure for enhanced performance traits such as growth rate, immunity and feed conversion efficiency. Additionally, microbiome transplantation is being explored as a way to restore healthy microbiota in fish post-antibiotic treatment or after environmental disruptions by Gomez and Balcazar (2008).

6. CONCLUSION

The diversity of the microbiome in fish is major role for maintaining physiological balance, enhancing immunity and providing resistance against pathogens (Llewellyn et al., 2014). This microbial community is influenced by several factors such as species, developmental stage, diet and environment conditions (Ghanbari et al., 2015). Therefore, a diversity of microbiome is development of probiotic and immunostimulant strategies to promote fish health and sustainable aquaculture practices (Ringo et al., 2020). The future research integrating advanced molecular technique will continue to uncover the functional roles of fish microbiomes and support the optimization of microbiome-targeted interventions.

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Diversity and Distribution of Genus *Trentepohlia* Mart. (Chlorophyta) from North Karnataka, India

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***Abstract*:** Diversity of the subaerial algae genus *Trentepohlia* Mart. was studied in 13 districts of North Karnataka, India. The presence of *Trentepohlia* species was examined in both forest and urban environments. The nine species (*Trentepohlia aurea* (Linnaeus) C Martius, *Trentepohlia odorata* (F. H. Wiggers) Wittrock, *Trentepohlia abietina* (Flotow ex Kützing) Hansgirg, *Trentepohlia rigidula* (J. Müller) Hariot, *Trentepohlia effusa* (Krempelhuber) Hariot, *Trentepohlia annulata* F. Brand, *Trentepohlia thevalliensis* M. V. N. Panikkar and P. Sindhu, *Trentepohlia chapmanii* Rindi and López-Bautista, *Trentepohlia infestans* Rindi and J. López-Bautista) and two varieties (*Trentepohlia abietina* var. corrugata (Leighton) Cribb, *Trentepohlia abietina* var. tenuis (Zeller) A. B. Cribb) of *Trentepohlia* were recorded from the different regions of North Karnataka. These documented species represent previously unreported taxa. Hence, these are considered as new distributional records from North Karnataka region. The survey of 13 districts conducted in the year 2022 – 2024 concluded that the physical factors like rainfall, temperature, relative humidity and light intensity plays important role in the growth of *Trentepohlia* species. The presence of *Trentepohlia* species was only in 3 districts (Dharwad, Belagavi and Haveri). Shannon diversity index was higher in Belagavi (2.02) followed by Dharwad (1.61) and the lowest diversity in Haveri (1.33) district.

***Keywords*:** Subaerial green algae, *Trentepohlia*, Diversity, Shannon index, North Karnataka.

1. INTRODUCTION

Previously genus *Trentepohlia* Mart. was assumed as *Byssus aurea* L. as in "Species Plantarum" Linnaeus described genus *Trentepohlia* as *Byssus aurea* L. but there is no proof to evident that, "*Byssus aurea*" was based on the concept of "*Byssus capillacea pulverulenta*" and later on, in honour to J. F. Trentepohl who was a German clergyman and botanist, C. F. Philipp von Martius named a particular type of subaerial green algae as *Trentepohlia* in 1817 (Rajapure and Singh, 2025). The subaerial green algae genus *Trentepohlia* Mart. belongs to the Kingdom Plantae, Division Chlorophyta, Class Ulvophyceae, Order Trentepohliales, and Family Trentepohliaceae. It is distributed across tropical, subtropical, and temperate climates (Liu et al., 2012). Species of *Trentepohlia* are easily recognizable on various substrata due to their distinctive orange to red coloration, which results from the accumulation of beta-carotene and hematochrome pigments (Rindi et al., 2008; Kharkongor and Ramanujam, 2015). Hence, these *Trentepohlia* species, although classified as microchlorophytes, exhibit a unique morphological phenotype that sets them apart from other green algae. This distinctive nature has led to their description as macrochlorophytes, due to their tendency to form, irregular vertical streaks of orange, red, or green colour on various natural and artificial substrata (Kharkongor and Ramanujam, 2015).

The identification of *Trentepohlia* species relies primarily on detailed morphological features. Key diagnostic traits include the shape and size of vegetative cells, the occurrence of characteristic hair-like cells known as setae, patterns of branching in the filamentous thallus, and the position, structure, and morphology of reproductive organs such as sporangia and gametangia (Liu et al., 2012; Saraphol, 2020). Despite these identifiable features, the genus *Trentepohlia* presents significant challenges. One of the major difficulties is high degree of polymorphism as the individuals within the same species can exhibit considerable morphological variability depending on environmental conditions or life cycle stages. This complicates the identification of species (Rindi and Guiry, 2002).

Scientific studies on the genus *Trentepohlia* have largely focused on regions such as Europe, Central America, South America, and some countries where subaerial green algae are relatively well-documented. However, tropical regions have proven to be particularly important in algal biodiversity research. Over the past two centuries, the majority of newly described subaerial algal species including those in the genus *Trentepohlia* have originated from tropical zones. This highlights the rich species diversity and ecological importance of these environments (Saraphol, 2020). Conducting the detailed studies on *Trentepohlia* species in tropical, subtropical, and temperate regions is very essential. These investigations provide the foundational taxonomic and ecological data needed to better understand the global distribution, evolutionary adaptations, and potential environmental roles of subaerial green algae. Aim of this study was to elucidate the diversity and some basic aspects of ecology of genus *Trentepohlia* in North Karnataka.

2. MATERIALS AND METHODS

2.1. Collection of *Trentepohlia* Species

Survey and collection of *Trentepohlia* species was obtained by scraping from natural substrata i.e. stem bark of trees and artificial substrata i.e. electric poles, concrete cementing walls of buildings and water tanks and kept in in air tight zip lock sterile plastic bags (Satpati and Pal, 2016) from different sampling areas (Belagavi – 15°51'N 74°30'E, Dharwad – 15° 27' 30" N 75° 00' 30" E and Haveri – 14.8° N 75.4°E) of North Karnataka. The parameters like latitude, longitude, temperature, relative humidity and light intensity of sampling sites were estimated by CTH 288 digital temperature, humidity meter and Lutron LX-101A and digital lux meter. The annual rainfall data report was recorded from government website Karnataka State National Disaster Management Centre (KSNDMC). The photographs of the algae along with its habitat have been recorded.

2.2. Identification of *Trentepohlia* Species

The collected algal samples were freeze-dried at −4°C (degree Celsius) and also preserved in 4% formalin in the laboratory (Satpati and Pal, 2016). The species were observed under compound microscope (Olympus OIC), morphometric evaluation by scaling of cell measurements using micrometry (Satpati and Pal, 2019). Identification of *Trentepohlia* species was carried out by using standard monographs, research papers (Cribb, 1970; Rindi and Guiry, 2002; Rindi et al., 2005, 2006 and 2008; Rindi and Lopez-Bautista, 2007; Allali et al., 2013; Satpati and Pal, 2016; Binoy et al., 2019; Saraphol, 2020) and herbarium of one of the species sent to BSI, Howrah for its identification.

2.3. Diversity Index

Diversity of genus *Trentepohlia* was calculated using Shannon-Weiner diversity index , Eq. 1. (Shannon and Weaver, 1963).

$$H' = -\sum_{i=1}^{s} pi \ln pi \ldots\ldots\ldots\ldots\ldots..(1)$$

Where,

H' - The species diversity index

s - The number of species

pi – The individuals of one particular species found (n) divided by the total number of individuals found (N)

ln – Natural log

3. RESULTS AND DISCUSSION

3.1. Effect of Physical Factors on Growth of *Trentepohlia* Species

Physical factors play the major role in the growth of *Trentepohlia* species. Among 13 districts of Karnataka only 3 districts show the diversity of *Trentepohlia* species. The rainfall, temperature, relative humidity and light intensity play crucial role in the growth of *Trentepohlia* species on its different substrata. Dharwad, Belagavi, and Haveri regions have favorable environmental conditions, hence, 9 species and 2 varieties of *Trentepohlia* were observed. In the remaining other 10 districts the *Trentepohlia* species were not observed due to the environmental conditions wherein, the rainfall, relative humidity was comparatively less and temperature, light intensity was comparatively more than Dharwad, Belagavi and Haveri districts. The environmental conditions may be unfavourable for the growth of *Trentepohlia*. In Alnavar forest of Dharwad and 2 sampling sites (Katridaddi and Jamboti) of Belagavi, the *Trentepohlia odorata* and *Trentepohlia rigidula* species were observed throughout the year because of the rich vegetation maintaining 24ºC - 27ºC temperature, 72% – 84% relative humidity and 4000 – 8000 lux light intensity. But in other districts such as Bidar and Kalaburgi, even though they have sufficient rainfall (838 mm and 813 mm), the other factors like temperature, relative humidity and light intensity were unfavourable for the growth of *Trentepohlia* species.

Earlier studies concluded that genus *Trentepohlia* is widely distributed across a broad range of climatic zones, including tropical, subtropical, and temperate regions (Liu et al., 2012; Satpati and Pal, 2016). This widespread occurrence indicates the genus ecological flexibility and adaptability to colonize diverse habitats. *Trentepohlia* species are frequently found growing on man-made substrates such as concrete surfaces, especially those that are fully exposed to direct sunlight. These exposed environments are often subject to high levels of solar radiation, temperature fluctuations, and limited moisture availability, making conditions challenging for many organisms to inhabit. However, *Trentepohlia* exhibits remarkable physiological adaptations that enable it to survive and even thrive under such extreme environmental stressors. These adaptations include mechanisms to tolerate desiccation (drying out due to water loss), endure thermal stress (such as high surface temperatures), and maintain metabolic activity under fluctuating light and humidity conditions (Abe et al., 1999).

3.2. Diversity and Distribution of *Trentepohlia* Species

Trentepohlia species were found to be present as orange, red and green patches on both the natural and artificial substrata i.e. stem bark of trees, electric poles, concrete cementing walls of buildings and water tanks. Out of total 108 sampling sites surveyed from the districts of North Karnataka, 32 sampling sites recorded the presence of *Trentepohlia* species. Hence, total 9 species and 2 varieties of *Trentepohlia* species have been identified (Table 2), collected from various regions of Dharwad, Belagavi and Haveri districts while species were not observed in remaining 10 districts during the survey. *Trentepohlia rigidula* (J. Müller) Hariot and *Trentepohlia odorata* (F. H. Wiggers) Wittrock were the 2 species observed common in all the 3 districts. *Trentepohlia abietina* (Flotow ex Kützing) Hansgirg was common in Belagavi and Haveri regions. *Trentepohlia chapmanii* Rindi and López-Bautista was common in the regions of Dharwad.

Table 1. Physical factors in the sampling sites of different locations

Site location	Rainfall (mm)	Temperature (ºC)	Relative humidity (%)	Light intensity (Lux)
Dharwad*	1051	24.3 - 28.6	62 - 84	3500 - 8550
Belagavi*	1194	24 - 28.8	62 - 84	4528 - 11200
Haveri*	1023	25 - 27.5	64 - 84	3900 - 7200
Gadag	399	29 - 34	52 - 57	18020 - 23500
Bidar	838	29.5 - 35	57 - 62	34500 - 48700
Kalburgi	813	27.1 - 30	47 - 49	38000 - 49000
Vijayapura	621	29 - 31	45 - 46	27500 - 48000
Yadgir	736	32 - 36	42 – 45	38000 - 48100
Raichur	637	32 - 36	42 - 45	38000 – 49200
Bagalkot	603	28.2 - 29.3	45 - 47	25400 - 47300
Koppal	598	28 - 34	49 - 52	23200 - 35600
Ballari	662.6	27 - 32	51 – 52	23500 – 38000
Vijayanagara	763.7	29 - 32	49 - 52	24000 - 42000

Favourable physical factors for the growth of Trentepohlia species

Table 2. Trentepohlia species in the districts of North Karnataka

***Trentepohlia* species**	**Dharwad**	**Belagavi**	**Haveri**
Trentepohlia aurea (Linnaeus) C Martius	_	+	_
Trentepohlia odorata (F. H. Wiggers) Wittrock	+	+	+
Trentepohlia abietina (Flotow ex Kützing) Hansgirg	_	+	+
Trentepohlia rigidula (J. Müller) Hariot	+	+	+
Trentepohlia effusa (Krempelhuber) Hariot	_	+	_
Trentepohlia annulata, F. Brand	+	_	_
Trentepohlia abietina var. corrugata (Leighton) Cribb	_	+	_
Trentepohlia abietina var. tenuis (Zeller) A. B. Cribb	+	_	_
Trentepohlia thevalliensis M. V. N. Panikkar and P. Sindhu	_	+	_
Trentepohlia chapmanii Rindi and López-Bautista	+	_	+
Trentepohlia infestans Rindi and J. López-Bautista	+	_	_

In Gadag, Bidar, Kalburgi, Vijayapura, Yadgir, Raichur, Bagalkot, Koppal, Ballari, Vijayanagara no species were observed

Keys to *Trentepohlia* species found in surveyed districts of North Karnataka

1. Thallus heterotrichous, distinguished into prostrate and erect filaments…………..........…..**2**

1. Thallus uniseriate…………………….….……..…**3**

2. Prostrate cells, globular or spherical to barrel shaped, erect cells cylindrical………...**2a, 2b, 4,5,6,7**

2a. Prostrate cells, globular or spherical to barrel shaped, erect cells rectangular and ovoid……………………..**8**

2b. Prostrate cells entangled into a mass of undifferentiated individual cells…………..……**9**

3. Prostrate and erect filaments are undifferentiated …………..**3a, 3b**

3a. Filaments and branched filaments all globular or elliptical with the width of 23.04 µm and length 26.88 µm, each cell separated by septa, presumptive gametangia is spherical 23.04 µm in diameter……………..*Trentepohlia rigidula*

3b. Branched filaments differentiated into primary long and secondary short filaments, cells cylindrical, width 7.68 – 9.6 µm, length 15.36 – 21.12 µm. apical cell without pectic cap. Sporangia very characteristically borne on lateral, terminal and intercalary position with different size 11.52 – 15.36 µm, 15.36 µm width, 17.28 µm length, 24.96 – 32.64 µm in diameter respectively…….......…*Trentepohlia thevalliensis*

4. Prostrate cells with width 10 – 13 µm erect cells cylindrical width 7 – 12.5 µm length 15.36 – 23.04 µm, branching densely entangled to produce compact mass, sporangia globular 14.5 – 22 µm in diameter borne on suffultory cell, slightly bent thin or broad….…*Trentepohlia infestans*

5. Prostrate cells of 7 – 13.44 µm in diameter, erect cells 3.84 – 5.76 µm in width and 19.2 – 21.12 µm in length, apical cell pointed with small pectic cap. Sporangia globular 19.2 µm in diameter borne on straight to slightly curved, flask shaped suffultory cell…………..…*Trentepohlia chapmanii*

6. Prostrate cell subglobose 13.44 – 24.96 µmin diameter, erect cells width of 7.68 – 13.44 µm and length 9.6 – 23.04 µm, apical cell with pectic cap. Stalked to sessile sporangia, globose to subglobose occurring laterally and terminally with a diameter of 10.5 – 11.52 µm, while globular gametangia are lateral, terminal and intercalary 13 – 15.36 µm in diameter……..…………...*Trentepohlia odorata*

7. Presence of horizontal prostrate cells from which erect cells arise vertically, branching present or absent, all cells are similar, bearing apical cell with pectic cap…..………**7a, 7b, 7c, 7d**

7a. Branching not observed, cells cylindrical to barrel shaped 5.76 – 7.68 µm in width and 15.36 - 16 µm in length, gametangia globular or ovoid 7.68 – 19.2 µm in diameter……………........*Trentepohlia abietina*

7b. Little or no branching, cells barrel shaped or inflated, rarely cylindrical, width 6 - 9 µm and length 12 - 26 µm, sporangia pedicellate 11 µm in diameter borne on slightly curved, flask shaped suffultory cell, gametangia globular 10 – 15 µm in diameter. Large apical cell with pectic cap…………………..…*Trentepohlia abietina* var. tenuis

7c. Cells with corrugate appearance, cylindrical or slightly swollen, slightly inflated, 5 – 15 µm width, 14 - 28 µm length, apical cell with pleomorphic pectic cap, gametangia globular 12 - 20 µm in diameter……………*Trentepohlia abietina* var. corrugata

7d. Profused branching, cells cylindrical, slightly inflated, width 19.2 – 30.72 µm and length 38.4 – 57.6 µm. Apical cell obtuse with blunt tips pectic cap. Gametangia globular to sub globular, slightly ellipsoidal 19.2 – 23.04 µm in diameter……………………..*Trentepohlia aurea*

8. Prostrate cells spherical 11.52 – 19.2 µm in diameter, erect cells rectangular and ovoid, width of 7.68 µm, length 15.36 – 19.2 µm, apical cell with pectic cap. Sporangia elliptical or flask shaped, 24.5 – 26.88 µm in diameter containing obliquely arranged zoosporangium at the top of the erect axis…………………*Trentepohlia annulata*

9. Erect cells arising from the pseudoparenchymatous mass of cylindrical cells, 3.84 – 5.76 µm in width and 19.2 µm in length, sparsely branched, apical cell tapering with pointed end. Reproductive structures sessile, globular, 7.68 – 15.36 µm in diameter..........*Trentepohlia effusa*

Description of Identified *Trentepohlia* species

1. Trentepohlia rigidula (J. Müller) Hariot, Queensland (1889)

Thallus uniseriate, The axis is upto 192 – 307.2 µm tall. The cells of filaments are elliptical or globular in shape and ranges 23.04 µm in width and 26.88 µm in length. Filaments are branched profusely. Each cell is separated by septa. The apical cell is oval ranges 19.2 µm in diameter. The presumptive gametangia is spherical in shape ranges 23.04 µm in diameter (Figure 1).

2. Trentepohlia thevalliensis M. V. N. Panikkar and P. Sindhu, Thevally, Kerala, India (1993)

The filaments are long bearing 20 – 25 cells. The primary branches are long and secondary branches are small. The cells are cylindrical, 7.68 – 9.6 µm wide and 15.36 – 21.12 µm long. Sporangia sessile, globular, lateral, terminal, or intermediate. The lateral sporangia 11.52 – 15.36 µm in diameter, terminal sporangia 15.36 µm wide and 17.28 µm long, the intermediate sporangia 24.96 – 32.64 µm in diameter. The apical cells without pectic cap 9.6 µm wide and 24.96 µm long (Figure 2).

3. *Trentepohlia infestans* Rindi and J. López-Bautista, Zone Industrielle, Collery Marengo, French Guiana (2007)

Thallus is heterotrichous, pseudoparenchymatous,

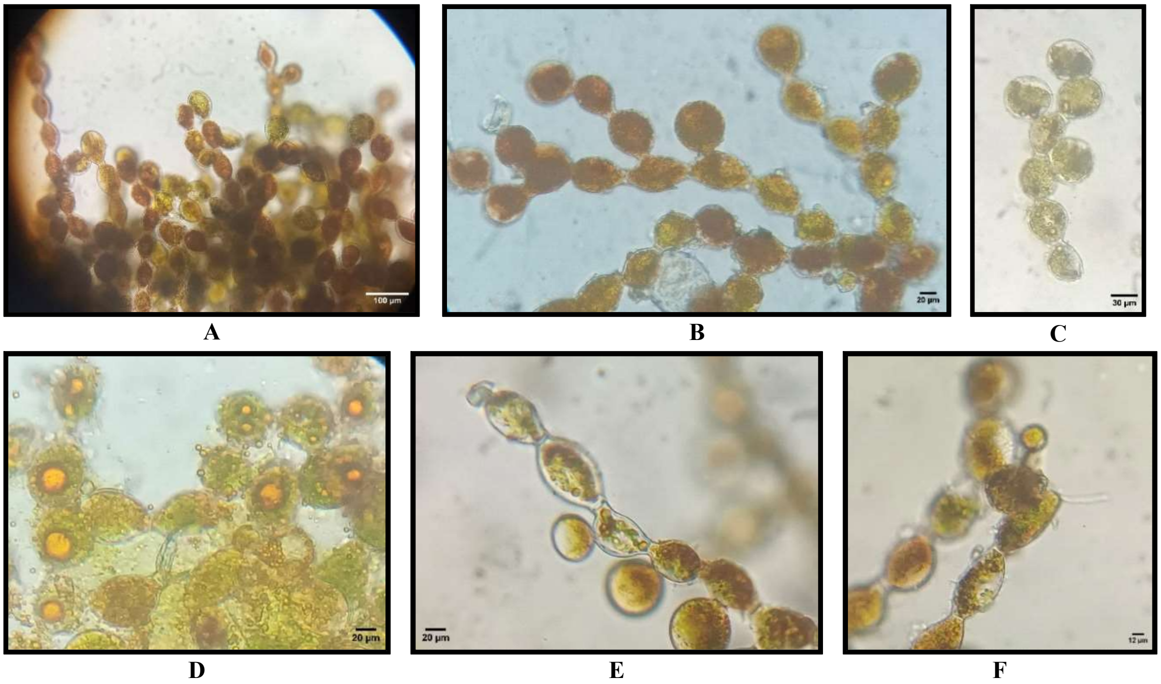

Figure 1. (A – F) Microscopic structures of *Trentepohlia rigidula* (J. Müller) Hariot **A** – Thallus **B** – Branched filaments **C** - Intercalary and Terminal Presumptive Gametangia **D** – Oil globules/lipid droplets **E** – Apical cap **F** – Stalked sporangia

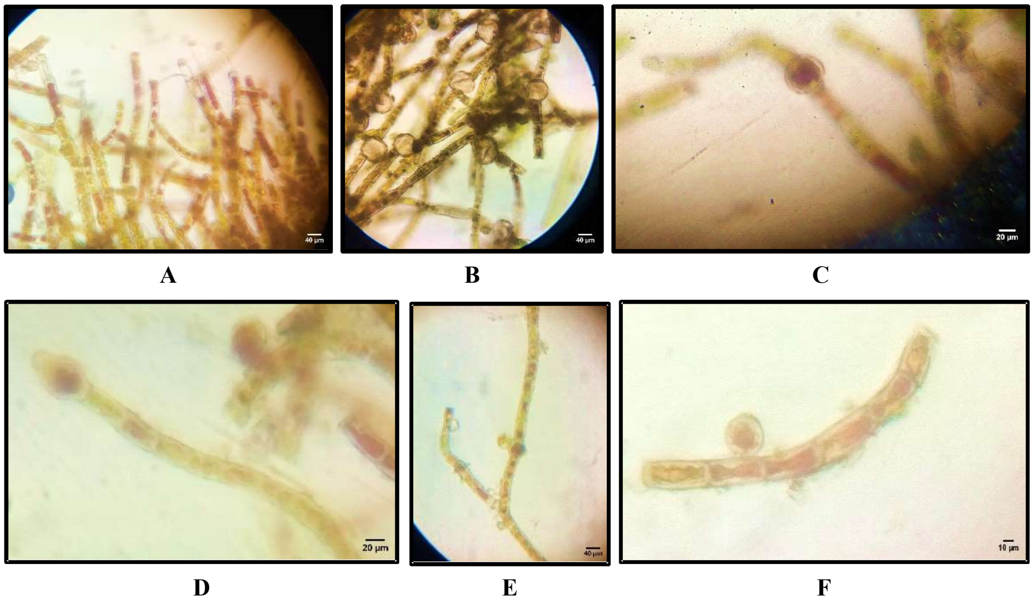

Figure 2. (A - F) Microscopic structures of *Trentepohlia thevalliensis* M. V. N. Panikkar and P. Sindhu **A** – Thallus **B** – Intermediate sessile Sporangia, **C and E**– Intermediate Sporangia **D** – Terminal Sporangia, **F** - Lateral Sporangia

consisting of separation between dense prostrate and erect parts. many individual filaments densely entangled to produce compact mass. The cells of the prostrate part were globular, elliptical, or barrel-shaped, 10–13 µm wide. The erect axes arise from the prostrate part. The cells of erect axes were cylindrical, 7–12.5 µm wide and 15.36 – 23.04 µm long. Presence of globular sporangia, 14.5 – 22 µm in diameter, borne on suffultory cell. The neck of the suffultory cell was slightly bent and thin as well as broad. Gametangia were not observed (Figure 3).

4. Trentepohlia chapmanii Rindi and López-Bautista, Fort Diamant, area of Rémire- Montjoly, French Guiana (2007)

Thallus heterotrichous. Prostrate part spreading, pseudoparenchymatous. Erect filaments arising from the prostrate parts. Cells of the erect filaments are cylindrical, 3.84– 5.76 µm wide and 19.2 – 21.12 µm long. Cells of the prostrate part are globular or elliptical 13.44 – 19.2 µm in diameter. Presence of globular sporangia, 19.2 µm in diameter. The apical cells are pointed and bear a pectic cap. The suffultory cell is straight or slightly curved, flask-shaped 24.96 µm long (Figure 4).

5. Trentepohlia odorata (F. H. Wiggers) Wittrock Fionia, Denmark (1880)

Thallus heterotrichous. Cells cylindrical, in erect filament, 9.6 – 23.04 µm long and 7.68 – 13.44 µm wide. The Cells of prostrate filaments are subglobose to ellipsoid 13.44 µm – 24.96 µm in diameter. Presumptive gametangia nearly spherical to elliptic, lateral, terminal, or intercalary 13 - 15.36 µm in diameter. Sporangia, globose to subglobose, lateral/ terminal, sessile and stalked, 10.5 – 11.52 µm

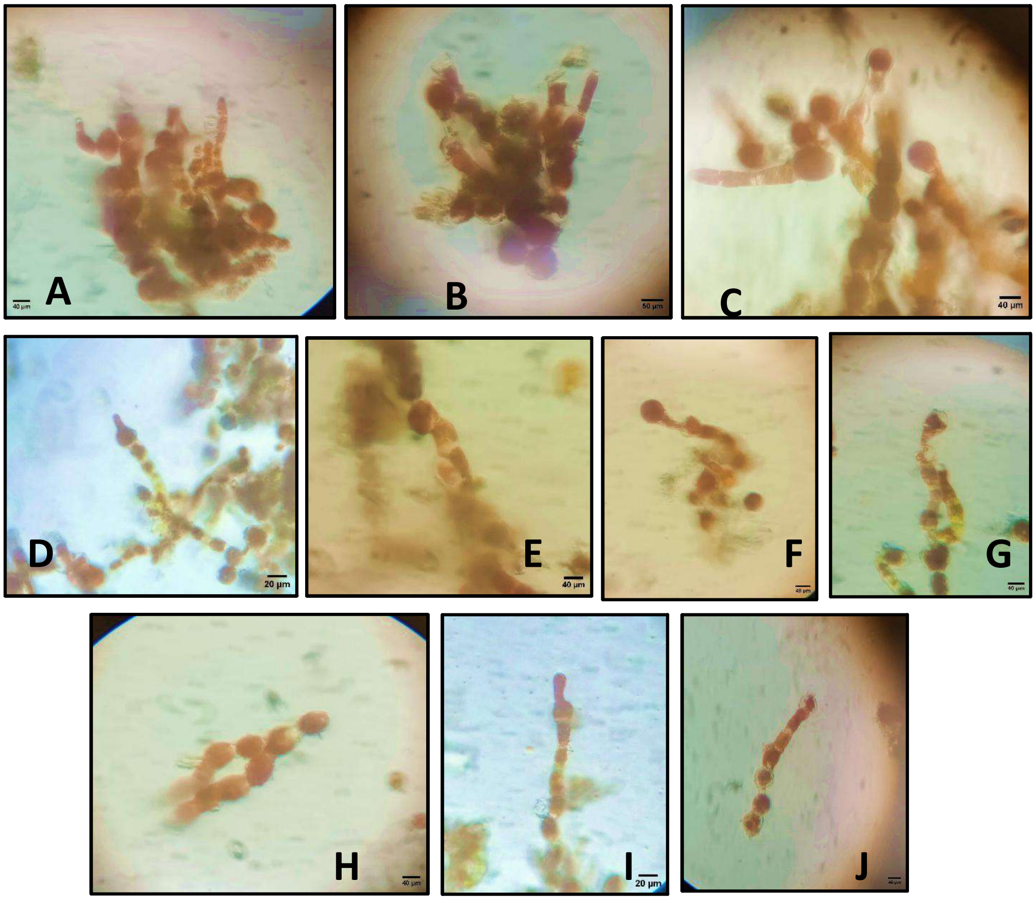

Figure 3. (A - K) Microscopic structures of *Trentepohlia infestans* Rindi and J. López-Bautista **A** – Individual cells entangled to form compact mass **B** – Thallus **C** – Sporangia formed on straight suffultory cell **D and I**– Zoosporangium has not yet been divided from the suffultory cell **E** – Sporangia formed on large suffultory cell **F** – Sporangia formed on slightly curved suffultory cell **G** – Sporangia formed on curved suffultory cell **H** – Branching of filaments **J** – Single filament

diameter. The suffultory cell is straight or slightly curved 11.52 µm long. Apical cells with pectic caps 15.36 µm wide and 19.2 µm long (Figure 5).

6. Trentepohlia abietina (Flotow ex Kützing) Hansgirg, Hirschberg, Bavaria, Germany (1886)

Thallus heterotrichous, pseudoparenchymatous. The erect axis 126 – 198 µm long. Cells of the erect axes are cylindrical or barrel-shaped 3.84 –7.68 µm wide and 15.36 - 16 µm long. Cells of the prostrate filaments are elliptical or cylindrical, or barrel-shaped, 5.76 – 7.68 µm wide and 15.36 - 16 µm long. Presumptive gametangia globular or ovoid, 7.68 – 19.2 µm in diameter. Sporangia and Suffultory cells not observed. Apical cell with pectic cap 15.36 – 19.2 µm long and 7.68 µm wide (Figure 6).

7. Trentepohlia abietina* var. *tenuis (Zeller) A. B. Cribb, Elephant Point, Pegu, Burma (1970)

Thallus heterotrichous. Erect axes 200-250 µm tall, with little or no branch, arising from limited prostrate parts. Cells barrel-shaped or inflated, rarely cylindrical, 2-3 times as long as wide (12 – 26 µm long), 6-9 µm wide. Gametangia globular, 10-15 µm in diameter. Sporangia pedicellate, 11 µm in diameter. Suffultory cell slightly curved, flask shaped. Apical cells larger with apical cap (Figure 7).

***8. Trentepohlia abietina* var. corrugata** (Leighton) Cribb Queensland, Australia (1970)

Thallus heterotrichous. The cells are cylindrical or

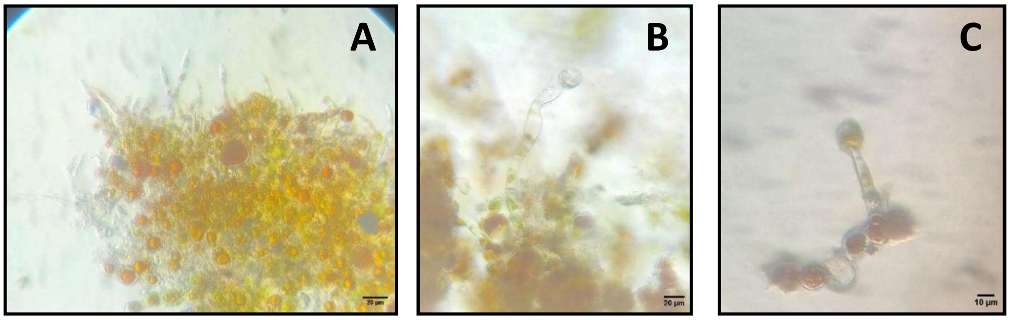

Figure 4. (A - C) Microscopic structures of *Trentepohlia chapmanii* Rindi and López-Bautista **A** – Thallus with Erect and Prostrate cells **B** – Sporangia formed on Suffultory Cell **C** – Terminal Sporangia

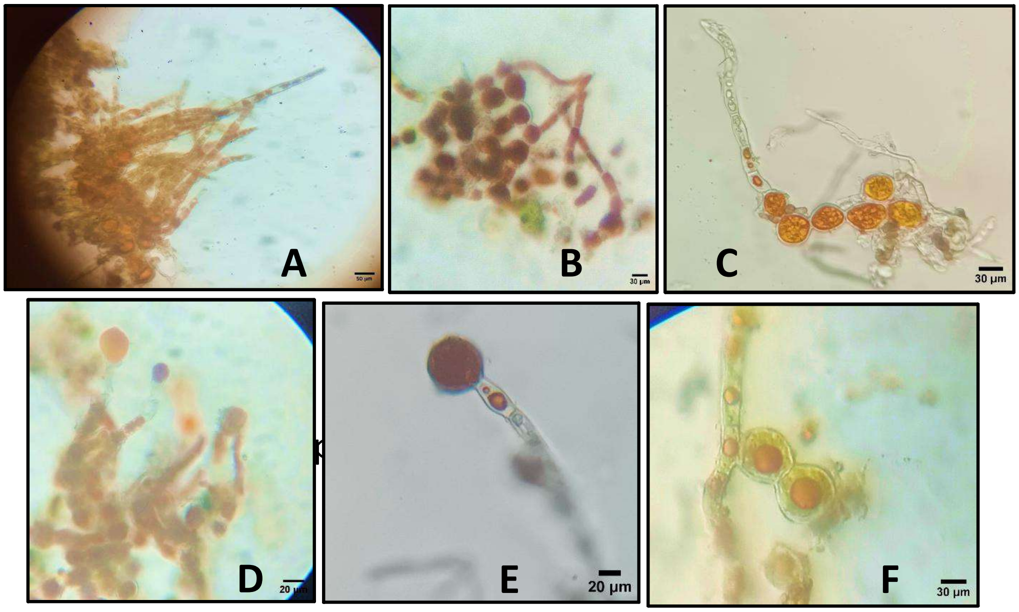

Figure 5. (A - F) Microscopic structures of *Trentepohlia odorata* (F. H. Wiggers) Wittrock **A -** Thallus with Erect and Prostrate Cells **B –** Apical cells with pectic caps **C –** Single Erect Filament Arising from the Prostate Cells **D** – Suffultory Cells (SC) **E** – Terminal Sporangium (S) **F -** Oil globules/lipid droplets

slightly swollen or slightly inflated, 5 –15 µm wide 14 – 28 µm long as wide. Cells with corrugate appearance. Branching of filaments. Gametangia are present but not abundant, globular, 12–20 µm wide. The zoosporangium is oval, 10–15µm in diameter. Apical cells with irregular caps (Figure 8).

9. Trentepohlia aurea (Linnaeus) C Martius, Wales: Caenarvon; Italy (1817)

Thallus heterotrichous, pseudoparenchymatous, 1.11-1.125 mm tall. Filaments are long, straight, slightly curved, more or less branched. Profused branching. The branched filaments are 390 – 450 µm long. Cells cylindrical or slightly inflated, 19.2 – 30.72 µm wide, and 38.4 – 57.6 µm long. Presumptive gametangia globular, sub globular, slightly ellipsoidal, 19.2 - 23.04 µm in diameter. The apical cells obtuse with pectic cap and are variable in shape with blunt tips (Figure 9).

10. Trentepohlia annulata, F. Brand, Germany (1902)

Thallus is formed by erect axes arising from the prostrate axes. Cells of erect axis are rectangular or ovoid 15.36 – 19.2 µm long and 7.68 µm wide. The prostrate cells are spherical 11.5 – 19.2 µm in diameter. Sporangium elliptical or flask-

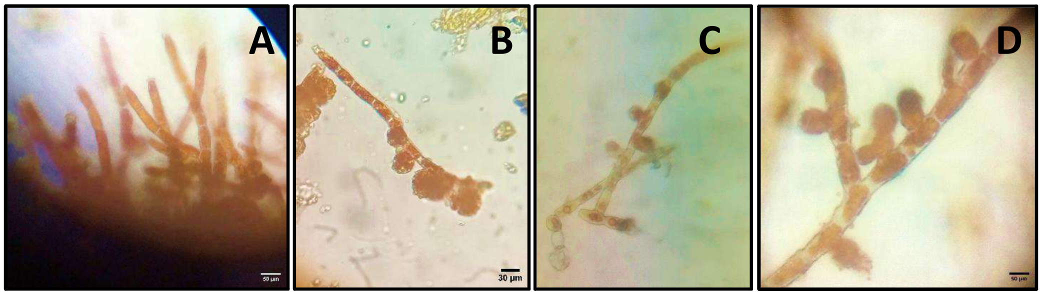

Figure 6. (A - D) Microscopic structures of *Trentepohlia abietina* (Flotow ex Kützing) Hansgirg **A -** Thallus **B -** Apical cells with pectic caps **C –** Single erect filament from the prostate cells **D** – Branched filaments with presumptive gametangia

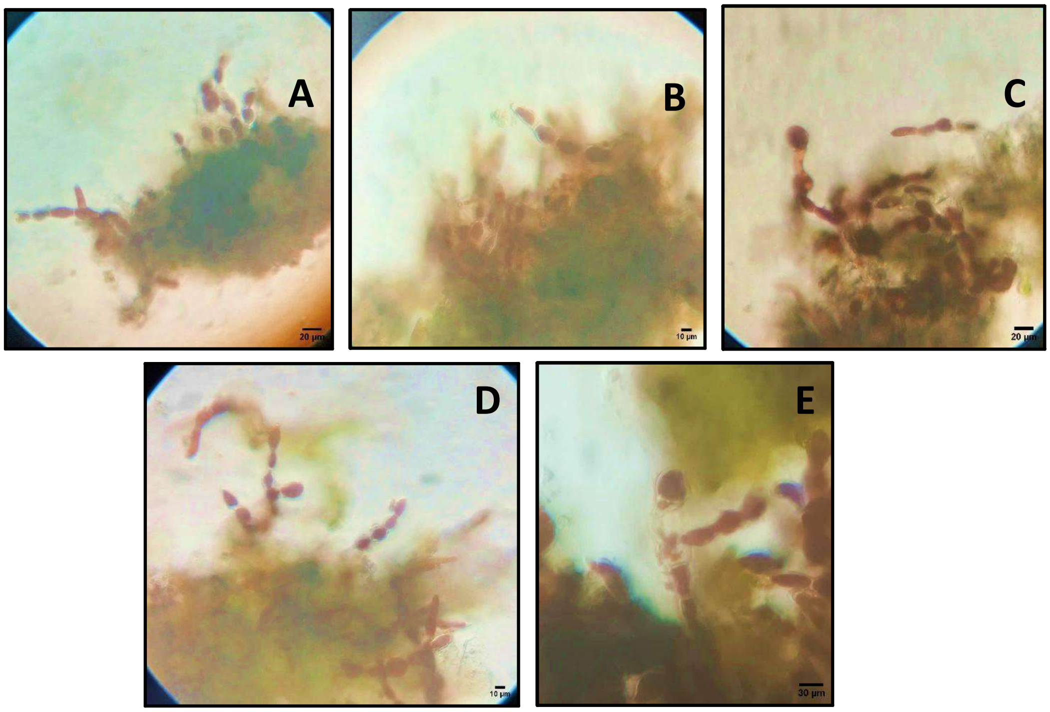

Figure 7. (A - E) Microscopic structures of *Trentepohlia abietina* var. *tenuis* **A –** Thallus **B –** Apical cell with Pectic Cap **C –** Sporangia on slightly bent suffultory cell **D –** Lateral Sporangia **E** – Sporangia on cup shaped Suffultory cell

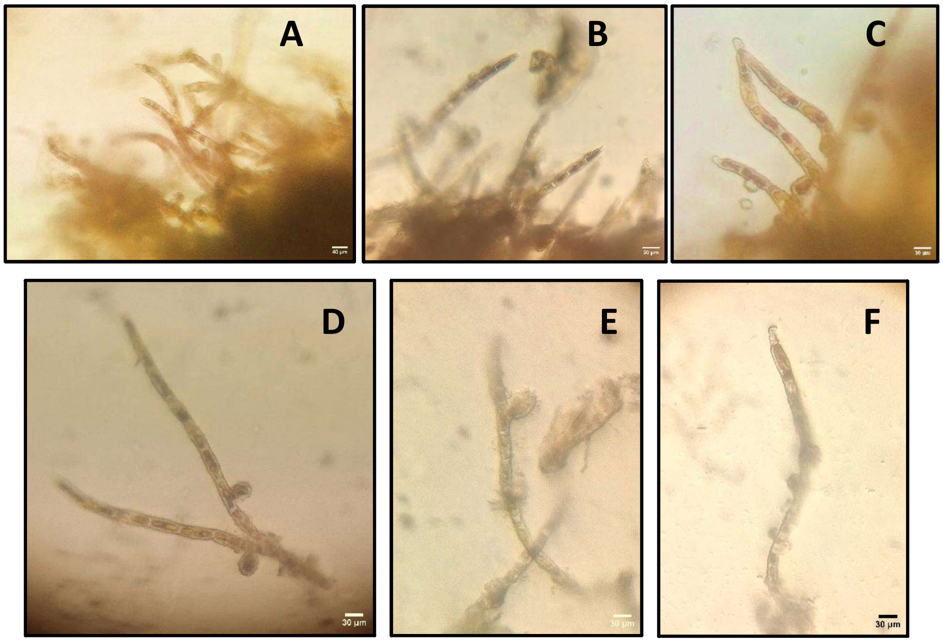

Figure 8. (A - F) Microscopic structures of *Trentepohlia abietina* var. *corrugata* **A** – Thallus **B, C and F** - Apical Cells with Irregular Caps **D** – Branched filaments with Lateral Presumptive Gametangia **E** – Single Filament with Zoosporangia

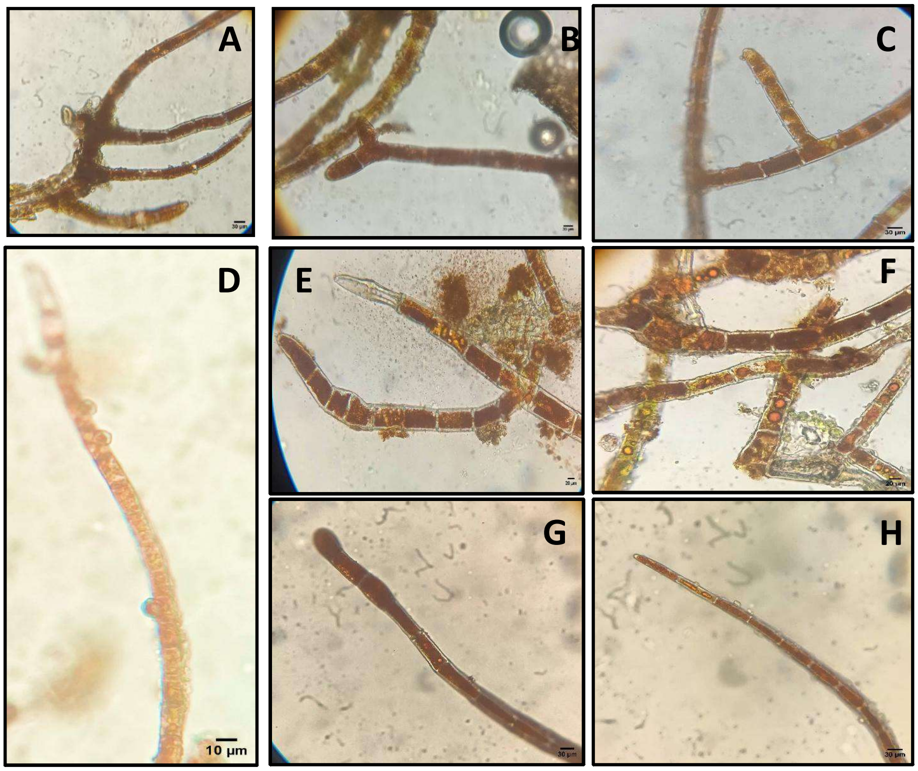

Figure 9. (A - H) Microscopic structures of *Trentepohlia aurea* Martius **A** and **C** – Profused Branching of Filaments **B** – Erect and Prostrate cells **D** – Presumptive Gametangia on Erect Filament **E** – Cells with rich carotenoid pigments **F** – Oil globules/lipid droplets **G** – Obtuse/ blunt tip **H** – Apical Cell with Pectic Cap

shaped 24.5 – 26.88 µm in diameter. Oblique arrangement of zoosporangium at the top of erect axes. The apical cell with pectic cap 15.36 µm long and 13.44 µm wide (Figure 10).

11. Trentepohlia effusa (Krempelhuber) Hariot, Brazil: Rio de Janeiro State (1890)

Thallus heterotrichous, consists of separation between dense prostrate parts and long sparsely erect parts. Erect axes 115.2 – 230.4 µm tall. Cells of the erect filaments are cylindrical with 3.84 – 5.76 µm wide, 19.2 µm long. Sporangia are sessile, globular 7.68 – 15.36 µm in diameter. Apical cells with tapering / pointed end (Figure 11).

Guiry and Guiry (2024) observed that genus *Trentepohlia* currently comprises of 53 species and 28 varieties worldwide. India has made a notable contribution to this global diversity, with the documentation of seven species that were first described based on specimens collected within the country and include *Trentepohlia gracilis, T. pathanamthittaensis, T. angadickalensis, T. thevalliensis, T. sundarbanensis, T. gaviensis*, and *T. keralensis*. These species were described by the Panikkar and Sindhu (1993); Satpati and Pal (2015 and 2019); and Binoy et al. (2019) based on the morphological observations and habitat specificity. In addition to these species, two varieties, *Trentepohlia aurea* Mart. var. tenuior and *Trentepohlia monilia* De Wildemann var. subspherica were reported from India. These varieties were documented by Bruhl and Biswas (1923) and Krishnamurthy (2000).

Several studies have contributed to the growing understanding of the global diversity and distribution of the genus *Trentepohlia*. In temperate regions, Rindi and Guiry (2002) reported the presence of four *Trentepohlia* species from urban habitats in western Ireland. This finding is significant as it highlights the ability of these algae to colonize artificial substrates in cooler, urban environments, indicating their ecological flexibility and tolerance to anthropogenic conditions.

In tropical South America, Rindi and Lopez-Bautista (2007) documented four *Trentepohlia* species from French Guiana, two of which were newly described and previously unknown to science. Expanding the biogeographical scope further, Rindi et al. (2005) identified five species from the Hawaiian Islands. The presence of multiple *Trentepohlia* species in these islands suggests that the genus has a remarkable dispersal ability and can establish populations in isolated island systems, possibly through airborne spores or anthropogenic vectors. In Central Africa, Allali et al. (2013) conducted an extensive survey in Gabon and recorded eleven species of *Trentepohlia*, the highest species richness among the studies cited. Gabon's equatorial climate, characterized by high humidity, stable temperatures, and dense forest canopies, likely provides ideal conditions for the growth and diversification of *Trentepohlia* species. More recently, Saraphol (2020) reported eight species from the Chiang Dao Wildlife Sanctuary in Thailand. The findings from this protected area highlight the importance of conservation areas in preserving algal diversity.

In India, the diversity and distribution of *Trentepohlia* species have been documented in various regional studies, reflecting the genus wide ecological range and occurrence in diverse climatic zones. In an early investigation, Panikkar and Sindhu (1993) reported eight species from the state of Kerala, emphasizing the favourable subaerial habitats provided by the humid tropical climate. Subsequently, Krishnamurthy (2000) recorded 14 species from Kolkata, West Bengal, contributing valuable data from an eastern subtropical environment. Tiwari et al. (2011) expanded on the Kerala records by identifying 18 species, the rich algal diversity in this southern coastal region. In contrast, Tamal et al. (2012) documented only a single species from West Bengal, and an additional survey by Satpati and Pal, (2013 and 2015) from the same region increased the known diversity to eight species. From state of Karnataka, Singh and Singh, (2017) recorded four species, providing insights into the genus occurrence in the regions of Karnataka. Binoy et al. (2019) reaffirmed Kerala's significance as a hotspot for *Trentepohlia*, reporting eight species. Collectively, these studies highlight the insufficient yet significant distribution of *Trentepohlia* across different biogeographical zones in India and emphasize the need for continued exploration and systematic documentation.

3.3. Shannon-Wiener Diversity Index

Shannon Weiner diversity index, summarizes that diverseness depends upon both the number of species and their relative individual distribution in the particular areas (Table 3). The total number of different species individuals present in an area is the measure of the total distribution of richness. Though the frequency of collection of samples in Belagavi district was lesser compared to Dharwad, yet Belagavi illustrated higher species diversity than Dharwad. The most abundant species recorded was *Trentepohlia odorata* in Belagavi which is diversified in all the 3 districts. The Dharwad collection was dominated by *Trentepohlia rigidula* with greater diversity. The Belagavi and Dharwad samples had a greater species diversity of 2.02 and 1.61 respectively. The Haveri collection numbered only fewer species with the diversity of 1.31. The reasons for these

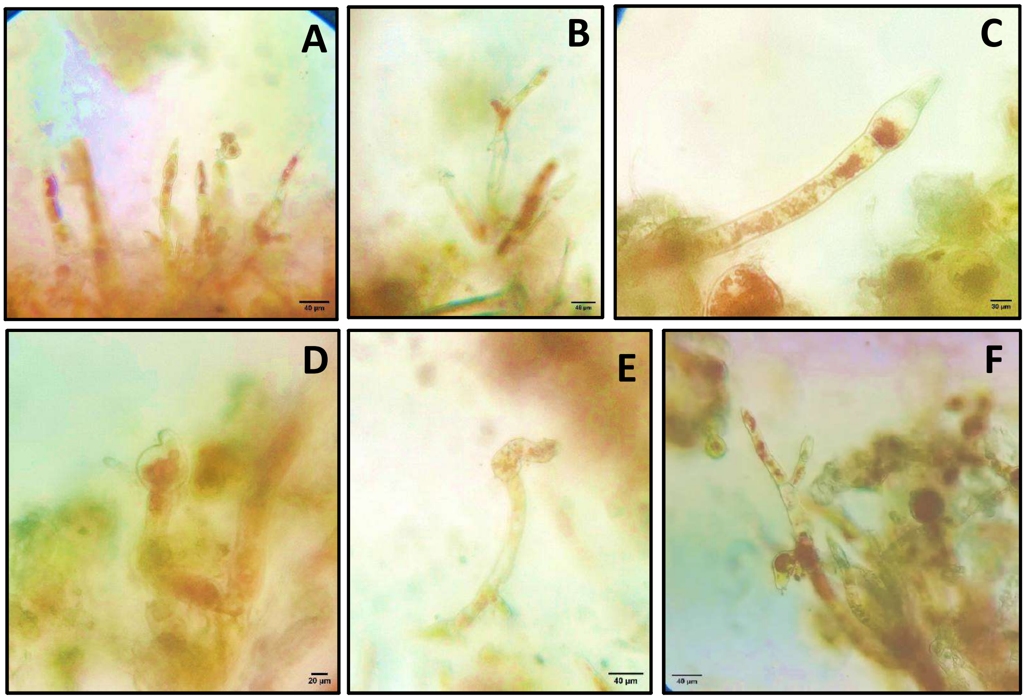

Figure 10. (A - F) Microscopic structures of *Trentepohlia annulata* **A** – Thallus **B** – Branching of Filament **C** – Apical Cell with Pectic Cap **D** –Development of Lateral Zoosporangium **E** – Oblique arrangement of Zoosporangium **F** – Lateral Zoosporangium

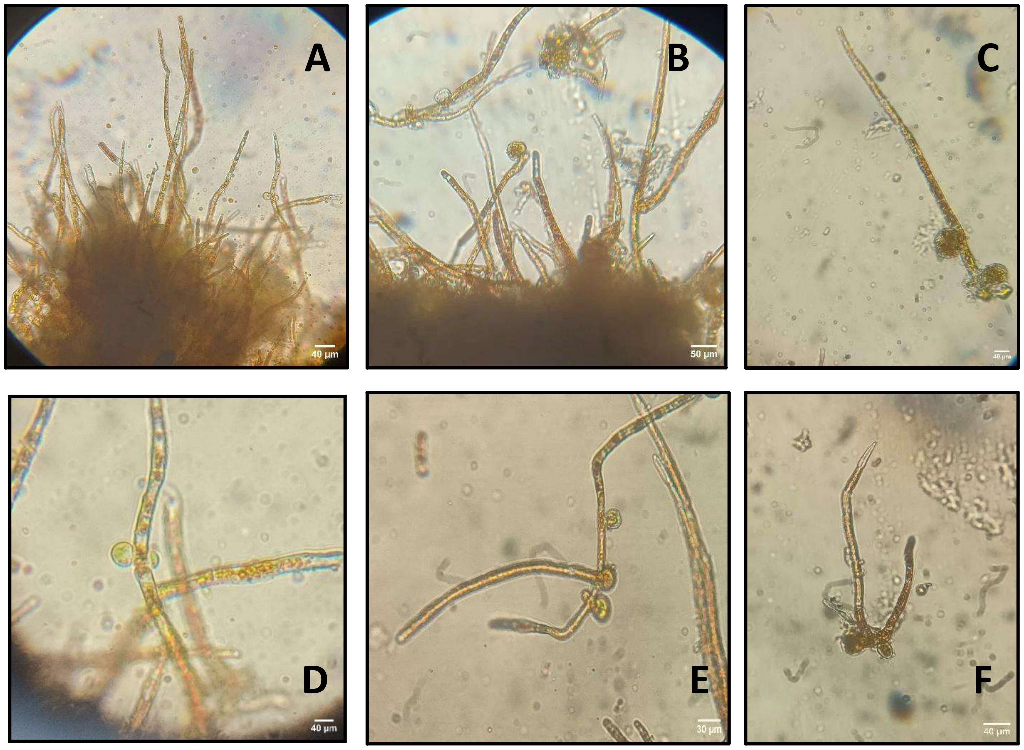

Figure 11. (A - F) Microscopic structures of *Trentepohlia effusa* **A** – Thallus differentiated into Erect and Prostrate parts **B, D and E** – Sessile Sporangia **C** – Single Erect filament with lateral sporangia **F** – Erect filaments arising from Prostrate Part

Table 3. Summary of *Trentepohlia* species surveyed in the sampling sites subjected to Shannon - Weiner diversity index

***Trentepohlia* species**	**Dharwad**	**Belagavi**	**Haveri**
Trentepohlia aurea (Linnaeus) C Martius	0	1	0
Trentepohlia odorata (F. H. Wiggers) Wittrock	1	4	1
Trentepohlia abietina (Flotow ex Kützing) Hansgirg	0	2	2
Trentepohlia rigidula (J. Müller) Hariot	10	3	1
Trentepohlia effusa (Krempelhuber) Hariot	0	1	0
Trentepohlia annulata F. Brand	1	0	0
Trentepohlia abietina var. corrugata (Leighton) Cribb	0	1	0
Trentepohlia abietina var. tenuis (Zeller) A. B. Cribb	1	0	0
Trentepohlia thevalliensis M. V. N. Panikkar and P. Sindhu	0	1	0
Trentepohlia chapmanii Rindi and López-Bautista	1	1	1
Trentepohlia infestans Rindi and J. López-Bautista	1	0	0
Shannon-Weiner Diversity Index	1.61	2.02	1.33

differences could be the variations in the favourable physical conditions of the particular regions for the growth of species.

4. CONCLUSION

The study reveals two characteristic environmental conditions in Karnataka which exhibits a tropical monsoon climate, whereas the northern region of the state generally experiences a subtropical climate characterized by warm to hot summers and mild winters, along with distinct wet and dry seasons. Presence of tropical monsoon climate favouring the growth of *Trentepohlia* species. Since geographically Dharwad and Belagavi comes under the Western Ghat belt, the number of species obtained was more compared to Haveri. The absence of *Trentepohlia* species in other districts was mainly due to low rainfall, low relative humidity, and high temperature and light intensity. All the species documented were previously unreported taxa for this region. These findings constitute new distributional records, indicating a broader biogeographical range than previously known and highlighting the unexplored biodiversity potential of North Karnataka.

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Authors' Contributions

Seema Jagannath Rajapure: Survey, Morphometric evaluation and identification, Data Curation, Original draft preparation, Review and Editing. Doris M. Singh: Conceptualization, Keys Preparation, Supervision

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare no use of AI tools in the writing process.

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Ecological Modelling and Indicator Assessment of Seasonal Fish Diversity in Bhaluka Beel, West Bengal: Integrating GAM, RDA, and CCA Approaches

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***Abstract*:** Understanding seasonal fish diversity in floodplain wetlands is crucial for effective conservation and management. This study assessed the fish assemblage structure of Bhaluka Beel, a monsoon-influenced floodplain wetland in Nadia District, West Bengal, India, using integrated ecological modelling approaches. Fish diversity was quantified across pre-monsoon, monsoon, and post-monsoon seasons alongside key physicochemical parameters, including temperature, dissolved oxygen, pH, free CO_2, alkalinity, and hardness. Generalized Additive Models (GAMs) revealed that temperature and free CO_2 negatively influenced Shannon-Weaver, Margalef's, and Pielou's indices, whereas pH exerted a positive effect. Species richness peaked post-monsoon, coinciding with stabilized water conditions after flood events. Multivariate ordination using Redundancy Analysis and Canonical Correspondence Analysis highlighted distinct seasonal community structures and identified dissolved oxygen and pH as key environmental drivers. Pre-monsoon indicator species included *Puntius ticto*, *Clarias batrachus*, and *Heteropneustes fossilis*. Functional guild analysis demonstrated a diverse assemblage of omnivores, carnivores, herbivores, and planktivores occupying pelagic, littoral, and benthic niches. The coexistence of tolerant and sensitive species reflects both ecological resilience and potential vulnerability to environmental stressors. This integrated modelling framework provides a robust tool for evaluating seasonal biodiversity patterns in tropical wetlands and offers practical insights for fishery management, biodiversity conservation, and water quality monitoring under monsoon-driven hydrological regimes.

Keywords: Multivariate ordination, Environmental gradients, Species assemblage, Wetland biodiversity, Trophic structure, Seasonal variation.

1. INTRODUCTION

Floodplain wetlands are among the most productive and biologically diverse ecosystems on Earth, offering critical habitats for a wide range of aquatic species, including fish, amphibians, birds, and invertebrates (Schofield et al., 2018). In tropical and subtropical regions, these wetlands are strongly influenced by monsoonal hydrology, resulting in seasonal pulses of inundation and drying that profoundly shape community dynamics and ecosystem functioning (Talbot et al., 2018). The periodic flooding not only enhances nutrient availability and habitat connectivity but also plays a key role in the life history strategies of many fish species, particularly those adapted to seasonal migration and spawning cycles (Van der Sleen and Rams, 2023). In India, floodplain wetlands such as beels, jheels, and chaurs constitute essential ecological and economic resources. These water bodies support subsistence and small-scale fisheries, contribute to groundwater recharge, and buffer against climate variability (Sarkar and Saha, 2021). However, anthropogenic pressures—such as land use change, overfishing, nutrient loading, and hydrological alteration—have severely impacted their ecological integrity. Understanding the patterns and drivers of fish diversity across seasonal cycles is thus critical for sustainable management and conservation planning (Zolfagharpour et al., 2022).

Traditional assessments of fish biodiversity in wetlands have relied on diversity indices and community composition analyses. While these provide valuable descriptive insights, they often fail to capture complex and nonlinear interactions between biotic communities and their environmental context (Rosenfeld, 2017; Stefani et al., 2024). To address this limitation, contemporary ecological research has increasingly adopted statistical modelling techniques, including Generalized Additive Models (GAMs), Redundancy Analysis (RDA), and Canonical Correspondence Analysis

(CCA), to explore species-environment relationships with greater precision (Lubinski et al., 2008, Alam et al., 2024; Vavalidis et al., 2025; Zou et al., 2025).

GAMs are particularly well-suited for modelling ecological data due to their flexibility in capturing nonlinear responses to environmental gradients (Clark and Wells, 2023). In aquatic systems, GAMs have been used to analyze temporal trends in species richness, detect thresholds of environmental tolerance, and predict the influence of physicochemical variables on biodiversity (Pilotto et al., 2023). Meanwhile, ordination techniques like RDA and CCA provide multivariate frameworks to assess how entire communities respond to environmental factors, making them valuable tools for habitat characterization and conservation prioritization (Shuai et al., 2018; Akhi et al., 2020).

This study focuses on Bhaluka Beel, a seasonally flooded wetland in the Nadia District of West Bengal, India. The beel represents a typical floodplain system with dynamic hydrology, varied physicochemical conditions, and diverse fish assemblages. Prior research in Bhaluka Beel has documented its ichthyofaunal richness and basic ecological parameters (Sarkar, 2023), but advanced modelling approaches to link environmental variability with fish community dynamics have not yet been applied. The present investigation was designed to develop a comprehensive ecological assessment framework for understanding seasonal fish diversity dynamics in Bhaluka Beel, West Bengal.

2. MATERIALS AND METHODS

2.1. Study Area: Bhaluka Beel is a perennial floodplain wetland situated in the Krishnagar-I Block of Nadia District, West Bengal, India (23.28°N, 88.32°E). This lentic water body is seasonally influenced by the adjacent Jalangi River system and experiences marked hydrological changes associated with the southwest monsoon. The wetland is shallow (average depth: 1.2-2.5 m), supports local fisheries, and is surrounded by agricultural land, which contributes to nutrient influx through runoff during rainy seasons. The study site was selected due to its ecological relevance, biodiversity richness, and previous documentation of ichthyofaunal diversity (Sarkar, 2023).

2.2. Sampling Design and Period: Stratified seasonal sampling with standardized multi-gear capture techniques was conducted seasonally during pre-monsoon (March-May), monsoon (June-September), and post-monsoon (October-January) periods from 2019 to 2022. Three representative stations within the beel were identified based on habitat characteristics, accessibility, and fishing pressure. Although GPS tagging was not applied due to logistical constraints, sampling sites were fixed using permanent physical landmarks to ensure repeatability.

2.3. Fish Collection and Identification: Fish specimens were collected using a combination of gill nets, drag nets, and traditional cast nets operated by local fishers. Sampling effort was standardized across sites and seasons. Collected specimens were identified using standard taxonomic keys (Jayaram, 2010) and cross-verified with FishBase (Froese and Pauly, 2025). Relative abundance was assessed semi-quantitatively using field scoring methods (+, ++, +++) based on catch per effort and validated against community observations.

2.4. Water Quality Analysis: Key physicochemical parameters—temperature (°C), pH, dissolved oxygen (mg/L), free carbon dioxide (mg/L), total alkalinity (mg/L), and hardness (mg/L)—were measured in situ using portable probes and standard analytical kits (APHA, 2017). Sampling was performed between 7:00 and 9:00 AM to minimize diel variability. All measurements were averaged over three subsamples per station to improve accuracy.

2.5. Diversity Indices Calculation:

Seasonal fish diversity was quantified using three standard ecological indices: Shannon-Weaver diversity index (H′), Margalef's species richness index (D), and Pielou's evenness index (J′).

The Shannon-Weaver diversity index (Shannon & Weaver, 1949) was calculated as:

$$H' = -\sum (p_i \ln p_i)$$

where p_i represents the proportion of individuals belonging to the ith species. This index integrates both species richness and relative abundance.

Margalef's richness index (Margalef, 1958) was computed as:

$$D = (S-1) / \ln N$$

where S is the total number of species and N is the total number of individuals.

Pielou's evenness index (Pielou, 1966) was derived as:

$$J' = H' / \ln S$$

where H' is the Shannon diversity and S is species richness. This index measures the uniformity of species distribution within the community.

These indices provide complementary views of community structure by integrating species richness, abundance distribution, and compositional evenness (Sarkar, 2022; Dada et al., 2025; Bastola et al., 2025).

2.6. Ecological Modelling Approaches:

Generalized Additive Models (GAMs) were employed to examine nonlinear relationships between diversity indices and environmental variables. GAMs extend generalized linear models by incorporating smooth functions of predictor variables (Wood, 2017). The general form of the GAM used in this study is (Eq.1):

$$g(\mu) = \beta_0 + s_1(X_1) + s_2(X_2) + ... + s_k(X_k) \ldots..(1)$$

where $g(\mu)$ is the link function, β_0 is the intercept, and $s_k(X_k)$ represents smooth spline functions of environmental predictors such as temperature, dissolved oxygen, pH, free CO_2, alkalinity, and hardness. GAMs are particularly suitable for ecological datasets exhibiting nonlinear responses (Clark & Wells, 2023).

Multivariate ordination analyses were conducted using Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA). RDA is a constrained linear ordination technique that relates species composition to environmental gradients (Legendre & Legendre, 2012). CCA, in contrast, assumes unimodal species responses along environmental gradients (Legendre & Gallagher, 2001). Prior to analysis, environmental variables were standardized to reduce scale effects. The significance of canonical axes was assessed using permutation tests.

2.7. Indicator Species and Functional Guild Classification: Species were assigned as seasonal indicators based on relative abundance differences across seasons. A species was considered an indicator if its occurrence score was highest in only one season. Functional guilds were categorized using ecological literature and FishBase data, assigning each species to trophic (herbivore, omnivore, carnivore, planktivore), habitat (benthic, pelagic, littoral),

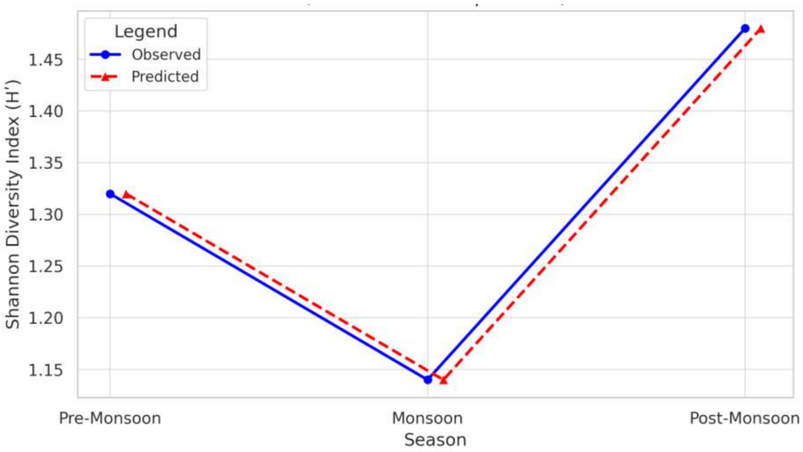

Figure 1. Observed vs. predicted Shannon–Weaver diversity index (H′) for fish communities in Bhaluka Beel

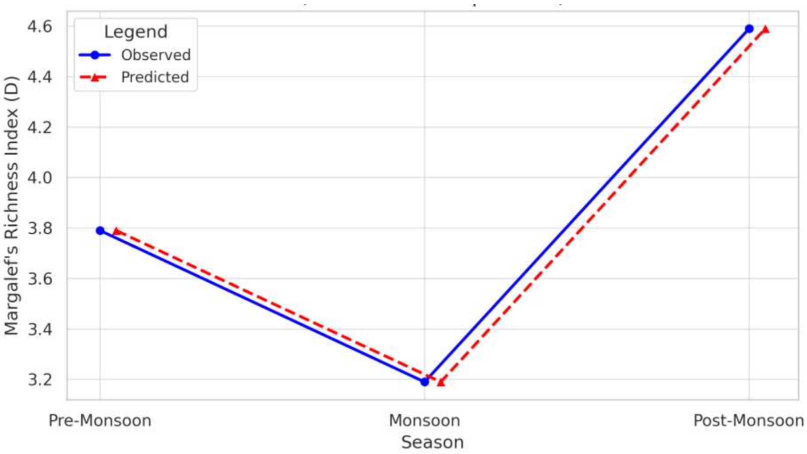

Figure 2. Observed vs. predicted Margalef's Species Richness Index (D) for fish communities in Bhaluka Beel

and tolerance (sensitive, moderate, tolerant) groups (Froese and Pauly, 2025).

3. RESULTS AND DISCUSSION

The ecological modelling framework applied in this study yielded comprehensive insights into the seasonal dynamics of fish diversity in Bhaluka Beel, a monsoon-influenced floodplain wetland. By integrating Generalized Additive Models (GAMs), Redundancy Analysis (RDA), Canonical Correspondence Analysis (CCA), indicator species analysis, and functional guild classification, the study elucidates both statistical patterns and ecological mechanisms shaping fish assemblages.

3.1. Seasonal Patterns in Diversity Indices

Seasonal analysis revealed consistent interannual patterns in fish diversity. Both Shannon-Weaver diversity (H′) and Margalef's richness (D) peaked during the post-monsoon phase (Figure 1-2), reflecting ecological recovery and recolonization following monsoonal flooding. In contrast, the monsoon season exhibited comparatively lower diversity values, likely due to habitat disruption, dilution effects, and hydrological instability (Chea et al., 2020; Saha et al., 2022; Liu et al., 2023). Evenness (Pielou J′) remained relatively stable across seasons (Figure 3), indicating consistent proportional distribution of individuals among species (Sarkar, 2023).

These seasonal patterns align with the flood-pulse concept, which posits that hydrological fluctuations regulate nutrient cycling, habitat connectivity, and biodiversity in floodplain systems (da Silva et al., 2013; Das et al., 2023). Post-monsoon stabilization of water levels likely enhances habitat heterogeneity and resource availability, supporting greater species richness and diversity.

3.2. Environmental Drivers and GAM Modelling

GAM results revealed significant environmental filtering effects (Table 1). Temperature and free CO_2 exerted consistent negative influences on diversity indices, whereas pH demonstrated strong positive associations. Dissolved oxygen exhibited non-linear responses, particularly during seasonal transitions, highlighting threshold effects.

The best model fit was observed for the Shannon index, which showed the highest explanatory power and lowest residual variance. Observed and predicted values closely matched across seasons (Figure 1), confirming model robustness. Margalef's richness also displayed strong

Table 1. Estimated coefficients from Generalized Additive Models (GAMs) predicting Shannon diversity (H′), Margalef's richness (D), and Pielou's evenness (J′) based on environmental variables in Bhaluka Beel

Predictor	Shannon (H′)	Margalef (D)	Evenness (J′)
Intercept	0.0122	0.0432	0.0052
Spline (Temperature)[1]	-0.0041	-0.0153	-0.0009
Spline (Temperature)[2]	-0.0107	-0.0400	-0.0024
Spline (Temperature)[3]	-0.0281	-0.0998	-0.0118
Spline (DO)[1]	-0.0047	-0.0174	-0.0011
Spline (DO)[2]	-0.0109	-0.0407	-0.0025
Spline (DO)[3]	0.0472	0.1685	0.0186
pH	0.1061	0.3774	0.0436
Free CO_2	-0.0824	-0.3134	-0.0123
Alkalinity	-0.0534	-0.1868	-0.0274
Hardness	0.0706	0.2403	0.0385

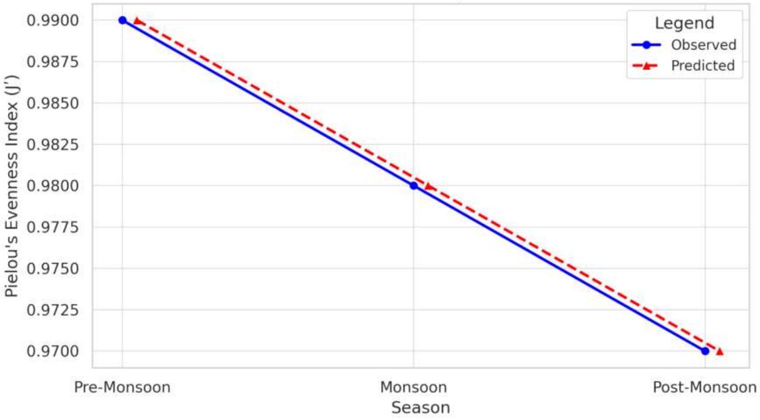

Figure 3. Observed vs predicted Pielou's Species Evenness Index (J′) for fish communities in Bhaluka Bee

seasonal predictability (Figure 2), with post-monsoon peaks corresponding to improved water quality conditions. Evenness (Figure 3), however, exhibited comparatively subtle responses, suggesting that community equitability remains less sensitive to physicochemical variability.

Ecologically, elevated temperature during the pre-monsoon period likely induces thermal and oxygen stress, disproportionately affecting sensitive taxa (Lianthuamluaia et al., 2024). Increased free CO_2, often linked to organic decomposition and microbial respiration, may further reduce habitat suitability (Cupp et al., 2020). Conversely, slightly alkaline pH conditions appear to enhance physiological performance and diversity (Napit and Pillai, 2024).

The effectiveness of GAMs underscores the importance of flexible modelling approaches capable of capturing non-linear species-environment interactions, particularly in seasonally dynamic systems (Sun et al., 2024).

3.3. Multivariate Community Structuring (RDA and CCA)

Ordination analyses further clarified multivariate ecological responses. RDA indicated that pH and dissolved oxygen were dominant environmental axes structuring fish assemblages, particularly in the post-monsoon season (Figure 4). In contrast, CCA effectively revealed unimodal species responses, with distinct clustering of pre-monsoon communities associated with elevated temperature and free CO_2 (Figure 5).

Pre-monsoon assemblages were dominated by air-breathing catfishes and tolerant omnivores, reflecting adaptive strategies under stress conditions. These findings corroborate earlier observations that ordination techniques effectively characterize niche differentiation and environmental filtering in floodplain ecosystems (Glińska-Lewczuk et al., 2016; Delong et al., 2019; Shields et al., 2021).

The separation of seasonal clusters confirms that physicochemical gradients strongly regulate compositional turnover and ecological niche partitioning.

3.4. Indicator Species and Seasonal Associations

Indicator species analysis identified *Puntius ticto*, *Clarias batrachus*, and *Heteropneustes fossilis* as strong pre-monsoon indicators (Table 2). These taxa are known for tolerance to low dissolved oxygen and elevated temperature, enabling persistence during water-stress periods (Alam et al., 2024).

Interestingly, no exclusive indicator species were

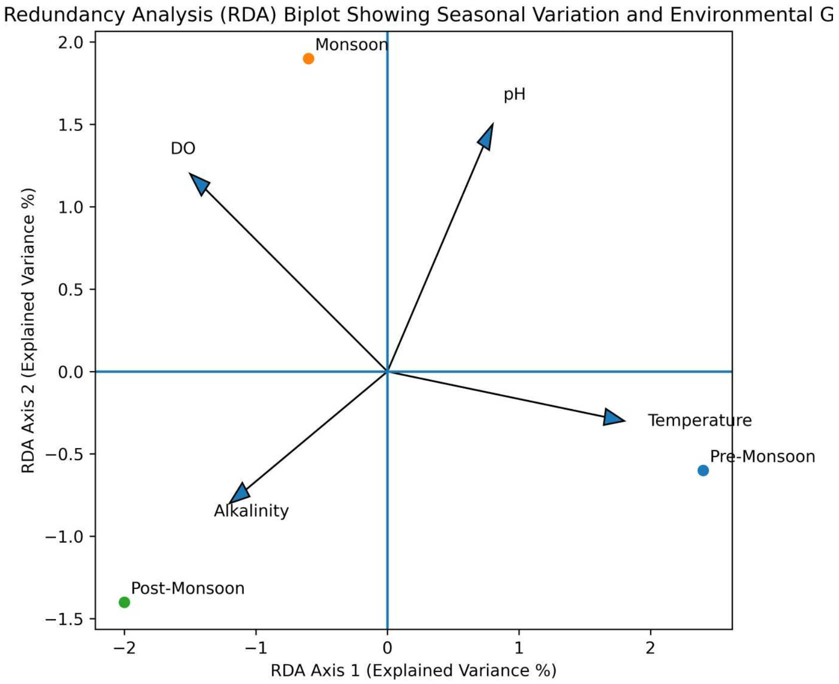

Figure 4. Redundancy Analysis (RDA) ordination plot showing the relationship between environmental variables and fish assemblage structure in Bhaluka Beel

detected for monsoon or post-monsoon seasons. This pattern likely reflects habitat homogenization during peak flooding, when increased connectivity facilitates dispersal and overlap among taxa (Espínola et al., 2017; Qiu et al., 2024). Thus, post-monsoon richness, although high, is broadly shared across species rather than dominated by season-specific specialists.

3.5. Functional Guild Composition and Ecological Implications

Functional guild classification revealed a diverse and ecologically versatile community (Table 3). Omnivores dominated the assemblage, followed by carnivores, herbivores, and planktivores. The coexistence of benthic and pelagic guilds highlights habitat heterogeneity within the wetland.

Table 2. Seasonal indicator fish species identified from Bhaluka Beel

Species	Pre-Monsoon	Monsoon	Post-Monsoon
Puntius ticto	3	0	1
Aorichthys (Sperata) aor	1	0	0
Clarias batrachus	3	2	2
Heteropneustes fossilis	3	2	2
Channa striata	3	2	2

3 (abundant); 2 (moderate); 1 (rare); 0 (absent)

Tolerance classification indicated the presence of both sensitive species (e.g., *Catla catla*, *Hypophthalmichthys molitrix*) and highly tolerant taxa (e.g., *Clarias batrachus*, *Cyprinus carpio*). While tolerant species enhance system stability under environmental stress, their increasing dominance may also signal ecological degradation, particularly under eutrophic or oxygen-limited conditions (Feng et al., 2023; Urbanski and Nogueira, 2024).

Projected increases in temperature and CO_2 under climate change scenarios may further shift community composition toward tolerant and air-breathing species, potentially reducing functional diversity (Dahms and Killen, 2023; Liu et al., 2025).

3.6. Integrated Ecological Interpretation

The integration of GAMs, ordination analyses, indicator species assessment, and trait-based classification provides a robust framework for understanding biodiversity dynamics in seasonally dynamic wetlands. The findings underscore the ecological importance of maintaining hydrological variability and water quality integrity to sustain diverse and functionally balanced fish assemblages.

This integrative modelling approach is transferable to other monsoon-driven wetlands across South Asia and can support adaptive conservation planning in regions facing accelerating climatic and anthropogenic pressures (Roy-Basu et al., 2020).

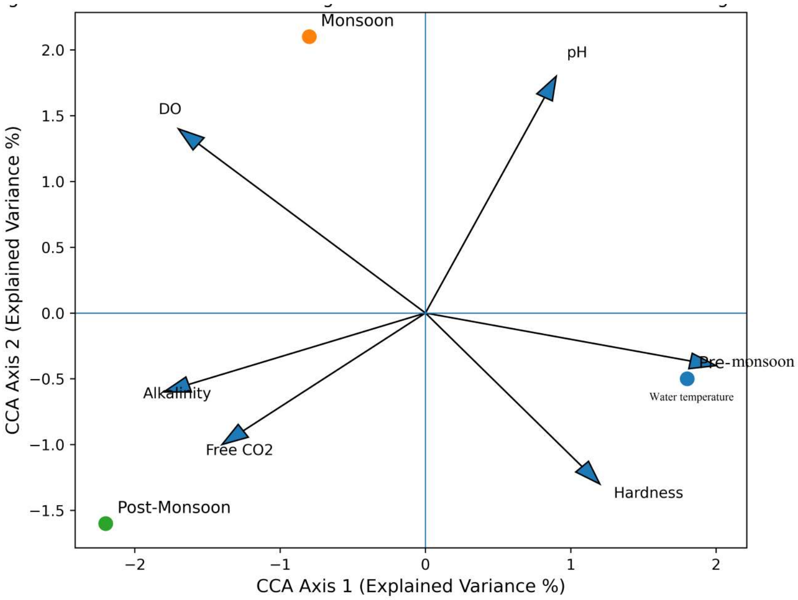

Figure 5. Canonical Correspondence Analysis (CCA) ordination plot showing the influence of environmental variables on fish assemblage structure in Bhaluka Beel across seasons

Table 3. Functional guild classification of selected fish species

Species	Trophic guild	Habitat guild	Tolerance level
Labeo rohita	Herbivore	Pelagic	Moderate
Labeo bata	Omnivore	Pelagic	Moderate
Labeo calbasu	Omnivore	Benthic	Moderate
Gibelion (Labeo) catla	Omnivore	Pelagic	Sensitive
Cirrhinus mrigala	Detritivore	Benthic	Moderate
Puntius sarana	Omnivore	Littoral	Sensitive
Puntius ticto	Omnivore	Littoral	Tolerant
Hypophthalmichthys molitrix	Planktivore	Pelagic	Sensitive
Cyprinus carpio	Omnivore	Benthic	Tolerant
Ctenopharyngodon idella	Herbivore	Pelagic	Moderate
Notopterus notopterus	Unknown	Unknown	Unknown
Notopterus chitala	Unknown	Unknown	Unknown
Mystus vittatus	Unknown	Unknown	Unknown
Aorichthys (Sperata) aor	Unknown	Unknown	Unknown
Wallago attu	Carnivore	Benthic	Moderate

4. CONCLUSION

This study provides an integrated ecological assessment of fish diversity in Bhaluka Beel, a monsoon-driven floodplain wetland in West Bengal, India. Seasonal surveys combined with statistical modelling and trait-based analyses revealed that temperature and free CO_2 act as stressors, whereas pH and dissolved oxygen promote richer, balanced communities. Generalized Additive Models highlighted nonlinear species-environment relationships, while RDA and CCA captured community structuring and niche differentiation across seasonal phases, particularly the ecological recovery in post-monsoon and stress-adapted pre-monsoon assemblages. Functional guild analysis demonstrated a diverse community dominated by omnivores alongside carnivores, herbivores, and planktivores, reflecting both resilience and vulnerability. Indicator taxa such as *Clarias batrachus* and *Puntius ticto* underline the monitoring potential. The findings advocate adaptive wetland management that safeguards hydrological variability and sustains biodiversity.

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Authors' Contributions

Chandan Sarkar: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Visualization, Writing - original draft, Writing - review & editing.

The author has read and approved the final manuscript.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

Data Availability

The datasets analyzed during the current study are available from the author upon reasonable request.

Declaration about use of AI tools

The author declares that artificial intelligence (AI) tools were used only for language refinement, formatting assistance, and improvement of clarity. All research design, data collection, analysis, interpretation, and scientific conclusions were independently developed by the author. The author takes full responsibility for the accuracy, integrity, and originality of the manuscript.

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Biometric Insights and Growth Patterns of the Endangered Golden Mahseer (*Tor putitora*) Inhabiting the Gobind Sagar Reservoir

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***Abstract*:** The aim of this study is to document the life history parameters of the endangered and widely distributed fish, *Tor putitora* (Golden Mahseer) in Gobind Sagar Reservoir, Himachal Pradesh toward effective conservation and management. Specimens were collected monthly at the two sites during 18 months (April 2023-September 2024). Biometric characteristics (morphometric and meristic parameters), length-weight relationship, length- length relationship, condition factor and truss networking analysis were evaluated. More than 70% of the species d in the reservoir were planktivores, which clearly indicates that the plankton plays an important role for the successful management of the reservoir with Intra and Interspecies competition among other planktivore fish species and targeted fish species. Mahseer species were significantly declined in this reservoir and reported fish weight varied between 425g and 2710g) and length between 31.9 cm and 63.1 cm (The calculated regression coefficient (*b*) were 3.162, indicating a positively allometric growth pattern-where body weight increases at a greater rate than length, resulting in a more robust body shape as the fish grows. The condition factor (*K*) and relative condition factor (*Kn*) reflected overall good health and growth performance. PCA of the truss network system showed that the first three components-PC1, PC2, and PC3-explained 78.38, 18.38, and 13.03% of the total variance, respectively. The information can provide an extensive knowledge of fish welfare, allowing for the development of a suitable and cost-effective plan to manage and conserve fish populations and biomass. The present findings would be of prime importance for conservation and management of *T. putitora* in the Himalayan aquatic ecosystems.

Keywords: *Tor putitora*, Gobind Sagar Reservoir, Truss networking analysis.

1. INTRODUCTION

Gobind Sagar Reservoir, a prominent aquatic ecosystem, supports a wide variety of fish, including 51 species native to the Sutlej River. This collection includes rare trout, snow trout, and numerous hill stream fish species (Bhatnagar, 1964). Among them, the golden mahseer, *Tor putitora*, stands out as a significant species in the region, often recognized as a prized game fish in India (Thomas, 1897). The landings in the Gobind Sagar reservoir are clearly declining due to intense competition from exotic carps (*Cyprinus carpio* and *Hypophthalmichthys molitrix*) (Johal et al., 2003). The majority of fish species found in the Himalayan region are small in size, and factors affecting their size, growth, and distribution includes water temperature, current velocity, type of substrate, food availability, and feeding patterns (Yousuf et al., 2003).

The mahseer belonging to the order cypriniforms, is native to this reservoir. The genus *Tor* contains a group of game/sport fish and is significant endemic to the Indian subcontinent and economically exploited in their natural habitat. The fish called the golden mahseer, or *Tor putitora*, found in South and Southeast Asia, including Afghanistan, Pakistan, India, Bangladesh, Nepal, Bhutan, Myanmar, Sri Lanka, western Iran and Thailand. It is generally located between 8°N and 36°N and 70 to 1,891 meters altitude (Jha et al., 2018). Despite its significance, this fish species is facing a decline in its wild populations caused by the anthropogenic activities. The status of species was listed Endangered (EN) in International Union for Conservation of Nature (IUCN) Red List with the species facing high threat from over fishing, habitat loss, decline in habitat quality leading to loss of breeding grounds and environmental damages such as construction of dams which has disabled their migration and had adverse effects on breeding (Jha et al., 2018).

Biometric characteristics are one of the common and cheap methods which is used for identification of stock. Morphometric variation for phenotypic stock discrimination in fisheries biology has attracted the attention of ichthyologists (Miyan et al., 2016; Dwivedi, 2019). In response to the shortcomings of traditional morphometric analyses, image analysis methods have also been developed to supplement the development and heterogeneity of morphometric approaches, advancing the potential for stock identification (Reyes-Valdez et al., 2011). The "Truss Network System" based on precise measured coordinates of morphometric reference points or "landmarks", is widely used for stock identification (Miyan et al., 2016). The truss network approach is a useful method to detect geographically diverging stocks. Isolation can generate morphological, meristic, and form differences among stocks of a species to the point of meeting criteria for recognition of populations of that species. The traits could have more importance for investigating short term computation off generated differences in the environment and the result may be useful for fish management (Cadrin, 2005). The current study conducted an effort to determine the *T. putitora* biometrics characteristics, length–weight relationship and truss networking analysis from the Gobind Sagar reservoir.

2. MATERIALS AND METHODS

2.1. Study Area and sampling

The present study was conducted in the Gobind Sagar Reservoir, and samples were collected from two sites. Site I was located at Bhakra Road, Bilaspur, Himachal Pradesh, at a latitude of 31°42'46"N and a longitude of 76°43'82"E. Site II was situated at Paroyan, Birhu Kalan, Una, Himachal Pradesh, at a latitude of 31°42'46"N and a longitude of 76°43'82"E.The present study was conducted for a period of 1.5 years from April 2023 to September 2024.

2.2. Collection of Fish Samples and Assessment of Biometric Data

The current investigation examined fish biodiversity and population structure in the Gobind Sagar Reservoir, highlighting the status of the native golden mahseer (*T. putitora*). In the present study, 50 fish specimens each of *T. putitora* were collected monthly from landing centers to cover the pre-spawning, spawning, and post-spawning phases. Length, weight and other biometric parameters have been estimated on the landing site using measuring board, Vernier calliper and weighing balance. Nineteen morphometric and 5 meristic characters were studied. Total length, standard length, head length, pre-orbital (snout length), eye diameter, and post-orbital length, pre-dorsal length, post-dorsal length, pre-pelvic length, pre-pectoral length, post-pelvic length, and pre-anal length were measured in the same manner, along the specimen left side. Measurements of morphometric parameters were taken to the nearest 0.1 cm with the aid of a wooden measuring board and measuring scale. Weight (g) was measured on a digital balance (accuracy ±0.01 g).

2.3. Length-Weight and Length-length Relationship

Cubes equations were used to compute the relationship between length and weight Froese (2006).

$W = aL^b$

Where,

W = weight of the fish (g)

L = length of the fish (mm)

a = constant

b = exponential coefficient

A linear function was used to the data to estimate the morphometric relationship between these factors (Ricker, 1975) (Eq 1).

$\text{Log } Y = \text{Log } a + b \text{ Log } X$……………..(1)

The length-weight relationship parameters 'a' and 'b' were determined using linear regression analysis, and the coefficient of correlation (r) and determination coefficient (r^2) were computed to determine the degree of link between two parameters.

Growth can be classified as positive allometric when $b>3$ and negative allometric when $b<3$ in length-weight relationships. The length-length relationships (LLRs) between Total length (TL) and Fork length (FL), Standard length (SL), Head length (HL) were calculated using linear regression analysis (FL). The equation 2 was used to express these length relationships.

$Y = a + bX$…………………..(2)

Where,

Y= a dependent variable (various body lengths)

X = an independent variable (total length)

a = constant (intercept)

b = regression coefficient (slope)

2.4. Condition factor (K) and Relative Condition Factor (Kn)

Condition factor (K) was calculated using the formula (Eq 3) of Froese (2006)

$K = W * 100 / L_3$………………………………(3)

Where,

W = weight of the fish (g)

L = length of fish (cm)

The ratio between the actual weight (observed weight) and the calculated weight based on the length-weight equation 4 was defined as the relative condition factor (Kn) (Froese, 2006).

$Kn = W/\hat{W}$

Where,

W= Observed weight (g)

$\hat{W}$= Calculated weight (log W * log L/ log L^2)………………………..(4)

2.5. Truss Network System

Fish specimens were routinely oriented on their right side on laminated graph paper with bodies and fins gently coaxed into a natural configuration. All samples were coded to protect their identity. The digital images were taken using an image acquisition system (Sony Cyber-shot DSC-W300 digital camera). After photographing, a sex was developed on each fish by external observation. TPSUTIL, version 1.52 (Rohlf, 2006; available at https://bit.ly/3fjhyDA) was used to convert JPEG images of photographed fish to TPS format. The program TPSDIG version 2.16 was applied to digitize 12 landmarks in the two-dimensional Cartesian coordinate format on each sample (Rohlf, 2006). Each specimen had its scale factor calculated using the set scale option for reference length.

The software Paleontological Statistics (PAST, v. 1.89) was utilized to produce truss-typed data by connecting each of the landmarks. Distances between landmarks generated 90 inter-landmark morphometric variables. Log transformation of the PAST data was used to maintain allometric relationships and similarity of variances (Hammer, 2001) and employed the following formula to account for size dependence (Eq5):

$Madj = M(L_s/L_0)^b$…………………………………(5)

Where, M was the original measure, L_0 was the fish's standard length, L_s was the average of standard length for all fish in each analysis, and b was calculated for each character from the data that was observed as the slope of the regression of log(M) on log(L_0) using all fish from each group.The final analysis excluded standard length (SL; character codes 1-6) as it was employed as a basis for transformation, providing 90 morphometric variables (Rohlf 2006).

Size-adjusted morphometric traits of *T. putitora* were compared with standard length to test significant differences, while sex was used as a class variable to assess male–female variation. Highly significant variables were subjected to principal component analysis (PCA) and discriminant function analysis (DFA) to examine morphometric variation. PCA reduced redundancy following Veasey et al. (2001) and Samaee et al. (2006), whereas LTD identified independent variables explaining inter-population differences. Principal components with eigenvalues >0.70 and factor loadings ≥0.30 were retained. Wilks' λ tested group differences, revealing significant morphometric variation ($P < 0.001$) across populations. The data from Discriminant Function Analysis (DFA) were used to calculate the percentage of correctly categorized cases. To estimate the predicted error rates of classification functions, cross-validation was performed by calculating the proportion of correctly classified cases. Morphometric data were analyzed using SPSS version 16.0.

Two-dimensional X-Y coordinate data for 12 landmarks on all 50 specimens were obtained using ImageJ version 1.50i (http://imagej.nih.gov/ij/) and exported in TPS format. Procrustes superimposition was applied to translate landmarks into shape, standardizing each specimen based on centroid size, which serves as an estimate of the total body size coordinates (Rohlf and Slice, 1990). Partial Least Squares (PLS) analysis identified significant differences ($P < 0.001$) between shape and size, and shape and sex. Relative warp analysis (TPSRELW) with wireframe visualizations illustrated form variations linked to seasonal migration. Canonical Variate Analysis (CVA) and Principal Component Analysis (PCA) were applied to assess and classify shape differences across populations. CVA-derived Mahalanobis and Procrustes distances confirmed significant inter-population variation All analyses, including visualization and statistical tests, were performed using MorphoJ version 1.06d, providing robust insights into morphological divergence and the main axes discriminating groups.

3. RESULTS AND DISCUSSION

3.1. Assessment of Historical Database stock of *T. putitora* in Gobind Sagar Reservoir

The fish biodiversity and stock structure of the Gobind Sagar Reservoir, focused on the status of the indigenous golden mahseer (*Tor putitora*). Eighteen commercially important species were identified based on abundance, economic value and market demand. Exotic species dominated the catch, with silver carp (*Hypophthalmichthys*

Figure 1. Locations of 12 landmarks used for shape analysis for *T. putitora*

molitrix) comprising 65.1%, common carp (*Cyprinus carpio*) 13.27% and bighead carp (*H. nobilis*) 6.58%. In sharp contrast, native species such as *T. putitora* constituted only 2.22% of the total fish population, while *Catla* and *Labeo rohita* made up 0.58 and 0.03%, respectively (Figure 2). This marked decline in *T. putitora* on the basis of number and weight of fish species which reflects habitat degradation, loss of spawning grounds, overfishing, and hydrological modifications restricting migration. Filter-feeding and benthic-foraging behaviors of exotic species have further altered nutrient balance, water clarity and native spawning habitats. Compared to historical records, the present findings indicate a serious ecological shift from native to exotic dominance (Strayer, 2009). These results highlight the urgent need for conservation, habitat restoration and management interventions to protect *T. putitora* and preserve indigenous fish biodiversity.

3.2. Biometric Characteristics

Fresh specimens exhibited distinctive greenish body coloration, transitioning to silvery along the flanks, with noticeable reddish-yellow or golden hues on the anal and pectoral fins. The species is characterized by a slightly prognathous upper jaw, with the lower jaw being marginally shorter. As per the meristic characters of fish studied, the dorsal fin ray of the specimens found to be 17; pectoral fin ray was 7-9, anal fin ray 8, pelvic fin ray count 10-11 while caudal fin ray count was 21-22. Based on this, the derived fin formula of *T. putitora* is D/17; P/7-9; V/10-11; C/22. All meristic characters observed in the present study were in the same ranges with few modifications (in no. of rays) as described by (Talwar and Jhingaran, 1991; Jayaram, 1999). In total, 19 morphometric and five meristic characters were documented for the selected species (Table 1). The total weight of fish ranged from 2710g (max) to 425g (min), while total length ranged from 31.9 cm (min) to 63.1 cm (max). Average standard length and fork length were 39.75 cm and 42.39 cm, respectively.

The biometric data of *T. putitora* indicates a healthy and balanced population structure. Juveniles (28% length, 34% weight) show strong growth and successful recruitment. Mature individuals make up the largest group (50% length, 44% weight), reflecting reproductive vitality, though slightly less developed in weight. Over-aged fish (22% for both length and weight) have reached a growth plateau, indicating natural aging with no excessive dominance, suggesting a well-distributed age composition (Table 2).

Studies suggest that mahseer attains sexual maturity at around 40-50 cm in length and 1-2 kg in weight, with larger females contributing significantly to population recruitment (Nautiyal, 2014). The gonadosomatic index (GSI), a key reproductive metric, tends to peak during the pre-monsoon and monsoon seasons, aligning with optimal spawning conditions such as increased water flow and temperature stability (Sharma et al., 2021). The higher body weight and

Table 1. Morphometric and meristic characters of *T. putitora* during April, 2023 – September, 2024

Measurements	Max.	Min.	Mean ±S.E.
Morphometric characters			
Total weight (g)	2710	425	1054.84±90.92
Total length (TL) (cm)	83.1	31.9	45.76± 1.067
Standard length (SL)	55.8	28.6	39.75±0.978
Fork length (FL)	58.5	29.1	42.39±1.009
Eye diameter (ED)	1.2	0.3	0.856±0.071
Dorsal fin length (DFL)	8.2	4.2	6.352±0.435
Pelvic fin length (PeFL)	8.5	2	4.111±0.351
Pectoral fin length (PFL)	6.2	5.1	5.621±0.175
Pre- Dorsal fin length (PDFL)	26.1	20.1	19.348±0.388
Pre- Pelvic fin length (PPeFL)	14.6	8.5	10.141±0.23
Pre- Pectoral fin length (PPFL)	31.3	15.5	20.98±0.526
Pre-orbital length (POL)	4.7	3	3.652±0.108
Post- orbital length (PoOL)	7.7	4.5	6.19±0.222
Snout length (SL)	4	2.1	3.656±0.162
Head length (HL)	18.2	5.5	7.623±0.199
Head diameter (HD)	5.2	3.1	4.508±0.281
Body depth (BD)	10.3	4.7	9.471±0.587
Anal fin length (AFL)	7.3	5.5	6.539±0.218
Pre-anal fin length (PAL)	43.4	26.7	30.566±0.79
Meristic characteristics			
Dorsal fin rays (DFR)	17	8	16.137±0.314
Anal fin rays (AFR)	8	8	7.431±0.117
Pectoral fin rays (PFR)	9	7	8.762±0.147
Pelvic fin rays (Pe.FR)	11	10	11.058±0.161
Caudal fin rays (CFR)	22	21	20.117±0.255

All measurements of length were made in centimeters. Mean values ± Standard Error are used to express the results (n=50)

Table 2. Stock structure of *T. putitora* in Gobind Sagar reservoir

Life stages	Length (%)	Weight (%)
Newly recruitment stage	28	34
Mature individuals	50	44
Over-aged size	22	22

larger length groups in the studied population indicate the presence of mature individuals with significant reproductive contributions. The presence of smaller individuals (31.9 cm, 425 g) suggests an active recruitment process within the population, demonstrating successful spawning and juvenile survival. However, growth rates can be influenced by environmental factors, including water quality, food availability, and anthropogenic.

All meristic parameters, including dorsal fin rays (DFR), pectoral fin rays (PFR), pelvic fin rays (PeFR), anal fin rays (AFR), and caudal fin rays (CFR), are consistent across species with different body lengths. Therefore, it is evident that meristic counts do not change as body length increases and are not impacted by body size). Morphometric characters like total length (TL), standard length (SL), fork length (FL), head length (HL), head depth (HD), snout length (SL), body depth (BD), and eye diameter (ED) all exhibited symmetrical growth when observed in different length groups. Bannikov and Tyler (2008) demonstrated that observable physical characteristics are essential for species recognition. Johal et al.(2003) reported 18 morphometric characters and 7 meristics characters from 16 specimens of *T. putitora* in the pong reservoir, Himachal Pradesh with 79.55 cm of maximum standard length followed by head length of 19.23 cm, maximum body width of 17.60 cm, which showed a high degree of association coefficient, although the head depth, caudal fin length, and caudal peduncle length showed the least correlation. In present study standard length ranged from 14 to 32 cm, while the fork length (FL) ranged from 15.2-34 cm. with correlations of 0.977 and 0.976, respectively, were the parameters that had the strongest relationship with total length. The meristic counts of *T. putitora* stayed constant as body length increased. These counts included the number of lateral line scales, pectoral fin rays, pelvic fin rays, dorsal fin rays, anal fin rays, and caudal fin rays.

3.3. Length – Weight Relationship

In the present investigation, the determined regression coefficient 'b' for *T. putitora* was found to be 3.162 whereas the logarithmic equation is calculated asLog W= -2.278+3.162 Log L, suggest that species are exhibiting a positively allometric growth pattern, in which weight increases with length and fish become more rotund as length increases (Figure 3).

Significant portion of the studied population comprises pre-mature and mature individuals, indicating a functioning breeding population within the ecosystem. Regression coefficient 'b' for *T. putitora* was found to be > 3; and

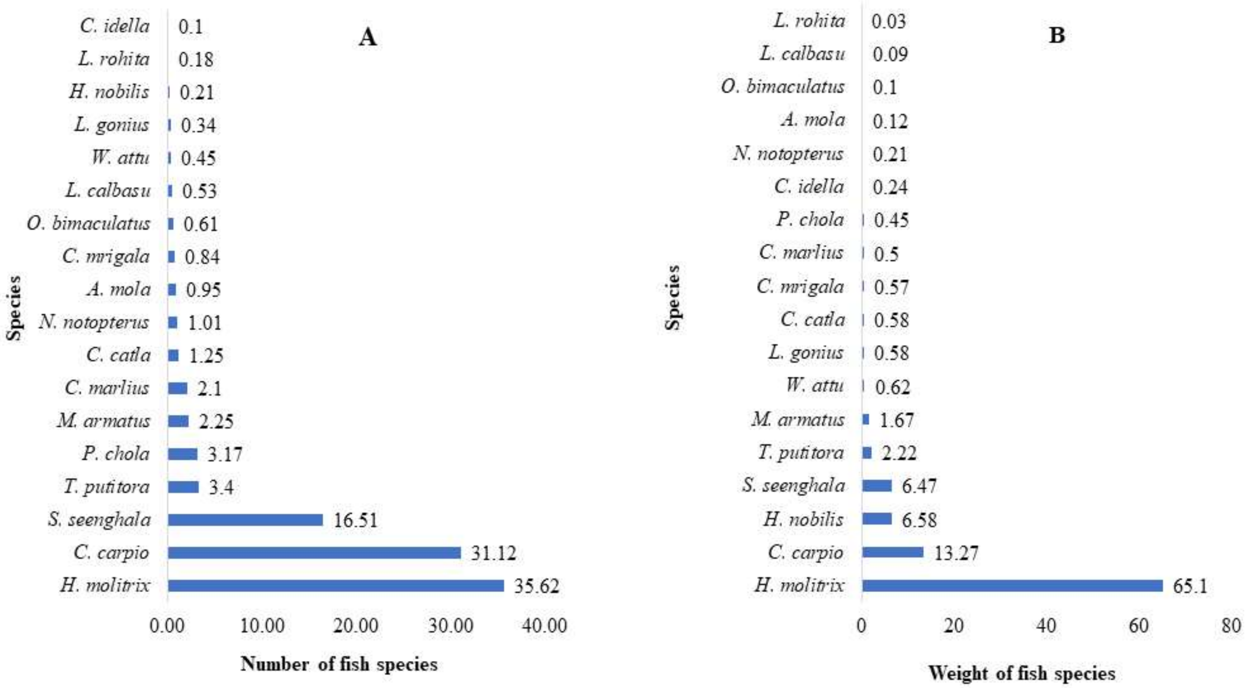

Figure 2. Fish catch composition based on number and weight (A&B) recorded in Gobind Sagar reservoir (April 2023 to September 2024)

exhibiting positively allometric growth pattern. Arslan et al. (2004) indicated that the "b" value in fish length-weight correlations is a crucial biological measure of growth pattern that takes into consideration dietary intake and shifting ecological conditions. Froese (2006) observed that fish maintains its specific gravity constant throughout its life by maintaining its shape and growing isometrically with regression coefficient 'b'=3. In the present study b value indicates as positive allometric growth pattern.

Similarly, *T. putitora* "b" value from the Ladhiya River in Uttarakhand was 2.99, indicating isometric growth through a strict cubic law (Patil et al., 2010). Ali et al. (2014) documented the isometric development pattern of golden mahseer from four distinct Himalayan River regions in India. Some of the samples grown more in weight than in length, suggesting that they were in very nutrient-rich environments when they were sampled (Froese, 2006).

3.4. Length - Length Relationship (LLRs) of *T. putitora*

All LLRs in *T. putitora* were highly correlated with 'r' values ranging from 0.816 to 0.992. The highest correlation between SL and FL (r^2=0.984) followed by TL-FL (and TL-SL (relationships. All LLRs were highly significant, with most of the coefficient of determination values being >0.666 (Figure 3). Various environmental (water depth, temperature, pH, turbidity) and biological factors (size, genetic profile) are responsible for variations between the body measurements (Ezeafulukwe et al., 2015; Ramasamy and Rajangam, 2016). Khan et al. (2012) correlated the length-length relationships of four species (*L. bata, C. punctata, O. pabda, M. armatus*) from river Ganga with coefficient of determination of >0.9, which was slightly different with findings of present study (r^2= 0.827-0.982).

3.5. Condition Factor and Relative Condition Factor

During the monsoon, the condition factor (K) and relative condition factor (Kn) values were highest, mean condition factor being 1.14 ± 0.07 and relative condition factor is 1.65 ± 0.009. The premonsoon season is also characterized with high values of Condition factor (K) = 1.06 ± 0.06 and Kn = 1.21 ± 0.003). However, sharp decrease was observed in post-monsoon season as the condition factor (0.91±0.05) and Kn (1.01±0.09) falls (Table 3).

During the monsoon, the condition factor (K) and relative condition factor (Kn) values were highest. This peak

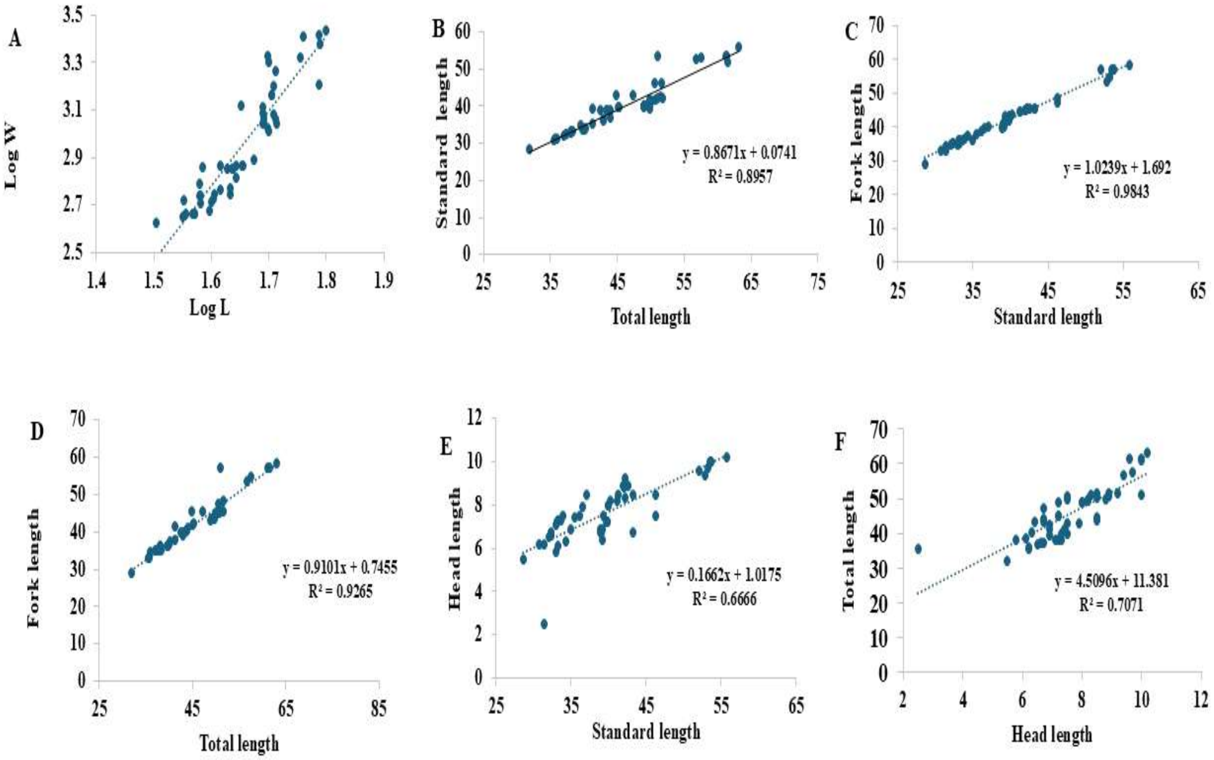

Figure 3. Length -weight and Length – length relationship (LLRs) of *T. putitora* from Gobind Sagar reservoir *(a.) Log length -weight relationship (b) LLRs of total length and standard length (c). LLRs of standard length and fork length (d). LLRs of total length and fork length (e). LLRs of standard length and head length (f). LLRs of head length and total length*

would correspond to increased feeding opportunities, favorable water conditions and active reproductive phases which all lead to heightened body condition (Araneda et al., 2008). The premonsoon season is also characterized with high values therefore, the preparation for spawning, the gain in energy reserve and the maintenance of good physiological status of the fish. However, sharp decrease was observed in post-monsoon season might be associated with the depletion of energy reserves after spawning, and perhaps with lower food supply or environmental stress. The winter period slightly recovers which can attribute to the lower metabolism and limited feeding activity in winter months. Altogether, this information provides evidence for *T. putitora* attaining maximum physical condition during the monsoon season, which seems to be a significant period of reproductive and growth activities, whereas post-monsoon, is a biophysically challenging period that requires carefully ecological management (Alam et al., 2014).

Seasonal changes may reveal the gonads and fatness state, whereas changes in condition factor values with length may reveal the size at the onset of maturity. o Balai et al. (2016), observed the Indian major carps (catla, rohu, and mrigal) from Jaisamand Lake were in good health, with K values ranging from 1.854to 1.186and Kn values between 1.005±and 1.008.

3.6. Truss Networking Analysis

In the present study, six variables with eigen values were derived from 19 morphometric measures using PCA analysis revealed that *T. putitora*, the first three principal components (PC1, PC2, and PC3) accounted for 78.377%, 18.38%, and 13.03% of the total variance, respectively. It was also observed that *T. putitora* had eigenvalues greater than one 12.83, 4.78, 3.38, 1.227, and 1.078, respectively (Table 4, Figure 4). The higher percentage of variance explained by PC1 in *T. putitora* (78.377%) exhibits a more distributed variance pattern across multiple principal components, indicating that its growth and body composition may be influenced by a combination of multiple factors such as habitat conditions, hydrodynamic forces, and trophic interactions (Nautiyal et al., 2008). Understanding PCA results in fish species is crucial for designing effective conservation and management strategies. The high variance explained by PC1 in *T. putitora* highlights key morphological and physiological traits that should be considered for breeding, conservation, and habitat restoration programs. The significant contribution of PC2 and PC3 in *T. putitora* further emphasizes the need for maintaining river connectivity and stable hydrological conditions to support its natural migratory and reproductive behaviors. Given the ecological importance of this species in freshwater biodiversity and fisheries, long-term monitoring of their morphometric and growth patterns using PCA can

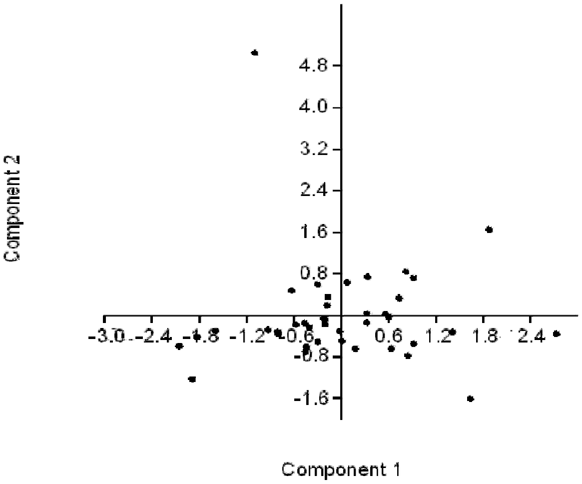

Figure 4. Principal component analysis of *T. putitora*

Table 4. Percentage of variance accounted by variables having eigen values more than 1of within group PCA for *T. putitora* from Gobind Sagar reservoir

Principle component	***T. putitora***	
	Eigen value	**% variance**
1	12.83	78.37
2	4.780	18.386
3	3.388	13.032
4	1.274	4.903
5	1.078	4.146
6	0.704	2.701

Table 3. Estimation of condition factor (K) and relative condition factor (Kn) of *T. putitora*

T. putitora	**Pre-monsoon**	**Monsoon**	**Post-monsoon**	**Winter**
Condition factor	1.06±0.06	1.14±0.07	0.91±0.05	0.79±0.07
Relative condition factor	1.21±0.003	1.65±0.009	1.01±0.09	0.98±0.001

Values are expressed as Mean±SE

provide valuable insights for sustainable aquaculture and conservation planning. Future studies should incorporate additional environmental variables such as water quality, temperature fluctuations, and genetic diversity to refine the PCA models and better understand species-specific adaptations. Rohlf (2006) suggested that a large portion of variance can be explained by the first few principal components (PC). According to the overall results of PCA, size is the primary factor that contributes to variability in both species, followed by shape. Principal Component Analysis (PCA) is a widely used multivariate statistical technique for identifying key variables that contribute to variations within datasets, particularly in biological and ecological studies (Jolliffe and Cadima, 2016). PCA helps in reducing the dimensionality of data while retaining the maximum variability, thereby identifying patterns in species morphology, growth, and environmental interactions (Legendre and Legendre, 2012).

4. CONCLUSION

This study demonstrated that the morphometric (19) and meristic (5) traits of *T. putitora* remain consistent across different size groups, indicating a stable body plan throughout ontogeny. The dominance of pre-mature and mature individuals confirms a self-sustaining breeding stock within the Gobind Sagar Reservoir. A regression coefficient 'b' >3 highlights a positively allometric growth pattern, suggesting improved weight gain with increasing length. Principal component analysis revealed that 78.4% of the variance in PC1 is linked to a single dominant factor, likely body shape, while PC2 and PC3 reflected secondary influences such as habitat fragmentation, seasonal flow changes and ecological fluctuations typical of a migratory species. By integrating growth condition, phenotypic variability and health status, the results provide essential baseline data for stock assessment, habitat management and sustainable harvest. These findings can guide conservation policies and adaptive management strategies for safeguarding this vulnerable species within the reservoir ecosystem.

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Authors' Contributions

Supreet Kaur conducted sampling and analysed the data and wrote the manuscript. Surjya Narayan Datta helped in sampling, interpreted the results and wrote the manuscript. Prabjeet Singh conceptualized the theme, helped in sampling; and Grishma Tewari helped in sampling.

Conflict of Interest

The authors declared that they don't have any conflict of interest among them and institute for conducting the experiment.

Declaration

The authors are hereby declaring that no AI tools have been used in the writing the manuscript.

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Physiological and Morphological Variation in *Withania somnifera* under Contrasting Agro-Climatic Conditions

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Abstract: *Withania somnifera* (Ashwagandha) is a medicinal plant from the Solanaceae family that is highly regarded for its root-derived withanolides. In order to identify the primary traits that influence productivity, this investigation evaluated physiological and morphological variation in two starkly different agro-climatic environments: Chennai (Tamil Nadu) and Jabalpur (Madhya Pradesh). Uniform agronomic practices were implemented during field trials in rainfed kharif conditions. The average plant height was 80.30 cm in Chennai and 88.53 cm in Jabalpur, while the root yield was 8.58 g and 10.57 g per plant, respectively. Regression analysis revealed that plant height and root area significantly influenced root yield in Chennai ($p < 0.05$), whereas leaf length was the only trait showing a significant association with root yield in Jabalpur ($p < 0.05$). Differences in shoot–root ratio between locations were also statistically significant, indicating environment-specific biomass allocation patterns. In Chennai, root area and plant height were identified as significant predictors of root yield, while leaf length was the sole significant factor in Jabalpur .Theincreased allocation of biomass to roots was indicated by lower shoot–root ratios. The results emphasize the genetic and phenotypic diversity of *Withania somnifera* and identify traits—particularly shoot–root ratio, root area, and leaf length-as potential selection criteria for breeding initiatives that are designed to enhance yield and stress tolerance.This study contributes to plant science by demonstrating how agro-climatic variability alters trait–yield relationships in a medicinal crop of high economic importance. It advances understanding of phenotypic plasticity and biomass allocation strategies in *Withania somnifera,* and establishes measurable traits-such as shoot–root ratio, root area, and leaf length-as reliable indicators of adaptive performance and yield potential.

Keywords: *Withania somnifera*, Agro-climatic adaptation, Physiological variation, Root yield, Breeding selection.

1. INTRODUCTION

Commonly referred to as Ashwagandha, Indian ginseng, or winter cherry, *Withania somnifera* (L.) Dunal is a perennial medicinal shrub from the Solanaceae family that has been utilized in Ayurveda for over 4,000 years as a Rasayana (rejuvenate tonic) to enhance resilience, vitality, and longevity (Ashwagandha extraction and its health benefits (Bokelmann, 2022). It is cultivated in Africa, the Middle East, and the Mediterranean regions, and it is native to the Indian subcontinent. It thrives in arid, subtropical climates (Akbar, 2020). The plant is highly regarded for its adaptogenic, neuroprotective, immunomodulatory, and anti-inflammatory properties, which are primarily attributed to steroidal lactones (withaferin A, withanolide A, withanone), as well as alkaloids and flavonoids (Jamalludin et al., 2022; Kumar et al., 2023). Pharmacological investigations have illustrated numerous mechanisms of action, such as the regulation of neurotransmitters, the hypothalamic-pituitary-adrenal axis, antioxidant activity, and cytokine balance (Singha et al., 2024; Shivananjappa et al., 2022). Traditionally, root and leaf preparations have been employed to alleviate tension, anxiety, reproductive disorders, arthritis, respiratory ailments, and metabolic dysfunctions (Pandya et al., 2022; Tuli et al., 2023). It is renowned for its adaptogenic, anti-inflammatory, and neuroprotective properties (Mir et al., 2012). The physiology of this plant involves the biosynthesis of bioactive secondary metabolites, including withanolides, alkaloids, and flavonoids, which are responsible for its pharmacological potential (Kumar et al., 2007). Its advantages in augmenting cognitive performance, reducing cortisol levels, supporting physical endurance, and improving sleep quality are further substantiated by contemporary clinical trials (Jędruszczak et al., 2023; Raj et al., 2022). In order to optimize its therapeutic potential, the necessity for standardized cultivation, phytochemical

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characterization, and region-specific selection is underscored by the increasing global interest in *Withania somnifera*.

Genetic diversity, environmental conditions, and agronomic inputs influence physiological and morphological characteristics, including the number of seeds per berry, the length of the leaf, the height of the plant, and the biomass yield. Amphimixis, open pollination, and facultative autogamy are the defining characteristics of reproductive biology, which guarantees reproductive resilience. The number of seeds per berry varies between 10 and 30, contingent upon the genotype and environmental conditions (Mir et al., 2012; Kumar et al., 2007). Nitrogen application at 120 kg/ha has been reported to optimize leaf area (Thakur et al., 2014), while optimal tillage depth and plant density can increase shoot biomass by over 20% (Kothari et al., 2003). Das et al. (2010) have also disclosed stable phenotypic variants, such as thick-leaf mutants with enhanced leaf dimensions, through induced mutagenesis studies. It is essential to comprehend the interplay between physiological processes, morphological variability, and environmental adaptability in *Withania somnifera* in order to optimize cultivation strategies, enhance phytochemical yield, and create stress-tolerant, high-yielding varieties that guarantee sustainable medicinal production.

2. MATERIALS AND METHODS

2.1. Site Location

Chennai (Tamil Nadu, India). Chennai is characterized by a tropical savanna climate with minimal seasonal temperature variation. In May–June, temperature maxima frequently exceed 38 °C, while winter minimums are typically around 22 °C. The northeast monsoon (Sept–Dec) is the primary source of precipitation, with an average annual rainfall of approximately 1,400 mm. Southwest rainfall (June–Sept) is also present. Jabalpur (Madhya Pradesh, India) is located at an elevation of approximately 412 meters. The city is characterized by a subtropical climate, with summer temperatures reaching as high as approximately 46°C and winter temperatures as low as 2°C. The majority of the annual rainfall, approximately 1,315 mm, is received between mid-June and September, with intermittent winter showers occurring in December and January.

Predominantly sandy loam to light red soil with excellent drainage and neutral to alkaline pH (7.5–8.0) are the soil characteristics of Chennai. The soil was well-aerated and had minimal moisture retention, which facilitated root growth. However, it is typically low in organic carbon and nitrogen. The soil at the experimental site in Jabalpur is sandy loam with a pH of approximately 7.5. The nutrients were moderate, with P and K at approximately 16 and 236 kg/ha, respectively. Nitrogen was low at approximately 206 kg/ha, and organic carbon was approximately 0.3%.

2.2.Crop Management and Cultivation

Uniform agronomic practices were implemented in the planting of *Withania somnifera* varieties in both Chennai and Jabalpur locations (Kumar et al., 2020; Sharma et al., 2017). Andexcellent tilth was established by pre-monsoon tillage. In accordance with the cultivation of the kharif season, direct sowing took place in July–early August following the initial rainfall. Irrigation was infrequent and was implemented solely during periods of drought, as both regions depend on rainfed conditions (Sharma et al., 2017). Observations: Vegetative characteristics include the number of branches, the duration of flowering and maturity, and the height of the plant. Plant reproductive characteristics include the number of fruit and the quantity of seeds produced per plant (Kulkarni et al., 2017; Nikam et al., 2013). Root characteristics include the number of primary and secondary roots, the length and diameter of the main root, and the weight of the fresh and dried roots. At the appropriate growth phases, all measurements were conducted in accordance with established protocols (Kumar et al., 2020), (Figure 1 a,b,c).Data on morphological and physiological traits and root yield were analyzed to determine the significance of differences between the two agro-climatic locations, Chennai (Tamil Nadu) and Jabalpur (Madhya Pradesh). Mean values were compared using Student's *t*-test to assess location-wise differences at a significance level of $p < 0.05$. Prior to analysis, data were tested for normality and homogeneity of variance to ensure compliance with test assumptions.

In addition, multiple regression analysis was performed separately for each location to identify traits significantly influencing root yield. Statistical analyses were carried out using standard statistical software, and results were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1.Physiological Variation and Trait–Yield Relationships in Chennai

Withania somnifera reached 50% flowering at 105.63 days and exhibited a mean plant height of 80.30 cm under Chennai conditions (Table 1). The plants reached maturity in 216.85 and yielded 43.15 fruit per plant. The mean seed yield was 13.54 g, with 250.75 seeds. The root length averaged 16.72 cm, while the root area exhibited exceptionally high variability (159.03 cm^2). The fresh root yield was 8.58 g.Root yield was significantly influenced by root area. The accumulation of root biomass is directly supported by the larger root systems, which likely enhance nutrient and water absorption. Additionally, root yield was substantially influenced by plant height (which may be attributed to its correlation with the overall photosynthetic capacity and assimilate availability for root growth. The number of days to maturity approached significance (indicating that certain genotypes may be able to develop roots more effectively as a result of extended vegetative periods. Root yield was not significantly influenced by other variables, such as root length leaflength, leaf area (F = 0.67; p = 0.750), biomass yield , and flowering time . The shoot-to-root ratio of 5.041, is lower than approximately 10.3 (Sangwan et al., 2013), suggests that the biomass is more effectively allocated to the roots, which is a beneficial characteristic for the production of medicinal roots. The significance of root architectural traits in Chennai's sandy loam and humid conditions is emphasized by this allocation pattern, in conjunction with significant root area effects.

3.2. Physiological Variation and Trait-yield Relationships in Jabalpur

The average height of the plants in Jabalpur was 88.53 cm, with 50% of the plants flowering at 105.58 days and reaching maturity at 213.35 days The seed count averaged 277.70, and the seed yield exhibited considerable variability (135.74 g). The mean berry production per plant was 43. The fresh root yield was 10.573 g, and the root area averaged 35.47 cm^2. The root length was 18.62 cm. The sole significant predictor of root yield was leaf length. This is likely due to the fact that larger leaves facilitate photosynthetic assimilation, which in turn allows for a more concentrated allocation of carbon to root development. Although not statistically significant, the F-values of root length and days to maturity were high, indicating that they may be of significance with a larger sample size. No significant impact was observed on root yield by other characteristics, including plant height, root area , or biomass yield.

The shoot-to-root ratio (4.761) was once again lower than the literature reports (~10.3), which corroborates the observation of a favourable resource allocation toward root biomass in both locations. Unlike Chennai, where root architectural characteristics were the most influential, Jabalpur's results indicate that leaf morphology is the primary factor influencing root productivity. This may be a reflection of the subtropical climate and soil nutrient profile, which encourage photosynthetically driven growth.

Breeding implications and comparative interpretation - A combination of potentially superior genotypes and favourable environmental conditions was suggested by the fact that both locations recorded greater plant height (80.30–88.53 cm) and fresh root yields (8.58–10.57 g) than previous studies (Sangwan et al., 2013: 34.08 cm height, 4.64 g root yield). Nevertheless, the determinants of root

Table 1. Physiological variation observed at Chennai (Tamil Nadu) and Jabalpur (Madhya Prdesh) conditions

Physiological character	Mean ± Std. deviation observed at Chennai	Mean ± Std. deviation observed at Jabalpur (Madhya Pradesh)
Plant height (cm)	80.30 ± 10.47	88.534 ± 11.71
50% flowering	105.63 ± 4.64	105.58 ± 7.097
Days of maturity	216.85 ± 4.66	213.35 ± 5.575
No. of berries/plant	43.15 ± 10.04	43.00 ± 11.16
No. of seeds / plant	250.75 ± 113.75	277.70 ± 71.82
Seeds yield per plant (in gm)	13.54 ± 4.078	135.74 ± 522.15
Root area	159.03 ± 557.44	35.47± 8.45
root length	16.72 ± 3.87	18.62 ± 5.26
Fresh root yield/ plant	8.58 ± 1.70	10.573 ± 2.13
Leaves length	7.78 ± 0.83	42.889 ±153.964
Leaves area	12.45 ± 3.18	13.135 ± 2.530
Biomass yield at maturity	67.72 ± 16.38	81.980 ± 22.96
Shoot root ratio	5.041 ± 1.29	4.761± 1.269
Root shoot ratio	0.210 ± 0.0511	.223 ± 055509

productivity varied significantly between Chennai and Jabalpur. In Chennai, root area and plant height were significant factors, while in Jabalpur, leaf length was a more significant factor.The significance of location-specific reproductive targets is underscored by these distinctions. To optimize root-yield enhancement programs, Chennai genotypes should be chosen for their expanded root systems and optimal shoot growth, while Jabalpur genotypes should be optimized for their larger, more photosynthetically efficient leaves. Stable flowering periods across sites suggest genetic uniformity, which facilitates synchronized seed production and harvesting.

4. CONCLUSION

This study demonstrates that the determinants of root yield in *Withania somnifera* vary distinctly across environments. Root architectural traits were the primary contributors to yield performance under Chennai conditions, whereas leaf morphological traits were more influential in Jabalpur. Both locations supported favourable biomass allocation patterns, indicating strong genetic potential for improvement. These findings highlight the importance of site-specific selection strategies in breeding programmes, emphasizing root-related traits for Chennai and leaf-related traits for Jabalpur to enhance productivity and stress resilience.

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Credit authorship Contribution Statement

Neha Singh: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. Nisha Singh: Data curation, review & editing. Anita R. J. Singh: Supervision, Validation, Writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest.

Funding Declaration

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Agroforestry for Sustainable Livelihoods: The Way Forward

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***Abstract*:** Agroforestry has been a practice since time immemorial but as a scientific discipline, it is relatively new compared to many other agricultural science disciplines. As a scientific discipline of enquiry, agroforestry evolved since the 1970s. It is a sustainable land management system which increases the overall production of the land by combining the production of trees, crops and or animals simultaneously or sequentially on the same unit of land. Agroforestry trees benefit the livelihoods of farmers in multifarious ways, including improved well-being, increased income and savings, decreased susceptibility to various whims, and sustainable resource usage. Furthermore, because of their capacity to store carbon and balance out the losses brought on by deforestation and forest degradation, agroforestry trees have recently gained more attention in the fight against climate change and adaptation. There has been an overall increase in the area under agroforestry at the global level in 2000-2010. The overall population in the world living in an agroforestry landscape has increased from 746.7 million to 837.6 million during 2000-2010, which implies that more farmers have realized the importance of agroforestry and consequently adopted it. Keeping in view the importance of agroforestry, there is a need to further accelerate its adoption and its intensification to increase the area under agroforestry for the overall benefit of the world farming community. In India, agroforestry has received the attention of policy makers as a sustainable land use as evident from the fact that India is the first country in the world to have a dedicated Agroforestry Policy. Despite its numerous benefits, several challenges must be addressed to accelerate the widespread adoption of agroforestry.

***Keywords*:** Livelihood, adoption, Sustainable development goals, Carbon sequestration, Climate change, Adaptation.

1. INTRODUCTION

Sustainable food systems face significant challenges due to expanding populations and declining agricultural land. Nearly 69% of India's population lives in rural areas, which are typified by poor access to safe drinking water, sufficient housing and sanitation, health care, education, and economic resources. Although governments' attempts to ensure sufficient and sustainable access to all means of livelihood have resulted in improvements in living standards, several gaps still exist (Patidar, 2019). Climate variability, water shortages, and declining land quality pose threats to rural livelihoods and global food security. Despite generating necessary meals, conventional agriculture frequently damages the environment by using excessive amounts of chemicals, water, and land, which results in biodiversity loss and climatic impacts. These problems highlight the necessity of multipurpose, sustainable agriculture systems that maintain social and environmental well-being while increasing productivity (Sudomo et al., 2023). The issue of livelihood security is of national importance in India, because a sizable section of the rural population is directly dependent on natural resources. Renewable resources are under extreme strain due to overexploitation, necessitating immediate action to restore ecological equilibrium. The National Environment Policy 2006, which centres sustainable development on human wellbeing, expressly acknowledges this concern. In order to meet socio-economic demands and mitigate the effects of environmental degradation, agroforestry provides a feasible route to resilience and sustainable resource use (Bansal et al., 2021). Around the world, agroforestry has become an essential land-use system that sustains the livelihoods of millions of rural communities. For small-scale farmers who mostly depend on natural resources for food, fuel, fodder, and additional revenue, it provides several ecological and financial advantages (Sahoo and Wani, 2019). Agroforestry offers a robust and sustainable substitute that improves environmental health and productivity as climate change, soil erosion, water scarcity, and growing input costs continue to pose challenges to traditional agriculture. Agroforestry's conservation and socioeconomic benefits have historically received little attention, despite its increasing significance.

Comprehensive, long-term data describing the evolution of agroforestry systems and the factors that have shaped their function in preserving biodiversity and improving livelihoods is still lacking (Pandit, 2014). It is essential to comprehend its function in contemporary farming methods, not only to increase output but also to favourably impact rural incomes and environmental sustainability. The demand for sustainable farming methods has been further heightened by population growth and growing competition for land.Deliberate tactics, such as the domestication of beneficial tree species, enhanced market accessibility, and favourable legislative settings, are necessary to strengthen agroforestry systems. By increasing food security and diversifying revenue streams, such systems can strengthen rural resilience, especially in areas susceptible to climatic fluctuation. Reviving rural economies and advancing sustainable land management require the incorporation of synergistic technologies within agroforestry frameworks (Sarveswaran et al., 2023). Agroforestry's potential as a climate-smart and livelihood-enhancing strategy is highlighted by international frameworks like the United Nations Sustainable Development Goals (SDGs), the Convention on Biological Diversity, the United Nations Framework Convention on Climate Change (UNFCCC), and the Convention to Combat Desertification. Although nations with national-level agroforestry programs, such as India and Nepal, have made significant policy strides, governance frameworks remain lacking in many other countries in the global South (Awazi et al., 2024). Agroforestry is becoming more and more acknowledged on a global scale as a means of achieving sustainable development objectives. Scaling up agroforestry as a global answer to climate resilience and rural development will require strengthening governance, policy coordination, and institutional support. This review is carried out to synthesize and interpret the contribution of agroforestry to sustainable livelihoods and to address the limitations in fostering sustainable livelihoods across diverse socio- ecological contexts.

2. ROLES OF AGROFORESTRY IN SUSTAINING LIVELIHOODS

Agroforestry systems play the following important roles in livelihoods of the farmers:

2.1. Increased Well-being

Through subsistence and revenue-generating pathways, farming systems that incorporate agro-ecological practices have been demonstrated to significantly reduce the negative externalities of farming while also improving indicators of household well-being, such as dietary diversity and nutrition. For instance, long-term research on an agroforestry program in Kenya discovered that the intervention had a favourable impact on revenue creation, fuelwood access, and household asset accumulation, especially in families headed by women (Milheiras et al., 2022).Agroforestry greatly increased financial capital in terms of timber, non-timber, and fuel wood income, according to a study conducted by Ahmad et al. (2021). Additionally, agroforestry farmer households (HHs) had significantly better natural capital (the amount of land and land ownership; the number of HHs growing vegetables, fruit crops, and medicinal crops); social capital (the number of social groups that HHs participated in and the number of HHs sharing crop seeds); and physical capital (buffalo plough, generators, and sprinklers) than conventional farmers. By adding diversity, taste, and nutritional value, as well as providing vital vitamins, minerals, proteins, and calories, tree-based foods improve diets. Fodder from dhaman (*Grewia optiva*), cultivated in agroforestry systems of the Western Himalayas, serves as a crucial supplement, reducing the scarcity of green fodder during winter (Sood and Mahajan, 2018). Furthermore, agroforestry reduces the reliance on forest-sourced fuelwood, traditionally collected by women thus, easing their workload and freeing time for leisure or other productive activities while simultaneously conserving natural forests.

2.2. Food and Nutritional Security

Agroforestry enhances food security by fostering ecosystem resilience and services derived from the functional diversity and species interactions within its inherent biodiversity (Nuwarapaksha et al., 2024). Agro-ecological agroforestry systems are more sustainable and capable of restoring degraded ecosystems, in addition to preserving agro biodiversity, allowing farmers and traditional communities to grow more resilient and self-sufficient. Therefore, food production in an agro-ecological system could ensure food sovereignty in developing countries. To ensure food security, rural poverty reduction, and sustainable development, agro-ecological practices by small farmers and vulnerable populations, such as the indigenous population, must be encouraged (Goncalves et al., 2021). By diversifying their crop bases, agroforestry systems lower the likelihood of crop failure due to poor weather conditions. These systems also help farms produce more by promoting beneficial processes like nutrient pumping, weed and pest control, nutrient cycling, biological nitrogen fixation, and increased soil moisture availability

(Sarvade et al., 2014). Combining forestry and agriculture will not only contribute to the availability of more nutrient-dense meals but also to the development of more private food production systems that are more resilient to environmental shocks. When properly implemented, integrating trees into farming methods guarantees that the most disadvantaged groups continue to have access to foods high in nutrients and supports the operation of natural food markets, supply chains, and distribution networks (Sahoo and Wani, 2019).

2.3. Employment and Income Generation

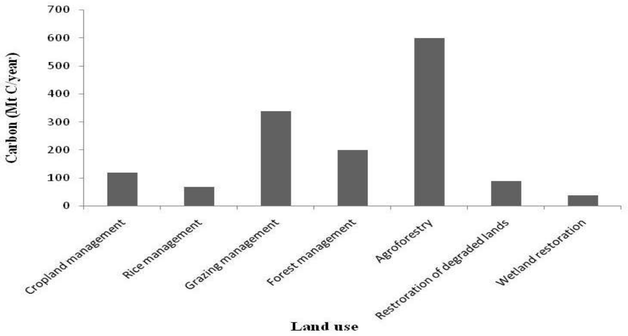
Agroforestry gives farmers a comprehensive understanding of ecological principles and climate change-adaptive management strategies, including food production, soil management, and tree and tree product management. It has been discovered that small farm households can improve and restore the rural farmland management system, as well as increase their output and income, by implementing cash trees and agroforestry. Research suggests that compared to arable systems, agroforestry operations yield a greater anticipated gross mixed revenue (Tebkew et al., 2024). In addition to providing essential raw materials for wood-based industries, trees grown in commercial agroforestry systems also increase farmers' incomes. For instance, Poplar cultivated in agroforestry systems across Punjab, Haryana, and parts of Uttar Pradesh serves as a key raw material for the paper industry, generating substantial income for farmers. Furthermore, trading carbon credits is a new source of income to farmers, diversifying their agricultural portfolio further. Policy analysis reveals that at prices of $100 per MgC, carbon sequestration in agroforestry systems would have the potential to raise per capita incomes of farmers by up to 15% (Antle et al., 2007). As a land use system, agroforestry has enormous potential to support rural development and livelihood security by creating jobs. One of the key ways to address the challenges of the future is to standardise, improve, and distribute agroforestry-based remediation technologies for problematic soils, as well as develop, standardise, and implement agroforestry models connected to the market to increase the productivity and profitability of small-holding farmers (Castle et al., 2021).

2.4. Sustainable Resource Use

In agricultural landscapes, trees and woodlands can preserve soil, protect crops from the direct or indirect effects of erosion, and help to maintain site productivity. By adding organic matter to the soil's top layer, trees increase the soil's organic matter and nutrient level. Through their deep tap root system, trees absorb nutrients from deeper soil layers and transfer them to the upper layer through the decomposition of litter. Trees therefore serve as a nutrient pump. Tree cover is essential to many agroforestry systems because it replenishes the soil's top layer with nutrients and increases site productivity (Dhyani et al., 2021). Additionally, tree leaves intercept rainfall, which lowers surface runoff and helps to maintain soil fertility. Further, a variety of tree species fix nitrogen from the atmosphere into the soil, increasing soil fertility. The examples of such nitrogen fixing trees include Khair (*Acacia catechu*), Kikar (*Acacia nilotica*), Shisham (*Dalbergia sissoo*) and Kachnar (*Bauhina variegata*) etc. The sustainability of agricultural production is aided by these improvements in soil characteristics and nutrient content. Additionally, trees help to moderate extreme weather conditions, such as temperature. As a result of these microclimatic changes the overall crop productivity increases.

2.5. Carbon Sequestration and Climate Change Adaptation

Agroforestry systems serve as effective carbon sinks, with significant potential for climate change mitigation and adaptation (Jose and Bardhan, 2012). Their carbon sequestration capacity is especially notable due to widespread applicability across agricultural and degraded lands. Smallholder tropical agroforestry systems can sequester 1.5–3.5 Mg C ha^{-1} yr^{-1} (Montagnini and Nair, 2004). Agroforestry offers significant potential for carbon storage and spans around 630 million hectares worldwide (IPCC, 2000). The many advantages of agroforestry are highlighted in recent IPCC reports, including food security, income creation, biodiversity conservation, carbon sequestration, climate adaptation, and the provision of ecosystem services (Quandt et al., 2023). Among the top 100 climate solutions, Project Drawdown found 11 agroforestry-based strategies, including bamboo, indigenous tree-based systems, silvopasture, intercropping, and multistrata agroforestry. Figure 1 highlights that agroforestry systems have a significantly higher capacity for carbon sequestration compared to conventional land use practices. According to (Zomer et al., 2022), just 10% more trees on agricultural land over ten years might sequester more than 18 PgC (1.83 PgC yr^{-1}). This is significantly higher than the net emissions from land use and forestry (1.6 PgC yr^{-1} in 2020) and the present annual carbon losses from tropical land-use changes (0.6–1.2 PgC yr^{-1}). According to the UN Decade on Ecosystem Restoration, small-scale improvements in agricultural tree cover can support global restoration objectives. Agroforestry, which offers both economic and



Source: (IPCC, 2007)

Figure 1. Carbon sequestration potential of different land use by 2040

ecological benefits as well as climate benefits, is acknowledged by the (IPCC, 2001) as a "no regrets" approach. Important systems that contribute to carbon sequestration include agrisilviculture (trees plus crops), silvipasture (trees plus livestock), and agri-silvipasture (trees plus crops plus livestock). Additionally, it has been discovered that agroforestry stores more carbon in deeper soil layers than treeless farming systems (Prabha et al., 2014).

2.6. Evolution of Agroforestry Research Over Decades

Since the late 1970s, when the term "agroforestry" gained international popularity, the number of publications has significantly expanded. Over the last four decades, most of the literature on agroforestry has focused on ecological issues; the field's ecological foundation is indicated by the prevalence of terms like forest, species, soil, and land. However, the language used to describe social dimensions has been extremely limited (Hastings et al., 2023). Trends in study topics have drastically changed throughout time. Early studies in the 1990s focused on traditional systems and biophysical assessments. Research has shifted to system structure and interactions by the 2000s, especially the competition between crops and trees for light, water, and nutrients. Studies examined how species interactions, such as those with shade trees or plants that repel pests, could enhance ecosystem productivity and services. In response to concerns about climate change, carbon sequestration became a significant issue with the aid of international frameworks like the Kyoto Protocol. The increasing loss of biodiversity has also led to the promotion of agroforestry as a strategy for habitat conservation. Since then, studies have expanded to examine ecosystem services, which are currently a major focus of agroforestry research (Liu et al., 2019). Over the years, important research clusters have been found using analytical techniques like LDA and HJ-Biplot. From 1993 to 2022, issues including biodiversity, climate change, food security, and organic soil carbon have not gone out of style. On the other hand, there is less attention in topics like aerial biomass and alley cropping (Montes et al., 2023). Even though agroforestry research has been conducted in India for over a century, the formal incorporation of agroforestry into national agendas began in 1983 when the Indian Council of Agricultural Research (ICAR) initiated the All India Coordinated Research Project (AICRP) on Agroforestry. Established at Jhansi in 1988, the National Research Centre on Agroforestry was renamed the Central Agroforestry Research Institute (CAFRI) in 2014. With 37 centres dispersed throughout 27 State Agricultural Universities and 9 ICAR institutes, AICRP currently covers a range of agro-climatic zones. The Indian Council of Forestry Research and Education (ICFRE) also contributes through its network of institutes (Handa et al., 2015). These changes show how agroforestry research has dynamically evolved from regional studies to globally important, multidisciplinary subjects centred on development, resilience, and sustainability.

2.7. Adoption of Agroforestry

Since its institutionalisation, agroforestry has been advocated as a significant sustainable land use choice by numerous national and international organisations. It has been a traditional land use in many regions of the world for ages. Despite this, until 2009, there was hardly any effort to calculate the global area covered by agroforestry adoption (Zomer et al., 2009). Agroforestry was divided into three groups by (Zomer et al., 2014) viz; tree cover > 10%, tree cover > 20%, and tree cover > 30%. Global Increase in Agroforestry Area by Tree Cover Density (2000–2010) suggests that there is a considerable increase in area adopted under agroforestry systems of varying tree cover densities (Figure 2). It has been estimated that a total of 1.8 billion people (24.6% of world's population) all over the world live in agricultural landscape (Zomer et al., 2014). Of these 746 million (41%) lived in agroforestry landscape with tree cover > 10% in 2000-2002 which increased to 837.6 million in 2000-2010. This represents an increase of 90.9 million (5%) during the period 2000-2010. The highest number of people lived in agroforestry landscape in East Asia, South Asia and Southeast Asia respectively.

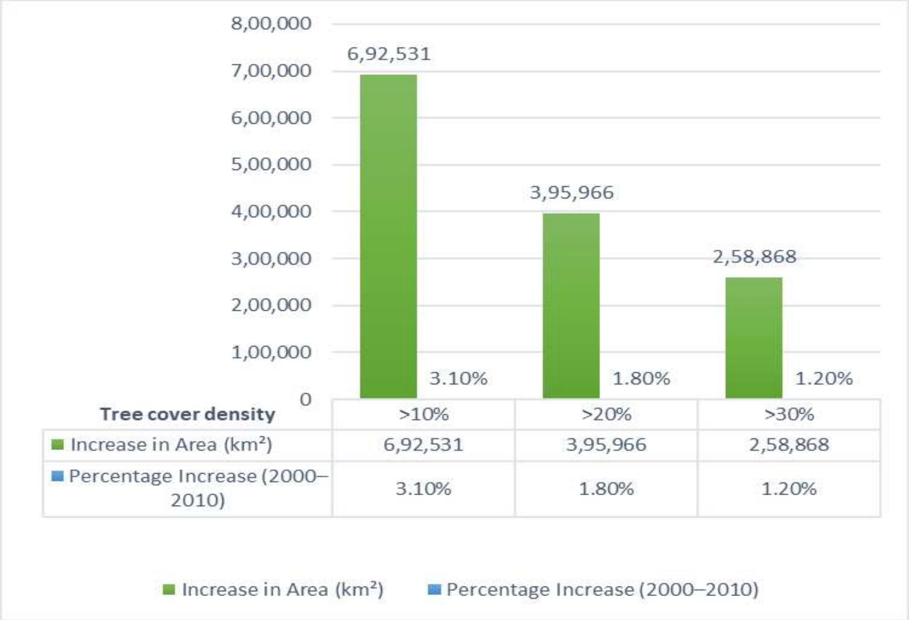
Therefore, it is implied that throughout the 2000–2010 decade, the area covered by agroforestry and its intensification with tree cover has grown. Additionally, there are now more people living in agroforestry landscapes worldwide. This suggests that farmers all around the world are becoming more and more interested in agroforestry as a land use method. Nevertheless, in order to improve farmer livelihoods and increase the environmental benefits at the local, regional, and global levels, efforts are needed to develop, accelerate, and intensify the adoption of agroforestry in all parts of the world. Various constraints need to be overcome to develop and promote agroforestry.These limitations differ not only across nations but also between regions of the world (Sood and Mitchell, 2011). The barriers to agroforestry adoption in India differ from one state to the next. There is limited additional research on the global, regional, and local trends in the area covered by agroforestry options after 2010.

Some of risks that landowners may face due to climate change and how agroforestry practices might be used to adapt to those risks (Table 1).

3. CONSTRAINTS IN PROMOTION AND CONDITIONS FOR AGROFORESTRY ADOPTION:

The broad constraints, especially with reference to India, in the development and promotion of agroforestry are as:

- Lack of institutional set up solely devoted to developing, managing and encourage agroforestry land use as a whole and there is a lack of coordination amongst various departments and organizations working individually and in isolation on different aspects (agriculture, forestry, and livestock) of agroforestry (Chavan et al., 2015).
- Poor or partial implementation of Indian National Agroforestry Policy (2014) by various states.
- Insufficient funding for agroforestry research and development due to lack of technical and economic data on different agroforestry models.
- Legal restrictions imposed by the governments on harvesting and intra and inter-state and inter country transport of agroforestry produce and inadequate attempts to ease out these restrictive regulations (Sood et al., 2018). A number of states have *de-jure* exempted several tree species from harvesting and transport restriction, however, *de-facto* such restrictions exist.
- Dearth of certified quality planting material and lack of certified tree nurseries
- Poor market linkages and infrastructure for proper sale of agroforestry produce.
- Insufficient work on standardization of post-harvest processing technologies for tree produce.
- Non-availability of economic data of different agroforestry models
- Poor extension mechanisms for the promotion of agroforestry.



Source: (Zomer et al., 2014)

Figure 2. Global increase in agroforestry area by tree cover density (2000–2010)

- Apprehension amongst the farmers that their land use will be changed after planting trees.
- Long time taken by the trees to produce desired tree product (long gestation period) compared to agricultural crops.
- Lack of research on reducing gestation period of many forest tree species and their poor domestication.
- Lack of institutional mechanisms to link agroforestry practitioners to carbon credits
- There are a number of conditions under which farm households may or may not adopt agroforestry implies that conditions like land tenure, tree ownership, management arrangements, security of future produce, right to harvest, transport and sell, prices and marketing conditions must be favorable to encourage agroforestry adoption (Table 2).

4. PROMOTION OF AGROFORESTRY: WAY FORWARD

The following strategies need to be adopted for promotion of agroforestry:

- Quantification of contribution of existing agroforestry to farmers' income. This will help in the framing strategies to promote agroforestry models which enhance the income of the farmers.
- Identification of superior germplasm of agroforestry tree species for production of quality planting stock having short gestation periods (GOI, 2014).
- Domestication of forest tree species
- Studies on tree-crop interactions, management practices and development of post-harvest technologies for tree produce and their value addition (Sood and Mahajan, 2018). This will assist the farmers in adopting better tree-crop combinations and management practices on their farms to enhance overall productivity of the land in a sustainable manner.
- Creation of awareness about agroforestry amongst the farmers and popularization of success stories on agroforestry adoption amongst the farmers and policy planners.
- Setting up of a separate institutional mechanism at global, regional and national levels for promotion of agroforestry and ensuring coordination amongst the stakeholders.
- Adoption of the recommendation of National Agroforestry Policy (2014) in India to promote agroforestry. Similar policies need to be framed at state levels.
- Liberalization of legal restrictive regime on harvesting, transport and sale of tree produce. Also, the officials or staff need to be made aware of already *de-jure* exemptions of various species from felling and transport rules so that this exemption can be *de-facto* implemented.
- Integrating agroforestry development with other rural

Table 1. Risks due to climate change and role of agroforestry in its adaptation

Risk	Adaptation	Agroforestry practice
Intense precipitation events	Slow water runoff to reduce flooding, soil erosion, and water pollution	Riparian forest buffers, alley cropping
Increased temperatures	Reduce heat stress on animals by providing shade	Silvipasture, Agrisilvipasture
Increased frequency and intensity of drought	Reduce evapotranspiration by reducing windspeed	Windbreaks (Agri-silviculture)
Increased storm intensity (wind & precipitation)	Protect crops from wind damage	Windbreaks (Agri-silviculture), alley cropping (Agrisilviculture)
Changes in growing season due to temperature and precipitation	Protect crops by creating microclimates	Windbreaks (Agri-silviculture), Agrisilvipasture, alley cropping (Agri-silviculture)
Winter storms and cold temperature extremes	Reduce cold stress on animals by providing shelter	Silvipasture, windbreaks (Agri-silviculture)
Increased insect and disease problems	Control pests by providing habitat for beneficial insects	Windbreaks (Agri-silviculture), riparian forest buffers, alley cropping (Agri-silviculture)
Increased possibility of crop failure due to other risks	Reduce total crop loss by increasing crop diversity	All agroforestry practices

Adapted and modified after: (USDA, 2015)

Table 2. Conditions for agroforestry adoption

Conditions	Adopt	Do not adopt
Land tenure	Secure	Insecure
Access to usufruct	Vested primarily in household	Subject to government control or taxation or ambiguous
Choice of tree species	To meet farmer's priority	To meet official's priority
Tree ownership	Owned by household by law or in practice	Owned by or shared with government or local authority or ambiguous
Management arrangements	Participative or semi-autonomous	Centralised and prescriptive
Security to future produce	Provided and binding	Uncertain
Rights to cut and fell trees	Unrestricted and practiced at will by owners	Restricted or believed to be restricted
Rights of transit to market	Unrestricted and practiced by at will by owners	Restricted or believed to be restricted
Marketing	Competitive	Monopolistic
Prices of tree produce	Known, stable or rising	Not known, unstable or dropping
Target concerns	Specific household needs	Social welfare
Production goal	Immediate household needs	Conservation

Based on: (Chambers et al., 1989); (Pasicolonet al., 1997); (Sood, 2003);(Sood et al., 2018)

development plans.

- Estimation of economics of different models of agroforestry so that it can help farmers to avail loans.
- Quantification and modeling of carbon sequestration potential of agroforestry at global level, regional and farm level. This will help in estimating the revenue potential to be earned through carbon credits.

5. CONCLUSION

In addition, production of tree and food products from the same area of land, agroforestry has gained popularity because of its role in climate adaptation and carbon sequestration. Compared to other land uses, agroforestry has the greatest potential to sequester carbon by 2040. This is due to the vast amount of land (630 x 106 hectares) that is accessible for agroforestry globally (IPPC, 2000). Globally, the amount of land covered by agroforestry has been trending upward since the 1990s. In India, the promotion of agroforestry has been viewed seriously as evident from the fact that India is first country in the world to adopt the National Agroforestry Policy. Despite this, obstacles to agroforestry's growth and intensification must be removed globally in order to enhance farmers' livelihoods and fulfil the practice's promise for local, regional, and global carbon sequestration. As a result, the loss from deforestation and degradation would be balanced, leading to sustainable land use and the adaptation and mitigation of climate change. To determine the size of the agroforestry area, investigations utilising remote sensing and GIS technology must be conducted on a regular basis. The policies solely devoted to agroforestry need to be enunciated by each nation to provide scaffolding to agroforestry development and research.

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Authors' Contributions

K.K Sood – Conceptualization, Resources, Writing-original draft, Supervision

Sandeep Sehgal – Conceptualization, Reviewing and Editing, Visualization

Vibhuti Rathore – Reviewing and Editing, Visualization

Conflict of Interest

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

Data Availability

Not Applicable

Declaration about Use of AI Tools

No use of AI tools have been used in the writing process.

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Hybrid Willow Cultivation: Maximising Yields through Variable Spacing and Coppice Managements

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***Abstract*:** The study was conducted in an experimental field at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, to assess the effect of spacing and clones on the growth and yield of hybrid willow. The experiment was set up in a factorial randomized block design with three hybrid clones (J-799, Kashmiri and J-194) planted at three spacings, 1 × 1 m, 1 × 2 m and 1 × 3 m. The highest survivability was observed for J-194 at 1 × 3 m spacing (86.04%), but the effects of spacing and clones were not significant. Spacing has a significant impact on the growth parameters of both trees and coppices. Kashmiri at 1 × 1 m spacing had the highest individual tree height (6.59 m) and the highest per hectare volume (189 m^3/ha), while J-194 at 1 × 2 m spacing recorded highest mean tree volume (2275.00 cm^3) and J-799 at 1 × 3 m largest mean diameter (6.94 cm). In coppice J-799 at 1 × 3 m spacing the highest collar diameter (3.03 cm), diameter at breast height (2.70 cm) and mean stem volume (2583.49 cm^3) were observed. Kashmiri at 1 × 1 m spacing showed highest leading coppice height (4.21 m) and J-799 at 1 × 1 m spacing the highest per-hectare volume (20.85 m^3/ha). The study shows that lower planting density favoured more lateral growth and individual volume production, whereas denser spacing was more favourable for height and volume per hectare.

Keywords: *Salix*, Willow, Coppice, Clones, Hybrid, Spacing.

1. INTRODUCTION

The genus *Salix*, in the family Salicaceae, contains 450-520 species worldwide (Wu et al., 2015). These are primarily deciduous trees and shrubs that thrive in moist soils across the Northern Hemisphere's temperate zones. Common names include 'willow', with specific narrow-leaved shrubs called 'osier' and broad-leaved species known as 'sallow'. In India, 33 willow species have been documented in the northern regions, of which 24 are explicitly found in the Indian Himalayan region. Willows exhibit remarkable adaptability, capable of growing in a wide range of soil conditions, including compacted, swampy, alkaline, or acidic soils, provided adequate moisture is available (Baum et al., 2009). Willows are highly versatile, fast-growing and extensively used in plantations worldwide (Chaudhary et al., 2013) and are good coppicers, frequently yielding 20 or 25 shoots from a single coppice stool (Anchal et al., 2022). They are notable for their rapid growth, excellent regenerative capabilities and high productivity within short rotation cycles and are widely used in wood and biomass production (Hernea et al., 2015).

Spacing is a crucial factor in project planning and execution in production forestry. Effective stand management requires careful control of growing stock spacing, plays vital role in resource availability and affects growth and yield (Stolarski et al., 2019). Moreover, spacing has significant silvicultural, economic and technological consequences, impacting productivity, costs and management practices (Moulin et al., 2015; Brito et al., 2019; Resquinet et al., 2019). Furthermore, spacing can alter wood properties by influencing tree growth and morphology (Hebert et al., 2016, Rocha et al., 2016). Optimum spacing is determined by the ability to produce the maximum yield with the desired size, shape and quality. However, it varies depending on the plant species, cultivars, site conditions and the genetic potential of the reproductive material used (Li et al., 2014; Brito et al., 2019; Huijuan et al., 2020; Fernandes et al., 2023). Numerous genetic improvement efforts have been undertaken in willow to develop improved clones for different end uses, such as bioenergy production, erosion control and phytoremediation (Larsen et al., 2014; Fehrenz et al., 2024). Willows can be grown at high densities and managed as short rotation coppice (SRC) due to their high coppicing ability. They are cultivated as an energy crop for

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biomass production and utilised as a renewable source of bioenergy (Fischer et al., 2015; Srivastava et al., 2025). In this context, the effect of spacing on the growth parameters and productivity of 3 clones and their coppice shoots formed after harvest was studied.

2. MATERIALS AND METHODS

The study was carried out at an experimental farm in Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), located at 30°51'05.0"N and 77°11'12.7"E with an altitude of 1060 metres above mean sea level. This site is situated within the mid-hill zone of Himachal Pradesh, India. The local climate is sub-temperate and sub-humid, with moderate summers and cool winters; May and June are the hottest months, while January and February are the coldest. Meteorological data, including rainfall, mean temperature and relative humidity, were recorded from January 2017 to December 2020 at the Meteorological Observatory of the Department of Environmental Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) (Figure 1). The prevailing weather conditions during the experimental period were favourable for willow growth. Yearly average meteorological data and averages during the growing period (March-October) are provided in Table 1.

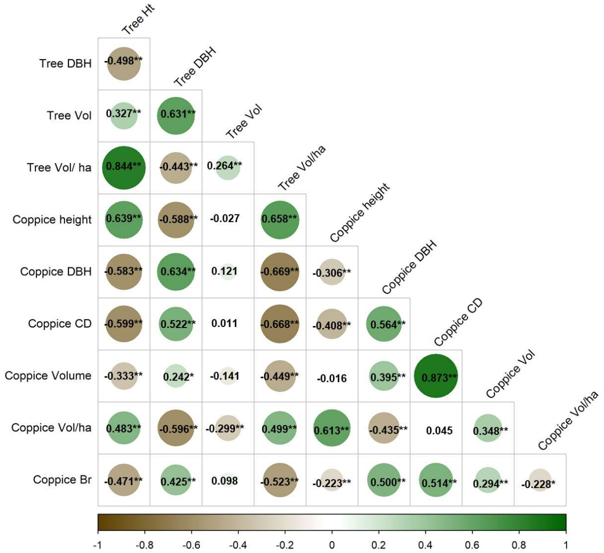

Figure 1. Correlation heat map of tree and coppice growth parameters

The soil depth at the site varies from very deep (>120 cm) to shallow (<20 cm), with the majority being moderately deep (50-80 cm). The soil at the experimental site was clay loam, with moderate fertility. The topsoil consists of clay (50%), silt (44-47%) and humus (3-6%). The experiment was set up in a factorial randomized block design with three clones, spacings and replications, with 18 trees for each combination (Table 2). After a growing period of three years, the survivability percentage (SV%) was calculated. The growth parameters of the surviving trees were measured and the trees were harvested, 15 cm from the base, to facilitate the growth of coppices on the stumps. The height of the tree (Tree height) was measured using a clinometer. Diameter at breast height (Tree DBH) of the trees was measured with the help of a tree caliper. Volume for each tree (Tree Vol) was calculated (Mahendran et al., 2018) and volume/hectare (Tree Vol/ha) was derived from it.

Leading coppices were selected from each stump and the remaining coppices were removed 2 months after harvest. The height of the coppice (Cop Ht) was measured with a measuring stick from the leading tip to ground level and the branches (Cop Br) were counted. The collar diameter (Cop CD) and diameter at breast height (Cop DBH) of the coppice shoot were measured with the help of a digital calliper. Volume for each coppice shoot (Cop Vol) was calculated similarly to that of the trees and coppice volume/hectare (Cop Vol/ha) was derived from it. The effects of the three spacings and clones on the growth parameters of trees and coppices were studied.

2.1. Data Analysis

Data were presented as means and all tests were statistically analysed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The primary and interactive influences of spacing

Table 1. Annual average of meteorological data and average of meteorological data during the growing period

Year	Annual			Growing period		
	Ppt (mm)	M T (°C)	RH (%)	Ppt (mm)	M T (°C)	RH (%)
2017	97.37	12.45	60.00	131.36	21.44	61.59
2018	40.27	18.13	61.08	46.91	21.28	64.37
2019	87.80	18.08	62.28	101.51	21.49	63.30
2020	87.21	19.05	61.25	98.46	21.75	63.25

Ppt (mm): Precipitation, M T (°C): Mean Temperature, RH (%): Relative Humidity

design and clones on growth parameters of trees and coppice shoots were determined using analysis of variance. Karl Pearson's correlations were calculated to determine the significance and strength of relationships among all variables.

3. RESULTS AND DISCUSSION

3.1 Survivability

After a period of 3 years of growth, the mean survivability (SV %) of the entire experiment was 67.79%, ranging from 50% for C1S2 to 86.04% for C3S3 (Table 3). Kirongo et al. (2013) had observed that wide spacing (2.5 x 2.5 m) gave significantly better survivals than closer spacing (1 x 1 m). Similar observations have been made in Douglas-fir (Curtis et al., 2016). The highest survivability was observed for C3 with the widest spacing (S3) in the current study. However, there were no significant differences among the spacings or clones.

3.2. Tree Growth Parameters

3.2.1. Height growth: The highest average tree height was observed in the C2 clone planted at S1, reaching 6.59 m. Conversely, the C1 clone at S3 exhibited the lowest average height, measuring 3.73 m (Table 3). The variation in tree height was significant among the three spacings and a progressive decrease in mean tree height was observed as planting spacing increased (S1 > S2 > S3). Among clones, tree height varied significantly only between C3 and C2 and between C2 and C1. Spacing has a profound influence on the form, growth and productivity of young plantations (Tun et al., 2018) by altering the resources each tree can access (Thomas et al., 2009). The mean tree height was higher in plots with closer plant spacing (S1) and decreased with increasing spacing. This phenomenon is a classic silvicultural response, where denser planting (S1) intensifies inter-tree competition for light. This competition promotes vertical growth, as trees grow faster to capture available sunlight, often at the expense of radial expansion. Similar observations were recorded in *Acacia mearnsii* (Amanuel et al., 2025) and in Teak (Kainyande et al., 2023). However, this effect of spacing on height might not be evident in the very early stages of growth and in some cases, spacing reduction can negatively affect height growth (Kirongo et al., 2012).

3.2.2. Lateral growth: The variation in tree DBH among the three spacings was significant, but the variations among the clones were not significant. The largest average tree DBH at breast height was in the C1 clone at S3, with a mean of 6.94 cm, whereas the C2 clone at S1 exhibited the smallest average tree DBH, at 5.32 cm (Table 3). In a direct and inverse relationship to tree height, tree DBH demonstrated an increase with wider planting spacing (S1 < S2 < S3). The results agree with Kirongo et al. (2012), Tun et al. (2018) and Kainyande et al. (2023) who observed that increasing spacing led to a subsequent increase in lateral growth. This can be attributed to the reduced competition for resources

Table 2. Spacing and clones used in the study

Spacing	Abbreviation	Clones	Abbreviation	Parents
1 × 1 m	S1	J-799	C1	*Salix matsudana* × *S. alba*
1 × 2 m	S2	Kashmiri	C2	*Salix alba* cv. *caerulea*
1 × 3 m	S3	J-194	C3	*Salix matsudana* × *S. arbutifolia* × *S. matsudana*

Table 3. Mean growth parameters of different clones planted at three spacings (Mean)

Clone	Spacing	Sv (%)	Tree height (m)	Tree DBH (cm)	Tree Vol (cm^3)	Tree Vol/ha (m^3/ha)
C1	S1	67.99	5.872^{b}	5.44^{c}	1707.98^{bc}	170.8^{ab}
C1	S2	55.36	4.817^{c}	6.24^{b}	1859.16^{bc}	97.61^{c}
C1	S3	73.54	3.732^{d}	6.94^{a}	1799.12^{bc}	59.96^{d}
C2	S1	74.34	6.589^{a}	5.32^{c}	1899.73^{bc}	189.97^{a}
C2	S2	50.00	5.045^{c}	6.23^{b}	1981.03^{ab}	99.05^{c}
C2	S3	60.74	4.204^{d}	6.83^{a}	1959.79^{abc}	65.32^{d}
C3	S1	77.31	5.201^{c}	5.56^{c}	1618.9^{c}	161.89^{b}
C3	S2	64.81	5.192^{c}	6.53^{ab}	2275^{a}	113.75^{c}
C3	S3	86.04	3.855^{d}	6.71^{a}	1757.38^{bc}	58.57^{d}

SV- Survivability percentage, Tree DBH- Tree Diameter at Breast height, Tree Vol- Tree volume, Tree Vol/ha- Tree Volume per hectare

with the stocking reduction (Đodan et al., 2024). Lower stocking allows greater resource availability per individual; trees can allocate more photosynthates to radial growth, resulting in thicker stems (Li et al., 2023).

3.2.3. Stem and stand volume: Tree volume varied significantly between spacings S1 and S2, and between S2 and S3. Among clones, tree volume varied significantly only between C2 and C1. The highest mean individual tree volume was observed for C3 at S2 (2275.00 cm^3) and the lowest was for the same clone at the densest spacing (S1), at 1618 cm^3 (Table 3). Consistently higher individual tree volumes for all clones were observed for the intermediate spacing S2. Reducing stocking has been observed to have a positive impact on individual tree volume (Héber et al., 2016). Similar observations were made by Zhang et al. (2020) and Stape et al. (2022) reported that the highest individual tree volumes occurred in stands with the largest spacing. However, this pattern is not evident in the current study, which might be due to the contributions of height and diameter towards volume and their inverse correlation among the different planting densities. Tree volume per hectare varied significantly among all the spacings. However, among clones, tree volume per hectare varied significantly only between C2 and C1. The highest mean tree volume per hectare was observed for the C2 clone at S1, reaching about 189 m^3/ha, while the lowest was for C3 at S3 (58.57 m^3/ha). C2 consistently outperformed the other clones in terms of Tree volume per hectare, across all spacings.Volume production per unit area is of paramount importance in commercial forestry, as it directly reflects the overall productivity of the stand. The consistent trend was observed where tree volume per hectare is generally highest at the densest spacing (S1) and progressively decreases with wider spacing (S3). The higher per-hectare volume has been observed in previous studies for younger or short-rotation stands with high stocking (Sang et al., 2021; Stape et al., 2022). There is a distinct and critical trade-off between individual tree volume and volume per hectare as planting spacing changes. This is primarily because, despite individual trees being smaller in denser stands, the significantly greater number of trees per hectare more than compensates, leading to a higher cumulative volume in younger stands.

3.3. Coppice Growth Parameters

3.3.1. Leading coppice height: Coppice height varied significantly between spacings S1 and S2 and between S1 and S3, but there was no significant difference among the clones. The maximum mean coppice height was observed for the C2 clone at S1, reaching 4.21 m and the C3 clone at S3 exhibited the least mean coppice height, at 2.68 m (Table 4). The trend for coppice height largely mirrors that of initial Tree height. i.e., denser initial spacing (S1) generally leads to taller coppice shoots. This is likely due to the competition for light among the coppice shoots of individual stools, along with the smaller initial spacing, which promotes vertical growth. However, studies with contradictory results have been reported. Ogunwande et al. (2022) reported that the leading coppice formed after cutting six-month-old *Albizia lebbeck* were tallest in the field with the least density (2 x 2 m). Intense competition can be deleterious to the overall growth when the stocking is very high (Berbec and Matyka 2020).

3.3.2. Lateral growth: The variation in coppice collar diameter and diameter at breast height among the three

Table 4. Growth parameters of the leading coppice shoots

Clone	Spacing	Cop Ht (m)	Cop DBH (cm)	Cop CD (cm)	Cop Vol (cm^3)	Cop Vol/ha (m^3ha^{-1})	Cop Br
C1	S1	3.92^{ab}	1.77^{de}	1.85^{cde}	2084.52^{abc}	20.85^{a}	20^{d}
C1	S2	3.28^{bc}	2.22^{bcd}	1.78^{cde}	1868.41^{bc}	9.34^{cd}	26^{bcd}
C1	S3	2.81^{c}	2.7^{a}	1.95^{a}	2583.49^{a}	8.61^{cd}	28^{bcd}
C2	S1	4.21^{a}	1.48^{e}	2.37^{e}	1508.77^{c}	14.71^{bc}	19^{d}
C2	S2	3.48^{c}	2.22^{abcd}	2.27^{bcd}	1819.14^{bc}	8.73^{d}	29^{abc}
C2	S3	3.03^{c}	2.64^{ab}	2.56^{ab}	2471.4^{ab}	8.24^{d}	34^{a}
C3	S1	3.51^{abc}	1.75^{cd}	3.03^{de}	1703.47^{bc}	16.81^{b}	22^{cd}
C3	S2	2.99^{bc}	2.31^{abc}	2.74^{bcd}	2128.57^{bc}	10.1^{cd}	29^{ab}
C3	S3	2.64^{c}	2.56^{a}	2.88^{abc}	2256.07^{abc}	7.52^{d}	29^{ab}

Cop Ht- Coppice height, Cop DBH- Coppice diameter at breast Height, Cop CD- Coppice collar diameter, Cop Vol- Coppice volume, Cop vol/ha- Coppice volume per hectare, Cop Br- Coppice branches

spacings was significant, while among the clones, coppice DBH varied significantly between C3 and C2. However, variation in coppice collar diameter among clones was not significant. The C1 clone at S3 had the largest mean collar diameter (3.03 cm; Table 4) and the highest mean coppice DBH (2.70 cm). Conversely, the C2 clone at S1 exhibited the smallest average Coppice collar diameter and coppice DBH at 1.78 cm and 1.48 cm, respectively. Plants with the widest spacing were observed to have the largest stem diameter, followed by intermediate spacing and the smallest diameter was observed in plants with the closest spacing (Ogunwande et al., 2022; Pradhan et al., 2023). This aligns with the results of this study. This trend of higher spacing producing coppices with larger stem diameter follows the pattern seen in tree DBH.

3.3.3. Stem and stand volume: Coppice volume had a significant difference only between the spacings S1 and S3, while coppice volume/ha varied significantly between the spacings S1, S2 and S1, S3. Among the clones, significant variation was observed between C3 and C1 and between C2 and C1 for coppice volume. The highest average coppice volume was for the C1 clone at S3, with 2583.49 cm^3 (Table 4). Conversely, the C2 clone at S1 exhibited the lowest average Coppice volume, at 1508.77 cm^3. Coppice volume generally increased with the spacing of the initial planting, except for C1, which showed a non-significant decrease from S1 to S2. Higher individual stem volumes have been reported in stands with lower density (Hébertet al., 2016; Huijuan et al., 2020; Sang et al., 2021). The coppice volume/ha varied significantly between C3 and C1 and between C2 and C1. The highest mean coppice volume per hectare was recorded for the C1 clone at S1, at 20.85 m^3/ha. C3 clone at S3 exhibited the lowest mean coppice volume/ha, at 7.52 m^3/ha. Similarly, to tree volume/ha, coppice volume/ha decreased with increasing initial planting spacing (S1>S2>S3). The C1 clone performed better for individual coppice volume and per-hectare volume production across most spacings. The contribution of stem number, due to higher planting density, is often greater than the individual stem volumes when calculating stand volume (Chane et al., 2022).

3.3.4. Branch number: Coppice branch number varied significantly between S1 and S3 and between S1 and S2; for clones, significant variation was observed only between C3 and C1. The maximum mean coppice branch number was recorded for the C2 clone at S3, reaching 34. This indicates that C2, when initially planted at the widest spacing, subsequently produced coppice shoots with the largest number of branches. Conversely, the C2 at S1 exhibited the lowest average number of 19 branches (Table 4). Similar to coppice DBH, coppice volume and coppice collar diameter, the number of branches generally shows an increasing trend with wider initial planting spacing (S1 < S2 < S3). This may be due to the lesser competition during the initial growth phase, resulting in larger and more vigorous stumps, which subsequently enable more robust basal shoot development upon coppicing. In line with this study's results, the number of branches and their size were reported to increase with reduced stocking (West and Smith 2020). With the increase in spacing, biomass accumulation has been reported to increase in the branches (Hébert et al., 2016)

3.4. Correlation Studies

The correlation coefficient reflects the level of correlation between the tree growth parameters and coppice growth parameters (Figure 1). Tree height and diameter at breast height (DBH) showed strong positive correlations with tree volume and tree volume/ha, indicating their direct contribution to volume. However, tree height and tree DBH were significantly negatively correlated. This is primarily due to the spacing effect, in which closer spacing promotes greater height growth than lateral growth and vice versa with increased spacing. Another notable effect is the strong negative correlation of tree diameter at breast height and coppice collar diameter with tree volume/ha and coppice volume/ha. This suggests that even though tree DBH and coppice collar diameter are contributing factors for volume estimation, the effect of the number of trees per hectare had a higher contribution to tree volume/ha. This has been observed in previous spacing studies, especially in short rotation crops (Sang et al., 2021; Chane et al., 2022). The coppice height showed significant positive correlations with tree height, tree volume and coppice volume/ha and significant negative correlations with tree and coppice DBH, collar diameter and branches. Unlike the significant positive correlation of tree height and volume, coppice height did not show any significant correlation with coppice volume. This might be due to the lateral growth in the leading coppice shoots after removing competing shoots from the stump. Regarding the efficacy of predictor variables, the analysis highlights that for coppice volume estimation, diameter-based measurements were far superior predictors than coppice height. DBH and collar diameter had a significant positive correlation with each other and also showed a significant positive correlation with tree DBH and coppice volume branch number. Furthermore, the size of individual coppice shoots was not a strong predictor of stand-level coppice volume/ha, suggesting that density is more influential for volume production at the stand level, at least at younger ages.

4. CONCLUSION

The study concludes spacing has a significant effect on growth parameters of 3-year-old hybrid willow clones and their coppices. The denser stocking has been shown to improve vertical growth, higher stocking density restricts lateral growth. Conversely, reducing planting density promotes lateral growth but reduces height growth. For higher stand volume, high-density stocking is recommended; wider spacing can be considered if higher-diameter growth is required. In general, Kashmiri clone consistently maintained higher volume per unit area across various spacings in trees compared to other clones; conversely. J799 clone performed better in most growth parameters across all spacings in coppice.

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Authors' Contributions

All authors contributed significantly to the development of this work. AM contributed to field data collection, data curation and the writing of the original draft. JPS contributed to the conceptualization, study design, methodology, supervision and validation. All authors (AM, JPS and BNP) contributed to the writing, review and editing of the manuscript, as well as to data analysis and interpretation.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Unraveling Genetic Variability in Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* (Guenee) using Mitochondrial Cytochrome Oxidase I (COI)

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***Abstract*:** The present study was undertaken to provide insights regarding population genetic structure of *Leucinodes orbonalis* from diverse locations. Molecular characterization of *L. orbonalis* populations collected from seven locations was carried using mitochondrial cytochrome oxidase I (COI). Collected specimens were subjected to DNA extraction, PCR amplification and sequencing of the targeted gene. The samples of *L. orbonalis* were collected from three locations of Himachal Pradesh, two locations each from Punjab and Haryana. The pair-wise genetic distance of *L. orbonalis* populations collected from different regions of Himachal Pradesh, Punjab and Haryana ranged from 0.0000 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations. The phylogenetic analysis revealed two distinct clades based on the COI gene of mitochondrial region of *L. orbonalis*. The population of *L. orbonalis* from Shimla (MW363534), Solan (MW344268), Una (MW363538), Karnal (MW363539), and Jalandhar (MW363537) were clustered together in clade I due to their close proximity, while population of *L. orbonalis* from Firozpur (MW363535) and Bhiwani (MW363536) were clubbed in clade II. The average nucleotide compositions were adenine (30.46 %), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93 %).

***Keywords*:** *Leucinodes orbonalis,* Mitochondrial cytochrome oxidase I (COI), Population, Phylogenetic analysis, Molecular characterization.

1. Introduction

Eggplant (*Solanum melongena* L.) is the major and economically important vegetable crop with high nutritive value and cultivated in the hot-wet climatic conditions in most parts of Asian countries (Thapa, 2010). In India, there are approximately 2500 varieties of brinjal with diverse shapes, ranging from oval or egg-shaped to long or club shaped. The color of the fruits ranges from white, yellow, green and purple to nearly black.

One of the major factors which is responsible for low productivity of brinjal is a variety of insect pests which not only reduce the yield but quality also. Among these, the brinjal shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera: Pyralidae) has been reported to be the serious pest which reduces the crop yield up to 60-70 per cent and inflicts the colossal loss in production (Sharma et al., 2017) and *L. orbonalis* is the most noxious and ubiquitous pest among the different insect pests of brinjal (Singh et al., 2019).

Insecticide treatments against this pest are not providing adequate control and repeated use of existing insecticides has resulted in the development of resistant biotypes by means of genotypic variations in these insects (Alyokhin et al., 2008). In response to the stresses, the populations of *L. orbonalis* have changed to adapt the ill-effects by changing their eco- behavioral pattern, feeding physiology and reproduction, in addition to the changes in their molecular machineries. It is therefore necessary to explore the genotypic variations that occur within the population of a species to design and formulate effective insect pest management strategies (Padwal and Srivastava, 2020). To minimize the hazardous effects of chemical pesticides, world agro-analysts intend to shift their insect pest management strategies solely towards biological control, a method which encompasses the broad range of concepts from using the different cultural practices and introduction of living organisms to control the pest (Baker et al., 2020).

Different researchers and scientists across the world are using molecular techniques like DNA barcoding for precise identification (Zhang et al., 2010; Shashank et al., 2015; Murali et al., 2017; Palraju et al., 2018). Amplified fragment length polymorphism-polymerase chain reaction (AFLP-

PCR) analysis was done to characterize 29 natural populations of *L. orbonalis* from different brinjal growing regions of India. Data analysis helps to determine the extent of inter- and intra- population variability amongst the different populations (Saini et al., 2006). Zhang et al. (2010) suggested a simple resampling approach for estimating several key sampling sizes for a DNA barcoding project.

Chakravarthy (2015) suggested that DNA barcoding is a major tool in fast and authentic identification of insects belonging to different orders and families. Murali et al. (2017) studied the molecular characterization of 21 population of *L. orbonalis* collected from Karnataka, Andhra Pradesh, New Delhi and Uttar Pradesh. They amplified CO I gene, the sequencing resulted in 600-636 bp and amino acid composition of nucleotide, which varied from 195-206 in different populations. Certain amino acids were either present or absent in different populations indicating their diversity.

Species level identification is dependent on different diagnostic characters. Morphology based identification is a hectic task due to time consumption. So, there is an unmet need to introduce the quick, economical and undisputed approaches to analyze the shoot and fruit borer taxonomically. This research was aimed to characterize populations of brinjal shoot and fruit borer, *L. orbonalis* for their genetic diversity from different location of Himachal Pradesh, Punjab and Haryana using universal primer (LCO1490/HCO2198) as a tool of the molecular marker.

2. MATERIAL AND METHODS

2.1. Survey and Collection of Insect Samples

Brinjal shoot and fruit borer (infested fruit and shoot) were collected from different locations of Himachal Pradesh, Punjab and Haryana (Table 1).

2.2. Maintenance of Population of *L. orbonalis* Collected from Different Locations

Larvae of *L. orbonalis* were collected in vials containing 90 per cent ethanol from each location. Collected specimens were brought to Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. After washing with alcohol larvae were transferred to clean glass vials with the help of forceps and were preserved in 90 per cent ethanol for the DNA analysis and stored in -20ºC. For DNA analysis, the larvae were used after the removal of the intestine.

2.3. Study on Genetic Variability

The protocols used to study genetic diversity of *L. orbonalis* from different locations were discussed under the following subheadings: a.) DNA extraction; b.) Preparation and running of Agarose gel; c.) PCR amplification; d.) Visualization of PCR product; e.) DNA Sequencing and analysis.

a. DNA extraction

Total genomic DNA of *L. orbonalis* was extracted from larvae was followed by using the HiPurA® Insect DNA Purification Kit. The DNA purification procedure using the miniprep spin column comprising of three steps *viz.,* adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. The eluate contained pure genomic DNA was stored for further use at -20°C in the deep freezer.

b. Preparation and running of agarose gel

For gel preparation of 0.8% concentration, 1.6g of

Table 1. Details of locations surveyed for sampling

States	District	Place of collection	Agro- climatic zones	Latitude	Longitude	Altitude (mamsl)
Himachal Pradesh	Shimla	Rohru	High hill	31° 12' 9.68" N	77° 45' 17.42" E	1554
	Solan	Nauni	Mid hill	31° 51' 44.75" N	77° 10' 9.15" E	1275
	Una	Rampur	Low hill	31° 27' 53.75" N	76° 16' 8.90" E	393
Punjab	Jalandhar	Phagwara	Central plain zone	31° 13' 26.4720" N	75° 46' 14.8728" E	234
	Firozpur	Dindsha	Western plain zone	30° 55' 24.3588" N	74° 36' 36.7704" E	182
Haryana	Karnal	Brass	Eastern zone	29° 41' 8.2644" N	76° 59' 25.9692" E	240
	Bhiwani	Chang	Western zone	28° 47' 56.5656" N	76° 8' 0.6504" E	225

Agarose powder (SeaKem® LE Agarose) was mixed with 200ml of 1X TAE (Tris-acetate-EDTA) buffer Solution in 250ml volumetric flask and the solution was heated until it was completely dissolved. For the visualization of nucleic acids, 2 µl Ethidium Bromide was added to the solution after the solution get cooled to 50-55°C. The solution was poured into the casting tray with the comb for solidification. After the gel gets solidified the comb was removed immediately. During the electrophoresis the gel was kept in the plastic tray, the wells were toward the negative electrode and the gel was submerged under 1X TAE buffer. For the detection of DNA in the sample, 2 µl of isolated DNA mixed with 2 µl 6X loading dye (Bromophenol Blue) was placed into the well carefully. Alongside the DNA samples, a DNA ladder was also placed in the well and the gel was run at 90 volts for 45 min to confirm the extraction. DNA was visualized by staining with ethidium bromide when ample migration has done (Corley, 2004).

c. PCR amplification

The PCR amplification of DNA samples was carried out in a thermocycler (ProFlex^{TM} Base) using 20 µl reaction volume. The reaction mixture contained PCR pre-mix which is composed of Taq Buffer, dNTPs, $MgCl_2$, Taq DNA Polymerase along with the pair of primers, distilled water and genomic DNA.

Primers: The PCR amplification of mitochondrial region (COX1) was done with the universal primer for arthropods LCO1490/HCO2198 (Table 2).

PCR amplification*:* The reaction mixture was added to PCR tubes and the thermal cycler was programmed for 35 cycles with one cycle of initial denaturation at 94°C for 3 minutes whereas denaturation, annealing and elongation were repeated 36 times for 1 minute each at 94°C, 52°C and 72°C respectively. A final extension at 72°C for 30 minutes was carried out.

d. Visualization of PCR product

PCR product 2 µl from each tube was mixed with 2µl loading dye separately on a parafilm sheet and then loaded on 1.5 percent agarose gel (submerged in 1X TAE buffer and stained with 2 µl ethidium bromide for the detection of DNA) along with 2 µl DNA ladder (Genei 3kb DNA ladder) to determine the size of the amplified products. Then the gel was run at 90 volts for 60 minutes. Later the image analysis and photography were done under UV light by using gel documentation system (BIO-RAD, Model- Universal Hood II) and Image Lab™ Software.

e. DNA Sequencing and analysis

PCR product 10 µl from each sample was sent to Eurofins Genomic India Pvt. Ltd., Bangalore, Karnataka for the sequencing. The Sanger dideoxy method was used for sequencing of *L. orbonalis* samples. The sequences were subjected to BLAST (Basic Local Alignment Search Tool) in search of the homology of the partial sequences and were compared with similar sequence from NCBI database in order to find their phylogenetic relationship. The phylogenetic tree was constructed based on the CO1 sequence of *L. orbonalis* with Neighbor-Joining (NJ) method by using Mega X software (Saitou and Nei, 1987).

f. Genetic divergence: The genetic divergence was shown in terms of the number of base substitutions per site from between sequences. Analyses were conducted using the Maximum Composite likelihood model (Tamura et al., 2004) and evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Submission of sequence: The aligned nucleotide sequence in FASTA format was submitted to NCBI with all the necessary details. BankIt was used for the submission of nucleotide sequences to NCBI (Wheeler et al., 2001; Benson et al., 2003), from where accession numbers for all the sequences were received.

3. RESULTS AND DISCUSSION

3.1. Genetic Variability

The universal COI primer sequence was successfully used to amplify the COX1 region from all the DNA isolates. The amplified PCR product of DNA isolates (Figure 1).

3.2. Analysis of Nucleotide Sequence

To find out the per cent similarities, the nucleotide

Base sequence of COI primer

Primer	Sequence (5´ to 3´)
Forward	GGTCAACAAATCATAAAGATATTGG
Reverse	TAAACTTCAGGCTGACCAAAAAATCA

Table 2. Primers along with their nucleotide sequences and properties

Primer	Sequence (5´ to 3´)	Molecular weight (g/mol)	Length (Base pair)	Temperature (°C)	GC content (%)
Forward	GGTCAACAAATCATAAAGATATTGG	7722.1	25	56.4	32.0
Reverse	TAAACTTCAGGCTGACCAAAAAATCA	7940.2	26	58.5	34.6

sequences of *L. orbonalis* were aligned with the reference of NCBI (National Centre for Biotechnology Information) using BLAST tool (Basic Local Alignment Search Tool) which revealed the taxonomic identifications *viz.,* genus, species, sub-species and family and a comparative analysis of test sequence and reference sequence (NCBI database) were provided. The mtCOI sequences of *L. orbonalis* from all seven locations of the present study were submitted to the NCBI GenBank vide accession numbers (Table 3).

The populations of *L. orbonalis* have changed to adapt the ill-effects by changing their eco- behavioral pattern, feeding physiology and reproduction, in addition to the changes in their molecular machineries. It is therefore necessary to explore the genotypic variations that occur within the population of a species to design and formulate effective insect pest management strategies. DNA-based identification system, which is in the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), can help in taxonomic classification of animal kingdom (Hebert et al., 2003). Chang et al. (2014) reported that *L. orbonalis* is the most detrimental South and Southeast Asian insect pest of eggplant. To help reduce the impact of this pest, population genetic diversity and structure of *L. orbonalis* were examined in eight populations from six countries using mitochondrial cytochrome c oxidase subunit I DNA sequences. Therefore, present investigation was undertaken on brinjal shoot and fruit borer, *L. orbonalis* for genetic diversity of different population.

For the genetic variability of *L. orbonalis,* the genomic DNA was collected from different locations of Himachal Pradesh, Punjab and Haryana and PCR analysis was performed using mitochondrial DNA specific primer. The universal base sequence of COI primers was successfully used for the amplification of COX1 region from all the DNA isolates and resultant amplicon of expected size around 600-700 bp was obtained and sequenced. Under analysis of nucleotide sequences, sequences of *L. orbonalis* were aligned with the reference of NCBI (National Centre for Biotechnology Information) using BLAST tool (Basic Local Alignment Search Tool) which revealed the taxonomic identifications. The accession number for all the samples were submitted.

3.3. Variation in Composition of Nucleotides

The present COI sequences of *L. orbonalis* from different regions of Himachal Pradesh, Punjab and Haryana showed significant variations in their nucleotide contents (Table 4). The average AT and GC content of all the specimen was 70.00% and 29.93%, respectively. The maximum AT content

Figure 1. Visualization of DNA samples on agarose gel electrophoresis (Where Ladder= 3Kb DNA ladder and DNA samples *viz.,* S11= Solan, S21= Una, S31= Shimla, H11= Bhiwani, H21= Karnal, P11= Jalandhar, P21=Firozpur

Table 3. GenBank accession no. of mtCOI sequences of the *Leucinodes orbonalis* collected from different locations

States	District	Place of collection	Submission ID	Gen Bank accession no.
Himachal Pradesh	Shimla	Rohru	SUB8692751	MW363534
	Solan	Nauni	SUB8691815	MW344268
	Una	Rampur	SUB8692807	MW363538
Punjab	Jalandhar	Phagwara	SUB8722720	MW363537
	Firozpur	Dindsha	SUB8722721	MW363535
Haryana	Karnal	Brass	SUB8722711	MW363539
	Bhiwani	Chang	SUB8722709	MW363536

(70.93%) was from the population of Haryana (Chang-Bhiwani) and the minimum AT content (69.45%) from the population of Himachal Pradesh (Rohru- Shimla). The highest GC content (30.53%) and lowest GC content (29.02%) was from Rohru- Shimla and Chang- Bhiwani, respectively. Overall, the average nucleotide compositions for *L. orbonalis* were found to be adenine (30.46%), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93%), respectively.

The highest adenine (A) was observed from the Phagwara- Jalandhar specimen (31.60%) and lowest (30.06%) from Rampur- Una specimen. The highest percentage of thymine (T) with a value of 40.27 per cent was recorded from Chang-Bhiwani specimen and lowest 38.52 per cent was from Phagwara- Jalandhar specimen. The highest percentage of guanine (G) was 14.28% in the Nauni–Solan specimen, while the lowest was 13.27% in the Chang–Bhiwani specimen. In contrast, cytosine (C) content was highest in the Rohru–Shimla specimen (16.31%) and lowest in the Chang–Bhiwani specimen (15.75%).

The sequence region showed AT rich nucleotide composition typical of insect mitochondrial gene (Crozier and Crozier, 1993). The average AT and GC content of all the specimen was 70.00 and 29.93%, respectively. Overall, the average nucleotide compositions for *L. orbonalis* were adenine (30.46%), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93%), respectively. The present observations on nucleotides composition of *L. orbonalis* slightly differed from the results of Shashank et al. (2015). They reported mean nucleotide compositions for *L. orbonalis* from different locations of India to be A (31.8 %), T (40.0 %), G (14.9 %) and C (13.3 %), respectively, which is in accordance with the results obtained in the present study.

3.4. Phylogenetic Analysis

The phylogenetic tree was constructed with Neighbour-Joining (NJ) method using Mega X programme (Saitou and Nei, 1987). This analysis recognized two distinct clades (Figure 2); the population of *L. orbonalis* from Shimla (MW363534), Solan (MW344268), Una (MW363538), Karnal (MW363539), and Jalandhar (MW363537) were clustered together in clade I due to their close proximity, while population of *L. orbonalis* from Firozpur (MW363535) and Bhiwani (MW363536) were clubbed in clade II. Second clade show some genetic difference in the phylogenetic analysis and molecular characters, which does not hold much significant importance in context to species evolution but their variability suggests the impact of geographical evolution of genetic behavior. However, both clades are of the same genera and species.

Under phylogenetic analysis of all the samples collected it was resulted that the pair-wise genetic distance of *L. orbonalis* populations collected from different regions of Himachal Pradesh, Punjab and Haryana ranged from 0 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations. Shashank et al. (2015) also reported genetic distance amongst *L. orbonalis* population that ranged from 0.00 to 0.016 in different geographical localities of India and inferred that there was no significant molecular diversity. Murali et al. (2017) reported the pair-wise genetic distance of *L. orbonalis* populations collected from different regions of India which ranged from 0.00 to 0.051 and inferred that there was no significant molecular diversity within *L. orbonalis* of different geographical locations of India with respect to COI but there is difference in genetic distance among the populations.

The pair-wise genetic distance (Table 5) of *L. orbonalis* populations collected from different regions of Himachal

Table 4. Average nucleotide composition of COX-1 gene sequences among all the *Leucinodes orbonalis* specimens collected from different locations

DNA isolates	A	T(U)	G	C	AT	GC
Rohru- Shimla	30.33	39.12	14.22	16.31	69.45	30.53
Nauni- Solan	30.10	39.78	14.28	15.82	69.88	30.10
Rampur- Una	30.06	39.86	14.16	15.90	69.92	30.06
Phagwara- Jalandhar	31.60	38.52	13.85	16.01	70.12	29.86
Dindsha- Firozpur	30.54	39.56	14.06	15.82	70.10	29.88
Brass- Karnal	30.50	39.43	14.16	15.90	69.93	30.06
Chang- Bhiwani	30.66	40.27	13.27	15.75	70.93	29.02
Average	30.46	39.57	14.02	15.93	70.00	29.93

Pradesh, Punjab and Haryana ranged from 0 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations.

The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). This analysis involved seven nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 478 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Significant genetic variation among the populations was observed by Murali et al. (2021) and Kar et al. (2021). A wide range of genetic variation occurs in the genome of *L. orbonalis* collected from different geographical locations. Murali et al. (2021) investigated the genetic variability of *L. orbonalis* in the region of Tamil Nadu, South India and during their study, 60 RAPD ten-mer primers were used. Among all the primers used, 10 primers generated reproducible and scorable banding profile. Highest genetic diversity over 80 per cent genetic polymorphism was shown

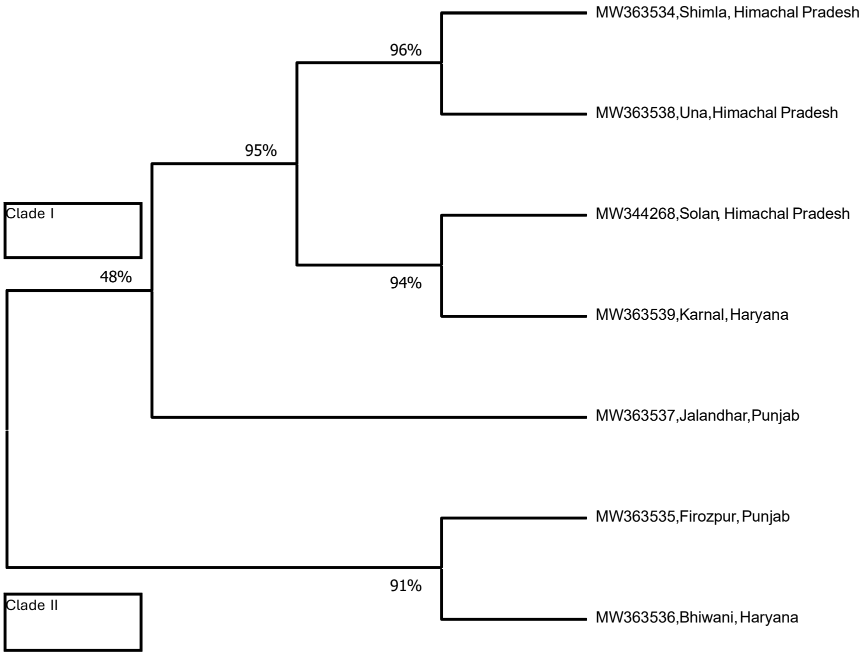

Figure 2. Phylogenetic tree of isolates of *Leucinodes orbonalis* collected from different locations of Himachal Pradesh, Punjab and Haryana

Table 5. Genetic divergence between COI sequences of nucleotide composition of COX-1 gene sequences among all the *Leucinodes orbonalis* specimens collected from different locations

Isolates	Locations	Isolates						
		1	2	3	4	5	6	7
1	Rohru- Shimla							
2	Nauni- Solan	0.0022						
3	Rampur- Una	0.0000	0.0022					
4	Phagwara- Jalandhar	0.0000	0.0000	0.0000				
5	Dindsha- Firozpur	0.0022	0.0044	0.0022	0.0000			
6	Brass- Karnal	0.0000	0.0000	0.0000	0.0000	0.0022		
7	Chang- Bhiwani	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

by primers *viz.,* OPG 7, OPG 8, OPS 2 and OPS 7. Hence, significant genetic variation was observed among the population. Kar et al. (2021) also studied genetic variability among geographically confined populations of *L. orbonalis* from 30 locations of Odisha using twenty random RAPD markers. The PCR amplification resulted into 118 bands by 10 primers and 116 bands were found to be polymorphic out of which 13 were unique bands. Genetic similarity varies from 0.37 to 0.93, with a mean of 0.65 which indicated a significant genetic variability among the populations. COI is widely used for determining the genetic diversity of *L. orbonalis* among the populations. Kariyanna et al. (2022) observed genetic diversity of *L. orbonalis* populations from different geographic locations across the world and observed that no significant variability was found but difference in the amino acid composition was resulted.

Several control strategies have been established for the management of *L. orbonalis.* But due to insecticidal resistance, various studies have reported on genetic diversity of *L. orbonalis* population of different locations so that a better understanding in the management strategies for these pests occurs. Thus, adoption of molecular marker method has been an effective tool in studying the genetic variability of *L. orbonalis* of different populations along with identifying and characterizing the insecticides resistance in populations of *L. orbonalis.*

CONCLUSION

This study revealed important insights into the genetic characterization of *Leucinodes orbonalis* populations across different regions. Molecular analysis showed the formation of two distinct clades: one comprising populations from Firozpur and Bhiwani with a similarity index of 91%, and the other including populations from Shimla, Solan, Una, Jalandhar, and Karnal with a similarity index of 95%. Pair-wise genetic distance among populations ranged from 0 to 0.0044, indicating low overall genetic divergence and a relatively homogeneous population structure. From a pest management perspective, this genetic uniformity suggests that control measures whether biological, chemical, or biotechnological—could be uniformly effective across regions without significant risk of localized resistance. Nonetheless, the slight divergence observed, particularly between the two clades, underlines the importance of ongoing regional monitoring to detect emerging variations. Integrating these molecular findings with ecological data, such as life fertility parameters, can contribute to the development of targeted, sustainable, and region-specific management strategies.

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Authors' Contributions

Rakesh Daroch conceived and designed the experiments. Vanshdeep Dhanda performed the experiments. Vanshdeep Dhanda and Rakesh Daroch analyzed the data. Vanshdeep Dhanda and Chetna Mahajan wrote the paper and contributed to the critical review and editing of the manuscript. All authors read and approved of the final manuscript.

Competing Interest

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

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Assessment of spatial distribution of fall armyworm, *Spodoptera frugiperda* (JE Smith) on maize (*Zea mays* L)

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***Abstract*:** Understanding insect spatial distribution patterns is fundamental to ecology, providing insights into community assembly, biodiversity maintenance and pest management strategies. The present study investigated the spatial patterns of larvae of fall armyworm on maize. The experiment was conducted at the Instructional farm, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan during Kharif 2019 and 2020.The larval population of *Spodoptera frugiperda* was recorded on sixty randomly selected plants at weekly interval. The data from original counts were arranged in frequency tables for fitting the different statistical parameters. Variance to mean ratio, exponent – K, patchiness index, clumping index, mean colony size, mean clump size, Iwao's patchiness regression and Taylor's power law were analyzed to test the dispersion behaviour of *S. frugiperda.* The result revealed that clumped or uniform or random distribution of *S. frugiperda* larvae in maize during both the year. The values of variance to mean ratio exceeded unity, the Lloyd's index was close to 1.0 and regression equation based on Taylor's Power law was computed as $\log S^2 = -0.507 + 1.398 \log \bar{x}$ and $\log S^2 = -0.141 + 1.108 \log \bar{x}$ in 2019 and 2020, respectively. All these values were indicated that the larvae of *S. frugiperda* showed clumped type of distribution in most of sampling date.

***Keywords*:** Clumped, Fall armyworm, Maize, Spatial distribution, *Spodoptera frugiperda.*

1. INTRODUCTION

Maize (*Zea mays* L) is an important cereal crop next to rice and wheat grown over a wide range of geographical and environmental conditions in India as compared to other cereal crops. Among various biotic factors, insect pests take a heavy toll of the crop thus bringing down crop yields. As many as 141 insect pests cause varying degrees of damage to the crop right from sowing till harvest (Reddy and Trivedi, 2008). The recently introduction of invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) is of serious concern for maize cultivation due to its notorious and polyphagous behaviour. The pest was first reported in West Africa in late 2016 (Goergen et al., 2016) by early 2017, the pest invaded sub–Saharan Africa. Moreover, this pest has been spread to many Asian countries. In India, it was reported for the first time on maize from Shivamogga district in Karnataka during May-June 2018 (Sharanabasappa et al., 2018). However, the presence of the pest has been reported from most growing region of the country (Rakshit et al., 2019). The knowledge on the behavior pattern of *S. frugiperda* and its bio-ecology are of major importance for development of strategies to manage this pest (Sarmento et al., 2006). The study of spatial distribution of *S. frugiperda* in maize cultivation is essential to develop a plan of integrated pest management *viz.,* by identifying whether pests are aggregated, random, or uniform, managers can move away from uniform, blanket-spray approaches to targeted, site-specific interventions, thereby ensuring the optimization of sampling and control strategies (Farias et al., 2001). Keeping the above facts in view, the experiment is conducted to understand the spatial behavior of *S. frugiperda* in maize field at Udaipur district of Rajasthan state.

2. MATERIALS AND METHODS

2.1. Location & Sowing of Maize

The experiment was laid out at the Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur. Maize variety Pratap Makka-3 was sown in the prepared field on the 5th July, 2019 and 2nd July, 2020 with row to row and plant to plant spacing of 60 cm × 25 cm, respectively.

2.2. Observation

The population of fall armyworm, *S. frugiperda* was observed on 15 randomly selected plants in each replication (four replications). In all a total of 60 plants were observed to record the population of *S. frugiperda* at weekly interval.

2.3. Statistical Analysis

The data from original counts were arranged in frequency tables for fitting the different statistical parameters. Variance to mean ratio (Southwood, 1978), exponent – K (Lloyd, 1967), patchiness index (Lloyd, 1967), clumping index (David and Moore, 1954), mean colony size (Tanigoshi et al., 1975), mean clump size (Arbous and Kerrich, 1951), Iwao's patchiness regression (Iwao, 1968) and Taylor's power law (Taylor, 1961) were analyzed to test the dispersion behaviour of this pest. α and Β which were obtained from the regression of the mean crowding against mean density refers to the basic component of dispersion and the pattern of distribution respectively (Iwao, 1968). The regression constants 'a' and 'b' of Taylor's power law also indicated the sampling parameter and the aggregation index respectively (Taylor, 1961). Different statistical analyses are used in spatial distribution to identify, quantify and interpret complex geographic patterns—such as clustering, dispersion or randomness—that simple visual mapping cannot explain.

The formulae of different statistical measures as follow (1-9):

$$\text{Dispersion index} = \frac{\text{Variance}}{\text{Mean}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

A ratio of 1 indicates a random (Poisson) distribution, >1 indicates a clustered or over-dispersed distribution, and <1 indicates a uniform or under-dispersed distribution.

$$\text{Dispersion pattern (K)} = \frac{(\bar{x})^2}{s^2 - \bar{x}} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, $\bar{x}$= mean density; s^2 = variance

$$CV = \frac{\sigma}{\bar{x}} \, x\ 100 \ldots\ldots\ldots\ldots\ldots (3)$$

Where, s = standard deviation; $\bar{x}$ = mean

$$\text{Mean Crowding Index (X)} = \bar{x} + \left(\frac{s^2}{\bar{x}} - 1\right) \ldots\ldots\ldots\ldots (4)$$

$$\text{Llyod's Patchiness Index} = \frac{X}{\bar{x}} \ldots\ldots\ldots (5)$$

Taylor's power law: This gives a relation between variance and mean.

$S^2 = a.x^b$.......(6) (Taylor, 1961) where 'a' is a constant depending upon experimental conditions and 'b' is the coefficient of contagion.

Iwao's patchiness index relates mean crowding (X*) to mean density as:

X*= α + βX (Iwao, 1968)......(7) where 'α' is the index of basic contagion and 'β' is the density contagiousness coefficient.

$$\text{Index of Clumping} = \frac{s^2}{\bar{x}} - 1 \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Mean Colony Size = 1 + mean crowding.... (9)

3. RESULTS AND DISCUSSION

Spatial distribution: The statistical parameters for testing distribution pattern of *S. frugiperda* larvae are presented in Table 1 and 2 during *Kharif,* 2019 and 2020, respectively. The data revealed clumped, uniform and random larval distribution. The values of variance to mean ratio exceeded unity in most of the sampling occasions indicating a contagious type of distribution during both the year. However, on 29th, 37th, 38th and 39th SWM in 2019 and on 29th, 38th and 39th SWM in 2020, the distribution was observed to be uniform. The larval distribution pattern on 36th SMW and 37th SMW was random during 2019 and 2020, respectively. The mean crowding index ranged from 11.50 to 31.62 and 7.79 to 37.26 during 2019 and 2020, respectively. The Lloyd's index was close to 1.0 during both the year that also

Table 1. Distribution pattern of *S. frugiperda* on maize during *Kharif,* 2019

SMW	Mean density ($\bar{x}$)	Variance (S^2)	Variance to mean ratio ($S^2/\bar{x}$)	Dispersion pattern (K)	Co-efficient of variation (CV)	Mean crowding index (X)	Lloyd's patchiness index ($X/\bar{x}$)	Index of clumping (IDM)	Mean colony size (C)	Pattern of distribution
29	16.00	4.67	0.29	-22.59	0.14	15.29	0.96	-0.71	16.29	U
30	29.50	45.67	1.55	53.83	0.23	30.05	1.02	0.55	31.05	C
31	31.25	42.92	1.37	83.71	0.21	31.62	1.01	0.37	32.62	C
32	11.50	16.33	1.42	27.36	0.35	11.92	1.04	0.42	12.92	C
33	12.50	16.33	1.31	40.76	0.32	12.81	1.02	0.31	13.81	C
34	25.50	46.33	1.82	31.21	0.27	26.32	1.03	0.82	27.32	C
35	12.75	18.92	1.48	26.36	0.34	13.23	1.04	0.48	14.23	C
36	20.25	20.92	1.03	615.09	0.23	20.28	1.00	0.03	21.28	R
37	22.25	14.92	0.67	-67.51	0.17	21.92	0.99	-0.33	22.92	U
38	19.00	15.33	0.81	-98.45	0.21	18.81	0.99	-0.19	19.81	U
39	12.00	6.00	0.50	-24.00	0.20	11.50	0.96	-0.50	12.50	U

U- Uniform; C- Clumped; R- Random; ($\bar{x}$) mean of 15 plants

indicated clumped type of distribution. The Iwao's patchiness regression was computed as $X^* = -0.328 + 1.022x$ in 2019 (Figure 1) and $X^* = -0.229 + 0.956x$ in 2020 (Figure 3). The index of basic contagion (a) was negative^{-} which indicated that aggregation was of individuals rather than colonies. The density contagiousness coefficient were $b = 1.022$ 1st year, being greater than unity suggested that the colonies were over dispersed. The regression equation based on Taylor's Power law was computed as $\log S^2 = -0.507 + 1.398 \log \bar{x}$ and $\log S^2 = -0.002 + 1.072 \log \bar{x}$ in 2019 and 2020 respectively (Fig. 2 & 4). The value of the index of aggregation (b) were more than unity in both the year, thus confirmed the aggregate nature of distribution.

The distribution pattern of *S. frugiperda* was contagious corroborated the findings of Melo et al. (2006), Farias (2001), Rios et al. (2014), Meena et al. (2019) and Hutasoit et al. (2020). The incidence of larvae of *S. frugiperda* in maize can show different distribution patterns: 'binomial-negative' or 'aggregated' when larvae are small, random, which is the most frequently reported and uniform (Melo et al., 2006). Farias (2001) observed that *S. frugiperda* showed aggregated distribution for the small caterpillars (high population density) in the field whereas, had random distribution of the large ones (low population density) showing more dispersed population of older larvae. Rios et al. (2014) reported that the aggregation indices

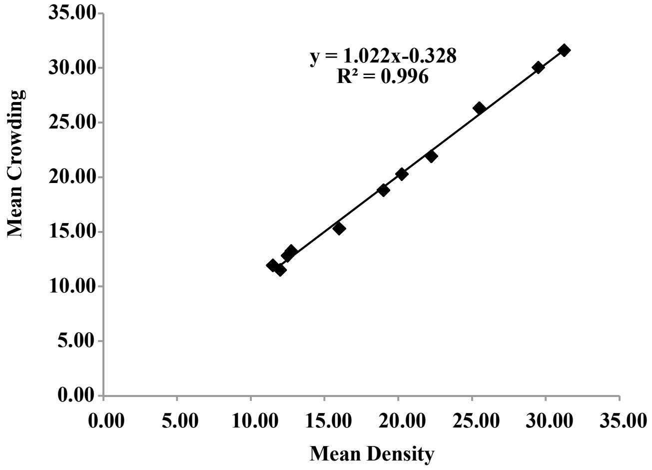

Figure 1. Iwao's Regression for mean crowding and mean density of *S. frugiperda* on maize during *Kharif* 2019

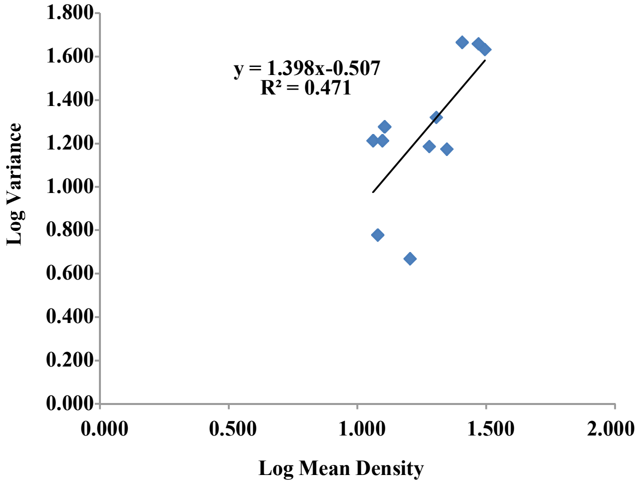

Figure 2. Regression of log variance on log mean density for *S. frugiperda* on maize during *Kharif*, 2019

Table 2. Distribution pattern of *S. frugiperda* on maize during *Kharif*, 2020

SMW	Mean density ($\bar{x}$)	Variance (S^2)	Variance to mean ratio ($S^2/\bar{x}$)	Dispersion pattern (K)	Co-efficient of variation (CV)	Mean crowding index (X)	Lloyd's patchiness index ($X/\bar{x}$)	Index of clumping (IDM)	Mean colony size (C)	Pattern of distribution
29	20.25	4.25	0.21	-25.63	0.10	19.46	0.96	-0.79	20.46	U
30	33.25	55.58	1.67	49.50	0.22	33.92	1.02	0.67	34.92	C
31	36.25	50.92	1.40	89.60	0.20	36.65	1.01	0.40	37.65	C
32	37.00	46.67	1.26	141.62	0.18	37.26	1.01	0.26	38.26	C
33	19.25	28.92	1.50	38.33	0.28	19.75	1.03	0.50	20.75	C
34	7.50	9.67	1.29	25.96	0.41	7.79	1.04	0.29	8.79	C
35	19.50	27.00	1.38	50.70	0.27	19.88	1.02	0.38	20.88	C
36	8.50	9.67	1.14	61.93	0.37	8.64	1.02	0.14	9.64	C
37	21.25	20.92	0.98	-1354.69	0.22	21.23	1.00	-0.02	22.23	R
38	22.25	16.92	0.76	-92.82	0.18	22.01	0.99	-0.24	23.01	U
39	14.50	9.67	0.67	-43.50	0.21	14.17	0.98	-0.33	15.17	U

U- Uniform; C- Clumped; R- Random; ($\bar{x}$) mean of 15 plants

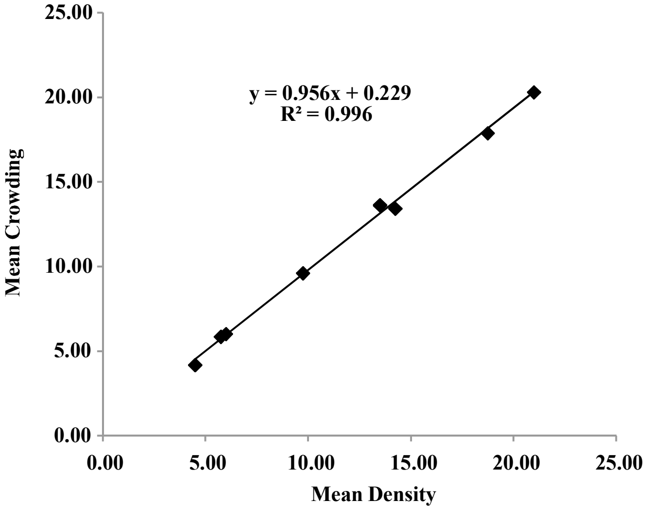

Figure 3. Iwao's Regression for mean crowding and mean density of *S. frugiperda* on maize during *Kharif* 2020

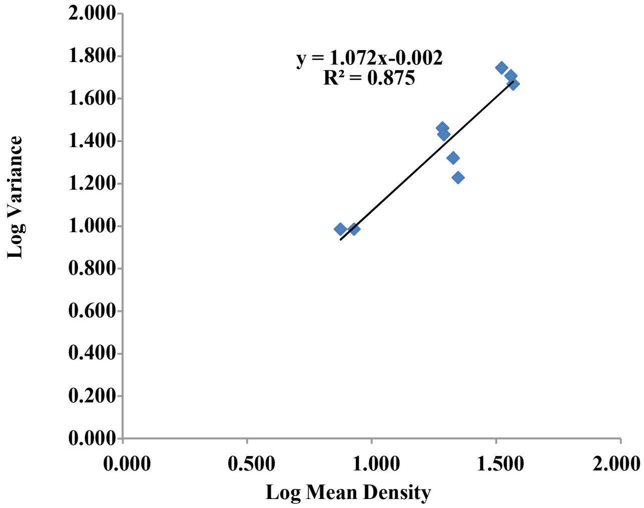

Figure 4. Regression of log variance on log mean density for *S. frugiperda* on *Kharif* maize 2020

(variance/mean ratio, Morisita's index, Green's index and Exponential k of a negative binomial distribution) indicated aggregate distribution for both small and large caterpillars. Meena et al. (2019) reported that the spatial distribution pattern of *S. frugiperda* larvae was uniform or close to random, rather than aggregated. Hutasoit et al. (2020) reported that the spatial distribution of *S. frugiperda* and result revealed that the intercept of regression values was greater than 0 and the regression slope value was around one, this indicated that armyworms spread in groups.

4. CONCLUSION

The study revealed that larvae of *S. frugiperda* had clumped, random and uniform type of distribution in different SMW. However, the values of variance to mean ratio exceeded unity and the Lloyd's index was close to 1.0 in most of SMW, which indicated that clumped type of distribution pattern was more prominent. Understanding these patterns is crucial for predicting pest spread and designing effective, region-specific control methods.

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Author's Contributions

KC Ahir, MK Mahla, S Ramesh Babu and B Singh prepared the initial manuscript draft and compilation of manuscript. All authors contributed to data entry, statistical analysis and figure preparation.

Conflict of Interest

The authors declare no conflict of interest.

Data availability

All data is present in the paper.

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Life Cycle and Productivity-Linked Parameters of Lac Insect, *Kerria lacca* (Kerr.) (*Rangeeni* strain; *Baisakhi* crop) on *Butea monosperma* (Lam.)

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***Abstract*:** A three-year study (2021–22, 2022–23, and 2023–24) was conducted to investigate the life cycle and productivity-linked parameters of lac insect, *Kerria lacca* (Kerr.) (*Rangeeni* strain: *Baisakhi* crop) on *Butea monosperma* (Lam.). The mean initial settlement density was 91.70 insects/cm², with a mortality rate of 7.72 percent. The mean sex-ratio (male percentage) was 12.20, and density at crop maturity was 4.82 insects/cm². The mean pre-sexual developmental period was 114.0 days, while the average longevity of female cell was 230.34 days. The mean female cell weight, resin output, and fecundity were 8.32 mg, 5.35 mg, and 146.91 eggs/female, respectively. The results confirmed *B. monosperma* as a suitable host for the *Rangeeni* strain during the *Baisakhi* season, ensuring satisfactory survival, fecundity, and resin yield.

***Keywords*:** *Kerria lacca*, *Rangeeni* strain, *Baisakhi* crop, *Butea monosperma*, Lac productivity, Biology.

1. INTRODUCTION

Lac is a natural resin secreted by the lac insect *Kerria lacca* (Kerr.) (Hemiptera: Kerridae), which plays a vital role in the livelihood of forest-dependent rural populations in India. The country contributes the largest share of global lac production, with two major strains, *Rangeeni* and *Kusmi* being cultivated on different host plants. Lac insects are predominantly associated with woody dicotyledonous angiosperms. Globally, more than 400 plant species have been reported to harbour lac insect, of which approximately 113 species in India serve as successful hosts. Among these, the principal host plants namely *Schleichera oleosa* (Lour.), *Ziziphus mauritiana* (Lam.), and *Butea monosperma* (Lam.) collectively account for nearly 95 percent of the country's commercial lac production (Kumar and Kumar, 2013).

In India, lac insects occur in two well-defined strains, *kusmi* and *rangeeni* distinguished by their preferred host plants and developmental durations. The *kusmi* strain performs optimally on *S. oleosa* (Kusum) and requires approximately six months to complete its life cycle, thereby yielding two high-quality crops annually (*Aghani* and *Jethwi*). In contrast, the *Rangeeni* strain proliferates on host plants other than Kusum, particularly *B. monosperma* (Palas) and *Z. mauritiana* (Ber), and is characterized by two distinct life cycles, *Katki* (four months) and *Baisakhi* (eight months) (Mohanasundaram and Sharma, 2018).

Butea monosperma (Leguminosae: Papilionoideae), a striking tree famed for its vibrant orange-red blooms, is popularly known as the Flame of the Forest. A member of the Fabaceae family, carries a rich diversity of regional names, including *palas, palash*, *mutthuga, bijasneha, dhak, khakara,* and *chichra***,** while internationally referred to as Bastard Teak, Bengal Kino**,** and Nourouc**.** This species occurs widely throughout India, Myanmar, and Sri Lanka, thriving in most ecological zones except the driest, highly arid tracts (Jaiswal and Singh, 2014). It is traditionally preferred for lac cultivation especially *Rangeeni* strain due to its widespread distribution, favourable bark texture, and high compatibility with lac insect biology (Sharma et al., 2015). However, lac productivity is influenced by several biological and environmental factors, including settlement success, developmental duration, mortality, and fecundity. The present investigation was carried out to study the life cycle and productivity-linked parameters of *K. lacca* (*Rangeeni* strain; *Baisakhi* crop) on *B. monosperma* over three consecutive years, under natural field conditions, to establish baseline biological and productivity data under Punjab conditions.

2. MATERIALS AND METHODS

The investigation was carried out on healthy *B. monosperma* trees of uniform age and vigour during the 2021-22, 2022-23 and 2023-24 *Baisakhi* crop (*Rangeeni* strain) at Regional field Gene Bank (30º89.33'N, 75º80.38'E), Department of Entomology, Punjab Agricultural University, Ludhiana.

2.1 Broodlac Inoculation

The broodlac from the previously maintained *Rangeeni* strain of *K. lacca* was used for inoculation. For this, broodlac sticks (10 cm each), having mature female (brood) cells, were tied to branches of trees. The nymphs were allowed to emerge from mature females for about two weeks and the crawlers were allowed to settle. Thereafter, *phunki* (left over sticks after the emergence of nymphs) were removed from the host trees. The inoculation was done during first week of November month every year. Once the crawlers emerged from brood cells, the following observations were recorded for different parameters:

2.2. Initial Settlement Density

Mean initial density (number per square cm) of settlement was recorded 7-10 days after the inoculation of brood lac. One square cm area of main stem infested with lac insect was selected on three sites (upper, lower, middle) on same tree and lac insect larvae settled were counted.

2.3. Initial Mortality (%)

To estimate initial mortality, the number of live lac insect larvae present within the previously selected one square cm areas at the upper, middle, and lower portions of the main stem was recorded 21 days after inoculation of brood lac. The initial mortality was calculated using following formula (eq 1):

$$\text{Initial mortality} = \frac{\text{Initial density - Density after 21 days of settlement}}{\text{Initial density}} \times 100$$

2.4. Duration of Pre-sexual Stages

Male and female cells differed markedly in shape and could be readily distinguished as male cells are elongated and cigar-shaped, whereas female cells are larger and globular. The time elapsed between date of inoculation to male and female cell differentiation was recorded.

2.5. Sex-Ratio (% of male insects)

After the differentiation of male and female cells, male cells were counted from three sites (upper, middle, lower) and percentage of male insects were calculated.

2.6. Longevity of Female Cell (days)

The time elapsed between the date of inoculation and crop harvesting (harvesting of brood sticks) was counted to work out the longevity/ life duration of the female lac insect.

2.7. Density at Crop Maturity (number per square cm)

Surviving female lac insects (after initial mortality and emergence of male lac insects) counted as above at crop maturity (appearance of yellow spot).

2.8. Fecundity (number of young ones produced by the female insect)

The collected mature female cells were stored individually into glass vials plugged with cotton for about a month and the emerged larvae were counted. Total count was taken as fecundity of the female lac insect.

2.9. Weight of the Female Cell and Resin Output (mg)

Weight of individual female lac insect was recorded after larval emergence has completed. The resin produced by an individual female cell recorded after removing the dead insect body from the cell. Fifty cells (10 each from five plants/replicate) were collected for this purpose.

2.10. Statistical Analysis

Mean values along with their standard errors were calculated using statistical functions of Microsoft Excel (Windows 10 Pro).

3. RESULTS AND DISCUSSION

3.1 Establishment, Mortality, and Survival Dynamics

Across the three *Baisakhi* crop cycles, variation was observed in the biological and productivity-linked parameters of lac insects (Table 1). The initial density of settlement ranged from 84.66 to 101.11 insects/cm², with the highest density during 2022-23 (101.11) and the lowest during 2023-24 (84.66). The overall mean settlement density (91.70) indicates favourable initial colonization and host suitability in all three seasons, though fluctuations likely reflect annual variability in climatic conditions and host plant physiology. Comparable settlement densities for *Rangeeni* lac on *B. monosperma* have been reported in earlier studies, confirming the species' consistent ability to support high initial nymph establishment (Sharma and Ramani, 2011; Mohanta et al., 2014; Swami et al., 2021; Biyani et al., 2022).

The mortality rate during settlement ranged from 5.82 to 8.77 per cent (Table 1). The relatively narrow range of mortality across seasons further indicates stable establishment conditions for the lac insect. Such mortality levels are typical for the *Rangeeni* strain on robust hosts and suggest favourable microclimatic and nutritional conditions during early development. The overall low mortality (7.72%) indicates a stable and supportive host-insect association, suggesting that *B. monosperma* provides favourable microclimatic buffering that enhances the survival of lac insects. Previous research has shown that *B. monosperma* generally supports lower early-instar mortality due to its stable physiological condition during the *Baisakhi* crop (Swami et al., 2021; Biyani et al., 2022).

The sex ratio (percentage of males) remained consistent across years, varying between 11.57 and 12.63 per cent (Table 1). Such a balanced and stable male proportion is favourable for normal population development, as extreme male-biased ratios can negatively influence brood lac production. The limited year-to-year variation suggests that sex differentiation was not significantly affected by environmental fluctuations. Similar sex-ratios have been documented for *Rangeeni* lac on *B. monosperma* and other compatible hosts by Biyani et al. (2022).

The survival at crop maturity also remained stable, ranging from 4.60 to 5.00 insects/cm², with a mean of 4.82 (Table 1). Although initial settlement densities were comparatively high, only about 5 insects/cm² survived to maturity, which is typical for lac populations where natural thinning occurs due to competition, host resource limitations, and natural mortality factors. The narrow survival range across all years indicates consistent crop performance and comparable host plant carrying capacity. The survival at crop maturity remained constant (4.60-5.00 insects/cm²), supporting the widely known phenomenon of natural thinning during lac development (Sharma & Ramani, 2011; Swami et al., 2021; Biyani et al., 2022).

Overall, the *Baisakhi* crop maintained stable survival, sex ratio, and low mortality across the three years, despite variations in initial settlement density. This stability highlights the resilience of the lac insect under the prevailing agro-climatic conditions and reflects strong host-insect compatibility and the suitability of this host for sustained lac production.

3.2. Duration of Developmental Stages

The duration of pre-sexual developmental remained highly consistent across the three *Baisakhi* crop years. The pre-sexual period ranged narrowly between 113.66 and 114.67 days, with a mean of 114.00 days (Table 2). This uniformity indicates that early developmental processes of the lac insect were not significantly influenced by inter-annual fluctuations in weather or host condition. These findings align with earlier reports that *B. monosperma* provides stable nutritional and physiological conditions conducive to pre-sexual development in the *Rangeeni* strain (Sharma and Ramani 2011; Biyani et al., 2022). The mean longevity of female was 230.34 days (Table 2). Longevity reflects the physiological robustness of the insect-host interaction, and the consistently high values recorded in this study reaffirm the ability of *B. monosperma* to sustain prolonged resin secretion and reproductive activity. These

Table 1. Initial density of settlement, per cent mortality, sex-ratio and survival at maturity of lac insect (*Rangeeni* strain; *Baisakhi* crop) on *Butea monosperma*

Parameters	*Baisakhi* crop*			
	2021-22	2022-23	2023-24	Mean
Initial density of settlement (no./cm^2)	89.33 (83-96)	101.11 (98-105)	84.66 (82-89)	91.70
Mortality (%)	8.77 (4-11)	8.56 (5-11)	5.82 (5-7)	7.72
Sex-ratio (% male insects)	12.40 (10.0-13.0)	11.57 (8.96-14.29)	12.63 (12.0-13.50)	12.20
Survival at crop maturity (no./cm^2)	5.00±0.23 (3-7)	4.60 (4.33-5.0)	4.86 (4.33-5.33)	4.82

Mean of 3 replications; Figures in parentheses are range values

Table 2. Duration of pre-sexual stages and longevity of female cells (*Rangeeni* strain; *Baisakhi* crop) on *Butea monosperma*

Parameters	*Baisakhi* crop*			
	2021-22	2022-23	2023-24	Mean
Duration of pre-sexual stages (days)	113.66 (110-116)	114.67 (113-116)	113.67 (112-117)	114.00
Longevity of female cell (days)	230.33 (216-245)	231.0 (219-243)	229.7 (215-244)	230.34

Mean of 3 replications; Figures in parentheses are range values

Table 3. Female cell weight, resin output and fecundity of lac insect (*Rangeeni* strain; *Baisakhi* crop) on *Butea monosperma*

Parameters	*Baisakhi* crop*			
	2021-22	2022-23	2023-24	Mean
Fecundity/female (no.)	139.7 (119-167)	161.17 (154-166)	139.87 (123-165)	146.91
Female cell weight (mg)	8.66 (6.30-9.85)	7.87 (7.40-8.68)	8.43 (7.50-9.32)	8.32
Resin output (mg)	5.81 (4.00-7.55)	4.74 (4.60-4.87)	5.51 (5.20-5.82)	5.35

Mean of 10 replications; Figures in parentheses are range values

observations corroborate earlier findings that emphasize extended female cell duration as a hallmark of favourable host suitability (Monobrullah et al., 2015; Biyani et al., 2022). Overall, key developmental timings, i.e. pre-sexual duration, male emergence initiation, and female cell longevity were remarkably stable across years for the *Rangeeni* strain on *B. monosperma*. This consistency reflects the suitability of the host species for lac insect development and highlights the host's reliability for maintaining synchronized development, healthy female longevity, and predictable crop progression. The results confirm the robustness of this host species for sustaining optimal lac insect development under *Baisakhi* crop conditions (Monobrullah et al., 2015; Swami et al., 2021).

3.3. Productivity-linked Traits

The reproductive and productivity traits of the *Rangeeni* strain on *B. monosperma* exhibited moderate but consistent patterns over the three *Baisakhi* crop seasons (Table 3). The fecundity fluctuated noticeably across seasons, maximum was 161.17 in 2022-23. The pooled mean fecundity was 146.91 indicating strong evidence of the biological suitability of *B. monosperma* as a host for the lac insect. High fecundity levels reflect favourable nutritional quality and host-mediated physiological support, which are essential for egg maturation and successful oviposition. The consistency of this fecundity across the study period indicates that *B. monosperma* not only sustains normal reproductive output but also provides a stable microenvironment conducive to maximizing the reproductive efficiency of the *Rangeeni* strain (Monobrullah et al., 2015; Swami et al., 2021). Such reproductive performance is a key determinant of long-term lac yield potential, thereby reaffirming the suitability of this host species for sustained and economically viable lac production.

The female cell weight remained relatively stable across the three seasons, with a pooled mean of 8.32 mg (Table 3) The consistency observed in female cell weight indicates reliable nymphal establishment and effective nutrient assimilation from the host. This uniformity indicates that *B. monosperma* maintains adequate vigour and nutritional quality throughout the *Baisakhi* crop cycle, thereby providing a stable resource base essential for optimal growth and development of the lac insect. The resin output exhibited only moderate variation, ranging from 4.74 to 5.81 mg across years (Table 3). Resin secretion is closely linked with female physiological status and host-insect interaction dynamics; therefore, stability in resin output suggests favourable host compatibility and suitable environmental conditions during the study period. The relatively higher resin yield observed in 2021-22 may be attributed to better initial settlement and slightly higher female biomass, a relationship previously reported by Sharma and Ramani (2011). Overall, the findings reinforce the significance of *B. monosperma* as a dependable host for *Rangeeni* lac production. The consistent female development and resin secretion across crop seasons indicate that the host-insect association remains strong, even under variable climatic conditions. The observed variations, though moderate, highlight the importance of continuous monitoring of environmental factors, host health, and strain performance to optimize lac productivity.

4. CONCLUSION

The present study established that *B. monosperma* is a highly suitable and reliable host for the *Rangeeni* strain (*Baisakhi* crop) of *K. lacca* under Punjab conditions. Consistent developmental durations, stable survival rates, and favourable productivity parameters confirm its strong host potential. The results also highlight the adaptability of the strain to the regional agroclimatic conditions. The findings provide essential baseline information optimizing lac cultivation practices and improving regional lac production in Punjab.

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Authors' Contributions

PSS designed the experiment, involved in execution, investigation and manuscript preparation; AT and SK performed the experiment, data analysis and involved in the preparation of tables and figures for the manuscript.

Conflict of Interest

The authors declare no potential conflict of interest.

Declaration of Generative AI and AI-assisted technologies

No use of AI tools have been used in the writing process.

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New Distribution of Bioluminescent Fungi, *Omphalotus olearius* and *Filoboletus manipularis* in Vansda National Park, Gujarat, India

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Abstract: Fungi play a crucial role in terrestrial ecosystems as decomposers, symbionts, and pathogens, contributing significantly to biodiversity. Among them, bioluminescent fungi, which produce light through a chemical reaction involving luciferase and luciferin, represent a remarkable phenomenon. These fungi are primarily found in the Basidiomycota division, with a few exceptions, and are believed to aid in spore dispersal by attracting insects. Despite their significance, bioluminescent fungi remain understudied, particularly in certain regions. This study documents the new distribution of *Omphalotus olearius* and *Filoboletus manipularis* in Vansda National Park, Gujarat, India. Observations conducted during field surveys from June to October 2024 revealed bioluminescent fungi growing on decaying substrates, emitting a characteristic green glow at night. This study highlights the need for further research on the ecological roles of bioluminescent fungi and calls for conservation efforts to protect these unique species in Vansda National Park, contributing to a broader understanding of fungal diversity in India.

Keywords: Bioluminescent fungi, Vansda National Park, Biodiversity, Conservation.

1. INTRODUCTION

Fungi play a crucial role in terrestrial ecosystems as decomposers, symbionts, and pathogens (Mueller et al., 2007). They are also among the most biodiverse groups of organisms on Earth (Hawksworth, 2001). Mushrooms, a type of macrofungi, belong to the phyla Ascomycota and Basidiomycota within the fungal kingdom.

Bioluminescent organisms represent one of nature's most remarkable phenomena, with various groups—including animals, plants, fungi, and bacteriaexhibiting the ability to produce light (Pandey and Sharon, 2017). In fungi, this trait is exclusive to the division Basidiomycota, except for *Xylaria hypoxylon*, which belongs to Ascomycota (Becker and Stadler, 2021). The bioluminescence process involves an enzyme luciferase, which catalyses the oxidation of the substrate luciferin in the presence of oxygen. This reaction releases excess energy as visible light typically perceived a pale green glow observable in complete darkness (Kaskova et al., 2017; Patil and Yadav, 2022). Research suggests may play a role in enhancing spore dispersal by attracting insects (Fleiss and Sarkisyan 2019; Patil and Yadav, 2022). This emitted light, often termed "cold light," produces minimal heat. To date, approximately 103 species of bioluminescent fungi have been documented (Desjardin et al., 2008; Chew et al., 2014; Desjardin et al., 2016; Weinstein et al., 2016; Karunarathna et al., 2020; Dauner et al., 2021; Oba and Hosaka, 2023). In India, notable records include *Nothopanus eugrammus* and *Omphalotus olearius* (Vrinda et al, 1999), followed by the discovery of *Mycena deeptha* in Kerala (Aravindakshan and Manimohan 2014), *Roridomyces cf. phyllostachydis* (Karunarathna et al., 2020), *Mycena chlorophos* (Arya et al., 2021; Koli et al., 2024; Jude et al., 2024), and *Armillaria mellea* (Patil and Yadav, 2022). This study aims to expand knowledge of fungal diversity in Vansda National Park, particularly bioluminescent fungi, which have not been previously documented in the region.

2. MATERIALS AND METHODS

During field surveys conducted from June to October 2024 in Vansda National Park, Gujarat, bioluminescent fungi were observed on decaying substrates, including branches, roots, and leaves. By day, the fungi displayed colours ranging from bright orange to yellowish-orange, white to cream, or pale pink. At night, emitted a characteristic green bioluminescence. Over a month-long observation period, six distinct locations of bioluminescent fungi were documented. Specimens were photographed using an iPhone 13 and identified based on existing literature. Further identification was carried out by cross-

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referencing the collected data with standard literature, monographs, and specialized books on mycology. Online databases such as www.mycokey.com and Fungi ID @ MycoAsia were also consulted for accurate species confirmation. All collected specimens were subsequently deposited in the Department of Biology, B.K.M. Science College, Valsad, Gujarat, India, ensuring proper curation for future research and reference.

3. RESULTS AND DISCUSSION

Field survey conducted in vansda national park during June-October 2024 resulted in the documentation of bioluminescent fungi from six locations. The geographic distribution and GPS coordinates of all bioluminescent fungal collection sites within Vansda National Park are presented in Figure 1. Two species were recorded growing on decaying woody substrates and leaf litter both species exhibited district green bioluminescence during nocturnal observation.

The macromorphological features and bioluminescent characteristics of the recorded species are illustrated in Figure 2. Dorsal, ventral, and luminescent views of *Omphalotus olearius* are shown in Figure 2A–C, while corresponding dorsal, ventral, and luminescent views of *Filoboletus manipularis* are depicted in Figure 2D–F'

3.1. *Omphalotus olearius*

Commonly known as the jack-o'-lantern mushroom, *Omphalotus olearius* produces clustered fruiting bodies measuring 7–20 cm across. Initially convex with an enrolled margin, the caps flatten with maturity, eventually developing an upturned, wavy edge. The surface is smooth and typically bright orange to yellowish-orange. Pileus 2-6.5 cm diam. fleshy, convex expanding plane, umbonate, depressed, umbilicate or even infundibuliform, surface chrome yellow, sunflower yellow or dark yellow becoming light yellow to amber yellow with radial innate fibril, dry, margin thin, often lobate and split, incurved, non-striate. Lamellae decurrent, pastel yellow to yellow up to 3 mm wide, luminescent in the dark, crowded, with lamellulae of varying lengths edge entire, concolorous. stipe 2.5-5.5 cm ×2-8 mm, central to excentric, often curved, cylindric or more often attenuated towards the base, solid, surface concolorous to the pileal surface, appressed fibrillose, dry. annuals nil. Smell pleasant, fruity. Context yellow up to 5 mm wide, tough, composed of interwoven 3-10 µm broad hyphae. Basidiospores 3.8-5.3 × 3-3.8 µm long subglobose to broadly ellipsoid, smooth, hyaline, inamyloid21. Voucher number: 242

3.2. *Filoboletus manipularis*

It is known as the reticulated luminous mushroom, *Filoboletus manipularis* forms clusters of fruiting bodies with relatively uniform morphology. At maturity, the pileus diameter ranges from 0.5 to 6.0 cm, with stipes measuring 2.0 to 7.0 cm in length. The pileus shape varies—conical, rounded, plane, or depressed, sometimes umbonate—while its colour ranges from white to cream, beige, or pale pink. Pileus 0.5-2.0 cm in diam., conico-campanulate to convex with conical umbo, hygrophanous, translucently reticulate, finely white pruinose, whitish, grayish to hyaline when moist, purely white to yellowish dried up. Hymenophore tubular, adnate or adnate-emarginate with a slightly

Figure 1. GPS Coordinates of Bioluminescent Fungi in Vansda National Park. Navsari, Gujarat

decurrent tooth. Tubes 1.5-4 mm long, arranged in radial rows, numbering 5-7 in a row, with angular-round pores 0.5-1 mm wide, white. Stipe 20-60 × 0.5-2.5 mm, cylindrical, thickened in the base, hollow, white to hyaline, completely white pruinose. Basidiospores 6.2-7.5× 4.4-5.1 µm, white, smooth, ellipsoid to broadly ellipsoid, amyloid22. Voucher number: 351

The documentation of Omphalotus olearius and Filoboletus manipularis in Vansda National Park significantly expands the known distribution of bioluminescent fungi in India. These findings are noteworthy because previous records of such species were limited to southern and northeastern regions of the country (Vrinda et al, 1999; Aravindakshan and Manimohan 2014; Arya et al, 2021). The present study confirms the presence of bioluminescent fungi in western India for the first time, highlighting Vansda National Park's potential as an underexplored biodiversity hotspot.

Fungal bioluminescence, though a well-documented phenomenon, remains ecologically enigmatic. One prevailing hypothesis is that light emission attracts insects, aiding in spore dispersal, thereby enhancing reproductive success (Fleiss and Sarkisyan, 2019; Patil and Yadav, 2022). The observed bioluminescence in Vansda National Park species, particularly visible on decaying wood and plant matter, supports the idea that these fungi play active roles in forest decomposition processes. Furthermore, their presence indicates healthy, undisturbed microhabitats rich in organic substrate and moisture critical requirements for the growth of such specialized fungi (Desjardin et al., 2008; Kaskova et al., 2017).

The ecological sensitivity of mushrooms makes them valuable bioindicators for environmental changes. Bioluminescent fungi, due to their unique visibility and habitat specificity, may serve as sentinel species for monitoring forest health (Becker and Stadler, 2021). However, deforestation, habitat fragmentation, and climate change pose serious threats to fungal biodiversity (Hawksworth and Lucking, 2017). The scarcity of comprehensive fungal surveys in India, especially within western states like Gujarat, underlines the importance of localized field research and documentation.

This study also demonstrates the effectiveness of simple field techniques visual observation in darkness, digital photography, and morphological identification based on established taxonomic keys for identifying macrofungi. Nonetheless, integrating molecular tools, such as DNA barcoding, in future research could provide more accurate

Figure 2. Bioluminescent fungi (A) Dorsal view of *Omphalotus olearius* (B) Ventral view of *Omphalotus olearius* (C) Luminescent view of *Omphalotus olearius* (D) Dorsal view of *Filoboletus manipularis* (E) Ventral view of *Filoboletus manipularis* (F) Luminescent view of *Filoboletus manipularis*

taxonomic resolution and uncover cryptic diversity (Nilsson et al., 2019). By documenting these species in Vansda National Park, this study contributes to the broader understanding of fungal distribution in India and emphasizes the need for conservation efforts to protect these unique organisms and their habitats.

4. CONCLUSION

The discovery of *Omphalotus olearius* and *Filoboletus manipularis* in Vansda National Park marks the first report of bioluminescent fungi from Gujarat, expanding their known range in India. These findings underscore the ecological richness of Vansda National Park and the importance of preserving its forested habitats. As bioluminescent fungi offer insight into forest health, ecological processes, and evolutionary adaptation, continued exploration and conservation of fungal diversity are essential. The present study also demonstrates that systematic field surveys, nocturnal visual observation, digital photography and detailed morphological analysis are effective and reliable techniques for the identification and documentation of bioluminescent macrofungi. This study advocates for increased fungal documentation efforts and habitat protection strategies to safeguard these unique and understudied organisms.

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Authors' Contribution

Conceptualisation: Dilipkumar Patel, Jigitsa Patel; Methodology: J. Patel, D. Patel; data Collection: J. Patel; Data Analysis: D. Patel, J. Patel; Writing Original Draft: J. Patel; Writing Reviewing and Editing: D. Patel. authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declares that they have no conflict of interest.

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Ameliorative Potential of Wheatgrass against Histological and Biochemical Alterations caused due to Chlorantraniliprole and Arsenic in Female Albino Rats

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Abstract: The ameliorative potential of wheatgrass juice was evaluated against the reproductive toxicity caused by combination of pesticide, chlorantraniliprole and heavy metal, arsenic in the female albino rats to address the growing concerns about simultaneous exposure to agrochemicals and heavy metals in the environment and assess their impact on female reproductive function. The rats were administered with chlorantraniliprole (1/2, 1/4 and 1/6 LD_{50}), arsenic(0.50 mg/L of water) and fresh wheatgrass juice (10 ml/kg b.w.) orally. The significant alterations such as increase in duration of estrous cycle, reduced number of follicles, increased atresia (%) in the ovaries, decreased diameter of uterine epithelium, myometrium and endometrium and scattering of lumen in the uterus; reduction in the protein and lipid content were observed in chlorantraniliprole (1/2, 1/4 LD_{50}) + arsenic treated rats as compared to control rats. However, less significant alterations were observed in the reproductive parameters of the rats treated with groups exposed to chlorantraniliprole (1/6 LD_{50})+ arsenic and only arsenic when compared to the control group. These changes were reduced with administration of fresh wheatgrass juice thus suggesting that wheatgrass acts as an ameliorative agent against chlorantraniliprole and arsenic induced female reproductive toxicity rats.

Keywords: Arsenic, Antioxidants, Biomarker analysis, Chlorantraniliprole, Wheatgrass.

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most commonly grown crops in India. The germination of wheat produces wheatgrass after 6-10 days which is rich in vitamins (A, B, C, E), chlorophyll, minerals, flavonoids, phenolics and enzymes like SOD, cytochrome oxidase that contribute to the therapeutic potential of wheatgrass (Tamraz et al., 2024). Wheatgrass is widely recognized for its anticancer potential, owing to its high chlorophyll content along with vitamin E, B and other essential minerals, that add to its medicinal value (Mishra et al., 2025). Since the atomic structures of chlorophyll in wheatgrass and haemoglobin in blood are nearly identical, except that magnesium is the central element in chlorophyll, while iron occupies this position in haemoglobin, this similarity allows wheatgrass to restore the body's alkaline balance, reduce acidity and promote the formation of healthy cells (Sharma et al., 2025). Wheatgrass also contributes to improved fertility and chances of pregnancy in women (Kaur et al., 2023). To satisfy the demands of the expanding population, agricultural production and use of pesticides and insecticides for reducing pest populations has been expanded in recent years which severely impacted the agricultural system (Bitschinski et al., 2024).

A new class of insecticides known as the Diamide Group-28 insecticides, was recognised in March 2010 under the Insecticide Resistance Management Global Guidelines of the Insecticide Resistance Action Committee (Du and Hu, 2023). These insecticides would target particular ryanodine receptors in insects, which by interacting with the calcium channels of the insect's cardiac and skeletal muscles impede the transmission of nerve impulses and offer protection (Ponepal et al., 2023). Chlorantraniliprole is applied on large number of crops such as brinjal, cotton etc. against pest species attacking these crops such as Lepidoptera. Chlorantraniliprole has a short half-life, its LD_{50} of 5000 mg/kg of body weight as per the USEPA exerts toxic effects on mammalian health (Wang et al., 2025). However, the environmental residues of chlorantraniliprole have been reported in crops such as rice, tomato, sugarcane and cotton (Mahato et al., 2023). Chlorantraniliprole accumulates in the muscle tissue of fishes and degenerates the detoxification pathways (Yin et al., 2023). It leads to an imbalance between pro-oxidants and antioxidants leading to severe reproductive diseases such as endometeriosis, polycystic ovarian syndrome etc. (Omar et al., 2022). Its exposure leads to severe damage to various body organs like kidney, liver, lungs etc. and causes neurological health effects including memory or learning disability, vision, impairment, signalling disability etc. along with causing immunological disorders such as hypersensitivity, asthma, and allergic reaction (Kimura et al., 2023). Its intrauterine exposure during pregnancy exerts its teratogenic effect on foetuses along with retarded morphological and mental growth (Kaabeche et al., 2024).

Heavy metals are metallic chemical elements having density higher than the density of water. Industrialization and urbanization have also led to an increased anthropogenic contribution of heavy metals within the environment (Kapoor et al., 2024). Arsenic (As) is one such heavy metal that is prevalent in water at concentrations higher than the permissible limit i.e 10 µg/L as recommended by WHO in most parts of the World with East Asian Countries being the most affected with over 230 million people at risk of drinking water exceeding the World Health Organization's (WHO) guideline of 10 µg/L (0.01 mg/L) (Shaji et al., 2021). Around 220 million individuals worldwide, particularly in Asia, may consume water with Arsenic above permissible limits (Jha et al., 2023). It has been identified as a carcinogen by the International Agency for Research on Cancer (IARC) for human skin, bladder and lungs. Arsenic exposure increases the evidence of stillbirth and even infertility in females due to the generation of free radicals. Arsenic owing to its lipophilic property gets bioaccumulated in organisms and is responsible for disrupting the biochemical and physiological functions in mammals (Mouttoucomarassamy et al., 2024; Ramesh, 2024).

The studies have been already conducted on the toxic effects on various systems being caused by chlorantraniliprole and arsenic individually as well as the protection provided by the wheatgrass in the various therapeutic studies in rats as a mammalian model (Mahboob et al., 2025; Mahmoud et al., 2025 and Raslan et al., 2025). However, no study has reported the combined toxic effect of chlorantraniliprole and arsenic on mammalian reproductive health and its possible amelioration with a natural herb such as wheatgrass. The current study therefore evaluates the ameliorative potential of wheatgrass against combined reproductive toxicity caused by the interaction of variable doses of chlorantraniliprole along with a fixed dose of arsenic by determining the alterations in the various parameters of female albino rats.

2. MATERIALS AND METHODS

2.1. Procurement of Animals

The sexually mature female albino rats were procured from the Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar after clearing the proposal through Institutional Animal Ethics Committee (IAEC), GADVASU. The rats were acclimatized for 10 days before using them for experimentation. The rats were maintained under controlled conditions of temperature (22±2°C) and provided with standard diet containing pelleted food and water. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee (IAEC) vide letter no GADVASU/2022/IAEC/63/11 dated 13/01/2022 approved this experimental protocol.

2.2. Treatment of Animals

Wheatgrass was grown in plastic trays and 100% wheatgrass juice (WJ) was extracted on the 9th day by grinding 3gm of wheatgrass in 2-3ml of water in the electric mixture and then adjusting the final volume to 10ml after sieving the mixture. The different doses of commercial formulation of chlorantraniliprole (CTPR) (18.5% SC) i.e. 1/2 LD_{50} (2500 mg/kg of b.w.), 1/4 LD_{50} (1250 mg/kg of b.w.), 1/6 LD_{50} (833 mg/kg of b.w.), arsenic (As) in the form of sodium arsenite @ 0.50mg/L in drinking water and wheatgrass juice @10ml/kg b.w. were provided to rats orally. The doses of chlorantraniliprole, arsenic and wheatgrass juice were considered based on earlier reported studies (Ghoneim et al., 2018; Mobdy et al., 2017; Moreno et al., 2016). The rats were treated for 28 days and divided into 8 groups with 6 rats in each group as: Group I: 1/2 LD_{50} chlorantraniliprole + arsenic; Group II: 1/4 LD_{50} chlorantraniliprole + arsenic; Group III: 1/6 LD_{50} chlorantraniliprole + arsenic; Group IV:Arsenic; Group V:1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice; Group VI :1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice; Group VII:1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice, Group VIII: Control.

2.3. Estrous Cyclicity

Three consecutive estrous cycles were monitored prior to treatment to confirm rats were cyclic. Throughout the treatment period, the four stages of the estrous cycle were identified by the vaginal smear cytology on the basis of cellular morphology such as the predominance of large nucleated epithelial cells in the proestrus stage, flattened anucleated cornified epithelial cells in the estrus stage, presence of both cornified epithelial cells and leukocytes in the metestrus phase and the abundance of small, round leukocytes in the diestrus stage. Consequently, the total duration of the estrous cycle was recorded.

2.4. Sacrifice of Animals

After the treatment, the rats were subjected to cervical dislocation and blood samples were collected directly from the ventricle by using syringes with heparin to prevent blood clotting. The animals were dissected and the reproductive organs i.e. ovary and uterus were excised, the adhering fat was cleaned off from the organs and these organs were preserved for further histological and biochemical analysis.

2.5. Homogenate Preparation

The tissue (ovary and uterus) were homogenized in a 50 mM phosphate buffer, 2ml (pH 7.4), and the tissue homogenate was centrifuged at 5000 r.p.m. for 20 minutes. The supernatant was collected and used for further biochemical analysis.

2.6. Evaluation of Histological Studies

The ovaries and uterus of the albino rats were cleared from the adhering tissue and fats and were thereby fixed in the Bouin's fixative for 24 hours. After fixing the organs in the Bouin's fixative, the tissues were processed for paraffin block preparation by acetone benzene schedule (Luna, 1968). Sections of approximate thickness of 5-6 μm were cut serially using a rotary microtome**.** Dewaxing and rehydration in descending series of ethanol to water followed with and stained with haematoxylin and counterstained with eosin. The microphotographs were analysed using Magvision software. The number of primordial, primary, secondary, pre-antral, antral as well as corpora lutea were observed in the ovarian sections (Pederson 1970). The percentage of atresia was calculated as follows (Eq.1):

$$\frac{\text{Number of atretic follicles in a stage}}{\text{Number of atretic + healthy follicles of stage}} \times 100$$

The uterine sections were observed for height of uterine epithelium, thickness of myometrium and endometrium, changes in the diameter of endometrial lumen and number of endometrial glands.

2.7. Biochemical Parameters

The biochemical parameters were analysed in the tissue homogenates of ovaries and uterus by the standard methods - Proteins (Lowry et al., 1951), Lipids (Folch et al., 1957), Phospholipids (Ames, 1966), Cholesterol (Zaltkins and Zak., 1968) and Free Fatty Acids (Lowry and Tansley., 1976).

2.8. Statistical Analysis

One-way analysis of variance was done by using Tukey's method in SPSS version 16.0 to compare mean differences among multiple groups, as this approach is appropriate for detecting intergroup differences while controlling Type I error (Tukey, 1949).

3. RESULTS AND DISCUSSION

3.1. Effect on the Estrous Cyclicity

Estrous cyclicity when studied was found to be disturbed in all of the chlorantraniliprole and arsenic treated rats. The control rats however showed normal cyclicity with all four phases of the cycle. The effect was more pronounced in ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats which demonstrated a significant decrease in the number of estrous cycles as compared to control rats and only arsenic treated rats. Rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in the number of estrous cycles as compared to control, however this result was non-significant as compared to only As treated rats. Wheatgrass however, showed protective effect as the number of estrous cycles varied non significantly from control in rats administered with wheatgrass (Table 1).

The duration of proestrus and estrus phase in the ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats was found to have significantly reduced as compared to control and only As treated rats, however rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in duration of proestrus and estrus phase as compared to control but varied non-significantly from only As treated rats which effectively reversed in rats treated with wheatgrass. The duration of metaestrus and diestrus stage observed in ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats was significantly high as compared to control. As treated rats and rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in duration of metaestrus and diestrus phase as compared to control but varied non-significantly from only As treated rats whereas the duration of metaestrus and diestrus stage in rats treated with wheatgrass showed non-significant difference as compared to control which suggested wheatgrass was an effective ameliorative agent (Table 1). These alterations resulted in a significant increase in the total duration of estrous cycle in the ($1/2LD_{50}$ chlorantraniliprole + As) treated rats as compared to control and only arsenic treated rats. The ($1/4LD_{50}$ and $1/6LD_{50}$ chlorantraniliprole + As) treated rats also significant increase in the total duration of estrous cycle as compared to control rats but varied non significantly with only As treated rats. Wheatgrass however, was able to potentially ameliorate the induced toxicity (Table 1). Sharma et al. (2020) investigated the reproductive toxicity caused by carbamates and organophosphate insecticides in female rats and reported significant disruption of ovarian function. Their findings demonstrated impaired follicular development and inhibition of ovulation, leading to alterations in the normal estrous cycle pattern. Prolongation of the diestrus phase was particularly evident, indicating endocrine imbalance and compromised reproductive cyclicity following insecticide exposure. Similarly, Omar et al. (2022) also found that rats treated with coragen showed significant changes in the duration of estrous cycle and a reduced estrus phase. Exposure to coragen as reported by Ponepal et al. (2023) significantly disturbed the estrus cyclicity is wistar rats by prolonging the duration of the diestrus stage which was also observed in the current study. Sprague Dawley rats when exposed to sodium arsenite showed prolonged oestrus cycle and halted diestrus phase owing to disruption in the level of estrogen and progesterone (Mir et al., 2023). Flavanols and phenols alter the intracellular signalling pathways of inflammation and apoptosis, thereby suppressing the pro-inflammatory gene Bcl expression thus inhibiting the apoptotic activities (Panda et al., 2021). As these constituents are also present in wheatgrass, thereby wheatgrass may also be able to mitigate the estrus cyclicity disturbances caused by combined action of chlorantraniliprole and arsenic.

3.2. Histological Changes

The light microscopic studies of the ovary showed various alterations in different group of rats (Table 2, Figure 1 A-H). The significant decreased number of follicles (primary, secondary, tertiary, pre-antral and antral), reduction in the diameter of the follicles and increased number of atretic follicles was observed in chlorantraniliprole (1/2LD$_{50}$ and 1/4LD$_{50}$) + As treated rats (Figure. B and C) as compared to control (Figure. A) and only Arsenic treated rats (Figure E).

The rats treated with chlorantraniliprole (1/6LD$_{50}$) + As showed significantly decreased number of normal follicles, decreased follicular diameter and significantly increased number of atretic follicles (Figure D) as compared to control but varied non-significantly from only As treated rats. The increase in the number of normal follicles, decrease in the number of atretic follicles along with increased diameter of follicles was observed in rats treated with chlorantraniliprole (1/2LD$_{50}$, 1/4LD$_{50,}$ 1/6LD$_{50}$) + As + wheatgrass as compared to only chlorantraniliprole + As treated rats with effective amelioration by wheatgrass in Chlorantraniliprole (1/6LD$_{50}$) + As + wheatgrass treated rats. The alterations such as necrosis and vacuolation in the interstitium of the ovarian tissue were observed in the chlorantraniliprole (1/2LD$_{50}$, 1/4LD$_{50,}$ 1/6LD$_{50}$) + As treated rats and only arsenic treated rats. However, no such damage of the ovarian tissue was observed in rats administered with wheatgrass along with chlorantraniliprole (1/2LD$_{50}$, 1/4LD$_{50,}$ 1/6LD$_{50}$) + As. The histological changes in the ovary such as reduced follicular number and diameter due to the combined effect of coragen and arsenic coincide with the previous studies wherein the female rats exposed to coragen also showed reduced number of follicles with arrested growth in the pre-antral stage,

Table 1. Effect on estrous cyclicity in albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	**Number of cycles (28 days)**	**Time duration of one estrous cycle (in days)**				
		Proestrus	**Estrus**	**Metaestrus**	**Diestrus**	**Total duration of estrous cycle**
1/2 LD $_{50}$ chlorantraniliprole + arsenic	4.57^{d}	0.17^{c}	1.21^{d}	0.97^{e}	2.68^{c}	5.03^{c}
1/4 LD $_{50}$ chlorantraniliprole + arsenic	4.75^{d}	0.19^{bc}	1.24^{d}	0.94^{de}	2.62^{c}	4.99^{b}
1/6 LD$_{50}$ chlorantraniliprole + arsenic	5.17^{c}	0.28^{abc}	1.27^{c}	0.89^{cd}	2.24^{b}	4.97^{ab}
Arsenic	5.14^{c}	0.24^{abc}	1.27^{c}	0.89^{cd}	2.17^{b}	4.97^{ab}
1/2 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	5.45^{b}	0.29^{ab}	1.29^{bc}	0.80^{abc}	1.76^{a}	4.98^{ab}
1/4 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	5.59^{ab}	0.32^{ab}	1.31^{ab}	0.78^{ab}	1.62^{a}	4.96^{a}
1/6 LD$_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	5.62^{ab}	0.37^{a}	1.33^{a}	0.75^{a}	1.59^{a}	4.97^{a}
Control	5.69^{a}	0.39^{a}	1.36^{a}	0.72^{a}	1.50^{a}	4.97^{a}

Values are expressed as mean values

Different superscript (a-f) in a column indicate significant difference ($p \leq 0.05$)

deposits of collagenous tissues in the corpora lutea. Oxidative stress increases the number of atretic follicles in the ovarian cortex along with disorganising the cellular arrangement in the stromal cells, causing loss of demarcation between the epithelial and stromal tissues in the uterus (El-Din et al., 2023). Hamdani and Yajurvedi (2017) also reported increase in the number of atretic follicles in the cortex area covered by surface epithelium due to exposure to cypermethrin. Xiao and Lai (2025) analysed the effect of oxidative stress on the histology of the ovaries induced by heavy metals and concluded the presence of widespread ovarian follicle atresia. Wu et al. (2025) observed the histological changes in the ovarian tissues of female wistar rats exposed to As and showed reduced number of follicles with arrested growth in the preantral stage which were in accordance with the findings of the current study.

The histological slides of uterus revealed the alterations in different group of rats (Table 3, Figure. 2 A-H). chlorantraniliprole (1/2LD$_{50}$ and 1/4LD$_{50}$) + As treated rats showed decreased uterine luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2B, 2C) as compared to control (Figure. 2A, Table 3) and only arsenic exposed rats (Figure. 2E, Table 3). However, rats treated with chlorantraniliprole (1/6LD$_{50}$) + As showed significant decrease in luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2D) as compared to control but varied non-significantly from only As treated rats. The rats treated with chlorantraniliprole (1/2LD$_{50}$, 1/4LD$_{50}$, 1/6LD$_{50}$)+ As + wheatgrass showed significant recovery in the uterine luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2F, 2G, 2H) as compared to only chlorantraniliprole + As treated rats with effective amelioration by wheatgrass against the combined toxicity due to chlorantraniliprole (1/6LD$_{50}$) and arsenic. Hassan et al. (2021) also reported that exposure to coragen caused accumulation of fibrous connective tissues in the stroma of uterus, follicles were also surrounded by pyknotic granulosa cells arranged in the form of symmetric rings below the basement membrane. Erthal et al. (2024) observed

Table 2. Effect on number and diameter of ovarian follicles in albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Number of ovarian follicles						Diameter of ovarian follicles			
	Primordial follicle	Primary follicle	Secondary follicle	Pre-antral follicle	Antral follicle	Atretic Follicle	Primary follicle	Secondary follicle	Pre-antral follicle	Antral follicle
1/2 LD $_{50}$ chlorantraniliprole + arsenic	5.33^{f}	0.33^{e}	0.33^{c}	0.33^{c}	0.33^{c}	3.66^{c}	31.37^{d}	76.38^{d}	152.70^{f}	217.21^{e}
1/4 LD $_{50}$ chlorantraniliprole + arsenic	7.33^{e}	1.66^{de}	0.66^{c}	0.33^{c}	0.33^{c}	3.33^{c}	34.19^{d}	80.17^{d}	174.27^{e}	231.24^{d}
1/6 LD$_{50}$ chlorantraniliprole + arsenic	10.33^{d}	2.66^{cd}	1.66^{ab}	0.66^{b}	0.66^{b}	2.33^{b}	38.93^{c}	85.39^{c}	196.48^{d}	256.27^{c}
Arsenic	9.33^{d}	2.00^{bcd}	1.33^{ab}	0.63^{b}	0.66^{b}	2.60^{bc}	38.62^{c}	85.22^{c}	196.16^{d}	257.30^{c}
1/2 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	12.66^{c}	3.33^{abc}	2.00^{a}	1.20^{a}	1.00^{a}	1.33^{ab}	42.11^{b}	90.36^{b}	212.87^{c}	275.29^{b}
1/4 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	16.33^{b}	4.00^{ab}	2.33^{a}	1.36^{a}	1.25^{a}	1.00^{ab}	46.77^{ab}	94.19^{ab}	236.97^{b}	296.31^{ab}
1/6 LD$_{50}$ chlorantraniliprole + arsenic + wheatgrass juice	17.66^{ab}	4.33^{a}	2.66^{a}	1.60^{a}	1.30^{a}	0.66^{a}	49.87^{a}	96.03^{a}	249.02^{a}	307.33^{a}
Control	19.00^{a}	4.66^{a}	2.66^{a}	1.66^{a}	1.33^{a}	0.66^{a}	50.23^{a}	97.45^{a}	257.11^{a}	309.89^{a}

See Table 1 for details

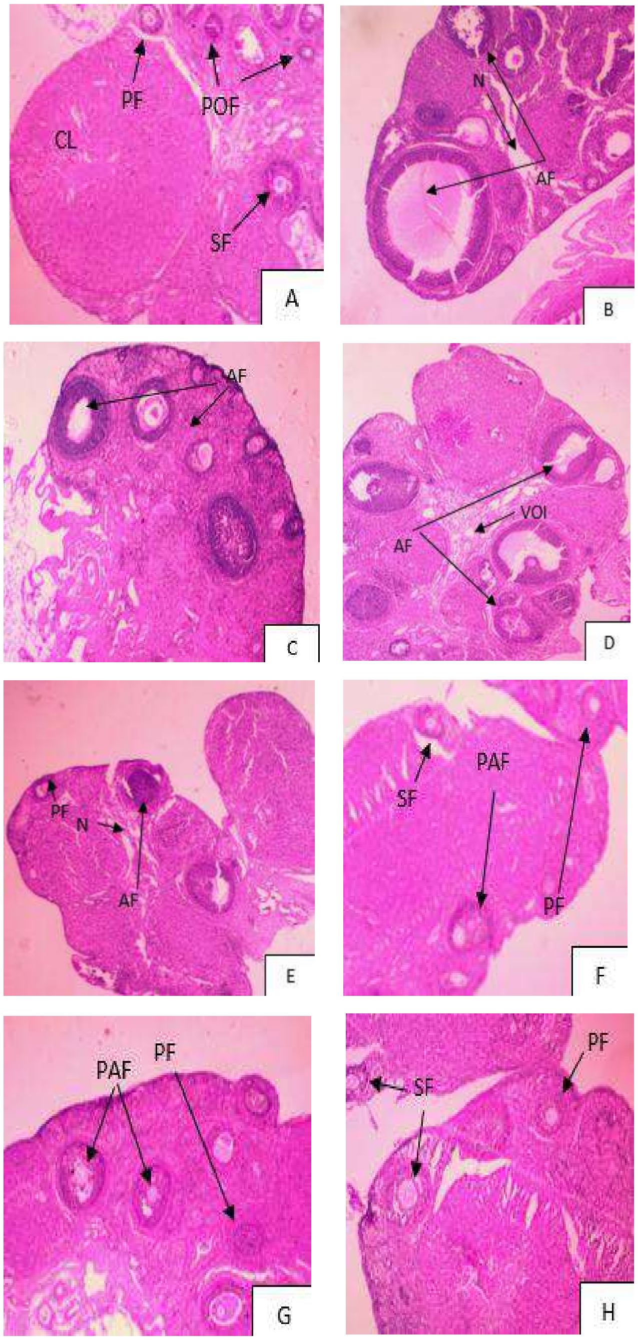

Figure 1. A-H: T.S. of ovary (X100) A Control B 1/2 LD $_{50}$ chlorantraniliprole + arsenic C 1/4 LD $_{50}$ chlorantraniliprole + arsenic D 1/6 LD $_{50}$ chlorantraniliprole + arsenic E Arsenic F 1/2 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice G 1/4 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice H 1/6 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice (PF-primary follicle, SF- secondary follicle, CL-corpus luteum, AF- atretic follicle, N-necrosis, V-vacuolisation, PAF-primary atretic follicle)

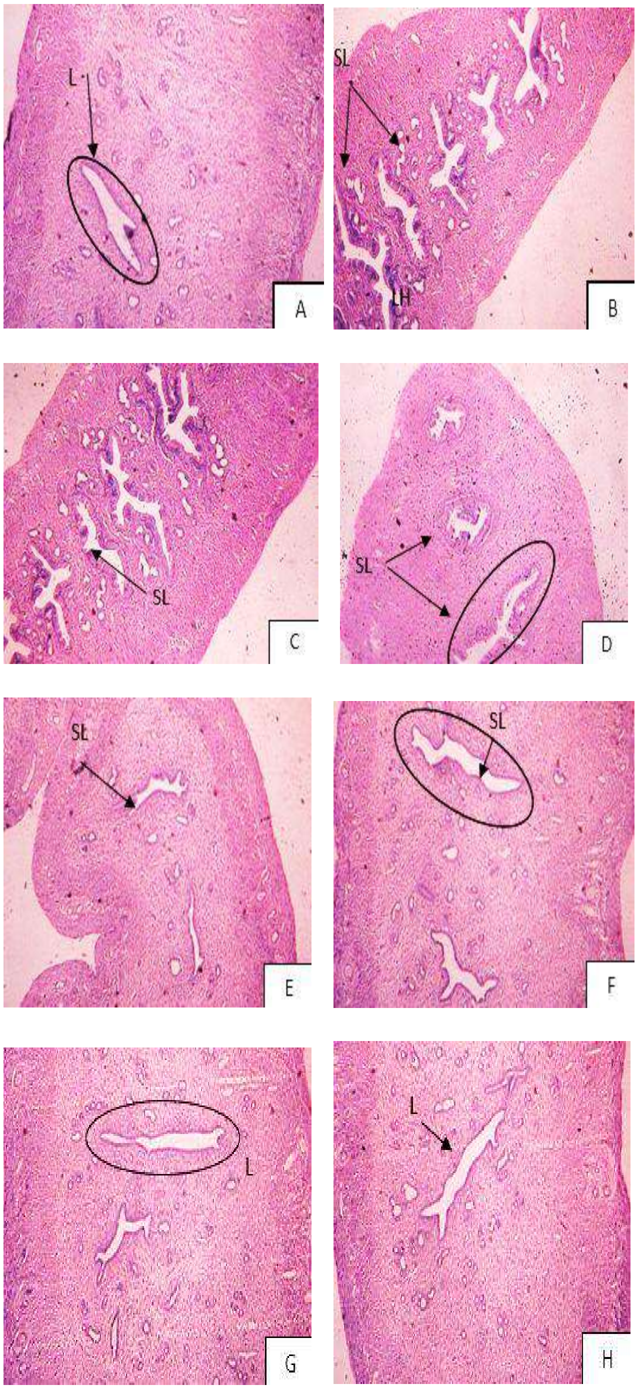

Figure 2. A-H: T.S. of uterus (X100) A Control B 1/2 LD $_{50}$ chlorantraniliprole + arsenic C 1/4 LD $_{50}$ chlorantraniliprole + arsenic D 1/6 LD $_{50}$ chlorantraniliprole + arsenic E Arsenic F 1/2 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice G 1/4 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice H 1/6 LD $_{50}$ chlorantraniliprole + arsenic + wheatgrass juice (L- Normal Lumen, SL- Scattered lumen)

that exposure of malathion in albino rats showed squamous hyperplasia and cornified vaginal and epithelium in the uterus along with endometrial and myometrial edema. Barbhuiya et al. (2021) studied that arsenic induced reproductive toxicity in female albino rats and observed that epithelial and myometrial diameter of uterus showed significant decrease along with a narrow and unfolded lumen. Methomyl exposure leads to reduced myometrial and endometrial diameter along with causing apoptosis in the uterine cells as suggested by Mosbah et al. (2024) which were also seen in the current study. Since wheatgrass is rich in active antioxidants it can potentially reduce the oxidative damage resulting in histological alterations and thereby showing the recovery which has been observed.

3.3. Biochemical Parameters

Biochemical parameters (Table 4) in the ovaries and uterus revealed significant reduction in the content of total soluble protein, total lipids, phospholipids and free fatty acids in chlorantraniliprole (1/2LD_{50} and 1/4LD_{50}) + As treated rats as compared to control and only arsenic treated rats whereas, rats treated with chlorantraniliprole (1/6LD_{50}) + As showed significant decrease in content of total soluble proteins, total lipids, phospholipids, free fatty acids as compared to control but varied non-significantly from only As treated rats. However, rats treated with chlorantraniliprole + As + wheatgrass as compared to only

Table 3. Effect on uterine parameters in the albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Diameter of uterine lumen (µm)	Endometrial thickness (µm)	Myometrial thickness (µm)
1/2 LD_{50} chlorantraniliprole + arsenic	42.57^{g}	261.60^{e}	53.99^{a}
1/4 LD_{50} chlorantraniliprole + arsenic	52.57^{f}	300.78^{d}	65.40^{e}
1/6 LD_{50} chlorantraniliprole + arsenic	58.39^{e}	342.17^{c}	82.99^{d}
Arsenic	57.17^{d}	339.93^{c}	85.06^{d}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	60.92^{c}	372.59^{b}	104.59^{c}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	64.30^{b}	404.62 ^{b}	142.83^{b}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	69.40^{a}	419.31^{a}	161.57^{a}
Control	70.85^{a}	426.80 ^{a}	172.47^{a}

See Table 1 for details

Table 4. Effect on the biochemical parameters in the reproductive organs of albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Total soluble protein (mg/g of tissue)		Total lipids (mg/g of tissue)		Phospholipids (mg/g of tissue)		Free fatty acid (mg/g of tissue)		Cholesterol (mg/g of tissue)	
	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus
1/2 LD_{50} chlorantraniliprole + arsenic	1.24^{f}	3.59^{e}	46.66^{c}	71.66^{c}	7.38^{d}	10.21 ^{f}	0.67^{e}	1.01^{e}	2.38^{d}	4.21^{f}
1/4 LD_{50} chlorantraniliprole + arsenic	1.96 ^{e}	4.35^{d}	49.33^{c}	75.33^{c}	8.31^{d}	14.63^{e}	0.81^{d}	1.31^{d}	1.91^{c}	3.62^{e}
1/6 LD_{50} chlorantraniliprole + arsenic	2.58 ^{d}	5.53^{c}	55.67^{b}	78.66^{b}	9.58^{c}	18.24^{d}	0.92^{c}	1.74^{c}	1.63^{bcd}	3.13^{d}
Arsenic	2.54 ^{d}	5.23 ^{c}	53.66^{b}	78.33^{b}	9.65^{c}	18.43^{d}	0.95^{c}	1.63^{c}	1.71^{cd}	3.23^{d}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	2.77^{bc}	6.71^{b}	55.31^{a}	81.66^{a}	14.49^{b}	22.5^{c}	1.15^{b}	2.20^{b}	1.58^{abc}	2.72^{c}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	2.95^{bc}	8.76^{b}	56.23^{a}	82.66^{a}	15.23^{ab}	24.67^{ab}	1.37 ^{a}	2.39^{ab}	1.47^{ab}	2.49 ^{ab}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	3.10^{ab}	9.09 ^{a}	57.06^{a}	83.21^{a}	15.39^{ab}	24.75^{ab}	1.41 ^{a}	2.58 ^{a}	1.41^{ab}	2.33 ^{a}
Control	3.27 ^{a}	9.54 ^{a}	58.69^{a}	84.3 ^{a}	15.44^{a}	26.28 ^{a}	1.46 ^{a}	2.56 ^{a}	1.36 ^{a}	2.26 ^{a}

See Table 1 for details

chlorantraniliprole + As treated rats suggested the ability of wheatgrass to protect against the combined toxicity of chlorantraniliprole and arsenic with maximum effect in chlorantraniliprole (1/4LD_{50} and 1/6LD_{50}) + As + wheatgrass treated rats. Similar observations were reported due to combined effect of arsenic and chlorpyirfos that resulted in decreased content of proteins as the ROS bind to thiol groups of amino acids and disrupt their macromolecular structures thus increasing the proteasomal degradation of proteins (Jasbeen and Noor, 2024). The decrease in the level of lipids and phospholipids observed was because of the polyunsaturated fatty acids (PUFAs) that are present in the cell membranes, are extremely sensitive to ROS as they modify the lipid composition, structure and dynamics of cell membranes because of lipid peroxidation and thereby decrease the content of total lipids and phospholipids that was observed by the combined effect of arsenic, cadmium and mercury (Nie et al., 2024). Shivyari et al. (2024) suggested that the level of free fatty acids decreases under oxidative stress as the triglycerides are taken up by the cells in the form of free fatty acids and further result in decreased polyunsaturated fatty acid among total free fatty acid content. Wheatgrass extracts also have flavonoid and phenolic content that inhibit ascorbate-Fe^{2+} induced lipid peroxidation in the liver and mitochondria of rats (Aldaamy et al., 2023). These changes are suggestive of why wheatgrass was able to effectively ameliorate the lipid peroxidation levels in the current study.

The content of cholesterol in the ovarian and uterine tissue in chlorantraniliprole (1/2LD_{50} and 1/4LD_{50}) + As treated rats showed significant increase as compared to control and only arsenic treated rats, whereas rats treated with chlorantraniliprole (1/6LD_{50}) + As showed significant increase in content of cholesterol as compared to control but varied non-significantly from only As treated rats. The groups administered with chlorantraniliprole + As + wheatgrass however, were able to mitigate the toxic effects of chlorantraniliprole and arsenic. The increased level of cholesterol reported in the present study is due to accumulation of cholesterol in the form of esters because of deficiency of the enzyme lecithin-cholesterol acyltransferase from the liver (Zhang et al., 2024).

4. CONCLUSION

The combination of variable doses of chlorantraniliprole and arsenic caused reproductive toxicity in albino rats by inducing the production of reactive oxygen species (ROS) which caused an imbalance in the functioning of enzymes involved in regulating the oxidative stress and thereby disrupt the other physiological functions of the reproductive system. Wheatgrass owing to its antioxidant properties is capable of balancing the increased oxidative stress and restoring the compromised reproductive functions caused by these prevalent environmental toxicants thereby depicting the role of wheatgrass as a promising ameliorative agent that can be explored for developing plant-based nutraceuticals or adjunct therapeutic formulations aimed at mitigating oxidative stress induced reproductive dysfunction caused by environmental toxicants.

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Authors' Contributions

SB: Data curation, Formal analysis, Investigation, Methodology, Writing the original draft, Writing- reviewing and editing. NK: Conceptualisation, Project administration, Supervision, Writing- reviewing and editing.

Conflict of Interest

The authors declare no conflict of interest.

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

No use of AI tools have been used in the writing process.

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Antioxidant Activity of *Cymbopogon martini* (Roxb.) Wats. Essential Oil from Different Ecotypes in Karnataka, India

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***Abstract*:** Palmarosa essential oil, derived from the *Cymbopogon martini* of the Poaceae family, has long been used in traditional medicine, particularly in India. Despite its widespread use, the antioxidant properties of Palmarosa oil remain underexplored. This study investigates its potential as an antioxidant, focusing on oil extracted from plants cultivated in the plains of South India. We employed two common methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the Nitric Oxide (NO) scavenging assay. Results indicated that Palmarosa oil displayed significant antioxidant effects, efficiently scavenging DPPH and NO free radicals. The IC50 values from the DPPH and NO assays suggest potent antioxidant activity, comparable to well-known antioxidants. These findings support the therapeutic value of Palmarosa oil, emphasizing its potential as a natural source of antioxidants for various health applications.

***Keywords*:** Antioxidant, DPPH assay, Nitric Oxide scavenging, Palmarosa essential oil, and Therapeutic applications.

1. INTRODUCTION

Medicinal plants have been integral to traditional medicine, offering remedies for ailments caused by microorganisms such as bacteria, fungi, and viruses (Ashraf et al., 2023; Breijyeh & Karaman, 2024). Among these plants, essential oils, particularly those derived from leaves, have attracted significant attention for their therapeutic properties (Bolouri et al., 2022). Palma Rosa oil, extracted from *Cymbopogon martinii*, is an essential oil known for its floral aroma, golden color (Figure. 1A and B), and medicinal benefits. Despite its traditional use in treating various conditions (Walters, 2022). The *Cymbopogon* genus, known for its fragrant oils, has been widely studied for its antibacterial, anti-fungal, and insecticidal properties, with compounds such as geraniol and citronellol identified as key bioactive agents (Dangol et al., 2023). However, the antioxidant activity of Palma Rosa oil, particularly its ecotype variations, remains poorly understood. Current studies on synthetic antioxidants have raised concerns over their side effects, making plant-based alternatives like Palma Rosa oil an area of growing interest. This research aims to address the gap by exploring the antioxidant potential of Palma Rosa oil from various ecotypes of Karnataka, South India. The study will employ established assays such as DPPH and Nitric Oxide scavenging to assess the oil's ability to neutralize free radicals and mitigate oxidative stress. The findings will contribute to a deeper understanding of the variations in antioxidant activity across different ecotypes, potentially influencing the therapeutic applications of Palma Rosa oil. The objective is to identify the most potent ecotype, paving the way for future research into its use as a natural antioxidant in medicine and related industries.

2. MATERIALS AND METHODS

2.1. Plant Collection and Documentation

The study was carried out in 2021 at the Department of Biotechnology and Genetics, M S Ramaiah College of Arts, Science, and Commerce, Bengaluru. Wild ecotypes of *Cymbopogon martinii* were collected and comprehensively documented. This documentation process included key details such as the collection date, exact location, and a reference map for easy access. In addition, habitat characteristics were recorded to capture the natural environment of the plants. Environmental factors such as temperature, altitude, longitude, humidity, rainfall, wind speed, and topography (latitude and altitude) were also noted to provide a clearer understanding of the growing conditions. Six distinct ecotypes of *C. martinii* were selected from different ecological regions in Karnataka, chosen

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based on variations in their environmental conditions. These ecotypes were labelled as Ecotype 1 (E1), Ecotype 2 (E2), Ecotype 3 (E3), Ecotype 4 (E4), Ecotype 5 (E5), and Ecotype 6 (E6) (Table 1). Each of these ecotypes was authenticated by submitting plant samples to the Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD) in Uttarahalli, Bangalore, India where voucher numbers were assigned for proper identification (Authentication/SMPU/RARIMD/BNG/2019-20/432, 433, 444, 445, 446, and 447). The collected samples of these medicinal and aromatic plants were studied and conserved at the National Bureau of Plant Genetic Resources (NBPGR), Delhi

2.2. Extraction of Essential Oil

The essential oil of *Cymbopogon martinii* (Palmarosa) was extracted using the steam distillation method by Koul et al. (2004). This method ensures that the volatile compounds, including the desired aroma and therapeutic properties, are preserved during extraction.

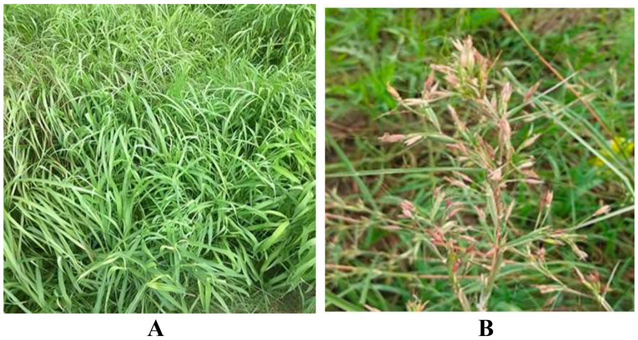

Figure 1A and 1B. Habitat and spikelet's of *Cymbopogon martini* (Roxb.) Wat

Preparation of plant material: Fresh leaves and stems of *Cymbopogon martinii* were harvested, washed thoroughly to remove any dirt or debris, and air-dried for a short period (1-2 hours). The plant material was cut into smaller pieces to facilitate better steam penetration during the distillation.

Steam Distillation: The prepared plant material was placed in the distillation chamber of a steam distillation apparatus with enough water to generate steam. The steam was passed through the plant chamber material, causing the essential oil to evaporate (Koul et al., 2004).

Collection: The essential oil was now cooled through a cooling system back into a liquid. The mixture of water and essential oil was collected in a receiving flask. Because essential oils are typically immiscible with water, they separate naturally, with the oil floating on top of the water.

Separation of oil: From the hydrosol (water phase) using a separator funnel. The hydrosol, often known as Palmarosa water or *Cymbopogon* hydrosol, can also be used in cosmetic formulations but is discarded or stored separately.

Storage: The essential oil was stored in airtight amber glass bottles to shield it from light and oxygen, which could potentially reduce its quality. These bottles were placed in a cool, dark environment to preserve the oil's efficacy.

2.3. Antioxidant Activity

The antioxidant activity of essential oils was assessed by two distinct methods: the DPPH assay (Khalaf et al., 2008), and the NO scavenging assay (Ghosh et al., 2010), For the DPPH radical scavenging assay, the ability of essential oils, and standard (Gallic acid) to donate hydrogen atoms or electrons was evaluated spectrophotometrically as per the method of Khalaf et al. (2008) with slight modifications. The stable radical DPPH was used as the reagent. Essential oil

Table 1. Environmental factors influencing plant habitat

Parameter	E1 Himavadgopala hill	E2 Siddarabetta	E3 Chikkaballapur	E4 Siddharuda mutt	E5 Karanji mantapa	E6 Koppa
Altitude	11454m	1200m	925m	720m	606m	565m
Latitude	11' 25' 53.12"N	14"37'12.00"N	12'58'20.7912"N	14'48'21.9712" N	13'20'17.7468" N	13'25'22.5874" N
Longitude	77'14'4.36"E	74050'60.00"E	77'34'50.3148" E	11'91'19.1297" E	77'6'5.0760" E	12'66'23.9852" E
Temperature (^{0}C)	18-34	21-28	21-31	20-30	18-35	18-35
Humidity (%)	71-85	68-82	68-80	70-80	70-80	70-82
Rainfall (mm)	791-800	700-860	680-900	680-880	600-700	1400-1500
Wind (Km/h)	8.41-14.1	13.80-14.50	12.3	12.38	6.8-9.2	6.8-9.2
Soil type	Red, loamy soil	Lateritic soil	Red loamy and black soils	Red sandy and black soils	lateritic gravelly soil	Red sandy & black soil

Source: Ministry of Environment, Forest and Climate Change, New Delhi, Government of India (E1 and E3); Tumkur Forest Department (E2); IIT Dharwad, Karnataka (E4); Karnataka Biodiversity Board (E5 & E6)

was diluted in methanol to obtain concentrations of 2.000, 1.000, 0.500, 0.250, 0.125, and 0.0625 mg mL^{-1}. A 0.0002% DPPH solution was prepared, and 2 mL was added to each test container containing varied amounts of essential oil. After thorough mixing the solutions were incubated in the dark for 30 min. The optical density was measured at 517 nm using a UV-visible spectrometer (Shimadzu UV-1280 wavelength scanning from 190-1100nm) with DPPH as a control. Inhibition of DPPH activity was calculated as (Eq. 1):

% inhibition of DPPH activity = A – B/A x 100

Where: A = Optical density of control; B = Optical density of sample

2.4. Nitric Oxide (NO) Scavenging Activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and measured using the Griess reagent, as described by Ghosh et al. (2010). At physiological pH, SNP undergoes a spontaneous reaction in aqueous solutions to release NO, which then reacts with oxygen to form nitrite ions. These nitrite ions can be quantified by responding with the Griess reagent. A solution containing 5 mM SNP in phosphate-buffered saline (PBS) was prepared and incubated at 25°C for 180 minutes. After incubation, the solution was mixed with the Griess reagent, and the absorbance of the resulting chromophore was measured at 546 nm using a spectrophotometer. Gallic acid was used as a standard reference. This method was adapted from Ghosh et al. (2010). The scavenging activity was calculated using the following formula (Eq. 2):

Nitric Oxide scavenged (%) = A – B/A x100

Where: A = Optical density of the control; B = Optical density of the essential oil sample

2.5. Calculation of Half-maximal Inhibitory Concentration (IC_{50})

The IC50 values were determined to evaluate the required to scavenge 50% of the radicals. A lower IC50 value indicates stronger antioxidant activity. To calculate the IC50, the percentage of inhibition was plotted against the sample concentration, and a regression analysis was performed to obtain the best-fit line. The IC50 was then determined from the curve where the percentage inhibition reached 50%.

2.6. Statistical Analysis,

The data were subjected to a two-way ANOVA (SPSS version) followed by Duncan's multiple range test(Duncan 1955) to identify significant differences between groups. Statistical analysis was conducted using SPSS version 30, and a p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The current study aimed to evaluate the *in vitro* antioxidant activity of *Cymbopogon martinii* (Palmarosa) essential oil, derived from different ecotypes in Karnataka, India. The antioxidant potential was assessed using two different assays: DPPH radical scavenging and NO scavenging methods.

3.1. DPPH Assay

The inhibition of DPPH radicals increased with the concentration of Palmarosa essential oil. Ecotype S6 exhibited the highest percentage of inhibition across all concentrations, reaching 70.70% at 500 µg (Table 2, Figure 7), indicating its potent antioxidant activity. This was followed by ecotypes S4 and S5 (Table 2, Figure 5 & 6) which also demonstrated relatively high inhibition percentages, especially at the higher concentrations.

The results suggest that the antioxidant activity of the essential oil increases with concentration, which is consistent with findings from other studies investigating the antioxidant properties of essential oils. For instance, *Rosmarinus officinalis* (rosemary) and *Lavandula angustifolia* (lavender) have also shown increased DPPH radical scavenging activity at higher concentrations (Hendel et al., 2024). Furthermore, the variation in activity among the ecotypes implies that environmental factors or genetic diversity might play a role in the antioxidant efficacy of

Table 2. Antioxidant potential of various *C. martinii* ecotype samples at different concentrations

Sample conc. (µg)	Percentage inhibition (DPPH assay)					
	S1	S2	S3	S4	S5	S6
100	1.7±0.20	9.3±0.20	31.1±0.20	23.9±1.00	16±0.09	38.2±0.24
200	2.6±0.30	16.6±0.50	33.8±0.60	43.2±0.32	27.7±0.60	50.2±0.24
300	2.8±0.10	21.6±1.10	41.3±0.31	57.9±0.70	40.9±0.92	50.9±0.80
400	3.2±0.20	26.76±0.20	57.2±0.32	69.7±0.60	52.2±0.28	59.9±0.70
500	4.9±0.20	32.6±0.70	64±0.12	71.2±0.21	56.6±0.40	70.7±0.60

Palmarosa oil (Jamwal et al., 2024).

3.2. Nitric Oxide Scavenging Assay

The NO scavenging assay is another crucial method for assessing antioxidant activity, as nitric oxide is a free radical involved in various physiological and pathological processes. The percentage inhibition of NO radicals also increased at 500 µg, sample S6 demonstrated the highest inhibition at 57.60% (Table 3, Figure 13), followed closely by S5 (57.30%, Figure 12). These findings are in line with the results from the DPPH assay, further supporting the strong antioxidant properties of the Palmarosa oil.

Sample S6 not only exhibited the highest DPPH radical scavenging activity but also showed a remarkably low IC50 value for the NO scavenging assay (8.125 µg, Table 4, Figure 13), indicating a higher potency of this ecotype in scavenging NO compared to others (Table 3). The IC50 values for the NO assay ranged from 319.50 µg for S1 to 8.125 µg for S6, with S6 demonstrating superior activity . Kim et al. (2022) also reported that essential oils from *Cymbopogon citratus* exhibited a natural source of readily available, low-cost extracts rich in antioxidants. The high potency of S6 suggests that this ecotype may be particularly valuable for therapeutic applications targeting oxidative stress and related conditions.

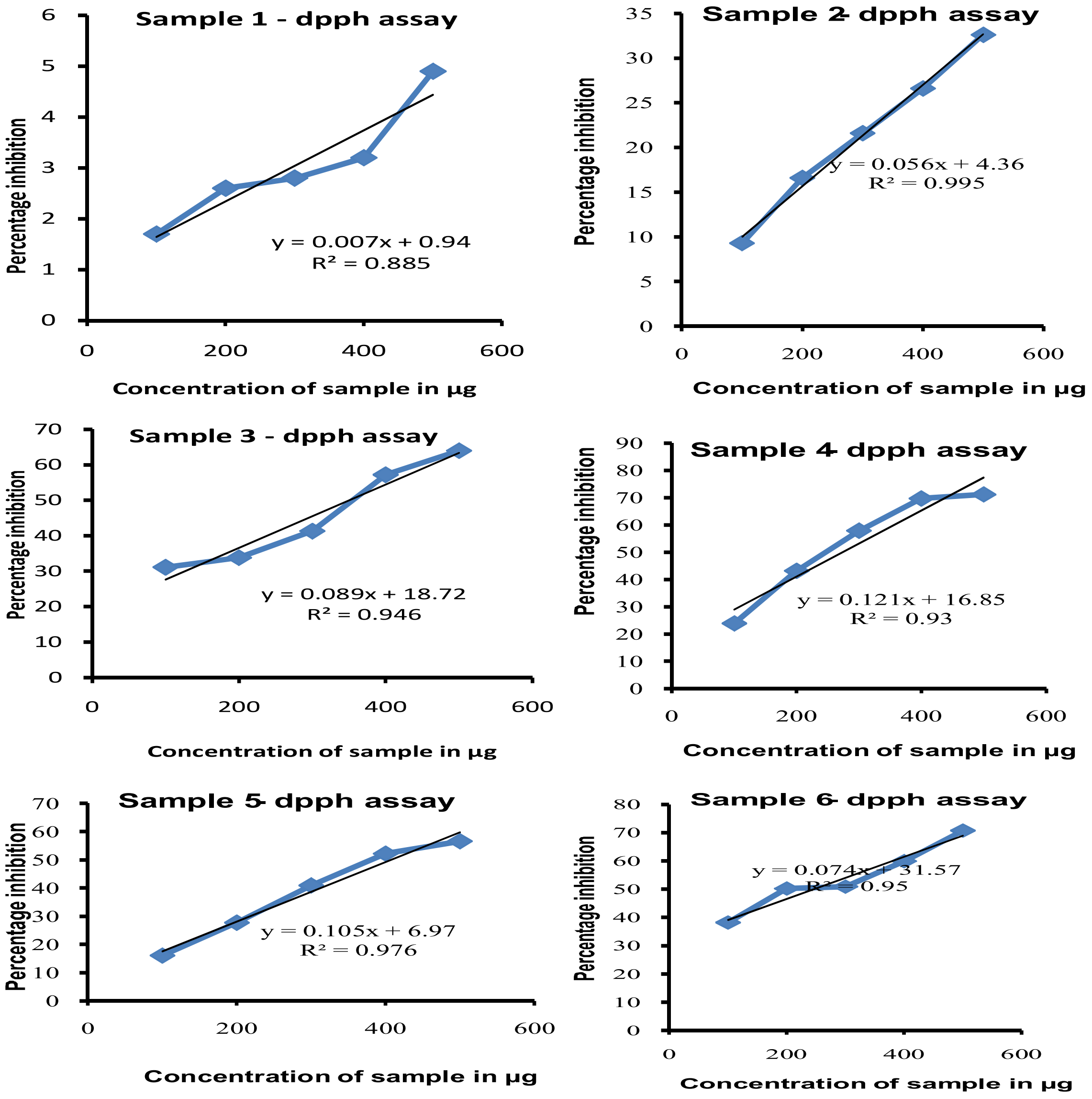

Figure 2-7. DPPH scavenging activity of *C. martini* ecotypes (E1-E6) at different concentrations

Table 3. Inhibition in Nitric Oxide (NO) by scavenging assay (%)

Sample conc. (µg)	**Percentage inhibition**					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
100	40.30±0.44	4.10±0.20	34.80±0.90	18.00±0.86	42.30±0.45	51.00±1.14
200	46.60±0.48	20.40±0.60	41.10±0.14	24.50±0.54	47.50±0.40	53.36±0.44
300	49.10±0.54	42.20±0.60	47.00±0.20	33.20±0.24	50.00±0.18	55.10±0.15
400	53.00±2.00	58.46±0.35	52.83±2.06	42.90±0.79	51.70±0.60	56.40±0.58
500	57.10±0.30	66.50±0.90	55.40±0.44	52.20±0.28	57.30±0.45	57.60±0.43

The values represent mean±standard error, with Duncan's multiple range test used to determine significant differences between groups based on 'F' ratios

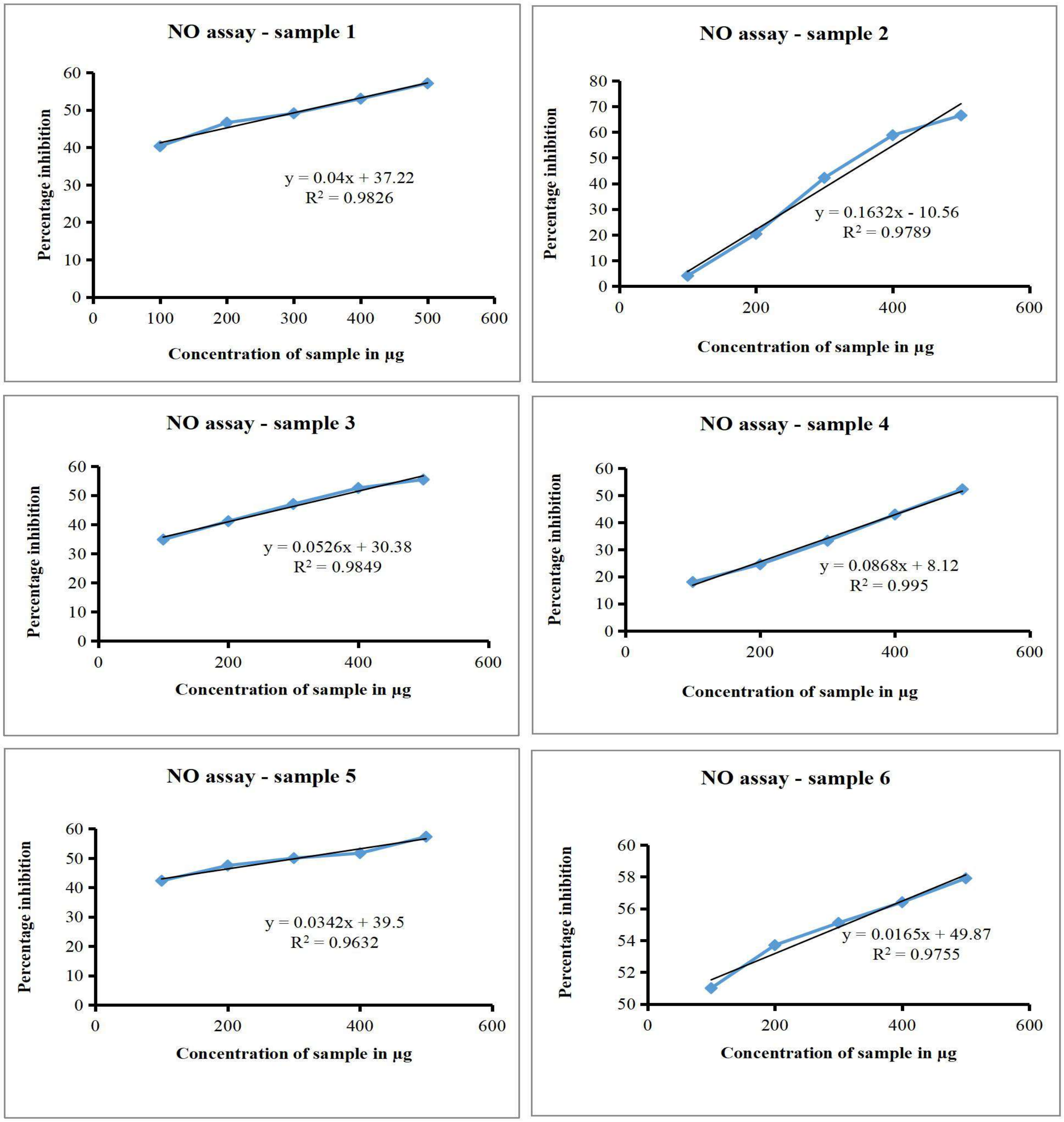

Figure 8-13. Bvaluation of nitric oxide scavenging activity in *C. martini Ecotypes* (E1-E6) across various concentrations

Table 4. IC50 values from DPPH and nitric oxide (NO) scavenging assays

Sample	DPPH IC50 (µg)	NO IC50 (µg)
S1	708.57±6.08	319.50±7.5
S2	815.00±14	371.53±3.07
S3	356.48±9.10	377.31±5.18
S4	273.32±4.03	488.66±9.83
S5	409.81±7.2	308.82±6.04
S6	249.05±7.05	8.125±0.04

3.3. IC50 Values

The IC50 values, which represent the concentration required to inhibit 50% of the free radicals, were calculated for both the DPPH and NO scavenging assays (Table 4). The lower the IC50 value, the stronger the antioxidant activity. For the DPPH assay, ecotype S6 had the lowest IC50 value at 249.05 µg (Table 4), indicating its superior ability to scavenge free radicals compared to the other samples. This was also true for the NO scavenging assay, where S6 demonstrated the lowest IC50 value (8.125 µg), a highly significant finding. In comparison, other ecotypes showed much higher IC50 values, with S1 and S2 showing the least efficient scavenging of both DPPH and NO radicals (Table 4). These findings align with previous research on the antioxidant activities of essential oils., *Cymbopogon citratus* (lemongrass) essential oil has shown similar results, with lower IC50 values correlating with higher antioxidant activity (Aldawsari et al., 2023). The results suggest that Palmarosa oil, particularly from ecotype S6, has strong potential as a natural antioxidant, possibly due to its high content of bioactive compounds such as terpenoids and phenolic compounds.

4. CONCLUSION

This study highlights the significant antioxidant activity of *Cymbopogon martinii* (Palmarosa) essential oil, particularly from ecotype S6, which exhibited superior radical scavenging activity and reduced power across multiple assays. The consistent findings across the DPPH and NO scavenging assays, and the low IC50 values, suggest that this essential oil could be a potent natural antioxidant. Given its potential to neutralize free radicals, Palmarosa essential oil may find applications in therapeutic fields of oxidative stress-related diseases. Further investigations into the chemical composition of the oil, particularly the identification and quantification of specific active compounds, could provide deeper insights into its medicinal potential.

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Conflict of Interest:

The authors declare that there are no conflicts of interest regarding the publication of 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 in the preparation of this manuscript.

Authors's Contributions

Dr. Vijayalakshmi: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing (90%)

S. Uma shivanna: Conceptualization (10%)

M.I. Farzana Tasneem: Investigation (10%)

Data Availability:

Authentication from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD) in Uttarahalli, Bangalore.

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Biochemical Basis of Resistance in *Oryza rufipogon* (Wild rice) against *Meloidogyne graminicola* and *Drechslera oryzae* in Punjab

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***Abstract*:** Rice (*Oryza sativa* L.), a staple food for over half of the global population, faces severe yield losses due to biotic stresses, notably the rice root knot nematode (*Meloidogyne graminicola*) and brown leaf spot pathogen (*Drechslera oryzae*). These pathogens significantly affect root function and overall plant health. This study investigates the biochemical basis of resistance in *Oryza rufipogon* (wild rice) accessions against these pathogens by analyzing key defense-related enzymes and compounds. Two resistant and two susceptible accessions of *O.rufipogon* were evaluated against *M. graminicola* alone, along with accessions resistant and susceptible to both pathogens. Biochemical parameters including PAL (phenylalanine ammonia lyase), TAL (tyrosine ammonia lyase), peroxidase, total phenols, ortho-dihydroxy phenols and total proteins were quantified post-inoculation. There were significantly higher activity of all enzymes and phenolic compounds in resistant accessions compared to susceptible ones under both single and combined pathogen inoculations. Increased activity of PAL and TAL suggests enhanced lignin biosynthesis, aiding structural defense. Elevated peroxidase activity indicates a stronger reactive oxygen species (ROS) detoxification response, while higher phenol levels point to the activation of redox-based defenses. The findings suggest that resistance in *O. rufipogon* is strongly associated with upregulation of specific biochemical pathways and can serve as a foundation for developing resistant rice cultivars. This study provides valuable insights into host-pathogen interactions and the role of biochemical markers in rice defense mechanisms.

***Keywords*:** Biochemical resistance, *Oryza rufipogon*, wild rice, *Meloidogyne graminicola*, *Drechslera oryzae*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most significant staple crops globally, feeding over half of the world's population and serving as a crucial source of calories and livelihood (FAO, 2021). However, rice production is continually threatened by various biotic and abiotic stresses with pathogens posing a major constraint. Among these, *Meloidogyne graminicola* (rice root-knot nematode) and *Drechslera oryzae* (causal agent of brown leaf spot) are increasingly recognized for their detrimental effects on rice growth and yield. *M. graminicola* is a soil-borne endoparasitic nematode that causes the formation of giant cells in rice roots, disrupting the vascular system and ultimately reducing nutrient and water uptake (Mantelin et al., 2017). This nematode poses a significant threat to both nursery-raised and direct-seeded rice systems, especially in rainfed and lowland ecologies. Similarly, *D. oryzae*, the fungal pathogen responsible for brown leaf spot, is a seed- and soil-borne disease known to cause considerable yield losses in susceptible cultivars, particularly under nutrient-deficient conditions (Surendhar et al., 2022).

In plant-pathogen interactions, biochemical defense responses are activated upon infection. These responses include the induction of phenolic compounds, defensive enzymes and protein synthesis, all of which play essential roles in plant resistance mechanisms (Saha et al., 2016). Phenolic compounds-particularly total phenols and ortho-dihydroxy phenols-are involved in reactive oxygen species (ROS) scavenging, strengthening of cell walls via lignification and direct antimicrobial activity (Pant et al., 2014; Senthilkumar et al., 2007). The accumulation at the infection site can lead to hypersensitive response and restricted pathogen growth (Ranchana et al., 2015). Among the enzymes implicated in plant defense, phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) serve as critical entry point enzymes into the phenylpropanoid pathway leading to the synthesis of lignin, flavonoids and other defense-related compounds (Barros et al., 2016). Similarly, peroxidases are involved in the detoxification of H_2O_2 and catalyze the polymerization of phenolic compounds into lignin, contributing to cell wall reinforcement and pathogen restriction (Falade et al., 2017).

The activity of these enzymes has been observed to be significantly higher in resistant genotypes of various crops, including rice, brinjal, and tomato, upon nematode or fungal infection (Nayak and Pandey, 2016).

Understanding the biochemical basis of resistance to both *M. graminicola* and *D. oryzae* is critical for developing durable disease-resistant rice varieties. Despite the economic significance of these pathogens, limited information is available on the biochemical and enzymatic changes associated with resistance in wild rice accessions such as *Oryza rufipogon*, a known reservoir of resistance genes (Kalaiarasan, 2009). The present study was undertaken to explore the biochemical basis of resistance to *M. graminicola* and *D. oryzae* in selected resistant and susceptible accessions of *O. rufipogon*. Specifically, the study aimed to quantify the activity of defense-related enzymes (PAL, TAL, and peroxidase), phenolic compounds (total phenols and ortho-dihydroxy phenols) and total soluble proteins post-infection. These insights may contribute to the identification of potential biomarkers for resistance breeding in rice.

2. MATERIALS AND METHODS

In order to understand the basis of rice root knot nematode (*M. graminicola*) and brown leaf spot (*D. oryzae*) resistance, biochemical analysis of two resistant and two susceptible accessions of *O. rufipogon* against *M. graminicola* and combined inoculation of *M. graminicola* and *D. oryzae* was done in 2020-2021 in Department of Basic Sciences of Punjab Agricultural University, Ludhiana. The accessions were: IR93070 and CR100381 (Resistant to *M. graminicola*), IR104844 and IR105494 (Susceptible to *M. graminicola*), IR104821 and IR105375 (Resistant to both pathogens *i.e. M. graminicola* and *D. oryzae*) and CR100381 and CR100006A (Susceptible to both *M. graminicola* as well as *D. oryzae*). The biochemical parameters include enzymes (phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and peroxidase), orthrohydroxy phenols, total phenols and total proteins. Biochemical assays were done on the roots of these genotypes. Sixty days following infection, the test plants were harvested. The soil was then carefully shaken away from the roots of the plants. The harvested rice roots were cleaned separately and dried with tissue paper before being examined for biochemical changes.

Extraction and assay of phenylalanine ammonia lyase (PAL) assay was performed as per method of Hadwiger and Schwochau (1971) while Tyrosine ammonia lyase (TAL) was done as per method given by Burrell and Rees (1974). Peroxidase extraction and assay was performed as method given by Shannon et al. (1966). The post-infectional changes in the level of ortho-dihydroxy phenols were studied using the colorimetric method of Nair and Vaidyanathan (1964). In addition to this, extraction of phenol and determination of total soluble proteins was also done.

2.1. Statistical Analysis

The differences among means were compared by Tukey method ($P<0.05$) using IBM SPSS software v.27.0.

3. RESULTS AND DISCUSSION

3.1. Biochemical Changes in Resistant and Susceptible Accessions of *O. rufipogon* after Inoculation of *M. graminicola*

3.1.1. Biochemical changes in enzymes: The data on effect of *M. graminicola* infestation on enzymes in roots of *O. rufipogon* depicts that the activity of all enzymes was more in resistant accessions as compared to susceptible ones. The activity of PAL was higher in both the resistant accessions. The PAL values in accession IR93070 and CR100381 (resistant to *M. graminicola*) was 0.072 and 0.068 u mol of t-cinnamic acid min^{-1} g^{-1} of FW respectively whereas the activity of this enzyme in susceptible accessions *viz*., IR105494 and IR104844 was 0.029 and 0.040 u mol of t-cinnamic acid min^{-1} g^{-1} of FW respectively.

The activity of tyrosine ammonia lyase (TAL) was highest in the resistant accessions IR93070 and CR100381. TAL activity in these accessions was 0.053 and 0.061 μ mol of t-cinnamic acid min^{-1} g^{-1} fresh weight (FW) respectively. In contrast, susceptible accessions showed markedly lower TAL activity. The minimum TAL activity was recorded in IR105494 and IR104844, with values of 0.011 and 0.014 μmol of t-cinnamic acid min^{-1} g^{-1} FW, respectively. Similarly, peroxidase activity was significantly higher in *O. rufipogon* resistant accessions than in susceptible ones. Peroxidase activity in IR93070 and CR100381 was 5.17 and 4.24 OD min^{-1} g^{-1} FW, respectively, whereas much lower activity was observed in the susceptible accessions IR105494 and IR104844, with values of 0.69 and 0.62 OD min^{-1} g^{-1} FW, respectively (Table 1).

3.1.2. Biochemical changes in total phenols (ug/g), Orthrohydroxy phenols (ug/g) and total proteins (mg/g): The activity of total phenols was significantly affected when *M. graminicola* was inoculated on resistant and susceptible accessions of *O. rufipogon*. After nematode inoculations, the total phenol activity was expressed more in roots of both resistant accessions as compared to susceptible. Value of total phenols was observed to be 214.12 and 236.20 ug/g in IR93070 and CR100381 accessions (Resistant) while the values were 69.76 and 73.31 ug/g in IR105494 and IR104844 accessions (Susceptible accessions). Rice roots treated with *M. graminicola* expressed significantly higher amount of orthrohydroxy phenols in resistant accessions of *O. rufipogon*. The amount of orthrohydroxy phenols in resistant accessions *viz*., IR93070 and CR100381 was observed to be 15.31 and 16.25 ug/g while it was observed to be comparatively less in susceptible accessions *viz*., IR105494 (4.75 ug/g) and IR104844 (3.99 ug/g). The activity of total proteins was also observed to be higher in resistant accessions as compared to susceptible accessions (Table 1).

3.1.3. Biochemical changes in resistant and susceptible accessions of *O. rufipogon* after combined inoculation of *M. graminicola* and *D. oryzae:* The activity of enzymes TAL, PAL and peroxidase in combined inoculation of rice root knot nematode and brown leaf spot was found to be higher in resistant accessions (IR104821 and IR105375) while decreased in susceptible accessions (CR100381 and CR100006A). The PAL values were 0.073 and 0.084 u mol of t-cinnamic acid min^{-1} g^{-1} of FW in resistant accessions *i.e.* IR104821 and IR105375 while PAL values decreased to 0.009 and 0.013 u mol of t-cinnamic acid min^{-1} g^{-1} of FW in susceptible accessions *viz*., CR100381 and CR100006A. The TAL also showed the similar activity and was more in both the resistant accessions and lower in susceptible accessions. Similarly the activity of peroxidase was higher in resistant accessions and lower in susceptible accessions.

The combined inoculation of both the pathogens also affected the activity of orthrohydroxy, total phenols and total proteins in resistant and susceptible accessions. The activity of all these parameters was more in resistant accessions and decreased in susceptible ones when combined inoculation of both pathogens was done. The orthrohydroxy phenols was 18.43 and 20.60 ug/g in IR104821 and IR105375 accessions and 2.49 and 2.92 ug/g in CR100381 and CR100006A accessions. The total proteins were also more in resistant accessions IR104821 and IR105375 (30.57 and 33.18 mg/g) as compared to susceptible accessions CR100381 and CR100006A (7.28 and 8.30 mg/g). Similarly, in case of total phenols the activity was higher in resistant accessions as compared to susceptible. The activity of all enzymes, orhtohydroxy phenols, phenols and total proteins was decreased more in the treatments where combined inoculation of both pathogens (*M. graminicola* and *D. oryzae*) was done as compared to nematode alone (*M. graminicola*) in both resistant and susceptible accessions of *O. rufipogon* (Table 2).

Rice root knot nematode (*M. graminicola*) and brown leaf spot pathogen (*D. oryzae*) significantly affect root function and overall plant health. Plants undergo morphological and biochemical changes in response to these pathogens. There was significant increases in enzymes (PAL, TAL, and peroxidase activity), orthrohydroxy phenols, total proteins and phenols in resistant accessions and significant decrease in susceptible accessions. Phenolic compounds in plants play a vital role in their defence system, particularly redox response and free radical scavenging (Saha et al., 2016). Accumulation of phenols at the site of infection is characteristics in plant defence response and cause rapid cell death and prevents penetration of pathogens (Pant et al., 2014). The increase in phenolics in resistant plants is due to high activity of α-glycosidase, which converts non-toxic phenolic glycosides to toxic phenolic which are inhibitory to the pathogen. These phenolic compounds are possibly converted by increased peroxidase

Table 1. Biochemical changes in roots of susceptible and resistant *O. rufipogon* accessions after inoculation with *M. graminicola*

Pathogen	Accessions	Phenol (ug/g)	Total proteins (mg/g)	PAL (u mol of t-cinnamic acid min^{-1} g^{-1} of FW)	TAL (n mol of p-coumaric acid min^{-1} g^{-1} of FW)	Orthohydroxy phenols (ug/g)	Peroxidase (OD min^{-1} g^{-1} of FW)
Nematode only	Resistant var I-IR93070	214.12^{ab}	30.27^{a}	0.072^{a}	0.053^{ab}	15.31^{b}	5.17^{a}
	Resistant varII-CR100381	236.20^{ab}	32.92^{a}	0.068^{a}	0.061^{a}	16.25^{b}	4.24^{a}
Nematode only	Susceptible var I-IR105494	69.76^{c}	11.55^{b}	0.029^{bc}	0.011^{c}	4.75^{c}	0.69^{b}
	Susceptible varII-IR104844	73.31^{c}	13.56^{b}	0.040^{b}	0.014^{c}	3.99^{c}	0.62^{b}

Means sharing common letters within columns do not differ significantly by Tukey's test at $P < 0.05$%

Table 2. Comaparative analysis of biochemical parameters in susceptible and resistant *O. rufipogon* accessions inoculated with *M. graminicola* and *D. oryzae*

Pathogen	**Accessions**	**Phenol (ug/g)**	**Total proteins (mg/g)**	**PAL (u mol of t-cinnamic acid min^{-1} g^{-1} of FW)**	**TAL (n mol of p-coumaric acid min^{-1} g^{-1} of FW)**	**Orthohydroxy phenols (ug/g)**	**Peroxidase (OD min^{-1} g^{-1} of FW)**
Nematode+ Brown leaf spot	Resistant var I-IR104821	211.29^{b}	30.57^{a}	0.073^{a}	0.058^{ab}	18.43^{ab}	5.12^{a}
	Resistant var II-IR105375	241.19^{a}	33.18^{a}	0.084^{a}	0.043^{b}	20.60^{a}	5.92^{a}
Nematode+ Brown leaf spot	Susceptible var I-CR100381	55.38^{c}	7.28^{c}	0.009^{d}	0.009^{c}	2.49^{c}	0.27^{b}
	Susceptible var II-CR100006A	57.55^{c}	8.30^{c}	0.013^{cd}	0.008^{c}	2.92^{c}	0.38^{b}

Means sharing common letters within columns do not differ significantly by Tukey's test at $P < 0.05\%$

activity to quinines in resistant cultivars and quinines are reported to be more toxic to microorganism. The quick breakdown of bound phenols and switching of phenols to alternative pathways leading to the creation of different compounds like lignin, which plays a crucial role in resistance, could explain the increase in phenolic compounds during infection (Senthilkumar et al., 2007).

Another mode of action of phenolic compounds may be related to the modification of the nematode physiology. RKNs secrete a pool of substances into the plant cell membrane to induce nematode feeding site formation (Williamson and Gleason, 2003, Cailaud et al., 2008). It has been proposed that such secretion may be induced by some phenolic compounds such as resorcinol, catechol, hydroquinone and caffeic acid (Jaubert et al., 2002). Ranchana et al. (2015) also concluded that the roots of nematode resistant tuberose genotype (Kahikuchi Single) retain highest phenolic content after inoculation indicating the use of resistant genotype as one of the parent to develop hybrids. Rani et al. (2008) also recorded that degree of resistance to root knot nematode is indicated by the increased phenolic content which may cause browning and resistant mechanism in the roots of tomato. Kalaiarasan (2009a) also observed higher total phenol content due to root knot nematode infestation. In addition to this, Bisen et al. (2015) also observed that higher amount of total phenol in rice leaves resulted lower disease incidence of *D. oryzae.*

Similarly, orthodihydroxy phenol content of different *O. rufipogon* accessions increased in the roots after inoculations. Nayak and Pandey (2016) observed enhanced peroxidase activity in nematode inoculated samples of both susceptible and resistant brinjal cultivars, although resistant cultivars had higher enzymatic activity. Ashfaq et al. (2021) also found that the brown leaf spot resistant phenotypes in rice line (PARC-7) reflect the higher accumulation of phenylalanine ammonia lyase, polyphenol oxidase and peroxidase and β-1, 3-Glucanase as compared to susceptible lines.

Peroxidases are regarded as detoxifying agents for H_2O_2 and have a precise metabolic function in the defence mechanism. In present investigation, peroxidase was also observed to be higher in resistant accessions of *O. rufipogon* than the susceptible accessions. This might be due to lignification of cell walls, which assists in delaying the penetration by the nematodes as mechanism of resistance. Increase in peroxidase activity may be attributed due to infection in plants as polymerization of cinnamyl lignification alcohols to lignin is catabolized by peroxidase lignification leading to disease resistance (Bhau et al., 2016). Pathogen infection results in overproduction of reactive oxygen species such as superoxide anion, singlet oxygen, hydrogen peroxidase and hydroxyl radical which are toxic (Sreedhar et al., 2013). Mahfouz et al. (2012) also reported enhancement in level of peroxidase after nematode infestation. Shamshad et al. (2024) also demonstrated that brown leaf spot resistant and moderately resistant rice genotypes have higher concentrations of the peroxidase enzyme. The findings imply that the peroxidase was crucial in the defense against brown spot disease.

Increase of PAL and TAL content was observed more in resistant accessions as compared to susceptible accessions of *O. rufipogon*. This enzyme plays a key role in the phenylpropanoid pathway, which involves the biosynthesis of polyphenols, flavonoids, and lignin precursors (Barros et al., 2016). Lignin is a component of the cell wall and vascular tissues. It confers mechanical resistance to cells, reducing nematode penetration (Ji et al., 2015; Gheysen and Jones 2006) and plant exposure to degradative enzymes released during the infection process (Gheysen and Jones 2006; Wuyts et al., 2007). Trans cinnamic acid, the product of PAL are lignin precursors and co factors of IAA oxidase and hence play a significant role in resistance reaction (Mishra and Mohanty, 2007). Studies have shown that plants resistant to *M. graminicola*, as well as rice plants treated with elicitors, deposit and accumulate lignin in the endoderm of cells surrounding the nematode feeding site, affecting nematode nutrition (Ji et al., 2015; Galeng Lawilao et al., 2018; Zhan et al., 2018). This process explains not only the reduction in J2 penetration in treated plants, but also the delay in nematode development. Umamaheswari et al. (2005) discovered that the defence enzymes PAL were more active in plants that had been treated with *M. incognita* when compared to plants that had not been treated. Ashfaq et al. (2021) also demonstrated that PAL activity was higher in leaves of brown leaf spot resistant genotypes and subsequently reduced as the resistance level decreased.

The increase in protein concentration caused by nematode infestation could be owing to the creation of novel enzyme proteins in infected plants, or it could be due to nematode contributions. Bisen et al. (2015) also observed that higher amount of soluble protein in rice leaves resulted lower disease incidence of *D. oryzae.*

4. CONCLUSION

The present study provides compelling evidence that biochemical defense mechanisms play a crucial role in conferring resistance against *M. graminicola* and *D. oryzae* in *O. rufipogon* accessions. Resistant genotypes exhibited significantly higher activity of defense-related enzymes-phenylalanine ammonia lyase, tyrosine ammonia lyase and peroxidase—as well as elevated levels of total phenols, ortho-dihydroxy phenols and total soluble proteins in comparison to susceptible accessions under both single and combined pathogen inoculations. The biochemical response was more pronounced in resistant accessions under combined pathogen stress, although a general decrease in all biochemical parameters was observed compared to single pathogen infection, indicating a complex and possibly competitive interplay between defense pathways activated by nematodes and fungi. These findings underline the importance of biochemical markers as indicators of resistance and offer valuable insights for breeding programs aimed at developing rice cultivars with durable and broad-spectrum resistance. The wild rice species *O. rufipogon* with its robust biochemical defense mechanisms, represents a promising genetic resource for introgressions resistance traits into cultivated rice varieties.

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Authors' Contributions

Anupam Sekhon and Narpinderjeet Kaur Dhillon jointly planned and executed the research work. Anupam Sekhon carried out data collection and statistical analysis. Narpinderjeet Kaur Dhillon supervised the entire study and critically reviewed the manuscript. All authors contributed to manuscript writing and approved the final version.

Conflict of Interest

Authors do not have any conflict of interest to declare.

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Nematicidal Potential of *Brassica* Species against *Meloidogyne incognita*: An In vitro Study

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Abstract: The root-knot nematode (*Meloidogyne incognita*) poses a significant threat to crop productivity, necessitating eco-friendly alternatives to synthetic nematicides. This study evaluated the *in vitro* nematicidal efficacy of aqueous leaf extracts from selected canola (Gobhi Sarson Canola 7- GSC 7, Raya Ludhiana Canola-RLC 3) and non-canola (Punjab Brassica Raya- PBR 357, Taramira Ludhiana Composite 2 - TMLC 2) *Brassica* varieties. Extracts were tested at four concentrations for their ability to inhibit egg hatching and induce second-stage juvenile (J_2) mortality over a 10-day exposure period. All varieties exhibited concentration and time-dependent nematode suppression. Among canola types, RLC 3 showed stronger inhibitory effects than GSC 7, maintaining substantial activity even at lower concentrations. Non-canola varieties outperformed the canola varieties, with TMLC 2 consistently demonstrating the highest level of inhibition and juvenile mortality. Complete egg hatch inhibition was recorded with TMLC 2 at S/2 within 24 hours, while J_2 mortality reached 100% at full-strength (S). PBR 357 also exhibited strong nematicidal activity, although slightly lower than TMLC 2. These findings suggest that non-canola *Brassica* varieties-particularly TMLC 2 harbours potent bioactive compounds and represent promising candidates for the development of plant-based nematode management strategies aimed at sustainable crop protection.

Keywords: *Brassica* spp., Canola, Egg hatching, Juvenile (J_2) mortality, *Meloidogyne incognita,* Non-canola.

1. INTRODUCTION

Plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* spp.), represent a major constraint to global agricultural productivity, causing estimated annual losses of over $100 billion worldwide (Jones et al., 2013). *Meloidogyne incognita*, the most widespread and economically damaging species, is notorious for its wide host range and aggressive colonization of plant roots, leading to the formation of characteristic galls that disrupt water and nutrient uptake (Sun et al., 2024).Traditional nematode management relies heavily on chemical nematicides; however, increasing environmental and health concerns, along with stricter regulatory frameworks, have prompted the search for safer and sustainable alternatives (Abd-Elgawad MMM, 2024; Desaeger et al., 2020; Chitwood, 2002).

Botanical nematicides, derived from plant metabolites, have emerged as promising candidates due to their natural origin, biodegradability, and low toxicity to non-target organisms (Mwamula et al., 2022; Akhtar and Malik, 2000). Members of the Brassicaceae family have attracted significant interest, primarily due to their production of glucosinolates and their hydrolysis products such as isothiocyanates, which exhibit broad-spectrum bioactivity, including nematicidal, antifungal, and insecticidal properties (Sharma et al., 2024; Zasada and Ferris, 2004). When the plant tissues of Brassica are disrupted, certain enzymes *viz*., endogenous myrosinase convert glucosinolates into toxic compounds, which are capable of impairing normal metabolism and reproduction of nematode (Chhajed et al., 2020; Lazzeri et al., 2004). The nematicidal potential of certain *Brassica* species is well established, variations among cultivars and extraction methods may influence efficacy.

We evaluated the *in vitro* nematicidal activity of aqueous leaf extracts from selected canola (*Brassica napus*; RLC 3 and GSC 7) and non-canola (*Brassica rapa* and *Eruca sativa*; PBR 357 and TMLC 2) varieties against root knot nematode (*M. incognita*). The findings are expected to contribute to the development of botanical-based control measures that are environmentally benign and compatible with sustainable agriculture practices.

2. MATERIALS AND METHODS

The study was conducted in the Nematology Laboratory,

Department of Plant Pathology, Punjab Agricultural University, Ludhiana. Healthy leaves were collected from sixty-day-old plants of *Brassica* species, comprising two canola varieties (RLC 3 and GSC 7) and two non-canola varieties (PBR 357 and TMLC 2). The leaves were washed meticulously with sterile distilled water to clear off surface residues or microbes. Aqueous leaf extracts were prepared separately for each variety by grinding 10 grams of fresh leaf tissue in 20 mL of distilled water using a sterile pestle and mortar. The homogenized sample was passed through four layers of muslin cloth to eliminate coarse debris, followed by centrifugation at 4000 rpm for 5 minutes. The supernatant was then filtered using Whatman No. 1 filter paper to obtain a clear extract. These aqueous extracts were stored in sterilized, covered flasks under refrigeration at 4°C and were used as stock solutions (denoted as S) for in vitro nematode bioassays.

2.1. Evaluation of Egg Hatching Inhibition

For the evaluation of egg hatching inhibition, egg masses of *M. incognita* were extracted from pure culture raised on susceptible brinjal cultivar 'Punjab Sadabahar' maintained in Research Farm, Department of Plant Pathology, PAU, Ludhiana. Brinjal plants were gently removed from the soil, and the galled roots were cleaned using sterilized distilled water. Uniform sized egg masses were hand pinched using a pair of forceps. The stock solutions were used to prepare four concentrations: full strength (S), half (S/2), quarter (S/4), and one-eighth (S/8). Total five treatments were tested including four concentrations and one untreated control (distilled water). Each treatment was replicated three times. Ten mL of each concentration was added to the respective Petri plates (50 mm diameter), and four egg masses were transferred into each plate. Plates containing sterilized distilled water served as the control. The Petri plates were incubated in a BOD incubator at 25 ± 1°C, and observations on egg hatching were recorded under a binocular microscope at 24, 48, 96, 144, 192 and 240 hrs after treatment. Percent egg hatching was calculated using the formula: % egg hatch = (T - C)/C × 100, where T represents the total number of juveniles in treatment and C represented the total number of juveniles in control.

2.2. Evaluation of Juvenile Mortality Inhibition

The egg masses were surface sterilized in 0.5% sodium hypochlorite (NaOCl) solution for 2-3 minutes and rinsed thoroughly with sterile distilled water. The sterilized egg masses were incubated on double-layer tissue paper supported on wire mesh in a modified Baermann funnel at room temperature (25 ± 2 °C). Freshly hatched second-stage juveniles (J_2) were collected daily from the funnel outlet into beakers, concentrated by allowing them to settle, and used immediately for juvenile mortality inhibition assays. Only active and healthy juveniles were considered for experiments. For the J_2 mortality experiment, approximately 100 freshly hatched second-stage juveniles of *M. incognita* were added to 50 mm Petri dishes containing 10 mL of each treatment solution (S, S/2, S/4, S/8). Control plates contained only sterilized distilled water. Each treatment was replicated four times. The plates were incubated at 25 ± 1°C in a BOD incubator and mortality of juveniles was assessed at 24, 48, 96, 144, 192 and 240 hrs post-treatment using a binocular microscope. Juveniles were considered dead if they failed to respond to probing with a fine needle. Percent juvenile mortality was calculated using the formula: % mortality = (T - C)/C × 100, where T represented the number of dead juveniles in the treatment and C represented the number of dead juveniles in the control.

2.3. Statistical Analysis

The experiments were laid out in a completely randomized design (CRD), and data on percent egg hatching and juvenile mortality were subjected to analysis of variance (ANOVA). Treatment means were compared to determine statistical significance at the 5% level by R Software version 4.1.1.

3. RESULTS AND DISCUSSION

3.1. Nematicidal Activity of Extracts of *Brassica* spp. (canola and non-canola) Extracts against Egg Hatching of *M. incognita*

Aqueous extracts derived from different *Brassica* varieties significantly inhibited the hatching of *M. incognita* eggs and the egg hatch inhibition increased with both concentration of the extracts and duration. Egg hatch percentage decreased consistently with increasing extract concentration. Maximum inhibition (100%) was observed at full-strength (S) concentration across all varieties within 24 hours. The canola variety GSC 7 exhibited strong inhibition, with 97.42%, 89.89%, 86.45%, and 75.91% inhibition at S, S/2, S/4, and S/8 concentrations, respectively, on the second day. Although egg hatching gradually increased over time, higher concentrations continued to suppress it effectively even on later days. After 144 hours, inhibition at S/8 concentration was 54.60% for GSC 7 and 70.26% for RLC 3. Among the canola varieties, RLC 3 exhibited complete inhibition on the first day and maintained higher effectiveness than GSC 7 throughout the observation period. Overall, RLC 3 was found to be the most effective in reducing egg hatch of *M. incognita*, indicating its strong nematicidal potential. Egg hatch inhibition of *M. incognita* was significantly influenced by variety (A), extract concentration (B) and duration of exposure (C). The significant A × B interaction indicated that varietal differences depended on extract concentration (Table 1). Both varieties exhibited comparable inhibition at higher concentrations. The efficiency of of RLC 3 became more pronounced even at lower concentrations. The B × C interaction showed that inhibitory effects declined more rapidly over time at lower concentrations. These interactions demonstrate that the nematicidal efficacy of canola *Brassica* extracts is jointly regulated by variety, concentration and exposure duration.

Non-canola *Brassica* cultivars (PBR 357 and TMLC 2) also exhibited a concentration and time dependent inhibition of *M. incognita* egg hatching (Table 2). Egg hatch percentage decreased with increasing extract concentration and this egg hatch inhibition increased gradually over time. Among the two, TMLC 2 showed consistently higher inhibitory effects than PBR 357. At full-strength (S)

Table 1. Effect of canola varieties (GSC 7 and RLC 3) on egg hatch inhibition of *Meloidogyne incognita*

Duration (hrs)	**Concentration**							
	S		**S/2**		**S/4**		**S/8**	
	GSC 7	**RLC 3**	**GSC 7**	**RLC 3**	**GSC 7**	**RLC 3**	**GSC 7**	**RLC 3**
24	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0
48	97.42±1.208	100.00±0	89.89±0.430	95.05±0.431	86.45±2.444	92.69±1.041	75.91±4.441	88.17±1.273
96	85.59±1.612	92.61±1.003	76.22±1.524	88.29±0.569	68.11±1.441	87.57±0.662	58.56±2.688	77.84±1.732
144	83.07±1.208	86.72±0.669	74.60±1.334	80.73±1.145	66.72±0.438	77.08±0.715	54.60±3.258	72.26±0.894
CD (P=0.05)								
A	1.038							
B	1.468							
C	2.075							
A×B	1.797							
B×C	2.542							

A = Variety (GSC 7 and RLC 3); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

Table 2. Effect of non-canola varieties PBR 357 and TMLC 2 on egg hatch inhibition of *Meloidogyne incognita*

Duration (hrs)	**Concentration**							
	S		**S/2**		**S/4**		**S/8**	
	PBR 357	**TMLC 2**	**PBR 357**	**TMLC 2**	**PBR 357**	**TMLC 2**	**PBR 357**	**TMLC 2**
24	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0	100.00±0
48	100.00±0	100.00±0	92.04±0.93	98.49±0.64	82.80±2.01	97.20±0.26	72.04±2.01	81.08±2.89
96	90.45±2.27	99.09±0.63	88.83±1.01	95.86±0.61	75.86±2.17	94.59±0.57	48.11±2.25	70.09±5.36
144	87.45±2.05	93.70±2.07	81.61±0.93	89.34±1.23	73.28±2.08	83.50±2.85	40.88±4.22	65.26±4.27
CD (P=0.05)								
A	0.092							
B	0.130							
A×B	0.184							
C	1.59							
A×C	0.225							
B×C	0.319							

A = Variety (PBR 357 and TMLC 2); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

concentration, both varieties recorded nearly complete inhibition (76%-100%) within the first 48 hours. Egg hatch inhibition in PBR 357 at S and S/2 concentrations remained statistically similar at both 24 and 48 hours. At the lowest concentration (S/8), TMLC 2 achieved 42.57% inhibition, while PBR 357 showed 33.11%, indicating a relatively lower efficacy. Interestingly, TMLC 2 exhibited complete (100%) inhibition at the S/2 concentration after 24 hours, which decreased to 66.76% by 240 hours, indicating an approximate 33% decline over time. Overall, TMLC 2 demonstrated greater efficacy than PBR 357 in reducing *M. incognita* egg hatching, particularly at higher concentrations and shorter exposure periods. Egg hatch inhibition of *M. incognita* by non-canola *Brassica* varieties was significantly affected by variety (A), extract concentration (B), and duration of exposure (C) (Table 2). The significant A × B interaction indicated that varietal differences were concentration dependent with both varieties showing comparable inhibition at higher concentrations but TMLC 2 maintained greater inhibition at lower concentrations. The A × C interaction showed that TMLC 2 retained inhibitory activity for a longer duration than PBR 357. The B × C interaction further demonstrated that egg hatch inhibition declined more rapidly over time at lower concentrations whereas higher concentrations sustained inhibitory effects for longer durations.

Among all the *Brassica* varieties tested, the non-canola cultivars demonstrated superior efficacy in inhibiting egg hatching of *M. incognita* compared to the canola varieties. Notably, the TMLC 2 extract emerged as the most potent, consistently exhibiting the highest level of egg hatch inhibition across all concentrations and time intervals, outperforming not only its non-canola counterpart PBR 357 but also all tested canola varieties. The present study clearly demonstrated that aqueous extracts of both canola and non-canola *Brassica* cultivars possess strong nematicidal activity against the egg hatching of *M. incognita*. Across all tested varieties, egg hatch inhibition increased consistently with extract concentration and exposure duration, with near-complete or complete inhibition observed at higher concentrations during early exposure periods. This uniform response across *Brassica* types indicates that egg hatching represents a highly sensitive developmental stage of *M. incognita* and can be effectively targeted using *Brassica*-derived bioactive compounds such as glucosinolates and their enzymatic hydrolysis products, particularly isothiocyanates which are released upon disruption of *Brassica* tissues. This enhanced activity can be attributed to their higher glucosinolate content and hydrolysis products such as isothiocyanates, which interfere with nematode development and physiology (Eugui et al., 2022). In contrast, canola varieties typically possess reduced glucosinolate levels due to selective breeding for improved palatability and oil quality, which likely accounts for their comparatively lower efficacy. Kumar et al (2019) demonstrated that dilution of botanical extracts led to reduced inhibition of *M. incognita* egg hatching. Their study, which included treatments with neem, cabbage, and cauliflower leaf extracts, highlighted that the most potent inhibition occurred at the highest extract concentration, particularly during early exposure, aligning with the trends recorded in the present work. These compounds are known to interfere with nematode embryonic development by penetrating the egg shell and disrupting essential metabolic processes. Recent studies have consistently reported that *Brassica*-based materials exert pronounced toxic effects on root-knot nematode eggs, supporting the rapid and substantial egg hatch suppression recorded in the present study (Dutta et al., 2019; Bui et al., 2021).

The clear concentration-dependent decline in egg hatching observed for both canola and non-canola cultivars aligns well with previous in vitro studies using botanical extracts. Higher extract concentrations likely release greater quantities of active metabolites, resulting in enhanced toxicity and prolonged inhibition of egg development. Similar dose-dependent suppression of *Meloidogyne* egg hatching has been reported by Das et al. (2021), who emphasized that reduced efficacy at lower concentrations is often associated with insufficient levels of nematicidal compounds to fully arrest embryogenesis. A gradual decline in inhibitory activity over time, particularly at diluted concentrations, was evident across both *Brassica* groups. This time-dependent reduction in efficacy is consistent with the transient nature of *Brassica*-derived volatiles. Isothiocyanates and related compounds are known to degrade or volatilize over extended exposure periods, especially in aqueous systems, leading to partial recovery of egg hatching. Such patterns have been well documented in recent studies, which reported strong initial nematicidal activity followed by reduced persistence over time (Bui et al., 2021; Petrikovszki et al., 2023).

Distinct varietal differences were evident within both canola and non-canola groups. Among canola cultivars, RLC 3 consistently exhibited higher egg hatch inhibition than GSC 7, while among non-canola cultivars, TMLC 2 outperformed PBR 357 across most concentrations and exposure periods. These differences are likely linked to variation in glucosinolate content and composition among *Brassica* genotypes. Previous studies have highlighted that plant genotype is a critical determinant of nematicidal efficacy, with certain *Brassica* cultivars maintaining higher activity even at lower concentrations or longer exposure durations (Dutta et al., 2019; Mwamula et al., 2022). Notably, the non-canola Brassica cultivars demonstrated overall superior efficacy compared to canola varieties, with TMLC 2 emerging as the most potent among all tested genotypes. This observation suggests that non-canola *Brassica* types may possess more favorable biochemical profiles for nematode suppression. Similar findings have been reported in earlier investigations, where non-canola *Brassica* species exhibited stronger biofumigation potential due to higher or more diverse glucosinolate concentrations (Dutta et al., 2019; Mwamula et al., 2022).

The significant interactions observed among variety, extract concentration, and exposure duration further indicate that nematicidal efficacy is governed by the combined influence of biochemical composition, dose, and persistence of active compounds. Higher concentrations sustained inhibitory effects for longer durations, while varietal differences became more pronounced under sub-optimal concentrations and prolonged exposure. Overall, the integrated results strongly support the potential of *Brassica* extracts, particularly from highly effective non-canola cultivars such as TMLC 2 and canola cultivar RLC 3, as promising botanical agents for suppressing *M. incognita* egg hatching under in vitro conditions.

3.2. Nematicidal Activity of Extract of *Brassica* spp. (canola and non-canola) on Second Stage Juveniles (J_2) of *M. incognita*

This experiment evaluated the effect of aqueous leaf extracts from various *Brassica* varieties-canola (GSC 7 and RLC 3) and non-canola (PBR 357 and TMLC 2)-on the mortality of second-stage juveniles (J_2) of *M. incognita* under *in vitro* conditions. All evaluated extracts caused a significant rise in juvenile mortality, which varied according to concentration and exposure duration. Among the canola varieties, RLC 3 was found to be the most effective, causing up to 98% mortality at full-strength (S) concentration after 10 days of exposure. At the lowest concentration (S/8), juvenile mortality was 58% in RLC 3 and 38.4% in GSC 7. Mortality at S/2 concentration increased from 45.2% to nearly 90% in GSC 7 and from 60 to over 90% in RLC 3 over the 10-day period. Similarly, at S/4 concentration, maximum mortality reached 77.2% for RLC 3 and 66% for GSC 7. A significant positive correlation was observed between concentration, exposure duration, and juvenile mortality. A significant A × B interaction indicated that varietal differences in juvenile mortality were concentration dependent. While both varieties caused comparatively high mortality at higher concentrations, the superiority of RLC 3 over GSC 7 became more pronounced even at lower concentrations particularly S/4 and S/8. The A × B × C interaction further demonstrated that these varietal differences across concentrations were influenced by exposure duration with RLC 3 maintaining higher mortality at lower concentrations over longer exposure periods. In contrast, the B × C interaction was non-significant, indicating that the relative effect of concentration on juvenile mortality remained consistent across exposure durations (Table 3).

In the non-canola group, both varieties were effective in reducing J_2 numbers with TMLC 2 showing stronger nematicidal activity. Mortality of second-stage juveniles (J2) of *M. incognita* was significantly affected by variety (A), extract concentration (B), and duration of exposure (C), with clear interaction effects. Across all concentrations and observation periods, TMLC 2 consistently induced higher juvenile mortality than PBR 357. At full-strength concentration (S), mortality in PBR 357 increased from 82.40% at 24 h to 97.20% at 240 h, while TMLC 2 increased from 84.00% to complete mortality (100%) over the same period. At S/2 concentration, mortality ranged from 50.40% to 89.60% in PBR 357 and from 74.40% to 98.80% in TMLC 2 confirming the superior efficacy of TMLC 2. At S/4 concentration, mortality in PBR 357 increased from 17.20% at 24 h to 64.40% at 240 h, whereas TMLC 2 showed higher values ranging from 42.80% to 86.40%. Similarly, at the lowest concentration (S/8), mortality increased from 8.00% to 59.20% in PBR 357 and from 18.40% to 68.80% in TMLC 2 over the exposure period. The significant A × B interaction indicated that varietal differences were concentration dependent, with differences between TMLC 2 and PBR 357 becoming more pronounced at lower concentrations (Table 4). The A × C interaction further showed that TMLC 2 maintained higher juvenile mortality over longer exposure periods compared to PBR 357. The B × C interaction demonstrated that mortality increased more rapidly at higher concentrations, while lower concentrations required longer exposure to achieve comparable effects. The significant A × B × C interaction confirmed that varietal differences in juvenile mortality across concentrations were further modulated by exposure duration.

From all the four varieties tested for mortality against J_2 of *M. incognita*, TMLC 2 was the most effective treatment across all concentrations and time points. The present study demonstrated that aqueous extracts from various *Brassica* species significantly increased the mortality of second-stage juveniles (J_2) of *M. incognita*, with effectiveness strongly influenced by both extract concentration and duration of exposure. All tested varieties exhibited nematicidal activity, confirming the presence of bioactive compounds capable of disrupting nematode viability. A clear dose and time dependent trend was observed, wherein juvenile mortality increased progressively with higher concentrations and prolonged exposure. Aqueous leaf extracts of both canola and non-canola *Brassica* varieties caused significant mortality of second-stage juveniles (J2) of *M. incognita* under in vitro conditions. Mortality increased consistently with extract concentration and duration of exposure. This clear dose- and time-dependent response indicates that *Brassica*-derived metabolites exert direct toxic effects on the infective juvenile stage of the nematode. High juvenile mortality observed at full-strength and half-strength concentrations suggests rapid penetration of toxic compounds through the nematode cuticle resulting in disruption of neuromuscular coordination and essential metabolic functions. Plant-derived sulfur-containing compounds, including glucosinolate degradation products, have been reported to induce paralysis and mortality in plant-parasitic nematodes by interfering with respiration and nervous system activity (Ntalli et al., 2017). Such mechanisms explain the rapid and high mortality recorded at higher concentrations in the present study. At lower concentrations, juvenile mortality increased gradually with prolonged exposure, indicating cumulative toxic effects rather than immediate lethality. Similar delayed but irreversible effects of botanical nematicides on *Meloidogyne* juveniles have been reported earlier where extended exposure compensated for reduced dosage (Oka, 2020). This supports the observed requirement for longer exposure periods to achieve substantial mortality at diluted extract concentrations. Marked varietal differences were evident among the tested *Brassica* cultivars. The consistently higher mortality induced by RLC 3 among canola varieties and by TMLC 2 among non-canola varieties suggests genotype-specific variation in the quantity and composition of nematicidal metabolites. Previous studies have demonstrated that *Brassica* genotypes differ significantly in their toxicity toward *Meloidogyne* juveniles due to variation in bioactive compound profiles (López-González et al., 2019). The superior and sustained efficacy of TMLC 2 across concentrations and time intervals highlights its strong nematicidal potential. Overall, the present findings confirm that aqueous *Brassica* extracts possess strong direct toxicity against *M. incognita* juveniles and that nematicidal efficacy

Table 3. Effect of canola varieties (GSC 7 and RLC 3) on mortality of second stage juveniles (J2) of *Meloidogyne incognita*

Duration (hrs)	**Concentration**							
	S		**S/2**		**S/4**		**S/8**	
	GSC 7	**RLC 3**	**GSC 7**	**RLC 3**	**GSC 7**	**RLC 3**	**GSC 7**	**RLC 3**
24	60.00±2.60	72.80±3.72	45.20±1.62	60.00±1.87	30.80±1.35	28.00±2.75	5.20±1.20	11.20±0.80
48	74.80±2.57	80.80±2.87	58.80±2.87	68.00±3.40	36.40±0.98	35.60±3.70	12.00±1.67	18.40±0.98
96	81.60±2.35	86.80±2.80	66.00±2.75	73.20±3.13	42.40±3.86	44.40±2.85	14.80±3.34	32.80±1.35
144	86.80±2.87	89.20±2.04	77.20±3.26	80.00±2.89	49.20±3.26	57.20±2.33	24.40±2.04	41.60±0.98
CD (P=0.05)								
A				0.103				
B				0.146				
C				0.207				
A×B				0.179				
B×C				NS				
A×B×C				0.358				

A = Variety (GSC 7 and RLC 3); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

Table 4. Effect of non-canola varieties (PBR 357 and TMLC 2) on mortality of second stage juveniles of *Meloiodogyne incognita*

Duration (hrs)	Concentration							
	S		S/2		S/4		S/8	
	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2	PBR 357	TMLC 2
24	82.40±1.47	84.00±1.09	50.40±3.70	74.40±1.93	17.20±2.33	42.80±2.32	8.00 ±1.26	18.40±1.60
48	84.40±2.40	88.80±1.20	56.00±4.33	82.80±1.20	24.00±1.89	51.60±3.81	16.40±1.32	23.60±2.40
96	88.40±2.48	94.40±1.16	65.20±2.24	86.40±1.16	33.20±2.05	58.80±3.61	28.40±2.27	29.60±1.93
144	94.00±2.19	98.40±1.16	71.60±4.11	91.20±1.20	44.80±2.57	68.40±2.40	37.20±2.05	39.20±1.62
CD (p=0.05)								
A				0.092				
B				0.130				
C				0.159				
A × B				0.183				
A × C				0.225				
B × C				0.318				
A × B × C				0.449				

A = Variety (PBR 357 and TMLC 2); B = Concentration (S, S/2, S/4 and S/8); C = Duration (hours); A × B represents the interaction between variety and concentration; B × C represents the interaction between concentration and duration; CD = Critical Difference at P = 0.05. Values are mean ± SE

is governed by cultivar, extract concentration, and exposure duration. These results support the potential use of highly effective *Brassica* cultivars as botanical sources for nematode management under in vitro conditions. These findings are also consistent with those reported by Feyisa et al. (2016), who evaluated botanical extracts including rapeseed (*Brassica napus*), *Lantana camara*, *Tagetes erecta*, and *Azadirachta indica* and reported maximum mortality (100%) in neem extract at the highest concentration after three days highlighting a similar pattern of increasing efficacy with higher dosage and longer exposure periods.

Overall, the data support the conclusion that *Brassica* extracts-particularly those from non-canola varieties like TMLC 2-hold considerable promise as bio-based alternatives to chemical nematicides. Their efficacy at higher concentrations and over extended periods suggests their potential for incorporation into sustainable nematode management strategies.

4. CONCLUSION

The present study clearly established the nematicidal potential of aqueous leaf extracts from various *Brassica* species against *M. incognita* under *in vitro* conditions. All tested extracts significantly inhibited egg hatching and increased juvenile mortality in a dose and time-dependent. Among the canola varieties, Raya Ludhiana Canola (RLC 3) was the most effective and Taramira Ludhiana composite 2 (TMLC 2) from the non-canola group consistently outperformed all other varieties to achieve complete juvenile mortality and maximum egg hatch suppression at higher concentrations and longer exposure durations. The superior efficacy of non-canola varieties can be attributed to their higher glucosinolates content and associated bioactive compounds. These findings confirm the potential of *Brassica* species, particularly TMLC 2, as effective botanical agents for the biological control of root-knot nematodes. This eco-friendly approach holds significant promise as a viable alternative to synthetic nematicides, contributing to sustainable agriculture and improved soil health.

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Conflict of Interest

The authors declare they have no conflict of interests.

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

The authors did not use generative AI or AI-assisted technologies in the writing of this manuscript.

Credit Authorship Contribution Statement

Harkanwal Pal Singh and Narpinderjeet Kaur Dhillon jointly planned and executed the research work. Harkanwal

Pal Singh and Anupam Sekhon carried out data collection for juvenile mortality, egg hatch inhibition and statistical analysis. Narpinderjeet Kaur Dhillon and Sukhjeet Kaur supervised the entire study and critically reviewed the manuscript. All authors contributed to manuscript writing and approved the final version.

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Amelioration of Salt Stress in Maize (*Zea mays* L.) by Seed Treatments with Glutathione

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Abstract: Salinity stress is one of the abiotic stress factors which reduces the crop production, and threat to global world food security. There is need to reduce the deleterious effects of salts in soil or irrigation water to minimize yield losses. The present study aims to evaluate the role of glutathione in alleviating the negative effects of salt stress in maize seedlings under controlled laboratory conditions. The study was conducted in complete randomized block design in quadruplicates. One hundred surface sterilized seeds of maize (*Zea mays* L.) cv J 1007 were treated with water (hydration) and solutions of glutathione (GSH) 100 and 500 ppm for 12 hours followed by surface drying. The treated seeds were subjected to salt stress levels of 0, 50, 75 and 100 mM NaCl. Ten days old seedlings were assessed for percent germination, seedling vigour indices and total soluble sugars and proteins. The results showed that both hydration and glutathione treatments significantly enhanced percent germination, length of lamina and sheath, vigour index I and II, total soluble sugars and total soluble proteins content than control. The glutathione 500 ppm is found to be effective as seed treatment in ameliorating deleterious effects of salt stress in maize than hydration and GSH 100 ppm.

Keywords: Glutathione, Maize, *Zea mays* L., Salt stress, Seed treatment.

1. INTRODUCTION

Maize (*Zea mays* L.) is a widely domesticated cereal crop which originated in America. It belongs to the family Poaceae. Currently, around 1148 Mt of maize is produced by around 170 countries from an estimate area of 185 Mha with average productivity is around 5.6 t/ha (Bamboriya et al., 2020). India ranks 4th in the area and 7th in the production among the maize growing countries. In India, maize is currently being cultivated on area of 9.6 Mha with 28.26 Mt production (Bamboriya et al., 2020). Among cultivated forage crops, maize is most suitable crop for fodder as well as silage because of its high yielding ability and excellent nutritional profile. Maize has a high TDN (total digestible nutrients) of around 85-90% and also contains comparatively higher amount of energy amongst all other cereal grains (Kaul et al., 2019). Maize is categorized as glycophytes, and it has potential to survive under low saline environments (≤ 2 DSm^{-1})(Himabindu et al., 2016). The early vegetative growth stage of maize is more sensitive to salt stress than reproductive stage.

Salt stress is one of the major abiotic stresses, which limits plant growth and yield especially in arid and semi-arid areas of the world (Hussain et al., 2019; Polash et al., 2019). Land clearing, unsustainable irrigation practices, poor drainage, intensive use of fertilizers and increasing pressures for bringing marginal lands contributed for soil salinity, and affected plant growth and production. Around 1125 million ha of land is under the effect of salt stress, of which approximately 76 million ha is affected by human induced sodification and salinization (Hossain, 2019). Excess salts affect the metabolism of soil flora and fauna, ultimately leading to the destruction of all soil life, converting productive and fertile lands into barren deserts.

In Punjab, the salt affected areas and water logged soils mainly lies in Bathinda, Muktsar, Ferozepur and Mansa districts. Salt affected soils lie along or across Bikaner canal, Bathinda and Kotla branches of Bhakra canal and Sirhind canal. Soil salinity of these regions may be attributed to the seepage from Rajasthan feeder canals and also appears to be the cause of waterlogging (Singh, 2013). This is affecting cropping patterns, crop productivity and soil fertility in different parts of Punjab.

The damaging effects of salt stress depend on various factors such as plant species and varieties, salt concentration in soil, stress type, environmental conditions and plant growth stages (Shahverdi et al., 2018). Salinity alters the plants apostrophe morphological and anatomical characteristics, physiological processes and plant metabolism which ultimately leads to loss in agricultural production and causes disturbances in plant metabolism, impairs cell membrane integrity, interrupts enzyme activities and induces sodium ions toxicity (Banerjee et al., 2019; Singh et al., 2020). Salinity stress causes ionic and osmotic imbalance in soil as well as disturbs plant cellular osmotic potential and K^+: Na^+ ratios that results in increased oxidative damages and hampered plant growth (dos Santos et al., 2022; Choudhary et al., 2023; He et al., 2023). Numerous studies demonstrated the negative effects of salt stress in terms of change in seedling vigour attributes, concentration of cellular metabolites and photosynthetic pigments, enzymatic and non-enzymatic antioxidants and yield attributes in various cereal crops (Singh et al., 2025a; Singh et al., 2025b; Chhatwal et al., 2025). Osmotic adjustment is an one of the important aspect to impart tolerance against abiotic stresses in plants. The change in the cellular content of osmolytes especially soluble sugars plays an important role in the adjustment of osmotic pressure under unfavorable conditions, and preventing the cells from dehydration and maintains the cellular integrity (Fu et al., 2017; Bhagat et al., 2025).

Glutathione (GSH) is the one of the most abundant and widely distributed water soluble non–protein thiol compound in cellular compartments of plants. Exogenously applied GSH under salt stressed conditions regulates both enzymatic and non-enzymatic antioxidants as well as osmoprotectants (Thind and Goyal, 2012). As non-enzymatic antioxidants, detoxify reactive oxygen species and attenuates the negative effects of oxidative stress in plants. The potential of glutathione in preventing lipid peroxidation and protecting the plasma membrane and other bio membranes has been also reported. Diverse roles of GSH in different metabolic activities showed that its metabolism has great impact in imparting plant tolerance against abiotic stresses through diverse ways (Ramzan et al., 2023). Numerous studies reported the exogenous application of glutathione through foliar or seed priming to enhance plant tolerance against aging and different abiotic stresses such as salt, heat and cold stress (Parcha and Gupta, 2017; Gaba et al., 2018; Chhatwal et al., 2025, Saeed et al., 2023 & 2025). Glutathione application as foliar spray @ 0, 0.4 and 0.8 mM in chilli pepper enhanced osmoprotectants, ascorbate, glutathione, capsaicin, and phenolic contents, as well as WUE under salt stress conditions (Al-Elwany et al., 2020). Thus the present investigation was planned to assess the effect of glutathione on seed vigour parameters of maize seedlings grown in salt stressenvironment under controlled laboratory conditions.

2. MATERIALS AND METHODS

2.1. Location and Experimental Set Up

The present study was conducted in Department of Botany, Punjab Agricultural University, Ludhiana. The seeds of maize (*Zea mays* L.) cv J 1007 were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The study was designed to investigate the role of glutathione (100 and 500 ppm) on germination percentage, seedling growth and seedling vigour, total soluble sugars and total soluble proteins in ten days old maize seedlings, subjected to salt stress under controlled laboratory conditions. The experiment was conducted in completely randomized block design and replicated four times.

Seeds were treated with 0.1 % mercuric chloride ($HgCl_2$) for surface sterilization followed by thorough washings with autoclaved water three-four times. For glutathione treatments, seeds were soaked in freshly prepared solutions of 100 and 500 ppm glutathione for 12 hours. Salinity treatments were induced by soaking the germination paper in the NaCl solution of desired salinity levels (0 (control), 25, 50, 75 and 100 mM NaCl). The untreated and treated seeds were kept on germination paper and allowed to germinate in an incubator, maintained at 25°C and 60±5 % relative humidity. Ten days old uniform seedlings were used to record following parameters:

2.2. Germination Percentage

Twenty seeds were placed in each petri plate for each treatment and replicated thrice. Seeds were considered germinated when the emergent root reached 2 mm in length. Final count of normal seedlings was recorded after 10 days of setting for germination.

2.3. Lamina and Sheath Length

The length of expanded part of the leaf i.e. lamina and sheath covering the shoot was noted and was expressed in millimeters. The mean of lamina and sheath length of ten seedlings was considered as one replication.

2.4. Vigour Index (VI)

The seedling vigour index was recorded (Abdul-Baki and Anderson, 1973)

Vigour Index I = Germination (%) x Seedling length (cm)

Vigour Index II = Germination (%) x Seedling dry weight (g)

The root and shoot length of ten representative seedlings was measured. The shoot length was recorded from the collar region to the point of attachment of cotyledons. Seedling length was recorded as sum of shoot length and root length. For seedling dry weight, the seedlings were oven dried at 70°C until the constant weight was obtained. The weight of same seedlings was recorded. The mean of seedling length and seedling dry weight of ten seedlings was considered as one replication.

2.5. Speed of Germination (Maguire, 1962)

Speed of germination was recorded by placing 20 seeds on top of paper in each petri plates. Data on the emergence of seedlings was recorded daily basis till the 100 percent germination or the final count *i.e.* 10th day.

Speed of germination = ∑ (n1/d1 + n2-n1/d2 +……nn-nn-1/dn) = Σ n / d

Where n = number of seeds germinated, d = number of days taken for germination

2.6. Total Soluble Sugars

0.1 g dry seedlings were homogenized in 80% ethanol followed by centrifugation at 5000 rpm for 15 minutes. The residue was re-extracted using ethanol and the supernatants were pooled and final volume was made 10 ml. The supernatant was used for estimation of total soluble sugars content using the method of Dubois et al. (1956). The standard curve of glucose (10-100 µg/ml) was prepared and used to estimate total soluble sugars content and recorded as mg/g dry weight.

2.7. Total Soluble Proteins

0.1 g fresh seedlings were macerated in 5 ml of 0.1 N NaOH followed by centrifugation at 5000 rpm. The extraction procedure was repeated twice and total volume was made 10 ml. 1 ml of protein extract and 1 ml of 15% trichloroacetic acid (TCA) was mixed and kept at 4°C for 24 hours. The same mixture was later centrifuged for 20 minutes at 5000 rpm. The precipitate so obtained were dissolved in 0.1 N NaOH and used for the estimation of total soluble proteins following the procedure of Lowry et al. (1951). The protein content was recorded as mg/g fresh weight.

2.8. Statistical Analysis

The Analysis of Variance (ANOVA) was done to find significant differences among treatments at 5% level of significance using SAS computer package.

3. RESULTS AND DISCUSSION

3.1. Percent Germination

Seed percent germination was significantly reduced by salt stress in untreated seeds and ranged from 95.00% (0 mM NaCl) to 76.67% (100 mM NaCl) (Table 1). The percent germination decreased rapidly as salt concentration reached 100 mM. GSH 100 and 500 ppm significantly improved the percentage germination of maize seeds. At 75 mM salt stress, it increased the percentage of germination from 88.33 to 93.67% (100 ppm GSH) and 94.33% (500 ppm GSH). GSH 500 ppm also showed significant increment in germination percentage from 76.67 to 83.33% at 100 mM salt concentration. Singh et al. (2025a) evaluated the percent germination of 78 *Berbet* introgression lines of wheat under salt stress and reported mean 31.60% reduction in percent germination under salt stress as compared to control. Pei et al. (2019) observed that GSH treatment significantly increased germination percentage of maize seeds under drought, salt and chilling stress.

3.2. Lamina and Sheath length

The decrease in lamina and sheath length was observed with increase in salt stress levels. This reduction was reversed when seeds were treated with water and GSH. The lamina and sheath length ranged from 1.2-3.1cm and 5.6-9.0 cm respectively in seedlings developed from untreated seeds under control and salt stress conditions. At 50 mM salt stress, sheath length ranged from 8.32 cm to 11.15 cm. GSH @ 500 ppm seed treatment showed significant improvement in sheath length in the seedling as an increment from 5.60 cm (control) to 9.27 cm at 100 mM NaCl stress. Sheath length is also an important parameter in indication of salt stress. The gradual reduction in sheath length was reported in oat seedlings subjected to salt stress (Kaur and Gupta, 2020). Decreased lamina and sheath length with increase in salt stress is attributed to lesser supply of metabolites to young parts of plant.

3.3. Seed vigour index I and II

Water and glutathione seed treatments improved the seedling vigour index I and II in maize at different salinity levels (Table 1). At 75 mM salt stress level, seedling vigour index I ranged from 1367.34-2678.51. The highest seedling vigour index I was observed with seed treatment GSH 500 ppm at 75 mM salt stress. Similarly, seedling vigour index II of untreated seeds ranged from 2.28-4.07 under control and salt stress conditions. At 100 mM NaCl, seedling vigour index II varied from 2.28-3.23. GSH 500 ppm increased the seedling vigour index II from 2.86 to 4.52 at 75 mM salt stress. Seed vigour is a physiological property of the seed which governs its capability as how fast it can produce a seedling in soil and to what extent the seed can tolerate different stress conditions. Seed vigour is directly related to the initial growth of the crop and affects the competitive ability of a plant against weeds (Dias et al., 2011). Increasing salinity levels decreased the seed vigour index in different genotypes of oats and wheat (Kaur and Gupta, 2020, Singh et al., 2025). Seed priming however increased seed vigour in sorghum (Komalasari and Arief, 2020).

3.4. Speed of Germination

Reduction in speed of germination was reported with the increasing salt stress levels (4.75-2.51 number of seeds/day) but it gradually increased with the application of treatments

Table 1. Influence of glutathione seed treatments on seed vigour parameters in maize cv J 1007 seedlings under salt stress conditions

Salt stress levels Treatment	0 mM NaCl (No stress)	50 mM NaCl	75 mM NaCl	100 mM NaCl
Germination percentage				
Control	95.00	93.33	88.33	76.67
Hydration	96.67	93.33	91.33	80.00
GSH 100 ppm	96.67	94.67	93.67	81.67
GSH 500 ppm	98.33	95.33	94.33	83.33
CD (p-0.05)	3.13	8.89	6.28	7.84
Lamina length (cm)				
Control	3.1	2.6	1.9	1.2
Hydration	4.2	3.8	10.3	6.3
GSH 100 ppm	8.6	8.2	5.9	3.0
GSH 500 ppm	12.8	10.9	12.7	7.4
CD (p-0.05)	0.46	0.42	0.55	0.54
Sheath length (cm)				
Control	9.0	8.32	7.31	5.60
Hydration	9.3	8.55	8.83	6.44
GSH 100 ppm	10.97	10.40	10.48	8.32
GSH 500 ppm	11.55	11.15	11.89	9.27
CD (p-0.05)	0.25	0.51	0.86	0.87
Vigour index				
Control	1888.61	1638.75	1367.34	972.94
Hydration	1989.47	1836.87	2009.86	1557.05
GSH 100 ppm	2389.68	2402.25	2349.09	2079.37
GSH 500 ppm	2731.60	2647.04	2678.51	2256.67
CD (p-0.05)	216.11	270.21	157.53	209.30
Vigour index II				
Control	4.07	3.61	2.86	2.28
Hydration	4.20	3.86	3.71	3.49
GSH 100 ppm	4.90	4.59	4.12	3.74
GSH 500 ppm	5.34	4.91	4.52	3.93
CD (p-0.05)	0.43	0.74	0.52	0.57
Speed of Germination (Number of seeds germinated/day)				
Control	4.75	4.66	3.47	2.51
Hydration	4.81	4.80	3.67	2.79
GSH 100 ppm	5.52	5.32	4.18	3.82
GSH 500 ppm	5.97	5.89	5.02	4.72
CD (p-0.05)	0.21	0.34	0.24	0.52

Table 2. Influence of seed treatments on total soluble sugars and total soluble proteins content in maize (*Zea mays* L.) cv J1007 seedlings under salt stress conditions

Salt stress level Treatment	0 mM NaCl (No stress)	50 mM NaCl	75 mM NaCl	100 mM NaCl
Total soluble sugars (mg/g DW)				
Control	30.32	44.28	51.81	60.16
Hydration	35.11	49.01	62.87	73.42
GSH 100 ppm	66.89	86.23	106.09	114.21
GSH 500 ppm	84.78	114.81	123.09	129.89
CD (p-0.05)	1.03	1.73	1.14	2.46
Total soluble proteins (mg/g FW)				
Control	16.45	19.71	25.57	30.25
Hydration	22.56	28.85	34.44	65.16
GSH 100 ppm	33.54	40.93	68.71	90.32
GSH 500 ppm	45.23	66.43	94.55	98.90
CD (p-0.05)	1.87	2.12	3.69	3.29

with GSH. Hydration seed treatment depicted a non-significant increase in speed of germination. The significant increase in speed of germination was observed with GSH 500 ppm seed treatment where it enhanced from 3.47 to 5.02 and 2.51 to 4.72 at 75 and 100 mM NaCl stress respectively. The significant reduction in germination velocity was found in dill, fenugreek, savory and dragonhead plants with increasing salt concentrations (Saberali and Moradi, 2019).

3.6. Total Soluble Sugars

Total soluble sugars content in seedlings developed from untreated seeds ranged from 30.32-60.16 mg/g DW under control and salt stress conditions. At 75 mM salt stress, hydration seed treatment increased the sugar content from 51.81 to 62.87 mg/g DW. The highest increase in sugar content was with GSH 500 ppm seed treatment which increased from 51.81 to 123.09 mg/g DW at 75 mM salt stress. Enhancement of total soluble sugars depicts their role in imparting tolerance by maintaining water potential under heat and salt stress conditions in wheat and Barley (Singh et al., 2025a; Singh et al., 2025b). The role of sugars to impart tolerance by foliar application of glutathione in salt stressed chickpea plants was reported by Sadak et al.(2017). The increased leaf soluble sugars under the impact of salinity, signified that maize employed soluble sugars for osmoregulation under salinity stress (El-Katony et al., 2019; Yu et al., 2025). Foliar application of glutathione helps to reduce the negative effect of salinity in sour passion fruit. The application of glutathione is reported to increase the total soluble sugars (de Souza et al., 2025) whichcontribute to the stress condition signaling process (Saddhe et al., 2021).

3.7. Total Soluble Proteins

Hydration and GSH 100 and 500 ppm seed treatment improved total soluble proteins content significantly under control (0 mM NaCl) and salt stress conditions. GSH 500 ppm increased the protein content from 25.57 (untreated seeds) to 94.55 mg/g FW at 75 mM NaCl (Table 2). Similar findings were reported by Jain and Vaishnav (2019) and Perveen and Nazir (2018) where the total protein content of the maize leaf segments was found to gradually increase with the application of NaCl. Ahmed et al. (2025) demonstrated that GSH increased the expression of stress responsive proteins and helped the plant to adapt to adverse conditions.

4. CONCLUSION

Salt stressed and water logged soils affect the plant establishment and growth by affecting various physiological processes. The damaging effects of salt stress can be mitigated by seed treatments with antioxidants like glutathione which improves the seed germination and vigour parameters under salt stress conditions.

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Authors' Contributions

Himani Chhatwal collected the data related to research work, performed data analysis and interpretation and prepared rough draft of manuscript; Namrata Gupta outlined and supervised the research work, interpreted the data and edited the manuscript; P. Goyal supervised the research work, assisted in interpretation of data and manuscript editing: Meenakshi Goyal helped in biochemical analysis and manuscript editing; Gurjit Kaur Gill performed conceptualized and provided the maize germplasm; Nitika Garg analyzed the data statistically and helped in manuscript preparation.

Conflict of Interest

The authors declare that they have no conflicts of interest of reported research work in this manuscript.

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Fluopyram Induced Oxidative Stress, Biochemical and Histopathological Changes in Brain and Blood of Albino Rats

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***Abstract*:** Fluopyram, a widely used fungicide in agriculture, has raised concerns regarding its potential toxicity and adverse effects on non-target organisms. The present study was designed to investigate the adverse effect of two doses of fluopyram (0.5 and 2.5 mg/kg body weight/day) on antioxidant enzymes and histopathological changes in the brain of male and female albino rats for 28 days and its amelioration with ginger extract. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were decreased and there was significant increase in lipid peroxidation (LPO) levels in the brain and blood plasma of male and female albino rats. Histopathological alterations in the brain revealed mononuclear cell infiltration, pyknotic neurons, degenerated neurons(DN), increased perinuclear space and hemorrhage in treated rats indicating fluopyram's neurotoxic potential. Rats treated with ginger extract and fluopyram showed restored antioxidant enzyme activities in blood and brain tissue and restored histopathological changes in brain. These pathophysiological alterations in blood and brain tissues could be due to potential toxic effects of fluopyram that is associated with generation of free radicals.

Keywords: Fluopyram, Albino rats, Ginger extract, Oxidative stress.

1. INTRODUCTION

Pesticides are chemical substances used to eradicate insect pests, weeds, fungus, and rodents. Plant growth regulators, fungicides, molluscicides, insecticides, herbicides, nematicides, fungicides and other substances are among them (Zhan et al., 2020; Bhatt et al., 2021a; Zhang et al., 2021). Fluopyram is a novel broad range fungicide created by Bayer Crop Science to manage plant pathogenic fungi like botrytis, black dot and white mold to fight fungal diseases in different fruits and vegetable crops (Tinwell et al., 2014). It prevents succinate dehydrogenase from accomplishing its function in the fungal mitochondrial respiratory chain and is classified as a succinate dehydrogenase inhibitor (SDHI) subclass of fungicides. Because of its widespread use, concerns regarding fluopyram's ecotoxicological effects have recently emerged (Matadha et al., 2019). From a human health perspective, it is crucial to recognize that numerous neurological disorders and various malignancies are associated with pesticide exposure (Yen et al., 2021). Fluopyram inhibits the mitochondrial complexes as it is an inhibitor of mitochondrial complex II, as these are the primary pathways in the brain tumors and diseases like parkinson's etc (Exner et al., 2012). In this context, assessing the toxicity of fluopyram on brain is very important.

Toxicological studies on fluopyram have highlighted its capacity to induce oxidative stress in various biological systems. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize these species with antioxidants (Sies, 2015). This imbalance can lead to cellular damage, inflammation and dysfunction, which are implicated in a variety of diseases and toxicological outcomes. In mammals, fluopyram exposure has been shown to lead to increased oxidative damage in multiple organs, including the brain and liver (Akinmoladun et al., 2020). The brain, being particularly vulnerable to oxidative stress due to its high metabolic activity and lipid-rich composition, is an important target for the toxic effects of environmental pollutants, including pesticides (Halliwell, 2006). Fluopyram-induced oxidative stress in the brain can result in neuro-degeneration, inflammation and impairment of cognitive functions (Kocaman et al., 2020). Similarly, fluopyram's effects on blood parameters have been investigated, with studies suggesting changes in hematological profiles, such as red and white blood cell counts and enzyme activities (Singh et al., 2019). Celik et al. (2012) conducted a study on rats by giving Luna Experience-

SC 400 (fluopyram and tebuconazole) and observed cytotoxic and genotoxic potential in rat bone marrow. Anti-platelet, antioxidant, anti-tumor, anti-retroviral, anti-hepatotoxic and anti-arthritic properties are a few of the pharmacological properties that have been shown by ginger (Kamtchoving et al., 2002). Studies on the impact of fluopyram on the blood and brain tissue however, are lacking. Therefore, the purpose of this study was to assess how the fluopyram affects the antioxidant potential and histology of brain of male and female albino rats and its amelioration with ginger extract as ginger extract is well known for its antioxidant, anti-inflammatory and neuroprotective properties.

2. MATERIALS AND METHODS

2.1. Chemicals

Fluopyram(N-[2-[3-Chloro-5-(trifluoromethyl)-2-pyridinyl] ethyl]- 2(trifluoromethyl) benzamide; CAS: 658066-35-4; 34.48% W/W SC) was purchased from the local market of Ludhiana Punjab, India, manufactured by Bayer Crop Science Ltd. under the trade name Bayer Velum Prime.

2.2. Experimental Design

In this study, a total of 24 mature male and 24 female albino rats weighing 130 to 170 g were used. Rats were housed in polypropylene cages and were given water and food *ad libitum*. The rats were acclimatized for a period of 2 weeks before the start of the experiment and were randomly divided into four groups; 6 rats in each group. Group I served as control, Group II and III received 0.5 and 2.5 mg/kgbw/day of fluopyram through oral gavage and Group IV received 100 mg/kg aqueous extract of ginger and 2.5 mg/kgbw/day fluopyram Table 1.

The rhizomes of *Z. officinale* were shade dried and crushed to powder. 125 g of the powder was macerated in 1000 ml of distilled water for 12 h. at room temperature and was then filtered to obtain the final aqueous extract. In this study each animal was orally given 1 ml of the final aqueous extract (Kamtchoving et al., 2002). Two doses of fluopyram were dissolved in 0.1 ml DMSO and were given by oral gavage for 28 days. The experiment was performed after the approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana, India under protocol No. (GADVASU/2023/IAEC/69/04 -17/05/2023).

After the treatment animals were put to death by cervical dislocation. Blood was collected by cardiac puncture and then centrifuged to obtain plasma and brain tissue was removed and weighed after dissecting the animals. The tissues were sheared in 0.9% saline (chilled) and homogenization was done in 0.1 M phosphate buffer(pH 7.4). The homogenates were centrifuged for 30 min at 1000 rpm at 4^0C to obtain supernatants. The plasma and tissue supernatants were used for the assay of different antioxidant enzymes.

2.3. Oxidative Stress Biomarkers in Blood and Brain

Superoxide dismutase(SOD) was determined according to method of Marklund and Marklund (1974), catalase (CAT) by Aebi (1983), glutathione peroxidase (GPx) by Hafeman et al. (1984) and lipid peroxidation(LPO) by Jollow et al. (1974) respectively. In the estimations of all the antioxidant enzymes the total soluble protein content was estimated by Lowry et al. (1951) taking BSA as standard.

Statistical Analysis: Software (SPSS) was used to analyze the data with post host Tukey's t-test was used for making comparisons between control and treated group of rats.

2.4. Histopathological Examinations

Brain tissue was cleared and fixed in 10% formalin for 24 hours. Then the tissues were dehydrated in different grades of ethanol, clearing was done in xylene and embedding was done in paraffin wax for the preparation of blocks. The 5-7µm thick sections were cut and stained in haematoxylin-eosin stain and mounted in DPX and slides were seen under

Table 1. Effect of fluopyram on net body weight and weight of brain of male and female rats

Group	Treatment	Male rats			Female rats		
		Initial weight (g)	Final weight (g)	Brain weight (g/100g body weight)	Initial weight (g)	Final weight (g)	Brain weight (g/100g body weight)
I	Control	151.66	-	4.28^a	130.00	-	4.32^a
II	0.5mg/kgbw/day	153.00	156.66	4.22^a	135.00	136.66	4.28^a
III	2.5mg/kgbw/day	153.00	156.66	3.90^a	140.00	146.00	4.24^a
IV	Ginger + 2.5 mg/kgbw/day	152.00	152.66	3.95^a	132.00	135.33	3.98^a

Values are expressed as mean±SE

^{a}represents no significant difference between treatments at $p \leq 0.05$ as compared to control

microscope. The histological alterations in brain were scored as follows: normal appearance(-), mild(+), moderate(++), severe(+++).

3. RESULTS AND DISCUSSION

No mortality was observed during the treatment period. Mean body weight of male and female albino rats did not vary significantly between rats of control and treated groups (0.5 and 2.5 mg/kgbw/day) (Table 1). Non-significant decrease in body weight was observed in male and female treated rats as compared to control rats. No significant difference was observed in the weights of brain of rats among all the treated and control groups. Different antioxidant enzymes were assessed in the plasma and brain of male and female rats exposed to fluopyram and the efficacy of ginger was evaluated at high dose post treatment of fluopyram. Rats of both sexes showed decreased catalase, SOD, GPx activity after being exposed to high dose of fluopyram, compared to the corresponding control groups in the plasma. The rats treated with ginger showed higher levels of catalase at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma when compared with the untreated and fluopyram treated rats (Table 2, 3). Rats of both sexes showed increased LPO levels after treatment with high dose of fluopyram, compared to the corresponding control groups in the plasma. The rats treated with ginger had lower LPO at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma (Table 2, 3) when compared with the untreated and fluopyram treated rats (low dose of fluopyram)

Male and female rats showed decreased catalase, SOD, GPx activity after being exposed to high dose of fluopyram, compared to the corresponding control groups in the brain tissue. The rats treated with ginger (ZO) had higher levels of catalase at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma when compared with the untreated and fluopyram treated rats (Table 5). LPO levels were also increased after being treated with high dose of fluopyram, compared to control groups in the brain tissue. The rats treated with ginger (ZO) indicated lower LPO at high dose, i.e., 2.5 mg/kg, in both male and female animals in the

Table 2. Effect of fluopyram on antioxidant parameters of plasma in male rats

Group	Treatment	Antioxidant parameters						
		Superoxide dismutase (SOD)	**Catalase (CAT)**	**Glutathione-S-transferase (GST)**	**Glutathione peroxidase (GPx)**	**Glutathione (GSH)**	**Glutathione reductase (GR)**	**Lipid peroxidation (LPO)**
I	Control	17.09^{b}	11.64^{c}	0.67^{b}	4.83^{a}	47.91	0.05^{a}	1.36^{b}
II	0.5mg/kgbw/day	13.88^{b}	10.40^{c}	0.66^{b}	4.23^{d}	46.78^{a}	0.04^{c}	7.53^{a}
III	2.5mg/kgbw/day	5.85^{a}	8.91^{a}	0.46^{a}	2.25^{a}	41.60^{a}	0.02^{a}	6.52^{c}
IV	Ginger+2.5 mg/kgbw/day	15.37^{b}	11.40^{b}	0.66^{b}	4.30^{b}	46.46^{b}	0.04^{b}	1.69^{b}

Values are expressed as mean±SE of six animals in each group

^{abc} represents significant difference between treatments at $p \leq 0.05$ as compared to control.

Units: SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GSH (nmol/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)

Table 3. Effect of fluopyram on antioxidant parameters of plasma in female rats

Group	Treatment	Antioxidant parameters						
		Superoxide dismutase (SOD)	**Catalase (CAT)**	**Glutathione-S-transferase (GST)**	**Glutathione peroxidase (GPx)**	**Glutathione (GSH)**	**Glutathione reductase (GR)**	**Lipid peroxidation (LPO)**
I	Control	18.34^{c}	11.64^{c}	0.84^{c}	4.87^{c}	47.9	0.06^{c}	1.85^{a}
II	0.5mg/kgbw/day	15.94^{b}	10.40^{b}	0.71^{c}	4.09^{c}	41.20^{b}	0.05^{c}	5.50^{b}
III	2.5mg/kgbw/day	5.89^{a}	5.55^{b}	0.45^{b}	3.86^{a}	40.46^{a}	0.02^{a}	7.82^{c}
IV	Ginger + 2.5 mg/kgbw/day	17.95^{b}	12.85^{c}	0.80^{b}	4.83^{c}	46.46^{c}	0.04^{b}	1.64^{a}

Values expressed as mean±SE

^{abcd} represents significant difference between treatments at $p \leq 0.05$ as compared to control.

Units: SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)

plasma (Table 4 and 5) when compared with the untreated and fluopyram treated rats(low dose of fluopyram)

Shah et al. (2010) observed that tebuconazole was given in four different groups (0, 100, 400 and 1600 ppm) for 13 weeks during a subchronic study conducted in rats. The results showed that one male and one female rat died during the study, that the mean body weight decreased in the high-dose group despite eating more food, and that the cytochrome P_{450} content and hepatic N-dimethylhylase activity increased. Gujral et al. (1978) observed- adult male and female rats showed reduced body weight, blood glucose, serum total cholesterol and serum alkaline phosphatase in response to ginger, demonstrating the hypocholesterolaemic effects of ginger.

Male and female rats have different responses to fluopyram due to hormonal differences, particularly the influence of sex hormones on oxidative stress and antioxidant enzymes. Estrogen showed neuroprotective effects and may help maintain antioxidant enzyme activity in females, while males may have a more pronounced reduction in SOD, CAT and GPx activity when exposed to fluopyram. The decrease in SOD, CAT and GPx activity was more in males as compared to females in plasma and brain of rats. However no significant difference was observed in between both the sexes.Due to factors like a high oxidative metabolic rate, a high ratio of membrane surface area to cytoplasmic volume, high levels of unsaturated lipids (mostly polyunsaturated fatty acids or PUFAs), high iron levels, increased free radical generation from dopamine metabolism and ineffective scavenging mechanisms, the central nervous system and specifically the spinal nerve, is particularly vulnerable to damage from free radicals (Abuja and Albertini, 2001).

The brain makes up only 2% of the body weight, but it has a high oxygen demand, around 20% of it. Higher concentrations of ROS are generated in the brain and glutathione-mediated detoxification is crucial (Abdollahi et al., 2004; Franco et al., 2010). The working of SOD and CAT

Table 4. Effect of fluopyram on brain antioxidant parameters of male rats

Group	Treatment	Antioxidantparameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Control	8.69^{c}	12.98^{d}	0.49^{c}	0.69^{a}	48.73^{c}	0.058^{c}	0.46^{a}
II	0.5mg/kgbw/day	7.07^{c}	10.02^{c}	0.43^{b}	0.68^{a}	41.32^{a}	0.061^{c}	0.52^{a}
III	2.5mg/kgbw/day	5.88^{a}	9.82	0.41^{a}	0.67^{a}	40.86^{b}	0.049^{b}	0.75^{b}
IV	Ginger + 2.5 mg/kgbw/day	8.79^{b}	11.48^{d}	0.48^{c}	0.69^{a}	46.77^{c}	0.05^{b}	0.47^{a}

Values expressed as mean±SE

^{abcd} ***represents significant difference between treatments at p≤0.05 as compared to control.***

Units: SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)

Table 5. Effect of fluopyram on brain antioxidant parameters of female rats

Group	Treatment	Antioxidantparameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Control	10.86^{d}	11.70^{c}	0.49^{d}	0.71^{a}	48.59^{d}	0.06^{b}	0.39^{a}
II	0.5mg/kgbw/day	8.69^{a}	8.52^{b}	0.43^{c}	0.69^{a}	41.32^{b}	0.049^{b}	0.75^{b}
III	2.5mg/kgbw/day	6.07^{a}	8.79^{b}	0.41^{a}	0.67^{a}	40.77^{b}	0.03^{a}	0.93^{d}
IV	Ginger+2.5 mg/kgbw/day	9.82^{d}	11.48^{c}	0.48^{d}	0.70^{a}	46.77^{d}	0.06^{b}	0.38^{a}

Values expressed as mean±SE

^{abcd} ***represents significant difference between treatments at p≤0.05 as compared to control.***

Units: SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)

is interrelated as superoxide molecule which is dismuted to hydrogen peroxide by the enzyme SOD followed by removal of hydrogen peroxide molecule by GSH-Px and CAT (found in peroxisome). The functions of GPx are performed in cytosol which limits its role in comparison to other antioxidant enzymes. Superoxide dismutases (SOD) helps in protecting the cells from molecular oxygen and also removes superoxide radicals and decreased SOD level in liver may lead to free radical damage at large scale. Decreased level of SOD will decrease the production of hydrogen peroxide and it also reduces the protection against free radicals. Decrease in the concentration of CAT will decrease the H_2O_2 concentration which in turn will decrease the free radicals. Glutathione peroxidase is known for the reduction of various hydro peroxides to H_2O through oxidation of reduced GSH into glutathione disulphide (GSSH). Keshav et al. (2024) reported the neurotoxic effect of dichlorvos in male and female Wistar rats and attenuation of toxicity was observed with ginger extract. Post treatment with ginger resulted in increased levels of SOD, CAT, GSH, GPx and GR and declined levels of LPO were reported. Increased lipid peroxidation seems to be indicator of several disorders in cells, tissues or organs and it might be involved in different diseased state of cell such as aging, nervous disorders etc. (Meng et al., 2002 a b). Sahoo et al. (2000) also reported neurotoxicity in rats following lindane exposure. The work by Dwivedi et al. (2010), showed a decrease in GSH level in the brain following exposure to monocrotophos and dichlorvos, is consistent with the lower GSH level seen in experimental animals.

Histologically, brain tissue section of control rats and fluopyram treated rats (0.5 and 2.5 mg/kgbw/day) showed mononuclear cell infiltration, pyknotic neurons, degenerated neurons(DN), increased perinuclear space,

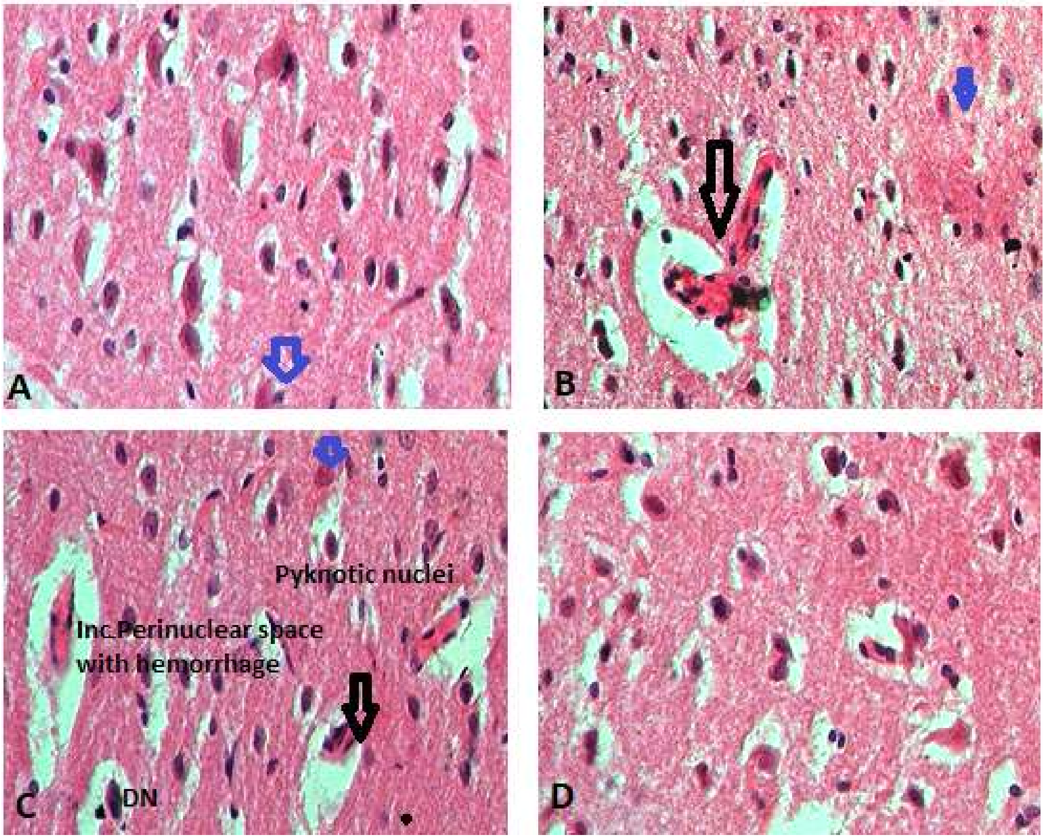

Figure 1. Brain Tissue Section from Control rats showed normal nerve or glial cells(A) In 0.5 mg/kgbw/day treated group brain showed mononuclear cell infiltration (black arrows), hemorrhage (blue arrows) (Fig 1 B) 2.5 mg/kgbw/day treated group showed pyknotic neurons, degenerated neurons(DN), increased perinuclear space and hemorrhage(blue arrows) (Fig 1 C) tissue section from rats treated with ginger extract and 2.5 mg/kgbw/day showed restored histology almost similar as that of control group observed by light microscopy with H&E (X400 magnification)

hemorrhage and tissue section from rats treated with 2.5 mg/kgbw/day and ginger extract showed restored histology almost similar as that of control group observed by light microscopy (Figure 1).

4. CONCLUSION

Decreased antioxidant enzymes influence the brain to increase free radical damage which in turn affects the defense system of body. The widespread application of fluopyram heightens the probability of environmental dispersal, resulting in the pollution of soil, water, and food products. Consequently, non-target organisms, such as animals, aquatic species, beneficial insects and soil microbes may experience direct or indirect exposure. It can be concluded from this study that ginger (post treatment) ameliorates the toxicity caused by fluopyram in brain of rats of both the sexes. This protection was provided by ginger as it improves the antioxidant defense system of rats. Therefore, daily dietary ginger intake can help mitigate the pesticide-induced toxicity. Further work is needed to understand the toxicological role of fluopyram on multiple organs of mammals.

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Authors' Contributions

PS planned the conceptualization and methodology of study. PS, NV and NK help in data curation, analysis and writing of manuscript. All the authors read and approved the manuscript.

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

The authors declare that generative AI and AI-assisted technologies were not used in the writing or preparation of this manuscript.

Conflicts of Interest

All authors declare there is no conflict of interest.

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Influence of Maturity of Stem cuttings and Plant Growth Regulators in Propagation of Orange Jasmine (*Murraya paniculata* L. Jack)

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***Abstract*:** *Murraya paniculata* (orange jasmine), is a versatile evergreen shrub belonging to family Rutaceae. It is widely used as cut foliage in floral arrangements and bouquet owing to its visual appeal. The current research was undertaken to determine the most efficient hormonal concentration for rooting of softwood and semi hardwood stem cuttings of *M. paniculata* at Kerala Agricultural University, Thrissur, Kerala, during the rainy season (July 2024). Sprouting (70 %), shoot length (6.44 cm), rooting (68.89 %), number of roots (13), root length (20.50 cm), and fresh weight of roots (1.07 g) were highest in softwood cuttings treated with IBA (1000 ppm). Early sprouting (8.67 days) was observed in softwood cuttings treated with IBA (2000 ppm). Total phenolic and flavonoid contents were lowest in softwood cuttings treated with IBA (1000 ppm) at 30 (phenol: 4.13 mg/g FW, flavonoid: 2.16 mg/g FW) and 45 (phenol: 3.45 mg/g FW, flavonoid: 1.73 mg/g FW) days after planting, while higher levels of phenols and flavonoids were observed in other treatments. Histological analysis showed that the adventitious roots of orange jasmine developed from cambial and xylem parenchyma cells, growing outward through the phloem, sclerenchyma, cortex, and epidermis, eventually emerging on the outer surface of the cutting at 45 days after planting. Softwood cuttings treated with 1000 ppm IBA proved to be the most effective for propagating *M. paniculata*.

***Keywords*:** Orange jasmine, *Murraya*, Plant growth regulators, Softwood cuttings.

1. INTRODUCTION

Orange jasmine is extensively used as cut foliage and fillers in floral arrangements, bouquets, wreaths, and interior decorations. Fully bloomed trees, adorned with white flowers, are highly ornamental and often recommended as specimen trees in gardens. Almost all parts of the plant serve various purposes, including ethnomedicinal uses, cosmetics, essential oil extraction, and insecticidal agents. Its leaves are oval-shaped, distinguished by their brownish-green colouring, bitter and spicy flavour, and aromatic fragrance. The leaf surface is smooth and glossy (Wardani et al., 2019). The flowers are pentamerous, bisexual, and have a delightful fragrance. Flowering occurs throughout the year, beginning in June during the rainy season. Orange jasmine can be grown from seeds, but seed propagated plants have long juvenile period before flowering and seeds are not available throughout the year. *M. paniculata* exhibits limited rooting ability which discourages its vegetative propagation. Due to the challenges in rooting of cuttings, it is necessary to standardize vegetative propagation methods to ensure multiplication of true-to-type progenies for the conservation and better exploitation of the diversity. Currently, planting materials are sourced from different centers, especially from Rajahmundry in Andhra Pradesh to different states including Kerala. To address these challenges, the current study was conducted to standardize vegetative propagation in orange jasmine using stem cuttings.

2. MATERIALS AND METHODS

Cuttings for the experiments were collected from mother plants of orange jasmine maintained at the shrubbery of the College of Agriculture, Vellanikkara (10^0 54'N latitude and 76^0 28'E longitude, with an elevation of 22.25 m above MSL), Thrissur during the monsoon season (June-August). Cuttings with two nodes were taken from the softwood and semi-hardwood portions of shoots which were collected from both the terminal and middle sections of the branches respectively. The chemicals used for this study were Indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), gallic acid, quercetin, sodium carbonate, folin-ciocalteu reagent, sodium hydroxide, aluminum chloride and sodium nitrate, which were of analytical grade purchased from Nice Chemicals Pvt. Ltd. Edappally, Kochi, Kerala.

Softwood and semi-hardwood cuttings of length (7-8 cm) and width (2.30-2.80 mm) were treated with different treatments such as distilled water (control), various concentrations of IBA and NAA alone and combinations of IBA and NAA (1:1) at concentrations ranging from 1000 to 3000 ppm and also tender coconut water. In control treatment, base of cuttings was immersed in distilled water for one minute (T_1). The base of the cuttings was immersed in growth regulator solutions (T_2 to T_{10}) for one minute while treatment with tender coconut water was continued for 10 minutes (T_{11}). After the treatment with distilled water/ growth regulator solutions/ tender coconut water, the cuttings were planted in 4x5-inch polythene bags filled with potting mixture consisting of soil, coir pith, and FYM in 2:1:1 ratio and placed inside ventilated rain shelter for sprouting and rooting.

2.1. Observations Recorded

Various growth parameters, including the number of days to sprout, percentage of cuttings sprouted, shoot length (cm), number of cuttings rooted, root number, root length (cm), and fresh root weight (g) were recorded at 90 days after planting.

2.2. Estimation of Total Phenolic and Flavonoid Content in the Basal Section of Cuttings

Gallic acid standards (1–15 ppm) were used for calibration, and the total phenolic content was calculated using the procedure outlined by Kharbangar et al. (2015) with minor modifications. The procedure outlined by Jing et al. (2015) was used for estimation of total flavonoid content.

2.3. Histological Observation of the Adventitious Rooting Zone

Adventitious rooting zone of cuttings in the most effective treatment (softwood cuttings treated with 1000 ppm IBA) was examined histologically after 30 and 45 days of planting. Leica sliding microtome (Model number: Leica SM 2010 R) was used to take the sections of 5 to 10 µm thickness. Appropriate portions were chosen, and the other stems were saved for later use. Following two to five minutes of staining with safranin at 10%, the sections were dried out using a range of graded alcohol solutions (30 to 95 and 100 per cent) and an alcohol-xylene mixture (50:50). Sections were examined under a compound microscope (4x and 10x magnification) (Model number: Leica ATC 2000). Images were taken using image analyser software Digi Pro.7.

2.4. Statistical Analysis

Data were analysed using GRAPES software developed by Kerala Agricultural University (Gopinath et al., 2021).

3. RESULTS AND DISCUSSION

3.1. Sprouting Potential of Cuttings

Significant difference was observed between softwood and semi hardwood cuttings treated with different concentrations as well as combinations of IBA and NAA in the sprouting characteristics (Table 1). Softwood cuttings treated with IBA 1000 ppm (S_1T_2) recorded highest sprouting percentage (70%) followed by semi-hardwood cuttings and softwood cuttings treated with IBA 2000 ppm which were on par with each other. Sprouting percentage was lowest in semi hardwood cuttings treated with distilled water (S_2T_1, 17.78%). Softwood cuttings treated with IBA 2000 ppm (S_1T_3) recorded minimum duration for sprouting (8.67 days) followed by semi-hardwood cuttings treated with IBA 2000 ppm (S_2T_3) and softwood cuttings treated with NAA 2000 ppm which were on par with each other with 10.67 days for sprouting. Semi hardwood cuttings treated with distilled water recorded maximum days for sprouting (21.67 days).

Sprouting of leaves from stem cuttings is influenced by several factors, including water, root development, nutrients, auxins, cytokinins, carbohydrates and proteins (Kurepa et al., 2019). Superiority of cuttings treated with IBA compared to NAA can be due to the prolonged auxin action and delayed degradation by auxin-degrading enzymes. IBA promotes cell division, leading to rapid callus formation in the cuttings. IBA improves the use of these nutrients and stimulates sprout growth by encouraging the breakdown and flow of nitrogenous chemicals and carbohydrates at the cellular level in the basal area of the cuttings at high concentrations and activity levels (Sabharwal, 2023).

Significant variation in shoot length was observed between the cuttings treated with different concentrations of growth hormones. Softwood cuttings treated with 1000 ppm IBA (S_1T_2) showed highest shoot length (6.44cm), followed by S_1T_6 and S_2T_3 (4.79 cm). In contrast, semi-hardwood cuttings treated with distilled water (S_2T_1) recorded shortest shoot length of 1.39 cm. Juvenility of the cells (more undifferentiated high active cells) and the availability of nutrients determine the sprout growth in the stem cuttings. Furthermore, CN ratio plays an important role in the development of sprouts in stem cuttings and it decline from root initiation to root emergence.

3.2. Rooting Behavior of Cuttings

Softwood cuttings treated with 1000 ppm IBA (S_1T_2) exhibited better root characteristics recording highest percentage of rooted cuttings (68.89%), number of lateral roots (13.00) and root length (20.50 cm) (Table 2, Figure 1). This was followed by semi hardwood cuttings treated with 2000 ppm IBA. Semi-hardwood cuttings treated with distilled water recorded the lowest percentage of rooted cuttings (17.78%), number of lateral roots (2.00) and root length (2.59 cm). Softwood cuttings taken from the upper stem develop better roots than hardwood cuttings from the basal stem (Pijut et al., 2011).

Auxins, whether they are surface-applied or naturally occurring, are essential for initiation of adventitious root growth on stems. External application of auxin transforms starch into simple sugars, which are necessary for the formation of new cells and the augmentation of respiratory activity in tissue regeneration during the growth of new root primordia. It also facilitates the hydrolysis and movement of carbohydrates and nitrogenous compounds at the base of the cuttings, stimulating cell division and enhancing root formation (El-Banna et al., 2023). Exogenous treatment of stem cuttings with auxin increases the number of roots and speeds up the rooting process by encouraging the formation of root primordia. IBA is the most commonly utilized auxin for adventitious root development and a more efficient regulator of plant growth. Treatment of cuttings with IBA causes greater hydrolytic activity and encourages the movement of carbohydrates from the leaves which aids in root growth (Bhatt and Tomar, 2010). Auxins encourage growth from the cellular stage to the organ level and eventually help in the development of the entire plant (Khudhur and Omer, 2015). IBA is less harmful to plants over a wider range of concentrations compared to NAA

Table 1. Effect of type of cuttings and growth regulators on sprouting potential in *M. paniculata* cuttings

Treatments	**Type of cuttings**	**No. of days for sprouting**	**Sprouting of cuttings (%)**	**Length of shoots (cm)**
T_1 – Control	S_1	18.67^{b}	25.55^{m}	1.44^{l}
	S_2	21.67^{a}	17.78^{n}	1.39^{l}
T_2 – IBA 1000 ppm	S_1	11.67^{ij}	70.00^{a}	6.44^{a}
	S_2	12.00^{i}	47.78^{def}	2.65^{fgh}
T_3 – IBA 2000 ppm	S_1	8.67^{k}	56.67^{bc}	4.23^{cd}
	S_2	10.67^{j}	57.78^{bc}	4.79^{b}
T_4 – IBA 3000 ppm	S_1	14.33^{gh}	52.22^{cd}	4.29^{c}
	S_2	13.33^{h}	44.44^{efg}	3.02^{ef}
T_5 – NAA 1000 ppm	S_1	12.00^{i}	43.33^{efg}	3.89^{d}
	S_2	14.33^{gh}	41.11^{gh}	2.90^{ef}
T_6 – NAA 2000 ppm	S_1	10.67^{j}	60.00^{b}	4.79^{b}
	S_2	14.33^{gh}	48.89^{de}	2.75^{fg}
T_7 – NAA 3000 ppm	S_1	15.33^{fg}	42.22^{fgh}	3.29^{e}
	S_2	16.33^{def}	34.44^{ijk}	2.82^{f}
T_8 - IBA+NAA (1:1)- 1000 ppm	S_1	16.00^{ef}	38.89^{ghi}	2.99^{ef}
	S_2	17.33^{cd}	30.00^{klm}	2.39^{ghi}
T_9 - IBA+NAA (1:1)- 2000 ppm	S_1	17.00^{cde}	36.67^{hij}	2.25^{ij}
	S_2	17.67^{bc}	26.67^{lm}	2.04^{ij}
T_{10} - IBA+NAA (1:1)-3000 ppm	S_1	15.33^{fg}	34.45^{ijk}	2.30^{hij}
	S_2	17.67^{bc}	27.78^{lm}	1.92^{jk}
T_{11} - Dipping in tender coconut water	S_1	16.33^{def}	32.22^{jkl}	2.12^{ij}
	S_2	18.67^{b}	24.44^{m}	1.62^{kl}
CD (p=0.05)	S	0.37	1.76	0.12
	T	0.86	4.13	0.28
	S × T	1.22	5.85	0.39

S_1- Softwood cuttings; S_2- Semi-hardwood cuttings; S- type of cutting; T- growth regulators

(Ashwath et al., 2023). Effectiveness of IBA, when compared to other growth regulators is attributed to the slower degradation rate of the chemicals by auxin-destroying enzymes (Reshma, 2017). The slow breakdown of IBA and its limited mobility leads to greater retention near the application site, making IBA one of the most efficient root stimulators.

Softwood cuttings treated with 1000 ppm IBA had more shoot length with foliage, which serves as the primary site for food production, which is then transferred to the roots for growth. Additionally, IBA promoted deeper root penetration into the soil, allowing the roots to absorb more nutrients, which ultimately resulted in increased root length. Kareem et al. (2013), also observed increased root length in softwood cuttings of guava var. Gola when treated with 0.4 g of IBA per 100 g of talc powder. Softwood cuttings treated with 1000 ppm IBA (S_1T_2) registered highest fresh root weight (1.07 g), followed by semi hardwood cuttings treated with 2000 ppm IBA (S_2T_3, 0.79 g) and softwood cuttings treated with 2000 ppm NAA (S_1T_6, 0.66 g). Alternatively, semi-hardwood cuttings treated with distilled water exhibited lowest fresh root weight (0.08 g). Early initiation of roots leads to more rapid uptake of nutrients, which may facilitate endosmosis of water and promote cell enlargement in the roots. As a result, longer roots are produced, increasing the fresh weight of roots (Sabharwal, 2023).

3.3. Effect of Auxins on Total Phenolic and Flavonoid Content

Semi-hardwood cuttings treated with IBA 2000 ppm (S_2T_3) recorded lowest total phenolic content (3.36 mg/g

Table 2. Effect oftype of cuttings and growth regulators on rooting potential in *M. paniculata* cuttings

Treatments	Type of cuttings	Cuttings rooted (%)	No. of roots	Root length (cm)	Fresh wt. of roots (g)
T_1 – Dipping in distilled water	S_1	24.44^{j}	3.00^{ijk}	3.39^{m}	0.13^{o}
	S_2	17.78^{k}	2.00^{l}	2.59^{n}	0.08^{p}
T_2 – IBA 1000 ppm	S_1	68.89^{a}	13.00^{a}	20.50^{a}	1.07^{a}
	S_2	53.33^{c}	5.66^{de}	10.69^{ef}	0.40^{ef}
T_3 – IBA 2000 ppm	S_1	53.33^{c}	5.66^{de}	11.42^{d}	0.45^{d}
	S_2	61.11^{b}	9.22^{b}	16.67^{b}	0.79^{b}
T_4 – IBA 3000 ppm	S_1	46.67^{d}	4.44^{fg}	11.01^{de}	0.43^{de}
	S_2	46.67^{d}	4.44^{fg}	10.19^{g}	0.38^{fg}
T_5–NAA 1000 ppm	S_1	40.00^{ef}	4.11^{fgh}	10.30^{fg}	0.36^{gh}
	S_2	41.11^{de}	4.78^{ef}	9.54^{h}	0.32^{hi}
T_6–NAA 2000 ppm	S_1	58.89^{bc}	7.89^{c}	13.53^{c}	0.66^{c}
	S_2	55.56^{bc}	6.33^{d}	11.45^{d}	0.43^{de}
T_7–NAA 3000 ppm	S_1	38.89^{ef}	4.44^{fg}	10.14^{g}	0.33^{h}
	S_2	36.67^{efg}	4.33^{fg}	8.45^{i}	0.28^{ij}
T_8-IBA+NAA (1:1)- 1000ppm	S_1	35.56^{efg}	3.89^{fghi}	8.67^{i}	0.29^{ij}
	S_2	34.44^{fg}	3.55^{ghijk}	7.61^{j}	0.28^{ijk}
T_9-IBA+NAA (1:1)- 2000ppm	S_1	34.45^{fg}	3.66^{ghij}	8.24^{i}	0.26^{jkl}
	S_2	31.11^{ghi}	3.33^{hijk}	7.21^{jk}	0.24^{klm}
T_{10}- BA+NAA (1:1)-3000ppm	S_1	32.22^{gh}	3.33^{hijk}	7.62^{j}	0.23^{lm}
	S_2	27.78^{hij}	2.78^{jkl}	6.92^{k}	0.19^{n}
T_{11}-Dipping in tender coconut water	S_1	27.78^{hij}	3.22^{hijk}	6.39^{l}	0.21^{mn}
	S_2	25.56^{ij}	2.66^{kl}	6.04^{l}	0.13^{o}
CD (p=0.05)	S	1.81	0.29	0.14	0.02
	T	4.24	0.68	0.32	0.03
	S × T	6.00	0.96	0.45	0.04

S_1- Softwood cuttings; S_2- Semi-hardwood cuttings; S- type of cutting; T- growth regulators

FW) which was on par with softwood cuttings treated with IBA 1000 ppm at 45 days after planting. On the contrary, semi-hardwood cuttings treated with distilled water (S_2T_1) had the highest total phenolic content (11.09 mg/g FW) (Table 3). Total phenolic content was reduced in all the treatments from 30 days after planting to 45 days after planting. Softwood cuttings treated with 1000 ppm of IBA had the lowest phenolic content at 30 days after planting (4.13 mg/g FW).

Early rooting in certain plants can be linked to the abundance of carbohydrates along with increased levels of phenolic compounds. During root initiation, these compounds are reduced, which promotes rooting in cuttings. De Klerk et al. (2011), observed that certain phenolic compounds can prevent the formation of roots by causing IAA to oxidize or decarboxylate, or by acting as precursors for the synthesis of lignin. In *Campomanesia phaea*, histochemical analysis revealed the presence of phenolic compounds in sclerenchyma tissue, which may negatively influence the adventitious root development (Santoro et al., 2022). Individual phenolic compounds may either stimulate or inhibit root formation (Izadi et al., 2016). Meta and ortho-diphenols, along with polyphenols, have the potential to inhibit auxin decarboxylation, thereby promoting rooting in cuttings. Sharma (2012) reported that etiolated plants with low total phenol levels have better root development, while woody plants with higher total phenolic content have slower root development.

Softwood cuttings treated with 1000 ppm IBA recorded lowest total flavonoid content (1.73 mg/g) at 45 days after

Table 3. Effect of type of cuttings and growth regulators on total phenol and flavonoid content in *M. paniculata*

Treatments	Type of cuttings	Total phenol content (mg/g FW)		Total flavonoid content (mg/g FW)	
		30 DAP	45 DAP	30 DAP	45 DAP
T_1 – Dipping in distilled water	S_1	11.96^{b}	10.61^{b}	5.21^{b}	3.74^{d}
	S_2	13.47^{a}	11.09^{a}	5.61^{a}	5.24^{a}
T_2–IBA 1000 ppm	S_1	4.13^{n}	3.45^{m}	2.16^{o}	1.73^{m}
	S_2	7.29^{j}	6.97^{i}	3.26^{jk}	3.00^{gh}
T_3–IBA 2000 ppm	S_1	5.91^{l}	5.61^{k}	$3.18k^{l}$	2.21^{kl}
	S_2	5.17^{m}	3.36^{m}	2.54^{n}	2.24^{kl}
T_4–IBA 3000 ppm	S_1	6.86^{k}	6.32^{j}	3.45^{ij}	2.35^{jkl}
	S_2	8.80^{i}	8.02^{g}	3.24^{jk}	2.78^{hi}
T_5–NAA 1000 ppm	S_1	7.62^{j}	7.53^{h}	3.66^{hi}	2.48^{ijk}
	S_2	9.47^{gh}	8.93^{f}	4.01^{g}	3.66^{de}
T_6–NAA 2000 ppm	S_1	5.26^{m}	4.78^{l}	2.78^{mn}	2.05^{l}
	S_2	6.54^{k}	5.36^{k}	2.94^{lm}	2.56^{ij}
T_7–NAA 3000 ppm	S_1	8.64^{i}	7.83^{g}	3.85^{gh}	2.65^{ij}
	S_2	9.82^{fg}	9.35^{e}	4.06^{fg}	3.58^{def}
T_8-IBA+NAA (1:1)- 1000 ppm	S_1	9.21^{h}	8.79^{f}	4.06^{fg}	2.73^{hi}
	S_2	10.11^{f}	9.43^{e}	4.33^{e}	3.77^{d}
T_9-IBA+NAA (1:1)- 2000 ppm	S_1	10.07^{f}	9.58^{e}	4.28^{ef}	2.97^{h}
	S_2	10.67^{de}	10.27^{cd}	4.46^{de}	4.20^{c}
T_{10}-BA+NAA (1:1)-3000 ppm	S_1	10.57^{e}	10.07^{d}	4.41^{de}	3.29^{fg}
	S_2	10.99^{cd}	10.51^{bc}	4.73^{c}	4.46^{bc}
T_{11}-Dipping in tender coconut water	S_1	11.22^{c}	10.35^{bcd}	4.62^{cd}	3.42^{ef}
	S_2	11.28^{c}	10.22^{cd}	4.78^{c}	4.65^{b}
CD (p=0.05)	S	0.11	0.09	0.08	0.09
	T	0.26	0.22	0.18	0.22
	S × T	0.36	0.30	0.25	0.31

S_1- Softwood cuttings; S_2- Semi-hardwood cuttings; S- type of cutting; T- growth regulators; FW- fresh weight

planting followed by softwood cuttings treated with 2000 ppm NAA (S_1T_6, 2.05 mg/g FW). Semi-hardwood cuttings treated with distilled water (S_2T_1) recorded higher total flavonoid content (5.24 mg/g FW). Softwood cuttings treated with 1000 ppm IBA had the lowest flavonoid concentration at 30 days after planting (2.16 mg/g FW). The total flavonoid content in all the treatments also decreased at 45 days after planting compared to 30 days similar to the trend observed in the phenol content.

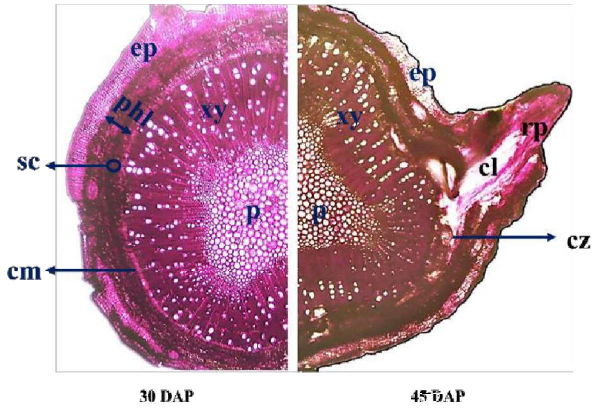
3.4. Morphological and Histological Changes in Cuttings during Rooting

Morphological and histological changes were observed at 30 and 45 days after planting in the best treatment viz., cuttings treated with IBA 1000 ppm. At 30 days after planting, the adventitious rooting zone showed a noticeable fading of green coloration and swelling and at day 45, this area faded further developing minor cracks, bursts, and small white callus tissues on the epidermis. Both woody and herbaceous plants naturally develop callus at the base of the cutting as a response of the cambium to the damage caused by cutting (Wroblewska, 2013). In contrast to this, in the present study, profuse callus formation was not observed in any of the treatments. Histological observations revealed that, adventitious roots of orange jasmine grew from cambial and xylem parenchyma cells and spread outward through the cortex, phloem, sclerenchyma, and epidermis before reaching the outer surface of the cuttings (Figure 2). Cambium layer or vascular tissues were identified as the origin of adventitious roots in many investigations. Cambium zone is the primary tissue where adventitious roots are most likely to form. However, it remains unclear which specific part of the new parenchyma belongs to the cambium, phloem, or xylem. In tetraploid *Robinia pseudoacacia*, IBA application enhanced cell divisions within the vasculature, with adventitious roots initiating specifically from the cambium layer (Uddin et al., 2024). Adventitious roots in woody plant cuttings can also develop in the phloem and pericycle. In *Rosa* species, roots developed in the cambial zone tissues near the pith rays, as well as within the phloem and pericycle (Monder et al., 2019). They observed the absence of a firm ring of sclerenchymatous cells in the adventitious rooting zone of cuttings in rose. In contrast to this, in this study, adventitious roots originated even in the presence of thick sclerenchymatous layer in the pericycle. Push of the root primordia for initiation of root resulted in rupture of cortex and epidermal cells (Figure 2).

a. Softwood cuttings

b. Semi-hardwood cuttings

Figure 1. Response of different type of cuttings and concentrations of plant growth regulators on the growth of *M. paniculata* at 90 days after planting



Abbreviations: ep- epidermis; phl- phloem; sc- sclerenchyma; cm- cambium; xy- xylem;
p- pith; cl- callus; rp- root primordia; cz- cambium zone; DAP – Days After Planting

Figure 2. Transverse sections of rooting zone of *M. paniculata* cuttings

4. CONCLUSION

The impact of several plant growth regulators on the emergence and establishment of softwood and semi-hardwood cuttings of *M. paniculata* was assessed. Softwood cuttings treated with IBA 1000 ppm (S_1T_2) resulted in maximum sprouting of cuttings, shoot length, percentage of rooted cuttings, number of roots, root length and fresh root weight, followed by semi hardwood cuttings treated with IBA 2000 ppm. Lower doses of growth regulators yielded better outcomes compared to higher concentrations and their combinations of growth hormones.

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Conflict of Interest

Authors do not have any conflict of interests to declare.

Authors' Contributions

Resmi Paul, Vishnu Raju and Mini Sankar developed the concept of the experiment. Yarrakula Venugopal executed the trial, collected and analysed the data and prepared the manuscript.

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Sustaining Soil Quality and Maize Productivity through FYM and Targeted Nutrient Application of Fertilization in Acid Alfisols of Northwest Himalayas, India

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Abstract: Long-term evaluation of nutrient management practices is vital for enhancing soil quality and sustaining crop productivity. A 13-year field experiment, established in 2007 in acid Alfisols of the Northwest Himalayas, assessed the effects of prescription-based fertilization and farmyard manure on soil properties and maize yield under continuous cultivation. The experiment followed a randomized block design with seven nutrient management treatments and one control, each replicated thrice. Post-harvest (2019) surface soil samples (0–15 cm) were analysed for key physical and chemical properties. The combined application of soil test crop response-based chemical fertilizers targeting a 4 t ha^{-1} yield along with 5 t ha^{-1} farmyard manure not only improved soil physical and chemical properties but also significantly increased nutrient availability. This treatment also resulted in the highest maize grain yield (4.27t ha^{-1}). The soil quality index increased from 0.72 under soil test-based fertilization to 0.82 under STCR without farmyard manure (FYM) and reached 0.95 with the integration of FYM. Strong positive correlation ($r^2 = 0.88$) between SQI and grain yield highlights the synergy between STCR-based nutrient management and FYM application. These findings demonstrate the potential of integrated nutrient strategies in enhancing maize productivity and sustaining long-term soil quality.

Keywords: Targeted nutrient application, Soil quality, Maize productivity, Prescription-based fertilization, Acid alfisols, North-West Himalayas

1. INTRODUCTION

As the global population rises, the pressure to enhance food production has driven the adoption of intensive agricultural practices, often reliant on synthetic fertilizers, pesticides, and monoculture systems to maximize short-term yields. However, this pursuit has frequently overlooked soil quality, which underpins long-term productivity. Fertilizer use, though vital, remains suboptimal in India as recommendations are largely based on broad agro-climatic zones, ignoring soil heterogeneity. Significant variability in soil texture and nutrient status within zones affects crop response, making uniform application inefficient. This leads to over- or under-fertilisation, causing soil degradation, acidification, micronutrient deficiencies, and groundwater contamination or nutrient depletion and loss of soil fertility (Lal, 2015). Prolonged dependence on synthetic inputs further deteriorates soil structure, organic matter, and microbial activity (Sarkar et al., 2016). While organic farming offers an alternative, its inconsistent nutrient release often fails to meet crop demand (Tilman et al., 2002).

To address these challenges, precision nutrient management approaches such as soil test crop response (STCR)-based fertilization integrated with farmyard manure (FYM) offer a sustainable solution. The targeted yield concept aligns fertilizer doses with attainable crop yields under specific conditions, ensuring efficient nutrient utilization and reducing environmental risks (Singh et al., 2015). Integrating FYM with fertilizers synchronizes nutrient supply with crop demand, fertilisers providing immediate nutrients, and FYM improving the soil's physical, chemical, and biological properties (Antil et al., 2011). Evidence shows that conjoint application enhances soil organic matter, nutrient availability, hydraulic conductivity, and microbial activity, while reducing compaction (Singh et al., 2015; Moharana et al., 2017; Parmar et al., 2023). Thus, STCR-based integrated nutrient management (INM) has emerged as a promising strategy for sustaining soil quality and crop productivity across diverse ecosystems.

Maize, the staple of North India's farming systems, occupies 9.89 M ha with an annual production of 31.65 Mt in India (GOI 2022), and 0.255 M ha producing 0.708 Mt in Himachal Pradesh (Anonymous, 2023). Given its role in food, feed, and industry, sustaining soil health in maize-based systems is critical. Soil quality, defined as the soil's capacity to sustain productivity and ecosystem functions (Doran & Parkin, 1994;), can be evaluated through key indicators such as pH, organic carbon, nutrient status, and microbial activity. Since soil quality changes occur gradually, long-term experiments are essential for understanding the sustainability of nutrient management practices (Krause et al., 2022; Das et al., 2023).

Despite growing evidence from rice and wheat systems (Mittal et al., 2018), studies on the long-term effects of STCR-based fertilization in maize, particularly under acid Alfisols of the NW Himalayas, remain scarce. Based on this gap, we hypothesized that integrating prescription-based fertilization with FYM over the long term would improve maize yield while sustaining soil quality. Therefore, the objectives of this study were: (i) to assess changes in soil physical and chemical properties under long-term STCR fertilization in maize, (ii) to evaluate the effect of FYM integration on soil quality and productivity, and (iii) to identify key soil quality indicators and their interrelationships.

2. MATERIALS AND METHODS

2.1. Site Location

The field experiment was conducted at the experimental farm of CSK HPKV, Palampur, India (32°6′ N, 76°3′ E; 1290 m amsl), representing the mid-hill wet temperate agro-climatic zone of North-West India. The region experiences mild summers and cool winters, with an average annual rainfall of 2500–3000 mm, about 75% of which occurs during the monsoon season (June–September) (Figure 1). The soil is classified as Typic Hapludalf (Alfisol) with silty loam texture. Initial soil physico-chemical properties (0–15 cm) are presented in Table 1.

2.2. Experimental Design and Treatments

A long-term maize–wheat cropping system experiment was initiated in kharif 2007 under the AICRP on Soil Test Crop Response (STCR). The experiment comprised seven nutrient management treatments and an unfertilized control, laid out in a randomized block design with three replications (Table 2). Fertilizer doses were calculated using STCR-based fertilizer adjustment equations for maize, with and without farmyard manure (FYM), targeting specific yield levels as proposed by Verma et al. (2007).

FYM was applied at 5 t ha^{-1} (dry weight basis) under integrated plant nutrient system (IPNS) treatments and contained 1.17% N, 0.62% P, and 0.78% K. Fertilizer requirements were computed using the following equations:

$FN=5.88T-0.23SN-0.90ON$

$FP=4.87T-1.22SP-0.81OP$

$FK = 3.66T - 0.49SK - 0.51OK$

where FN, FP, and FK represent fertilizer N, P_2O_5, and K_2O (kg ha^{-1}), respectively; T is the targeted yield (t ha^{-1}); SN, SP, and SK denote soil-available nutrients; and ON, OP, and OK represent nutrient contributions from FYM.

2.3. Field Techniques

Maize hybrid 'Kanchan' was sown on 20 June 2019 using a

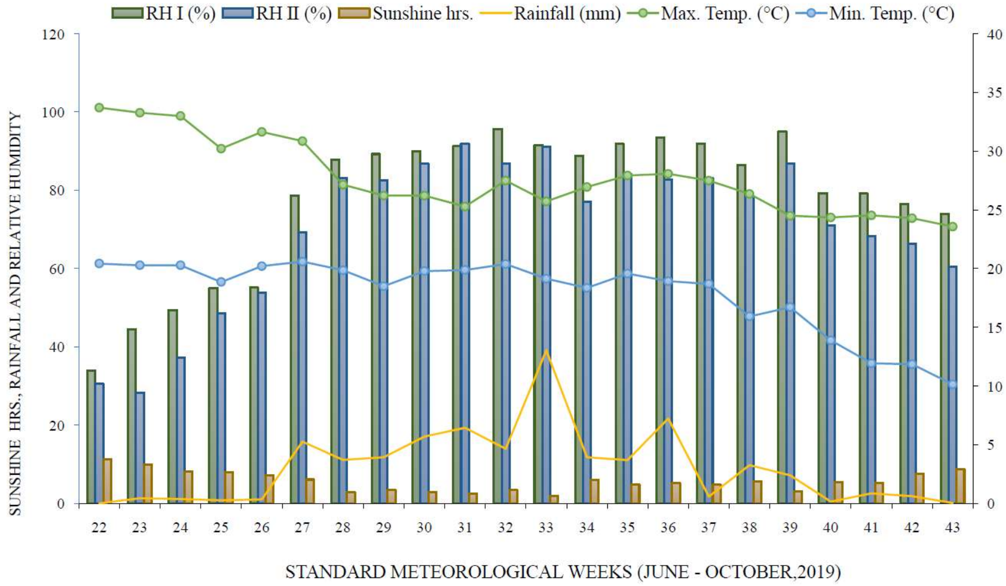


Figure 1. Weekly mean meteorological data during the experimental period

seed rate of 20 kg ha^{-1} at 60 × 40 cm spacing in plots of 5 × 3 m. Fertilizers were applied as per treatment specifications. Full doses of P_2O_5 and K_2O and one-third of N were applied basally at sowing, while the remaining N was top-dressed in two equal splits at knee-high and tasseling stages. FYM (5 t ha^{-1}) was incorporated in treatments targeting 3.0 and 4.0 t ha^{-1} yields. The crop was harvested at physiological maturity on 22 October 2019, and grain and stover yields were recorded.

Table 1. Initial physico-chemical properties of the soil prior to the commencement of the experiment (2007)

Soil characteristics	Value
Mechanical composition	
Sand (%)	22.5
Silt (%)	43.6
Clay (%)	31.7
Texture	Silty clay loam
Bulk density (Mg m^{-3})	1.28
Water holding capacity (%)	47.8
Particle density (Mg m^{-3})	2.68
Porosity (%)	48.9
pH (1:2.5)	5.2
EC (dS m^{-1})	0.19
Organic carbon (g kg^{-1})	7.2
Available nutrients	
N (kg ha^{-1})	236
P (kg ha^{-1})	41
K (kg ha^{-1})	272
S (kg ha^{-1})	15.1
Ca (c mol(p^{+})kg^{-1})	0.68
Mg (c mol(p^{+})kg^{-1})	0.84
DTPA exractable (mg kg^{-1})	
Fe (mg kg^{-1})	23.2
Mn (mg kg^{-1})	21.4
Zn (mg kg^{-1})	1.25
Cu (mg kg^{-1})	0.75

2.4. Soil Analysis

Surface soil samples (0–15 cm) were collected post-harvest in 2019 from five random locations within each plot and composited. Samples were air-dried, ground, sieved (<2 mm), and analyzed for physical and chemical properties using standard methods (Table 3).

2.5. Minimum Data sets and Soil Quality Index (SQI)

The soil quality index (SQI) was computed following the four-step approach described by Andrews et al. (2002a). Principal component analysis (PCA) was used to identify the minimum data set (MDS) from soil variables showing significant treatment effects. Principal components with eigenvalues >1 and explaining ≥5% variance were retained. Within each PC, variables with factor loadings within 10% of the highest value were selected, and redundant variables were eliminated using correlation analysis. Selected indicators were scored using a "more is better" approach and weighted according to the proportion of variance explained by each PC. The SQI was calculated as (eq 1):

$$SQI = \sum_{i=1}^{n} Wi \times Si \quad \text{.....................................(1)}$$

Where Wi is the weight of the variable derived from PCA and Si is the score of the variable.

2.6. Statistical Analysis

Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) was used to compare means and Duncan's Multiple Range Test (p=0.05) was calculated running online OPSTAT software (Sheoran et al., 1998). Pearson correlation analysis was employed using the corrplot package in R (version 4.4.0) (Wei and Simko, 2021) to visualize the linear

Table 2. Treatment-specific quantities of fertilizers and FYM applied in the study

Treatments	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	FYM (t ha^{-1})
T_1=Unfertilized/unmanured control	0.00	0.00	0.00	-
T_2=Farmers' practice (25 % general recommended dose of N to maize + 5 t ha^{-1} FYM)	30.00	0.00	0.00	5
T_3=GRD (general recommended dose of 120 N, 60 $P_2O_5$and 40 K_2O kg ha^{-1} to maize)	120.00	60.00	40.00	-
$T_{4=}$ STB (NPK on soil test values)	150.00	45.00	40.00	-
T_5=STCR-based fertilizer NPK alone for grain yield target of 3.0 t ha^{-1}	110.12	85.93	0.00	-
T_6=STCR-based fertilizer NPK + 5 t ha^{-1} FYM for grain yield target of 3.0 t ha^{-1}	53.29	43.52	0.00	5
T_7=STCR-based fertilizer NPK alone for grain yield target of 4.0 t ha^{-1}	166.10	118.27	8.09	-
T_8=STCR-based fertilizer NPK + 5 t ha^{-1} FYM for grain yield target of 4.0 t ha^{-1}	109.67	81.61	0.00	5

relationship between individual soil properties and maize grain yield. The PCA was performed in XLSTAT 2024 and PCA biplots were obtained from PAST version 4.03. Bar diagrams and linear regression graphs were prepared in Microsoft Excel 2019.

3. RESULTS AND DISCUSSION

3.1. Soil Physical Properties

Different fertilization strategies significantly influenced soil physical properties, with pronounced improvements under FYM-integrated treatments. Bulk density was highest in the control (1.36 Mg m⁻³) and lowest under the STCR-based treatment targeting 4 t ha^{-1} with FYM (T8; 1.20 Mg m⁻³), which was statistically at par with the 3 t ha^{-1} target supplemented with FYM (Table 4). The reduction in bulk density under FYM treatments can be attributed to enhanced soil organic matter accumulation, improved aggregation, and increased pore development, resulting in better aeration (Gangwar et al., 2006; Islam et al., 2012; Mahmood et al., 2017). Particle density followed a similar trend, with the highest value in the control (2.61 Mg m⁻³) and the lowest in T8 (2.51 Mg m⁻³). Treatments receiving fertilizers, with or without FYM, recorded significantly lower particle density than the control and were statistically comparable among themselves. This decline is likely associated with increased microbial activity and organic matter decomposition, which promote soil aggregation and porosity through microbial by-products (Whalen and Chang, 2002; Sepehya et al., 2012). Soil porosity and water holding capacity were markedly improved under FYM-integrated STCR treatments. The highest porosity (52.0%) and water holding capacity (56.6%) were observed in T8, compared to the lowest values in the control (47.8% and 46.6%, respectively). These improvements reflect the positive role of FYM in enhancing soil structure, aggregate stability, and pore continuity, thereby increasing moisture retention (Bhattacharya et al., 2008; Nagwanshi et al., 2018).

3.2. Soil Chemical Properties

3.2.1. pH, EC and OC: Treatments targeting grain yields of 3 and 4 t ha^{-1} with FYM, along with farmers' practice, recorded slightly higher soil pH (5.43–5.45) compared to the control (Figure 2). This marginal increase in pH under FYM-amended treatments is likely due to the chelation of

Table 4. Long-term effects of STCR-based target yield approach on soil physical properties

Treatment	Bulk density (Mg m^{-3})	Particle density (Mg m^{-3})	Porosity (%)	Water holding capacity (%)
T1	1.36^{d}	2.61^{c}	47.8^{e}	46.6^{f}
T2	1.23^{a}	2.53^{ab}	51.6^{ab}	52.4^{cde}
T3	1.30^{c}	2.57^{bc}	49.0^{d}	49.8^{e}
T4	1.27^{b}	2.56^{b}	50.4^{c}	50.6^{de}
T5	1.26^{b}	2.55^{ab}	50.8^{c}	52.8^{cd}
T6	1.22^{a}	2.52^{ab}	51.7^{ab}	56.2^{ab}
T7	1.25^{b}	2.55^{ab}	51.0^{bc}	53.7^{bc}
T8	1.20^{a}	2.51^{a}	52.0^{a}	56.6^{a}

Different lowercase letters within the same row indicates significant difference (Duncan's multiple range test, p<0.05)

Table 3. Methodology employed for determination of various soil properties

Chemical properties	Method/Extractant	References
OC (g kg^{-1})	Wet Digestion	Walkley and Black (1934)
Soil pH	(1: 2.5, soil : water)	Jackson (1973)
Soil EC	(1: 2, soil : water)	Jackson (1973)
Available N (kg ha^{-1})	Alkaline permanganate	Subbiah and Asija (1956)
Available P (kg ha^{-1})	0.5N $NaHCO_3$ (pH 8.5)	Olsen et al. (1954)
Available K (kg ha^{-1})	1N Neutral NH_4OAc	Black (1965)
Available S (kg ha^{-1})	I N $CaCl_2$	Williams and Steinbergs (1959)
Exch.Ca & Mg ({cmol (p^{+}) kg^{-1}})	I N NH_4OAC	Jackson (1973)
DTPA-Fe, Mn, Cu and Zn (mg kg^{-1})	DTPA extraction	Lindsay and Norvell (1978)
Physical Properties	**Method/Extractant**	**References**
BD (Mg m^{-3})	Core Sampler	Singh (1980)
PD (Mg m^{-3})	Pycnometer method	Gupta and Dhakshinamoorthy (1980)
Porosity (%)	Empirical method	Gupta and Dhakshinamoorthy (1980)
WHC (%)	Keen's moisture box	Piper (1950)

exchangeable Al^{3+} by organic ligands released during FYM decomposition, thereby reducing soil acidity (Choudhary et al., 2019). Soil electrical conductivity (EC) varied significantly among treatments, with the lowest value in the control (0.17 dS m^{-1}) and the highest in the STCR-based treatment targeting 4 t ha^{-1} with FYM (0.23 dS m^{-1}). The increase in EC under fertilizer and FYM application may be attributed to enhanced base saturation and greater availability of soluble ions, a trend commonly reported following organic matter additions (Gonzalez et al., 2010; Durani et al., 2017). Soil organic carbon (SOC) content increased markedly with FYM integration, ranging from 6.6 g kg^{-1} in the control to 8.9 g kg^{-1} under the 4 t ha^{-1} target yield with FYM (Figure 2). This improvement reflects continuous carbon inputs from FYM along with crop residues, root biomass, and exudates over the long term, leading to sustained SOC accumulation (Verma et al., 2012; Choudhary et al., 2019).

3.2.2. Available NPK: Available nitrogen varied significantly among treatments, with the highest value recorded under the STCR-based treatment targeting 4 t ha^{-1} with FYM (313.6 kg ha^{-1}) and the lowest in the control (145.5 kg ha^{-1}) (Figure 3). STCR-based target yields of 30 and 40 q ha^{-1} without FYM showed higher available N than the general recommended dose and soil test-based treatments, while FYM integration further enhanced N availability by 2.8–3.9% over their non-IPNS counterparts. The improvement in soil N status under FYM-amended treatments can be attributed to enhanced microbial activity and increased mineralization of organically bound nitrogen, leading to a sustained build-up of available N (Kumari and Tripathi, 2018). Available phosphorus ranged from 21.0 kg ha^{-1} in the control to 74.2 kg ha^{-1} in the 4 t ha^{-1} target yield treatment with FYM (Figure 3). Farmers' practice markedly increased soil P compared to the control, while STCR-based fertilization without FYM outperformed the general recommended dose and soil test-based application due to balanced nutrient supply. Integration of FYM further

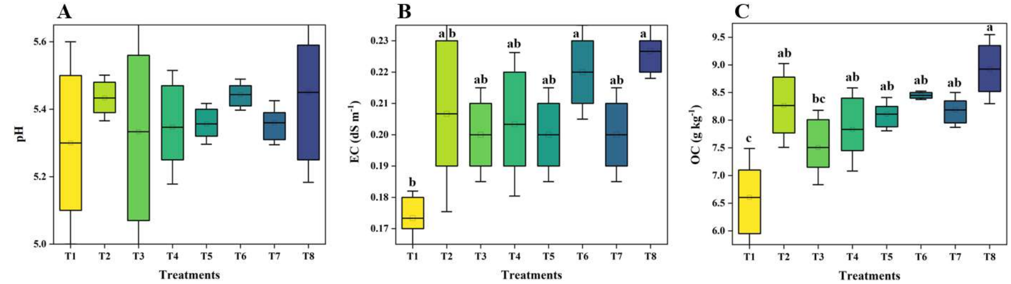


Figure 2. Long-term effects of STCR-based target yield approach on **(A)** soil pH, **(B)** electrical conductivity (EC) and **(C)** organic carbon (OC) content. Box plots show the median, interquartile range, and variability. Vertical bars represent the standard error of the mean. Bars followed by different lowercase letters are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT)

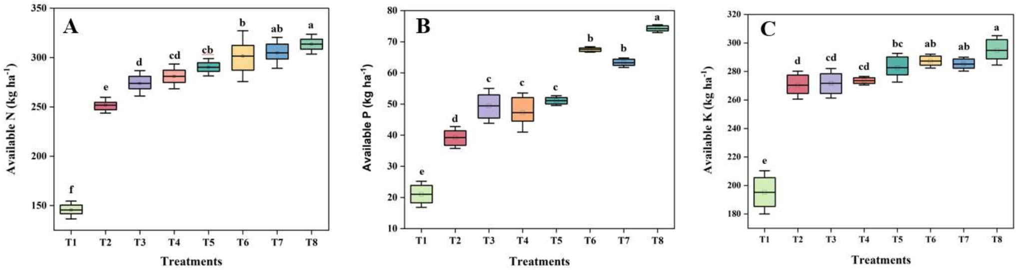


Figure 3. Long-term effects of STCR-based target yield approach on available macronutrient status in soil: **(A)** nitrogen (N), **(B)** phosphorus (P) and **(C)** potassium (K). Box plots show the median, interquartile range, and variability. Vertical bars represent the standard error of the mean. Bars followed by different lowercase letters are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT)

enhanced P availability across target yield treatments, likely due to the release of organic acids during FYM decomposition that facilitated phosphorus solubilization (Prasad et al., 2010; Kumari & Tripathi, 2018). Potassium availability followed a similar trend, with the highest value observed under the 4 t ha^{-1} target yield with FYM (294.8 kg ha-1) and the lowest in the control (195.2 kg ha-1). FYM-integrated STCR treatments significantly improved available K over conventional fertilizer practices, possibly due to reduced K fixation and enhanced release from clay–organic matter interactions (Sharma et al., 2001). In contrast, continuous nutrient removal without external inputs resulted in depletion of available NPK in the control treatment (Yadav et al., 2019).

3.2.3. Secondary and micronutrients: In addition to primary nutrients, fertilization strategies significantly influenced secondary and micronutrient availability. Available sulphur ranged from 13.8 kg ha^{-1} in the control to 23.7 kg ha^{-1} under the STCR-based treatment targeting 4 t ha^{-1} with FYM (Table 5). FYM integration consistently enhanced soil S availability compared to non-IPNS treatments, primarily due to mineralization of organically bound sulphur and the use of single super phosphate, which supplies soluble S (Babu et al., 2017). Exchangeable calcium (Ca) and magnesium (Mg) showed a clear increasing trend with fertilizer application, with the lowest values in the control and markedly higher concentrations under FYM-integrated STCR treatments. While sole fertilizer treatments resulted in moderate improvements, the incorporation of FYM increased Ca and Mg availability by 15–36% and 17–38%, respectively, compared with GRD and STB treatments (Table 5). These improvements may be attributed to indirect Ca supply through fertilizers, enhanced nutrient mobilization by deeper root systems, and increased microbial activity associated with organic matter addition (Shivashankar et al., 2018).

Table 5. Long-term effects of STCR-based target yield approach on exchangeable calcium (Ca), magnesium (Mg) (c mol(p^{+}) kg^{-1}), and available sulphur (S) (kg ha^{-1})

Treatment	Ca	Mg	S
T1	0.54^{d}	0.77^{f}	13.86^{f}
T2	1.39^{bc}	0.92^{e}	17.37^{e}
T3	1.28^{c}	0.96^{de}	18.07^{e}
T4	1.42^{bc}	1.05^{c}	20.00^{d}
T5	1.54^{bc}	1.03^{cd}	21.23^{c}
T6	$1.64a^{bc}$	1.23^{b}	22.63^{b}
T7	1.69^{ab}	1.10^{c}	22.46^{b}
T8	1.93^{a}	1.51^{a}	23.68^{a}

Different lowercase letters within the same row indicates significant difference (Duncan's multiple range test, p<0.05)

Micronutrient availability responded strongly to FYM integration. The highest DTPA-extractable Fe (31.4 mg kg^{-1}) and Mn (27.2 mg kg^{-1}) were recorded under the 4 t ha^{-1} target yield with FYM, reflecting substantial increases over non-IPNS treatments (Table 6). Enhanced Fe and Mn availability is likely due to organic acid release and chelation processes during FYM decomposition, which promote micronutrient solubilization under acidic soil conditions (Prashanth et al., 2019). Similarly, Zn availability increased progressively with FYM addition, reaching a maximum of 1.45 mg kg^{-1} under the 4 t ha^{-1} target yield with FYM, owing to direct nutrient inputs and reduced fixation through organic complexation (Chauhan et al., 2018). Copper followed a comparable trend, increasing from 0.44 mg kg^{-1} in the

Table 6. Long-term effects of STCR-based target yield approach on DTPA-extractable micronutrients

Treatment	DTPA extractable micronutrients (mg kg^{-1})			
	Fe	Mn	Zn	Cu
Control	14.4	16.1	0.81	0.44
Farmers' practice	26.3	23.0	1.33	0.76
General recommended dose of fertilizers	23.1	20.9	1.21	0.73
Soil test-based fertilizer application	24.0	21.6	1.27	0.75
Target yield 30q ha^{-1}	26.8	23.2	1.34	0.77
Target yield 30q ha^{-1} with FYM @ 5t ha^{-1}	29.6	25.5	1.41	0.79
Target yield 40q ha^{-1}	27.7	23.7	1.37	0.78
Target yield 40q ha^{-1} with FYM @ 5t ha^{-1}	31.4	27.2	1.45	0.82
CD (p=0.05)	3.0	4.1	0.19	0.08

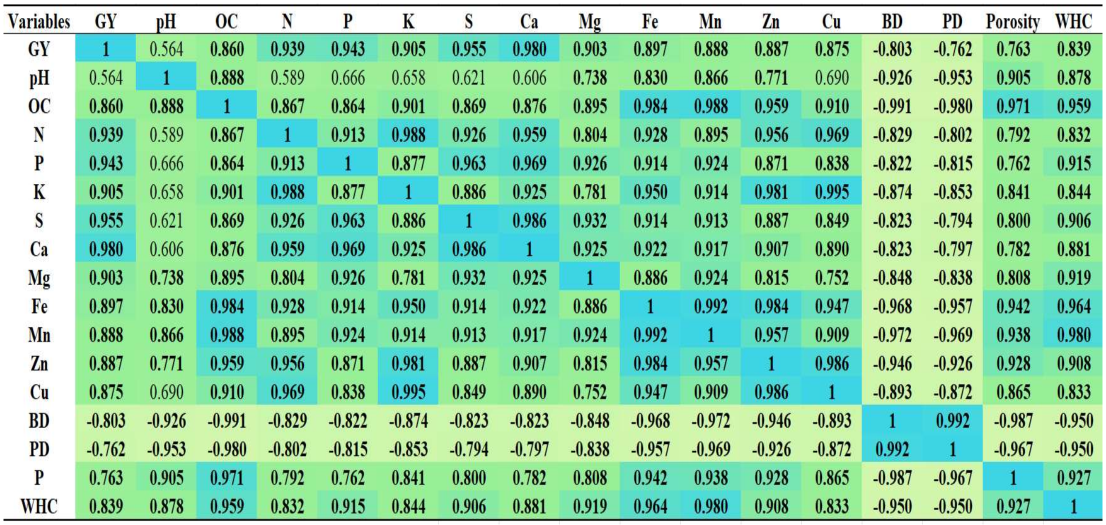
control to 0.82 mg kg^{-1} under FYM-integrated STCR treatment, likely due to the formation of stable organo-Cu complexes and enhanced microbial activity (Bolan and Duraisamy, 2003; Meng et al., 2005).

3.3. Pearson's Correlation

Maize grain yield showed significant positive correlations with available nitrogen (r = 0.763) and water holding capacity (r = 0.838), indicating the importance of nutrient availability and soil physical conditions in determining crop productivity (Figure 4). The integrated application of FYM and chemical fertilizers likely improved the soil physical environment, thereby promoting better crop growth, whereas no significant relationship was observed between grain yield and soil bulk or particle density. Grain yield was also positively correlated with soil EC (r = 0.733) and organic carbon (r = 0.860), while no significant association was observed with soil pH. The positive relationship between EC and yield may be attributed to the fine-textured nature of the soils, which enhances nutrient retention and availability. Improved SOC levels under integrated nutrient management, driven by sustained inputs of organic residues and root biomass, contributed to enhanced nutrient supply and crop performance. Strong positive correlations were observed between grain yield and available N, P, and K, with r values of 0.939, 0.942, and 0.905, respectively. In addition, secondary nutrients (S, Ca, and Mg) and micronutrients (Fe, Mn, Zn, and Cu) exhibited significant positive relationships with yield (Figure 4). These results underscore the cumulative benefits of integrated nutrient management in improving nutrient availability across multiple pools, thereby enhancing maize productivity, consistent with earlier findings (Moharana et al., 2017).

3.4. Selection of MDS and Principal Component Analysis

Following laboratory analysis, soil quality indicators were subjected to principal component analysis (PCA), and components with eigenvalues >1 were retained (Figure 5A). The first two principal components (PC1 and PC2) explained 89.5% and 5.3% of the total variance, respectively, together accounting for 94.9% of the variability in soil properties (Table 7). PC1 was strongly associated with multiple chemical, physical, and biological indicators, indicating its dominant role in explaining soil quality variation under different nutrient management practices. Based on high factor loadings, vector lengths, and orientations in the PCA biplot (Figure 5B), six variables; organic carbon (OC), available phosphorus (P), exchangeable calcium (Ca), manganese (Mn), water holding capacity (WHC), and microbial biomass carbon (MBC) were identified as the most influential contributors to soil quality. These indicators showed strong positive associations, suggesting that improvements in fertility, physical structure, and biological activity are closely



Variables	GY	pH	OC	N	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	BD	PD	Porosity	WHC
GY	**1**	0.564	**0.860**	**0.939**	**0.943**	**0.905**	**0.955**	**0.980**	**0.903**	**0.897**	**0.888**	**0.887**	**0.875**	**-0.803**	**-0.762**	**0.763**	**0.839**
pH	0.564	**1**	**0.888**	0.589	0.666	0.658	0.621	0.606	**0.738**	**0.830**	**0.866**	**0.771**	0.690	**-0.926**	**-0.953**	**0.905**	**0.878**
OC	**0.860**	**0.888**	**1**	**0.867**	**0.864**	**0.901**	**0.869**	**0.876**	**0.895**	**0.984**	**0.988**	**0.959**	**0.910**	**-0.991**	**-0.980**	**0.971**	**0.959**
N	**0.939**	0.589	**0.867**	**1**	**0.913**	**0.988**	**0.926**	**0.959**	**0.804**	**0.928**	**0.895**	**0.956**	**0.969**	**-0.829**	**-0.802**	**0.792**	**0.832**
P	**0.943**	0.666	**0.864**	**0.913**	**1**	**0.877**	**0.963**	**0.969**	**0.926**	**0.914**	**0.924**	**0.871**	**0.838**	**-0.822**	**-0.815**	**0.762**	**0.915**
K	**0.905**	0.658	**0.901**	**0.988**	**0.877**	**1**	**0.886**	**0.925**	**0.781**	**0.950**	**0.914**	**0.981**	**0.995**	**-0.874**	**-0.853**	**0.841**	**0.844**
S	**0.955**	0.621	**0.869**	**0.926**	**0.963**	**0.886**	**1**	**0.986**	**0.932**	**0.914**	**0.913**	**0.887**	**0.849**	**-0.823**	**-0.794**	**0.800**	**0.906**
Ca	**0.980**	0.606	**0.876**	**0.959**	**0.969**	**0.925**	**0.986**	**1**	**0.925**	**0.922**	**0.917**	**0.907**	**0.890**	**-0.823**	**-0.797**	**0.782**	**0.881**
Mg	**0.903**	**0.738**	**0.895**	**0.804**	**0.926**	**0.781**	**0.932**	**0.925**	**1**	**0.886**	**0.924**	**0.815**	**0.752**	**-0.848**	**-0.838**	**0.808**	**0.919**
Fe	**0.897**	**0.830**	**0.984**	**0.928**	**0.914**	**0.950**	**0.914**	**0.922**	**0.886**	**1**	**0.992**	**0.984**	**0.947**	**-0.968**	**-0.957**	**0.942**	**0.964**
Mn	**0.888**	**0.866**	**0.988**	**0.895**	**0.924**	**0.914**	**0.913**	**0.917**	**0.924**	**0.992**	**1**	**0.957**	**0.909**	**-0.972**	**-0.969**	**0.938**	**0.980**
Zn	**0.887**	**0.771**	**0.959**	**0.956**	**0.871**	**0.981**	**0.887**	**0.907**	**0.815**	**0.984**	**0.957**	**1**	**0.986**	**-0.946**	**-0.926**	**0.928**	**0.908**
Cu	**0.875**	0.690	**0.910**	**0.969**	**0.838**	**0.995**	**0.849**	**0.890**	**0.752**	**0.947**	**0.909**	**0.986**	**1**	**-0.893**	**-0.872**	**0.865**	**0.833**
BD	**-0.803**	**-0.926**	**-0.991**	**-0.829**	**-0.822**	**-0.874**	**-0.823**	**-0.823**	**-0.848**	**-0.968**	**-0.972**	**-0.946**	**-0.893**	**1**	**0.992**	**-0.987**	**-0.950**
PD	**-0.762**	**-0.953**	**-0.980**	**-0.802**	**-0.815**	**-0.853**	**-0.794**	**-0.797**	**-0.838**	**-0.957**	**-0.969**	**-0.926**	**-0.872**	**0.992**	**1**	**-0.967**	**-0.950**
P	**0.763**	**0.905**	**0.971**	**0.792**	**0.762**	**0.841**	**0.800**	**0.782**	**0.808**	**0.942**	**0.938**	**0.928**	**0.865**	**-0.987**	**-0.967**	**1**	**0.927**
WHC	**0.839**	**0.878**	**0.959**	**0.832**	**0.915**	**0.844**	**0.906**	**0.881**	**0.919**	**0.964**	**0.980**	**0.908**	**0.833**	**-0.950**	**-0.950**	**0.927**	**1**

Values in bold are significant at level p=0.5

Figure 4. Pearson correlation matrix showing relationships among grain yield (GY) and various soil physico-chemical properties (pH, electrical conductivity (EC), organic carbon (OC), available macronutrients (N, P, K, S), secondary nutrients (Ca, Mg), micronutrients (Fe, Mn, Zn, Cu), and soil physical properties (bulk density (BD), particle density (PD), porosity, and water holding capacity (WHC)

interlinked. Following established criteria for soil quality assessment and essentiality (Arnon & Stout, 1939; Andrews et al., 2002b), one representative variable each from primary (P), secondary (Ca), and micronutrients (Mn), along with key physical (WHC) and biological (MBC) indicators, was selected for the minimum data set (MDS). Organic carbon was additionally retained due to its sensitivity to long-term FYM application and its central role in regulating soil physical, chemical, and biological processes (Lal, 2008; Dhull et al., 2004).

In PC2, soil pH was the only variable retained due to its highest factor loading, with no other indicator falling within the 10% selection threshold (Table 7). Given the acidic nature of the study soils, pH was considered a critical indicator directly influencing nutrient availability and crop productivity. The Pearson correlation analysis further supported the inclusion of the selected indicators (Figure 4). In contrast, physical properties such as bulk density (BD) and particle density (PD) exhibited shorter vector lengths and orientations opposite to fertility and biological indicators in the PCA biplot (Figure 5B), indicating that integrated nutrient management reduced soil compaction and improved structural conditions conducive to root growth and microbial activity. The PCA biplot also revealed clear treatment clustering, with FYM-integrated STCR treatments positioned along the positive axis of PC1 and closely associated with key soil quality indicators, reflecting enhanced fertility, biological activity, and maize yield. Conversely, the control and non-STCR treatments were grouped along the negative axis of PC1, indicating poorer soil quality. Accordingly, the final minimum data set (MDS)

Table 7. Principal Component Analysis (PCA) of soil quality indicators showing eigenvalues, percentage variability and cumulative variance explained by the first two principal components (PC1 and PC2), along with factor loading values for each soil property

Principal components	**PC1**	**PC2**
Eigenvalue	17.023	1.016
Variability (%)	89.592	5.347
Cumulative %	89.592	94.939
Soil quality indicators	**Factor loading values for each Principal component**	
pH	0.837	**-0.525**
OC	**0.985**	-0.121
N	**0.922**	0.370
P	**0.932**	0.204
K	**0.935**	0.278
S	**0.931**	0.244
Ca	**0.936**	0.309
Mg	**0.919**	-0.001
Fe	**0.996**	0.011
Mn	**0.997**	-0.057
Zn	**0.971**	0.103
Cu	**0.930**	0.220
BD	**-0.968**	0.206
PD	**-0.957**	0.252
P	**0.938**	-0.240
WHC	**0.973**	-0.146
MBC	**0.964**	-0.076
DHA	**0.915**	-0.230
LC	**0.966**	0.089

Boldface component loadings were considered as highly weighted. Among them bold-underlined values were retained in minimum data sets

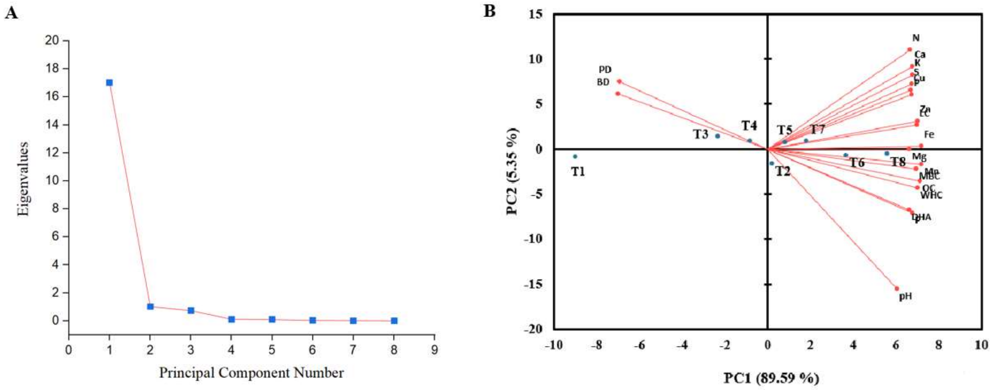


Figure 5. (A) Scree plot showing eigenvalues corresponding to each principal component, indicating that the first two components explain most of the variability. **(B)** PCA biplot illustrating the distribution of treatments (T1 to T8) and the loading values of different soil variables. PC1 (89.59%) and PC2 (5.35%) together account for 94.94% of the total variance. Arrows represent the direction and magnitude of each variable's contribution to the components

comprised OC, P, Ca, Mn, WHC, MBC, and pH.

For SQI computation, all indicators were scored using a "more is better" approach (Table 8). Weights were assigned based on the proportion of variance explained by each principal component: PC1 contributed a total weight of 0.94, distributed equally (0.15 each) across six indicators, and PC2 assigned a weight of 0.05 to pH. Indicator scores were normalized using their respective maximum values, and the weighted scores were summed to derive the soil quality index:

$$SQI = (0.15 \times S_{OC}) + (0.15 \times S_{P}) + (0.15 \times S_{Ca}) + (0.15 \times S_{Mn}) + (0.15 \times S_{WHC}) + (0.15 \times S_{MBC}) + (0.05 \times S_{pH})$$

Where S is the score of the selected variable

3.5. Grain Yield and Soil Quality Index

All fertilization treatments significantly increased maize grain yield compared to control, which recorded the lowest yield (1.36 t ha^{-1}), while the STCR-based treatment targeting 4 t ha^{-1} with FYM achieved the highest yield (4.27 t ha^{-1}) (Figure 6A). Both general recommended dose and soil test-based fertilization improved yield over farmers' practice, with greater increment observed under the targeted yield approach. The superior performance of FYM-integrated STCR treatments may be attributed to improved root growth, balanced nutrient supply, and enhanced nutrient uptake, collectively supporting higher photosynthetic efficiency and crop productivity (Kumar and Santhi, 2017; Suresh and Santhi, 2018). In addition, soil test-based fertilizer recommendations promote efficient and economical fertilizer use, supporting sustainable crop production (Tegegnework et al., 2015).

Soil quality index (SQI) values ranged from 0.49 in the control to 0.95 under the 4 t ha^{-1} target yield with FYM, representing a 94% improvement over the control (Table 8). STCR-based treatments consistently recorded higher SQI than general recommended and farmers' practice treatments, with further enhancement observed following FYM

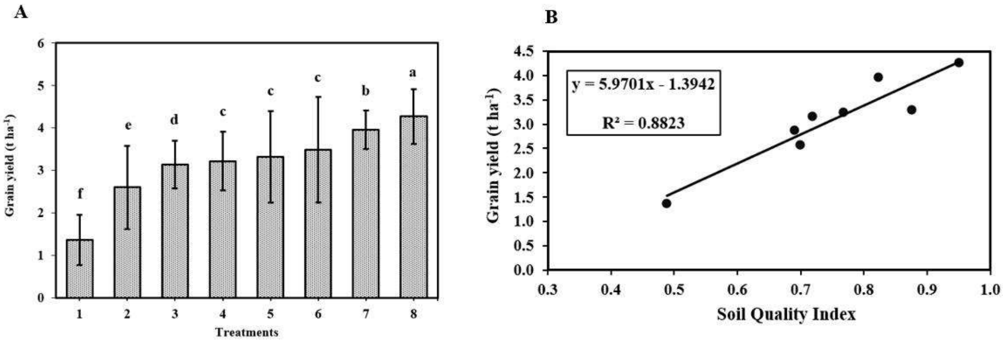


Figure 6. (A) Long-term effects of STCR-based target yield approach on grain yield (t ha^{-1}) of maize. Results are presented with standard error of mean (Sem ±). Bars with different lowercase letters indicate statistically significant differences among treatments at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT). **(B)** The linear relationship between maize grains yield (t ha^{-1}) and soil quality index

Table 8. Soil quality index (SQI) and normalized scores (S) with their respective weights (W) for selected soil quality indicators under different nutrient management treatments

Treatments	OC		P		Ca		Mn		WHC		MBC		pH		SQI
	S	W	S	W	S	W	S	W	S	W	S	W	S	W	
Control	0.74	0.15	0.28	0.15	0.28	0.15	0.59	0.15	0.82	0.15	0.21	0.15	0.97	0.05	0.49
FP	0.93	0.15	0.53	0.15	0.56	0.15	0.85	0.15	0.93	0.15	0.54	0.15	0.99	0.05	0.70
GRD	0.84	0.15	0.67	0.15	0.66	0.15	0.77	0.15	0.88	0.15	0.46	0.15	0.97	0.05	0.69
STB	0.88	0.15	0.64	0.15	0.74	0.15	0.79	0.15	0.89	0.15	0.53	0.15	0.98	0.05	0.72
T30	0.91	0.15	0.69	0.15	0.80	0.15	0.85	0.15	0.93	0.15	0.61	0.15	0.98	0.05	0.77
T30FYM5	0.95	0.15	0.91	0.15	0.85	0.15	0.94	0.15	0.99	0.15	0.86	0.15	0.99	0.05	0.88
T40	0.92	0.15	0.85	0.15	0.87	0.15	0.87	0.15	0.95	0.15	0.70	0.15	0.98	0.05	0.82
T40FYM5	1.00	0.15	1.00	0.15	1.00	0.15	1.00	0.15	1.00	0.15	1.00	0.15	1.00	0.05	0.95

integration. In contrast, omission of FYM resulted in a 7–23% reduction in SQI across targeted yield treatments, underscoring the importance of organic amendments in sustaining soil quality. Even farmers' practice improved SQI by 43% over the control, highlighting the long-term benefits of FYM addition (Shahid et al., 2013; Ram et al., 2016; Singh et al., 2015; Sankhyan et al., 2024). Organic carbon (OC) and water holding capacity (WHC) emerged as the most influential contributors to SQI, followed by Mn, Ca, P, microbial biomass carbon (MBC), and pH (Figure 7). The strong positive relationship between OC and WHC (r = 0.95) reflects the role of FYM in enhancing soil aggregation and moisture retention. MBC, due to its sensitivity to management practices, further contributed to nutrient cycling and soil functioning (Chinnadurai et al., 2014). Given the acidic nature of the soils, pH also played a critical role in regulating nutrient availability and soil processes (Amoakwah et al., 2023). A strong linear relationship between maize grain yield and SQI ($R^2 = 0.88$) confirms that integrated use of organic and inorganic nutrient sources is essential for improving productivity while sustaining long-term soil quality (Figure 6B).

4. CONCLUSION

The outcomes from this 13-year study on STCR based target yield model, effectively reinforce this perspective, demonstrating that integration of FYM (5 t ha^{-1}) with STCR based nutrient recommendations for target yield of 4 t ha^{-1} not only maximized maize productivity but also substantially improved soil physical and chemical properties. This strategy not only meets the productivity goals for maize but also plays a vital role in maintaining soil quality thus making prescription-based integrated nutrient management a promising approach. Promoting such an approach particularly in mid hills of North-west Himalayas could lead to more precise and balanced fertilizer recommendations, enhanced nutrient use efficiency and conservation of soil health, besides economizing the use of costly fertilizer inputs.

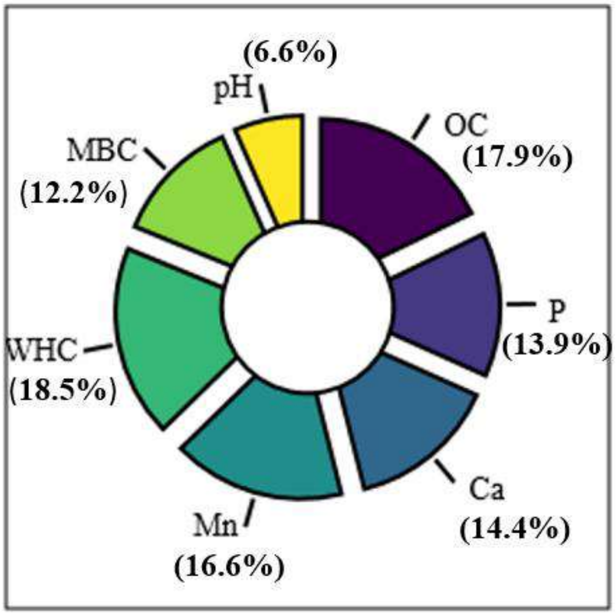


Figure 7. Relative contribution (%) of individual indicators to the soil quality index

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Credit Authorship Contribution Statement

Varun Parmar: Conceptualization, Writing original draft, Methodology, Investigation, Formal analysis, Data curation. Naveen Datt: Conceptualization, Validation, Supervision & Funding Acquisition. Shahi Pal Dixit: Validation, Funding Acquisition Rushali: Editing, Review and Methodology.

Disclosure Statement

The authors report there are no competing interests to declare.

Data availability statement

The data with respect to this study is available from the corresponding author upon reasonable request.

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Performance Evaluation of a Power-Operated Bamboo Drip Irrigation System Using *Schizostachyum pergracile* in South Gujarat, India

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***Abstract*:** This study assesses the performance of a bamboo drip irrigation system at the Bambusetum, College of Forestry, NAU, Navsari, Gujarat, India. Parameters evaluated include the coefficient of uniformity, coefficient of variation, friction loss, pressure-discharge relationship, discharge rates, surface wetting patterns, and cost-effectiveness. The system utilized bamboo as a conveyance material, with medi-infusion tubes and bamboo microtubes of varying diameters serving as emitters. PVC pipes with drippers were used as the control at operating pressures of 1.3, 1.4, and 1.5 kg/cm^2. The bamboo-based system exhibited discharge rates ranging from 1.17 to 18.68 l/hr. CU ranged from 50.89 to 89.77%, and CV values from 0.04 to 0.65. Despite variability in some treatments, the results demonstrate that bamboo systems can deliver adequate uniformity with suitable emitter selection. Friction loss increased with rising pressure, while surface wetting patterns were influenced by microtube diameters. Though cost savings were marginal (₹370 per 122 m²), the system demonstrates economic viability when scaled and supported by local, low-cost materials. The bamboo systems showed slightly lower discharge uniformity, but remain a sustainable, affordable solution for subsistence farming and agroforestry in water-scarce regions where uniformity is less critical.

Keywords: Bamboo drip irrigation, Surface wetting patterns, Emission uniformity, Sustainable irrigation.

1. INTRODUCTION

Water scarcity is an escalating concern globally, affecting various sectors of human life and natural ecosystems (Rosegrant *et al.,* 2002). The rapid depletion of irrigation water resources is a significant contributor to this issue, compounded by growing populations and accelerated economic development in numerous nations. This challenge is particularly severe in arid and semi-arid regions, including India, where water resources are limited, and demand continues to rise (Biswas, 2001; Rosegrant et al., 2002). Agriculture remains the sector with the highest water consumption globally. Consequently, the development and implementation of water-efficient and conservation-focused agricultural practices are vital for promoting sustainable development within the agricultural sector (Li et al., 2023).

Among the water-efficient practices, drip irrigation has emerged as one of the most effective solutions to address water scarcity. Drip irrigation involves the efficient and frequent delivery of water and nutrients directly to the crop root zone through emitters or drippers, significantly reducing water wastage (Ding et al., 2019; Bajpai & Kaushal, 2020; Moursy et al., 2023). The adoption of drip irrigation systems substantially improve water use efficiency by 60-200%, conserve water by 20-60%, and reduce fertilizer usage by 20-33%, enhances crop quality and increases crop yields by 7-25% compared to conventional irrigation methods (Guo & Li, 2024). Drip irrigation systems are particularly valued for their ability to deliver uniform water distribution across agricultural fields, ensuring reduced water loss from deep percolation, soil evaporation and runoff maintaining consistent moisture levels critical for healthy crop growth (Gil et al., 2008; Guo & Li, 2024).

Despite these advantages, conventional drip irrigation systems rely on pipes primarily made of polyvinyl chloride (PVC). These materials are often imported, making them expensive and unaffordable for small-scale farmers (James, 1988; Fipps, 2003). Additionally, the production and disposal of PVC pipes pose environmental concerns due to their non-biodegradable nature. Given these challenges, the use of bamboo as an alternative material for constructing drip irrigation systems presents a promising solution. Bamboo is widely recognized as a "non-timber renewable forest product" and encompasses 1,575 species, which are used in a diverse range of applications (Paudyal et al., 2019).

The utilization of bamboo for water management is not a novel concept. In Meghalaya, a northeastern state of India, bamboo pipes have been used for water conveyance systems for over 200 years (Ryngnga, 2018). Sharma (2014) conducted an analysis of the physical properties of *Schizostachyum pergracile*, a bamboo species predominantly found in Manipur and extensively cultivated in Gujarat, revealing its suitability for use as pipes due to its favourable dimensions and structural characteristics. Subsequently, Agossou et al. (2017) highlighted the broader utility of bamboo, emphasizing its natural abundance, biodegradability, and cost-effectiveness as key attributes that position it as a sustainable and eco-friendly alternative to plastic components in drip irrigation systems, particularly in resource-limited contexts. Introducing bamboo as an alternative to conventional materials for drip irrigation systems offers significant benefits for small-scale farmers. Bamboo is cost-effective, environmentally friendly, and less polluting compared to PVC and asbestos-cement pipes.

The primary objective of this study is to evaluate the performance of bamboo, particularly *Schizostachyum pergracile*, in a controlled low-pressure drip irrigation system, exploring its potential as a sustainable alternative for small-scale farming.

2. MATERIALS AND METHOS

The methodology involves a detailed sequence of analyses, beginning with an overview of the study area, followed by a description of the drip irrigation system. This includes the experimental setup and an evaluation of the system's hydraulic performance through the assessment of various uniformity parameters such as the coefficient of uniformity, coefficient of variation, friction loss, pressure-discharge relationship, emitter discharges, and surface wetting patterns. Finally, the economic aspects of laying the drip irrigation system are analysed to provide a comprehensive understanding of its feasibility and efficiency.

2.1. Study Area

The study was carried out at Bambusetum, College of Forestry (ACHF), Navsari Agricultural University located at 20°55'21.29" N latitudes, 75°54'16.00" E longitudes (Figure 1).

2.2. Bamboo Drip Irrigation Setup

Schizostachyum pergracile was chosen as the species for the construction of the bamboo drip irrigation system, taking into account its suitability owing to its straight culms, breakable internodes, hollowness, and degree of availability (Ryngnga, 2018). The irrigation system was evaluated in a subunit comprising a mainline (20 m in length) and six

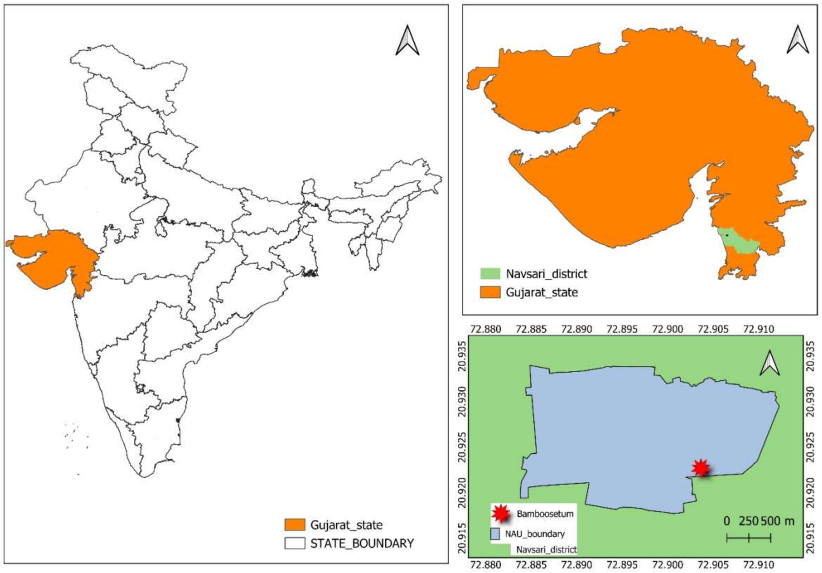
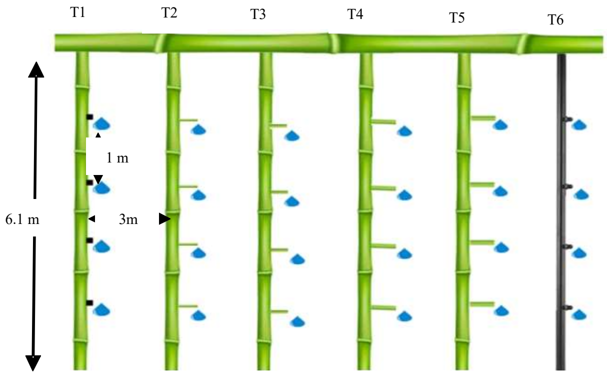


Figure 1. Location map of study area

lateral lines, each 6.1 m long and spaced 3 m apart, made of bamboo and set up on a flat surface. The septa of the nodes were removed using a metal rod and a hammer. Emitters were spaced 1 m apart on each lateral, with six drip points drilled using a sharp drill (Figure 2). The mainline and laterals were joined using a special adhesive blend of bamboo dust and Fevicol (serial no: 77345418) in equal proportions. For enhanced strength and waterproofing, an additional coating of Bondtite (parts A and B) was applied. The formal longevity testing was not conducted, similar composite bonding methods using wood adhesives have demonstrated adequate durability in short-term field use under low-pressure irrigation (Ella et al., 2013).

There were two types of emitters used in this study. The first comprised six medi-infusion tubes inserted into the first lateral (T1) (Mofoke et al., 2004; Awe & Ogedengbe, 2011). The second to fifth laterals (T2 to T5) employed bamboo microtubes with internal diameters of 0.3 cm, 0.4 cm, 0.5 cm, and 0.6 cm, respectively, as emitters. A PVC lateral with conventional emitters served as the control (T6). All emitters were inserted into pre-drilled drip points on the respective laterals. Water was supplied using a 1.0 HP centrifugal motor pump, commonly used in small-scale irrigation setups. Operating pressures were recorded at the mainline inlet using a pressure gauge and corresponded to stabilized levels of 1.3, 1.4, and 1.5 kg/cm² achieved by adjusting the pump output. These pressure levels were used to assess emitter discharge and hydraulic performance under controlled low-pressure conditions representative of field-relevant scenarios. A test run was conducted prior to data collection to ensure proper functioning and to correct any leakage. The study was conducted for almost a period of 3 months. During the field assessment, catch cans were positioned beneath the emitters, and the system was operated for 5 minutes. The emitter discharge collected in the catch cans was then measured using a measuring cylinder based on volume. The discharge rate of the emitters was calculated through volumetric measurements over the specified time period.



T1: Bamboo lateral with six medi infusion tube emitters

T2: Bamboo lateral with six bamboo microtube of 0.3cm emitters

T3: Bamboo lateral with six bamboo microtube of 0.4cm emitters

T4: Bamboo lateral with six bamboo microtube of 0.5cm as emitters

T5: Bamboo lateral with six bamboo microtube of 0.6cm as emitters

T6: PVC pipe lateral with 6 drippers used as control

Figure 2. Layout of the drip irrigation system

2.3. Hydraulic Performance of Drip Irrigation System

The hydraulic performance of the drip irrigation system was evaluated using various uniformity parameters, including the coefficient of uniformity (CU), coefficient of variation (CV), friction loss (Hf), pressure-discharge relationship, emitter discharge, and surface wetting patterns. Discharge was recorded from each emitter under three pressure levels, resulting in 18 observations per treatment and 108 total observations across all treatments. These values were used to calculate mean discharge, CU, and CV and other parameters. In this study, coefficient of uniformity (CU) and coefficient of variation (CV) were used to evaluate the hydraulic performance of bamboo and PVC-based laterals. Additionally, the economics of installing the bamboo-based system was analysed and compared with a conventional PVC drip irrigation system.

2.4. Coefficient of Uniformity (CU)

The coefficient of uniformity (CU), defined by Christiansen (1942), measures the average deviation from the average depth of water application. It is influenced by factors such as operating pressure, emitter spacing, land slope, pipe size, and discharge variability. The formula for CU is (Eq. 1):

$$\mathrm{CU} = 1 - \frac{\sum_{\mathrm{i}=1}^{\mathrm{n}} qi - \bar{q}}{\mathrm{N}\bar{q}} \quad ....(1)$$

Where CU is the coefficient of uniformity, qi = emitter discharges in l hr^{-1}, q = average discharges in l/hr and N= total number of emitters in a lateral.

2.5. Coefficient of Variation (CV)

Flow variations among emitters of the same type can occur due to factors such as clogging from particulate matter, chemical precipitates, and organic growth, leading to non-uniform water application. Analysing these variations helps in the proper design of the drip system. The coefficient of variation (CV), a statistical parameter, was used to evaluate emitter discharge variations across laterals under different pressures. It was calculated using the formula defined by Keller and Karmeli (1974).

$$\mathrm{CV} = \frac{\mathrm{S}}{\bar{\mathrm{q}}} \;\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where CV stands for coefficient of variation, S stands for standard deviation of all emitters and q stands for average emitter discharges expressed in l/hr.

2.6. Friction Loss (Hf)

Friction loss or head loss (Hf) occurs due to the materials used in the drip irrigation system and the insertion of laterals and emitters. In bamboo drip systems, it is attributed to the bamboo material and the insertion of components. The Darcy-Weisbach equation (Michael, 2009), which is effective for small-diameter laterals, was used to calculate friction loss for average emitter discharges under pressure values of 1.3, 1.4, and 1.5 kg/cm^2. The equation 3 is as follows:

$$\mathrm{Hf} = \frac{\mathrm{fL}}{\mathrm{D}}\,\frac{\mathrm{V}^2}{2\mathrm{g}} \;\ldots\ldots\ldots\ldots (3)$$

Where Hf stands for head loss expressed in meters, f stands for friction factor which is 0.02 for bamboo (Merriam & Keller, 1978), L stands for length of the pipe expressed in meter, D stands for diameter of the pipe expressed in meter, V represents fluid flow velocity expressed in m/s and g represents acceleration due to gravity (9.8m/s).

2.7. Pressure Discharge Relationship

Pressure-discharge models were developed to optimize emitter selection and operating pressures. Data for pressures (1.3, 1.4, and 1.5 kg/cm^2) were measured, and emitter volumes were recorded. Using Microsoft Excel, an exponential function was fitted, and R² values were calculated to assess the correlation between pressure and discharge. Dripper flow rate is expressed as a function of pressure in the following manner (Eq. 4):

$\mathrm{Q} = \mathrm{Kp}^{\mathrm{X}}$ .....(4) (Keller & Karmeli, 1974)

Where Q stands for dripper discharge expressed in l hr^{-1}, K stands for coefficient of proportionality, P stands for operating pressure expressed as kg/cm^2 and X stands for emitter discharge exponent.

2.8. Discharge of Emitters

The discharge rate of each emitter was measured by collecting water in catch cans for 5 minutes, recording the volume in millilitres using a measuring cylinder, and repeating the process three times for accuracy. The values, converted to litres per hour, were averaged and calculated for pressures of 1.3, 1.4, and 1.5 kg/cm^2 (Michael, 2009), with pressure measured using a gauge connected to the main valve with the following equation 5:

$$\mathrm{Q} = \frac{\text{volume of water collected}}{\text{time}} \;\ldots..(5)$$

2.9. Surface Wetting Pattern

The surface wetting pattern was evaluated by measuring the horizontal wetted diameter at 10, 20, and 30 minutes after irrigation using a standard measuring tape. The wetted area was approximated as an ellipse using the formula A = πAB (Keller & Bliesner, 1990), where A and B represent half of the measured horizontal diameters along two axes (Eq.6).

$$\mathrm{A} = \pi * \mathrm{A} * \mathrm{B} \ldots\ldots(6)$$

Where, π (pi) value is 3.14, A stands for half of the horizontal diameter and B stands for half of vertical diameter.

2.10. Economics of Laying the System

The economics of the bamboo drip irrigation system was compared to a hypothetical conventional system using identical area, pipe length, lateral length, and number of emitters. Feasibility was assessed based on costs for cutting, assembling, and installation to determine its practicality for the forested watershed of South Gujarat.

3. RESULTS AND DISCUSSION

3.1. Coefficient of Uniformity (CU) and Coefficient of Variation (CV)

The PVC control (T6) exhibited the highest uniformity (CU ≥ 96.24%) and lowest variation (CV ≤ 0.05), (Table 1)

Table 1. Coefficient of uniformity and coefficient of variation in different treatments

Treatment	Coefficient of uniformity (%)			Coefficient of variation		
	Pressure range (kg/cm^2)					
	1.3	1.4	1.5	1.3	1.4	1.5
T1	89.77	88.40	87.99	0.1	0.15	0.14
T2	54.63	52.29	56.15	0.52	0.59	0.65
T3	51.21	51.21	50.89	0.52	0.63	0.59
T4	58.89	58.89	56.35	0.62	0.48	0.25
T5	81.59	81.59	56.35	0.36	0.25	0.55
T6	96.24	96.24	90.38	0.05	0.05	0.04

consistent with global standards for efficient micro-irrigation (Bralts et al., 1981; Solomon, 1985). Among the bamboo treatments, T5 (0.6 cm bamboo microtubes) performed best (CU: 81.59%, CV: 0.25–0.55), likely due to reduced internal friction and smoother discharge paths. These findings align with earlier observations that larger emitter diameters improve flow stability and uniformity in low-cost systems (Ella et al., 2013; Sokol et al., 2019). T1 (medi-infusion tubes) also performed reliably, with CU > 87%, showing potential for hybrid applications using commercial components in traditional materials. T2 and T3, which used smaller diameter bamboo emitters (0.3 and 0.4 cm, respectively), demonstrated the poorest performance. This may be attributed to higher head losses and clogging susceptibility due to narrower internal paths-issues well-documented in uniformity studies of non-compensating emitters (Gil et al., 2008).

Overall, while bamboo systems cannot yet match the uniformity of standard PVC setups, treatments like T5 and T1 show promising performance, especially under controlled pressure conditions. Moreover, these systems offer a sustainable and low-cost alternative, particularly for smallholder or hill farming regions (Jeeva et al., 2006; Maurya & Singh, 2021).

3.2. Flow Dynamics and Efficiency

The T6 (PVC lateral) had the lowest friction loss (0.0015–0.00168 m), highest discharge coefficient (6.606), and a stable pressure–discharge exponent of 0.886 (Table 2). Exponents between 0.4 and 1.0 indicate predictable and hydraulically stable flow. In contrast, bamboo microtubes-especially T2 (0.3 cm)-had higher friction losses and unstable exponents (>10), reflecting pressure-sensitive discharge (Kirnak et al., 2004). T5 (0.6 cm) performed better, with lower friction loss (0.0202–0.0344 m) and a moderate exponent (6.169), consistent with findings that larger diameters and smoother surfaces improve uniformity (Obio et al., 2013; Chen et al., 2022). Modifications like bore standardization, internal polishing, or resin/wax coatings can reduce resistance and improve flow stability (Awe & Ogedengbe, 2011). Although tested under moderate pressure (1.3–1.5 kg/cm²), the high CV in bamboo treatments reflects discharge inconsistency more than pressure effect. Prior studies confirm that optimized bamboo systems can function efficiently under low-pressure, gravity-fed conditions, making them suitable for smallholder use (Awe & Ogedengbe, 2011; Obio et al., 2013).

3.3. Discharge of Emitters

There were significant differences in emitter discharge across treatments (Table 3). The PVC pipe drippers (T6), with a rated discharge of approximately 8 l/hr, maintained consistent output across pressures (7.94-8.42 l/hr). This stability can be attributed to low friction loss, high manufacturing precision, and pressure-regulated discharge, typical of commercial emitters (Bralts et al., 1981; Keller &

Table 3. Average discharge of emitters (l/hr) with pressure (kg/cm^2)

Treatment	Average discharge of emitters (l/hr) with pressure (kg/cm^2)		
	1.3	1.4	1.5
T1	1.17	1.56	1.28
T2	3.15	2.61	0.52
T3	5.81	2.95	12.34
T4	11.48	7.84	18.68
T5	14.21	8.01	18.54
T6	7.94	8.28	8.42
CD (p-0.05)	4.56	2.78	7.13
C.V. (%)	52.54	44.91	60.21

Table 2. Friction loss and pressure discharge relationship of the emitters

Treatment	Friction loss (mm)			Developed model	Pressure discharge		
	Pressure range (kg/cm^2)				Discharge Coefficient	Exponent	R²
	1.3	1.4	1.5				
T1	0.03327	0.05915	0.03982	$Q=0.654P^{2.030}$	0.654	2.030	0.95
T2	0.03176	0.02180	0.00087	$Q=0.018P^{12.88}$	0.018	12.88	0.84
T3	0.02564	0.00661	0.11567	$Q=0.186P^{10.12}$	0.186	10.12	0.99
T4	0.03280	0.01530	0.08685	$Q=1.366P^{6.039}$	1.366	6.039	0.88
T5	0.02020	0.00642	0.03438	$Q=1.640P^{6.169}$	1.640	6.169	0.96
T6	0.00150	0.00163	0.00168	$Q=6.606P^{0.886}$	6.606	0.886	0.99

Bliesner, 1990). T6 delivered uniform wetting, aligning with its low CV and high CU observed earlier. In contrast, bamboo microtubes showed greater variability. For instance, T2 (0.3 cm) experienced a sharp discharge decline from 3.15 to 0.52 l/hr with increasing pressure. This may be due to increased head loss, localized clogging, or flow turbulence in the narrow lumen at higher velocities, phenomena well-documented in drip systems using unrefined or small-diameter materials (Kirnak et al., 2004, Gil et al., 2008). Similarly, the sharp increase in discharge observed in T3 and T4 under rising pressure may result from sudden shifts from laminar to transitional or turbulent flow in inconsistently polished bamboo tubes. Minor changes in surface roughness or lumen shape can dramatically alter flow behavior, leading to the observed non-linearity-especially in naturally variable materials like bamboo. On the other hand, T5 (0.6 cm) exhibited the highest discharge (14.21–18.54 l/hr), indicating that larger internal diameters reduce resistance and enhance flow capacity (Chen et al., 2022). Skaggs et al. (2004 , 2010) demonstrated that wider emitters promote greater horizontal soil wetting, which complements findings that bamboo microtubes, when properly dimensioned and polished, can mimic the performance of PVC systems (Bidondo et al., 2010; Awe & Ogedengbe, 2011)

3.4. Surface Wetting Pattern

Surface wetting patterns differed markedly across treatments (Table 4). T5 (0.6 cm bamboo) produced the widest horizontal spread-up to 6032 cm^2 at 1.5 kg/cm^2 in 30 min-while T2 (0.3 cm) managed only ~1948 cm^2, reflecting its lower discharge and higher flow resistance. These findings align with studies showing that higher emitter discharge leads to increased lateral spread, especially in sandy loam soils where infiltration capacity is high and vertical penetration exceeds lateral expansion (CGWB, 2013; Naglic et al., 2014; Onwuegbunam et al., 2019). The wetted diameters observed in sandy loam setups ranged from 15 to 23 cm in 30-75 min, with limited depth spread supporting the trends (Table 4). The study measured only horizontal wetted area as vertical infiltration data were not collected. This methodological limitation will be addressed in future investigations. The high discharge can risk surface ponding in slower soils, no ponding was observed, suggesting that the sandy loam in this trial accommodated the flow without exceeding its infiltration capacity. From an agronomic standpoint, a broader wetted surface improves moisture availability in the root zone, benefiting shallow-rooted crops and enhancing nutrient uptake efficiency (Kandelous & Simunek, 2010). Hence, emitter selection and spacing must be tailored not only for hydraulic performance but also for crop-specific rooting depth and water requirements to maximize irrigation efficiency (Bozkurt et al., 2006). Optimizing bamboo microtubes by increasing diameter and reducing internal roughness has clear benefits, delivering wider and more uniform surface coverage. However, future validation should include vertical infiltration behaviour and soil-specific performance, especially when considering irrigation in different soil textures.

3.5. Economics of Laying the System

The cost analysis indicates that bamboo drip irrigation is marginally more economical than conventional systems, with a total cost of ₹17,280 per 122 m^2 compared to ₹17,650 for conventional drip (Table 5). The slightly lower initial investment (₹16,530 vs. ₹16,720) and maintenance costs (₹331 vs. ₹349) contribute to this difference, while water and labour expenses remain constant. Although the immediate financial savings are modest, bamboo offers certain practical advantages such as local availability, biodegradability, and reduced material dependence on plastics. In regions where bamboo is abundant and locally treated to enhance

Table 4. Surface wetting pattern across different treatments

Treatment	Area (cm^2) covered under the surface wetting pattern of 1.3kg/cm^2			Area (cm^2) covered under the surface wetting pattern of 1.4kg/cm^2			Area (cm^2) covered under the surface wetting pattern of 1.5kg/cm^2		
	10 min	**20 min**	**30 min**	**10 min**	**20 min**	**30 min**	**10 min**	**20 min**	**30 min**
T1	461.58	659.40	967.12	372.87	631.92	1001.66	439.60	678.24	879.20
T2	760.05	1285.27	1832.57	888.54	1639.90	1889.99	607.96	1113.15	1948.19
T3	854.08	1601.63	2136.00	795.36	1236.35	1609.25	907.36	1848.45	2431.44
T4	1732.25	2748.15	2092.38	505.54	1287.85	1880.88	1002.34	2431.44	3590.93
T5	1753.29	3047.03	4039.61	1810.83	2776.02	3202.70	1298.79	3360.12	6032.14
T6	961.62	1103.71	1318.80	827.39	1103.71	1350.20	753.60	960.84	1193.20

Table 5. Comparative statement of cost of bamboo drip and conventional drip

Particulars	Bamboo drip irrigation	Conventional drip irrigation
Initial investment (Rs/122m^2)	16,530	16,720
Maintenance cost (Rs/122m^2)	331	349
Water cost (Rs)	230	230
Labour cost (Rs)	700	700
Total cost (Rs)	17280	17650

durability, components may be reused across short-term cycles-particularly in gravity-fed systems where pipes are aboveground-leading to lower per-season costs. PVC systems are more durable and standardized, bamboo-based systems may still serve as a low-cost, transitional alternative for smallholder or resource-constrained contexts with access to bamboo. Previous studies have highlighted its application in traditional irrigation in such regions, supporting its use with appropriate design and handling practices (Swami, 2021, Pan et al., 2023). However, the environmental sustainability of bamboo-based systems must be weighed against their limited lifespan under field conditions. Studies indicate that untreated bamboo is susceptible to biodegradation and fungal attack, often deteriorating within 6–24 months (Kaur et al., 2016 and Liu, 2023). The preservative treatments using crude lake salt and borax–boric acid have been shown to significantly extend the service life of bamboo under fungal exposure (Mwanja et al., 2024), making such systems more viable for repeated seasonal use. These findings highlight the need for proper treatment and maintenance protocols if bamboo-based drip systems are to be considered for wider adoption.

4. CONCLUSION

This study evaluated the hydraulic performance, surface wetting patterns, and cost effectiveness of bamboo microtube-based drip irrigation systems under varying lateral diameters. The bamboo systems are capable of delivering water, they currently fall short of standard performance benchmarks. The high discharge rates, poor emission uniformity, and high variability were observed, especially in treatments with smaller diameters. These inconsistencies limit the reliability of the system under variable pressure conditions. Although larger-diameter bamboo laterals showed improvements in discharge rate and lateral wetting area, these gains were not sufficient to achieve acceptable uniformity or flow stability. Furthermore, only the horizontal spread of moisture and absence of vertical infiltration restricts the completeness of the wetting pattern analysis. Additionally, the high discharge rates may risk surface ponding, which runs counter to the principles of efficient drip irrigation. There was some potential for economic benefit in bamboo-abundant regions, the savings alone do not justify widespread adoption given the hydraulic limitations.

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Authors' Contributions

Haripriya S: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing the original draft. P.K. Shrivastava: Conceptualization, Supervision, Validation, Writing – review & editing. Nirav Pampaniya: Data analysis and tabulation. Dileshwar Nayak: Technical support, Instrumentation. Jayendra Chawda: Field experimentation, Data collection. Jayesh Pathak: Supervision, Project administration

Conflict of Interest

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

Declaration of Generative AI and AI-assisted technologies

The authors declare that no generative AI tools were used in the preparation of this manuscript.

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Predicting Wheat Yield by Principal Component and Regression Techniques based on Morpho-Physiological and Quality Traits

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Abstract: The study reviewed morpho-physiological and quality traits of 194 wheat genotypes to identify the traits determinant to wheat yield exploring principal component and regression techniques. The experiment was performed at Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana) during 2023-24 crop season. Principal component analysis identified eight components that cumulatively explained 66.30 % of the total variation of 25 morpho-physiological and quality traits. The first principal component was associated with grain yield, grain filling duration, harvest index and 1000-grain weight and also showed strong association with quality traits *viz*., crude protein, gluten content and sedimentation value. The coefficient of multiple regression (R^2) explained 86.74% of the variability and showed positive correlation between the dependent and independent variables. The stepwise regression analysis identified four traits - harvest index, biological yield per plot, chlorophyll content, and days to 50% heading - as significant contributors to grain yield in wheat. These variables were sequentially retained in the model based on their statistical significance indicating substantial proportion of the variation in yield. Hence, these traits emerged as the most reliable predictors of grain yield and may serve as important selection criteria in wheat improvement programmes.

Keywords: Wheat, Principal component, Regression, Yield traits.

1. INTRODUCTION

In India, wheat is the second most important staple food crop after rice, occupied over 32.76 million hectares and produced 117.51 million tonnes with national average productivity of 35.87 quintals per hectare (ICAR-IIWBR, 2025). While sustaining and further enhancing wheat crop productivity is central to national food security under population pressure and erratic climatic conditions. Grain yield, being complex quantitative trait, is governed by the interaction of several morpho-physiological and phenological attributes, making direct selection for yield less effective.

Understanding the complex interaction between grain yield and its component traits is therefore essential for identifying reliable selection criteria in breeding programmes. Multivariate statistical approaches provide an effective framework for disentangling such complex interrelationships among traits and for identifying those variables that contribute most significantly to yield variation. Among these, principal component analysis (PCA) has been widely used to reduce data dimensionality and to identify major sources of variation by grouping correlated traits into a smaller number of independent components (Das et al., 2017). PCA thus helps in minimizing redundancy and highlighting key traits influencing yield performance.

Regression analysis, particularly stepwise regression, is another powerful multivariate tool used to quantify the contribution of individual plant traits to grain yield and to identify an optimal subset of predictors. In cereal crops, yield is influenced by several interrelated attributes, and regression-based approaches assist breeders in prioritizing traits with higher predictive ability and selection value (Budka et al., 2015). Previous studies in wheat have demonstrated the usefulness of regression techniques for identifying yield-determining traits; however, many such studies were limited either in the number of genotypes evaluated or in the range of traits considered, and often relied primarily on weather-based predictors (Przulj and Momcilovic, 2011; Kumar et al., 2014).

Although climatic variables play a major role in determining crop yield, reliance solely on weather-based models neglects the contribution of intrinsic plant characteristics that directly reflect genetic potential and physiological efficiency. Integrating morpho-physiological and quality traits into yield prediction models can therefore provide a more biologically meaningful and breeder-relevant understanding of yield formation. However, comprehensive studies combining large genotype sets with multiple statistical techniques remain limited, particularly for major wheat-growing regions of India.

In view of these considerations, the present study was undertaken using 194 diverse wheat genotypes to (i) assess the interrelationships among key morpho-physiological and quality traits, (ii) identify the major sources of variation using Principal Component Analysis, and (iii) develop an efficient yield prediction model through stepwise regression analysis. The results are expected to aid in identifying reliable trait-based predictors of grain yield and to support more effective selection strategies in wheat improvement programmes.

2. MATERIALS AND METHODS

2.1. Experimental Area

The experiment was performed during crop season 2023-24 at Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (Latitude 29°10′N, Longitude 75°46′E and Altitude 215.2 m).

2.2. Experimental Material and Design

The study included a set of 194 wheat genotypes (Table 1), planted and evaluated in Augmented Design. Each genotype was planted in two rows spaced at 20 cm apart, occupied a plot size of 2.5 m × 0.4 m. The experiment was conducted under timely sown and irrigated conditions, and the recommended agronomic package of practices (Anonymous, 2023). The observations were recorded for 25 morpho-physiological and quality traits (Table 2).

2.3. Methodology

Canopy temperature (°c) was recorded using a portable infrared thermometer (IRT) 'Model AG-42, Teletemp crop Fullerton'. The difference between canopy temperature and ambient temperature was calculated to determine canopy temperature depression. Observations were taken at anthesis and subsequently at 15 days after anthesis. Relative Water Content (%) was calculated using the method outlined by Barrs and Weatherley (1962). Chlorophyll content (SPAD value) was recorded using a portable optical chlorophyll meter (Model No. Minolta SPAD 502 Plus) at anthesis. The quality traits *viz*., crude protein (%), gluten content (%) and sedimentation value (ml) were estimated by Infratec 1241 grain analyzer. Hectolitre weight (Kg/hl) was measured with the help of test weight instrument developed at IIWBR, Karnal. It represents the weight of 100 litres of wheat and provides a general estimate of grain soundness, density and flour yield.

2.4. Statistical Analysis

The principal component analysis (PCA) was employed to reduce the number of variables into a few correlated components and to identify traits that load the most in explaining the observed variability of original set in order to know the variables (traits) associated with grain yield. Further, to quantify the contribution of particular variable toward grain yield, regression analysis was performed. The recorded data was analyzed for principal component and regression using R Studio software (2025.05.01+513) (R Core Team, 2025).

3. RESULTS AND DISCUSSION

3.1 Identification of Major Sources of Variation

In the present investigation, PCA was performed on 25 morpho-physiological and quality traits of 194 wheat genotypes (Table 2). The first eight principal components, each with an eigenvalue greater than one, collectively explained 66.30% of the total variation embedded in the wheat genotypes. The remaining components contributed only marginally to the variation and were therefore of limited practical value for wheat improvement.

Among these, the first principal component (PC1) accounted for 15.61% of the total variation, followed by PC2, PC3, PC4, PC5, PC6, PC7 and PC8. Earlier studies also indicated similar findings (Bhatti et al., 2022; Abdelghany et al., 2023; Atsbeha et al., 2023; Khan et al., 2023). Kumar et al. (2021) analyzed 440 bread wheat genotypes for 12 agro-morphological and physiological traits and reported first five components with eigenvalues exceeding one that explained 63.84% of the total variation. Comparable contributions of principal components have also been described in wheat by Prakash et al. (2023); Arshad et al. (2025) and Yadav et al. (2025).

3.2. Assessment of the Interrelationships among Key Morpho-physiological and Quality Traits

The factor loadings of individual traits on the eight principal components indicated the strength of association of each trait with the corresponding principal component. PC1 was primarily associated with yield-related traits such as grain yield, grain filling duration, harvest index, and 1000-grain weight, along with quality attributes such as crude protein, gluten content, and sedimentation value (Table 2). PC2 was defined by number of grains per spike, spikelets per spike, and flag leaf breadth, and also showed strong loadings for phenological traits such as days to heading, anthesis, and maturity. PC3 was dominated by flag

Table 1. List of wheat genotypes used in the present study

Genotypes	Genotypes	Genotypes	Genotypes
DDW 48	GW 1339(d)	HS 679	PBW 821
BFKW 7	GW 1346(d)	HS 681	PHSL 10
BNSR 6	GW-2014-596	HS 661	QLD 118
CG 1029	GW 499	HUW 338	QLD 120
CG 1036	GW 509	IC 212176	QLD 121
CG 1040	HD 2967	IC 376265	RWP-2019-38
CG 1023	HD 3369	IC 78841	HI 1617
GW 477	HD 3386	IC 252459	WSM 138
DBW 166	HD 3407	IC 252454	RAJ 4541
DBW 187	HD 3411	IIWBR DN 502	RAJ 3448
DBW 243	HD 3437	IIWBR PHY 2	RAJ 3765
DBW 278	HD 3438	HPW 360	RAJ 4238
DBW 302	HD 3440	JKW 261	RW 5
DBW 308	HD 2864	K 1616	RWP-2017-21
DBW 316	HD 2932	K 1317	RWP-2018-31
DBW 318	HD 3086	RWP-2019-41	RWP-2018-32
DBW 325	HD 3406	KARAN POSHAN 1	RWP-2018-3
DBW 342	HD 3293	KARAN POSHAN 2	Sonalika
DBW 357	HI 1544	KRL 283	UAS 3022
DBW 370	HI 1650	KRL 35	UAS 478 (d)
DBW 371	HI 1653	KRL 99	UAS 481 (d)
DBW 372	HI 1654	LBP-2017-2	UASQ 332 (d)
DBW 377	HI 1655	LBP-2023-24	Unnat PBW 550
DBW 400	HI 1665	LBP-2023-25	UP 2909
DBW 107	HI 8818 (d)	KHTW 1	UP 2938
DBW 110	HI 8826 (d)	MACS 4100 (d)	UP 2944
DBW 168	HI 8827 (d)	MACS 6768	VL 3024
DBW 173	HI 8830 (d)	MACS 6795	VL 3028
DBW 252	HI 8839 (d)	MACS 4058(d)	VL 3035
DBW 303	HI 8840 (d)	MP 1323	VL 2028
DBW 221	HI 8846 (d)	MP 1378	VL 2041
DBW 327	HI 8847	GW-2010-288	VL 3010
DBW 222	HI 1633	MP 3535	VL 2015
DBW 322	HI 1636	MPO 1357 (d)	VL 3022
DBW 332	HI 1634	NIAW 4028	VL 3020
DBW 296	HI 8627	QLD 122	VL 3021
DBW 328	HI 8802(d)	PBS-NGSN-23-01	WAP 2320
UP 2903	HI 8805(d)	PBS-NGSN-23-02	WAP 2321
HUW 838	HI 8823(d)	PBW 766	WAP 2210
GW 513	HI 8808	PBW 803	WAP 2211
DBW 88	GW 1348 (d)	PBW 824	WAP 2212
DBWH 221	HPW 484	PBW 835	WCF-12-19
DDW 47 (d)	HPW 487	PBW 869	WCF-12-208
DTW 119	HPW 489	PBW 870	WCF-12-61
DWAP 1608	HPW 493	PBW 875	WH 1402
GW-2019-957	HPW 368	PBW 902	WH 1403
GW 528	HPW 373	PBW 723	WHD 965 (d)
GW 532	HS 545	PBW 752	
GW 547	HS 628	PBW 771	

leaf length and spike length, while PC4 was associated with plant height and awn length. PC5 was characterized by chlorophyll content, relative water content, and peduncle length, whereas PC6 was strongly associated with canopy temperature depression and biological yield. PC7 showed a strong relationship with hectolitre weight, and PC8 with number of tillers per meter.

The clear grouping of traits within components illustrates the successful reduction of 25 correlated variables into eight independent dimensions, which together explained more than two-thirds of the total variability. Similar clustering of traits on principal components in wheat has been reported by Shiramakrishnan et al. (2016); Dagnaw et al. (2023) and Vijeth et al. (2025).

Table 2. Variance attributable to principal components with loading of different variables

Variance	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Eigen value	3.90	3.11	2.47	1.77	1.54	1.42	1.22	1.15
Percentage of variance	15.61	12.43	9.88	7.07	6.16	5.69	4.88	4.58
Cumulative percentage of variance	15.61	28.04	37.92	44.99	51.15	56.84	61.72	66.30

Factor loadings

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
DH	0.474	0.694	-0.460	-0.026	0.004	0.116	0.003	-0.049
DA	0.497	0.683	-0.477	-0.041	0.005	0.112	-0.054	-0.039
DM	-0.059	0.473	-0.381	0.110	0.320	-0.183	0.438	0.174
PH	0.086	-0.114	0.265	-0.514	0.450	0.078	-0.222	0.172
AL	-0.146	0.297	-0.243	0.428	-0.087	-0.042	-0.110	0.372
FLL	0.160	0.352	0.473	0.185	0.247	-0.237	-0.173	0.102
FLB	0.064	0.518	0.240	-0.015	0.154	-0.207	-0.075	-0.136
NT/M	-0.133	0.130	0.041	0.342	0.313	-0.253	0.201	-0.509
SL	0.009	0.270	0.555	-0.487	-0.288	-0.090	0.026	-0.139
PL	-0.013	0.263	0.284	0.174	0.469	0.011	0.214	0.233
NS/S	0.101	0.499	0.420	-0.473	-0.038	0.034	0.136	0.108
NG/S	-0.122	0.439	0.134	-0.409	0.145	-0.107	0.119	0.105
BY	-0.404	0.387	0.341	0.264	0.072	0.446	-0.026	-0.360
GY	-0.580	0.424	0.350	0.277	-0.087	0.385	0.091	-0.120
GFD	-0.581	-0.427	0.265	0.121	0.209	-0.250	0.355	0.157
HI	-0.492	0.075	0.122	0.023	-0.267	0.003	0.299	0.314
TGW	-0.523	0.120	-0.041	0.056	0.077	0.213	-0.291	0.329
CTD 1	-0.183	-0.231	0.018	-0.128	0.109	0.551	0.068	-0.157
CTD 2	-0.158	0.046	-0.174	-0.055	0.159	0.470	-0.019	0.248
CC	0.235	0.205	0.283	0.178	-0.539	0.082	0.164	0.254
RWC	0.081	0.230	0.343	0.300	-0.396	-0.258	-0.221	0.008
CP	0.819	-0.269	0.290	0.180	0.091	0.152	0.149	0.057
GC	0.723	-0.074	0.389	0.266	0.072	0.273	0.128	0.082
SV	0.799	-0.275	0.237	0.177	0.140	0.097	0.055	0.108
HW	-0.148	0.066	0.211	0.236	0.260	-0.106	-0.613	0.105

DH-Days to 50% heading, DA-Days to anthesis, DM-Days to physiological maturity, PH-Plant height (cm), NT/M-Number of effective tillers per meter, SL-Spike length (cm), PL-Peduncle length (cm), FLL-Flag leaf length (cm), FLB-Flag leaf breadth (cm), AL-Awn length (cm), NS/S-Number of spikelets per spike, NG/S-Number of grains per spike, BY-Biological yield per plot (g), GY-Grain yield per plot (g), GFD-Grain filling duration, HI-Harvest index (%), TGW-1000 grain weight (g), CTD 1-Canopy temperature depression at anthesis(°C), CTD 2-Canopy temperature depression at 15 days after anthesis (°C), RWC-Relative water content (%), CC-Chlorophyll content (SPAD value), CP-Crude protein (%), GC-Gluten content (%), SV-Sedimentation value (ml) and HW-Hectolitre weight (kg/hl)

In the biplot analysis, the variables contained in different quadrants depicts the correlation among them, facilitate in determining the variables contributing to yield. The angle between vectors defines the relationship of variables, when the angle between vectors is < 90 (acute) degrees indicated the positive association and when the angle is > 90 (obtuse) degrees that explained negative correlation. First two principal components explained 28.04 % variability, were exploited to construct biplot for comparing relationship between variables (Figure 1). The variables occupied their position in a particular quadrant signifies positive association among them. The biplot displayed positive association of grain yield with variables placed in 3rd quadrant. In addition, grain filling duration, flag leaf length and breadth, days to heading and anthesis, spike length, number of spikelets per spike and relative water content also exhibited positive association with grain yield, as indicated by acute angles between their vectors. The remaining variables were negatively correlated with grain yield, deciphered by obtuse angles between their vectors. Similar approach has also been followed in wheat by Vijeth et al. (2025) for clear visualization of variable representation based on PCA.

3.3. Development of Yield Prediction Model

To further dissect the contribution of individual traits to grain yield, stepwise regression analysis was performed (Table 3). Seven traits were retained in the final model: days to 50% heading, biological yield per plot, harvest index, 1000-grain weight, canopy temperature depression at 15 days after anthesis, chlorophyll content, and sedimentation value. Among these, harvest index, biological yield, chlorophyll content, and days to 50% heading showed

Sr. No.	Variables
1	DH
2	DA
3	DM
4	PH
5	AL
6	FLL
7	FLB
8	NT/M
9	SL
10	PL
11	NS/S
12	NG/S
13	BY
14	GY
15	GFD
16	HI
17	TGW
18	CTD 1
19	CTD 2
20	CC
21	RWC
22	CP
23	GC
24	SV
25	HW

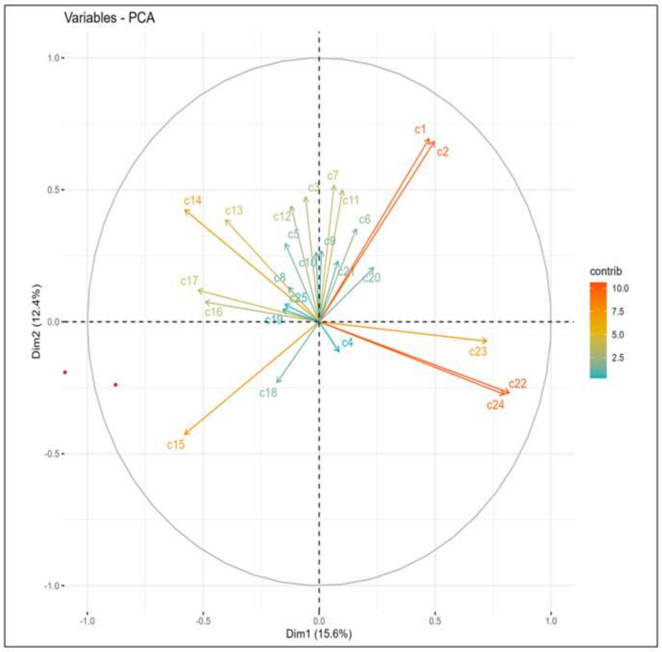

Figure 1. Biplot of morpho-physiological and quality traits studied (DH-Days to 50% heading, DA-Days to anthesis, DM-Days to physiological maturity, PH-Plant height (cm), NT/M-Number of effective tillers per meter, SL-Spike length (cm), PL-Peduncle length (cm), FLL-Flag leaf length (cm), FLB-Flag leaf breadth (cm), AL-Awn length (cm), NS/S-Number of spikelets per spike, NG/S-Number of grains per spike, BY-Biological yield per plot (g), GY-Grain yield per plot (g), GFD-Grain filling duration, HI-Harvest index (%), TGW-1000 grain weight (g), CTD 1-Canopy temperature depression at anthesis(°C), CTD 2-Canopy temperature depression at 15 days after anthesis (°C), RWC-Relative water content (%), CC-Chlorophyll content (SPAD value), CP-Crude protein (%), GC-Gluten content (%), SV-Sedimentation value (ml) and HW-Hectolitre weight (kg/hl)

significant regression coefficients, suggesting that improvement in these traits would substantially enhance grain yield. The model explained 86.74% of the variation in grain yield; adjusted R^2, indicating robustness and absence of overfitting. These findings emphasize the priority of selection for harvest index, biological yield, chlorophyll content, and favorable heading time in wheat breeding programs. Comparable yield predictors have been highlighted in previous studies (Abderrahmane et al., 2013; Nasri et al., 2014; Kumar et al., 2016; Fouad, 2018; Eid, 2020; Pirzado et al, 2021; Karuna et al., 2024).

Based on stepwise regression, the following predictive model for grain yield (y) was obtained (eq. 1):

$y = -620.8+1.49DH + 0.27BY+11.61HI+2.2CC$…………………..(1)

where DH = days to 50% heading, BY = biological yield per plot, HI = harvest index, and CC = chlorophyll content.

Scatter plots of these predictors against grain yield (Figure 2) confirmed their strong associations, consistent with patterns observed by Marzougui and Chargui (2018). Similar regression-based yield prediction approaches in wheat have also been reported by Bhutto et al. (2016), Shastry et al. (2017), Hu et al. (2018) and Shar et al. (2024).

AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) values indicated model fit and complexity, with sharp decreases signifying major

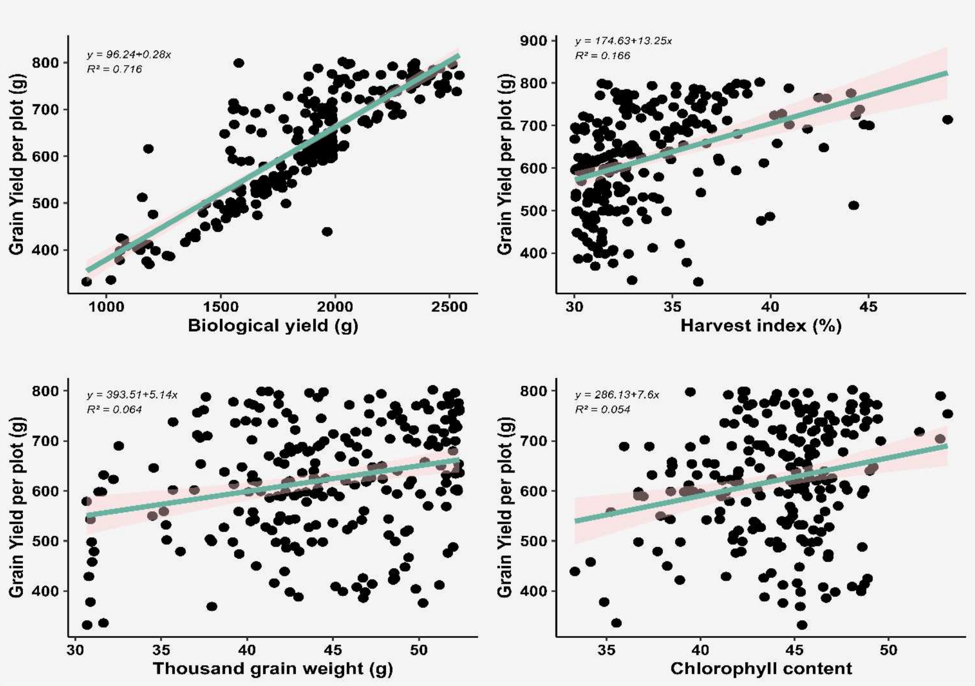

Figure 2. Scatter diagram based on simple regression of grain yield with important variables

Table 3. Stepwise regression analysis

Variables	Regression coefficients	Estimate Std. Error	t value	Pr (>|t|)	Significance
(Intercept)	-620.8	79.01	-7.86	0	***
DH	1.49	0.63	2.37	0.02	*
BY	0.27	0	32.67	<0	***
HI	11.61	0.82	14.22	<0	***
TGW	0.94	0.55	1.73	0.08	.
CTD2	5.15	3.12	1.65	0.09	.
CC	2.2	0.86	2.56	0.01	*
SV	0.57	0.31	1.86	0.06	.

Significance: '' 0.001 '**' 0.01 '*' 0.05 '.' 0.1; Multiple R^2: 0.8674; Adjusted R^2: 0.8632***

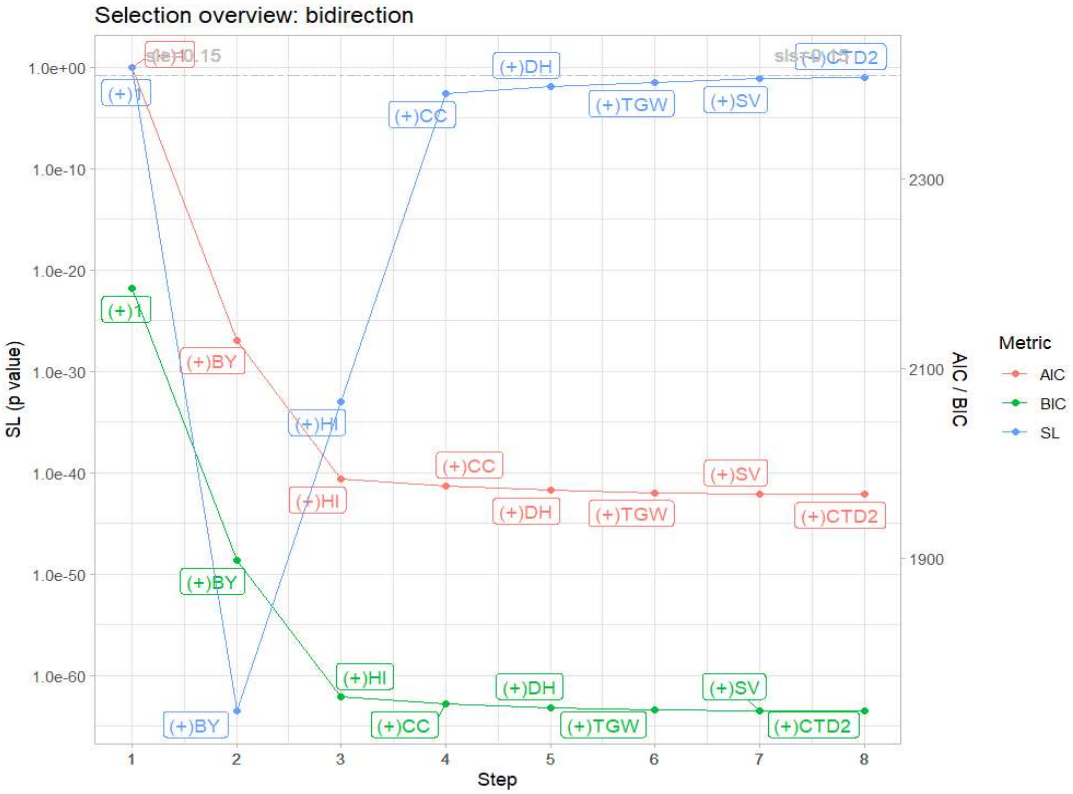

Figure 3. Stepwise regression illustrating trait selection in both forward and backward direction

contributors (Figure 3). Biological yield was the strongest predictor, entering first with the largest reduction in AIC/BIC, followed by harvest index. Days to 50% heading and chlorophyll content improved the model moderately, whereas the remaining traits contributed less substantially. Previous studies have also explored the use of principal components as predictors for yield forecasting in wheat (Goyal and Verma, 2018; Chetna et al., 2022).

4. CONCLUSION

Principal component identified eight components with eigen value exceeding one that altogether explained 66.30 % of the total variation of 25 morpho-physiological and quality traits of 194 genotypes. The first principal component was associated with grain yield, grain filling duration, harvest index and 1000-grain weight and also showed strong association with quality traits *viz*., crude protein, gluten content and sedimentation value. The coefficient of multiple regression showed a positive correlation between the dependent and independent variables, explaining 86.74% of the variability. Similarly, the modified (Adj.) indicated that, even with the modification, the regression model closely reflects the variance in the data set without changing the parameter. Stepwise regression revealed that biological yield, harvest index, chlorophyll content and days to 50% heading proved to be the main predictors of wheat yield.

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Authors' Contributions

P: Execution of field experiment, data recording and compilation, YK: Conceptualization, manuscript writing & finalization, DK: Statistical analysis. All the listed authors read and approved the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Declaration of Generative Ai and Ai-Assisted Technologies in the Writing Process

The authors declare that none of AI tools have been used in the writing process.

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Flood Risk Assessment Using GIS and Remote Sensing Technology in Telangana State, India

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***Abstract*:** Recurring flash floods pose a serious hazard in several parts of Telangana State, India, necessitating a comprehensive assessment of flood-prone areas. This study aims to delineate flood hazard risk zones across Telangana using a multi-criteria decision analysis framework integrated with Remote Sensing (RS) and Geographic Information System (GIS) techniques. Key flood influencing parameters; including slope, elevation, aspect, rainfall, drainage density, land use/land cover, soil type, normalized difference vegetation index (NDVI), hillshade, flow accumulation, and topographic wetness index (TWI) were analysed and integrated through a weighted overlay approach. Approximately 40.52% (402 km^2) of the area falls within high to very high flood risk categories, while 39.83% (951 km^2) was identified as low to very low risk. Specifically, high-risk and very high-risk zones cover 188 km^2 (18.10%) and 213.7 km^2 (22.42%) of the region, respectively. The generated flood hazard map provides valuable spatial insights for disaster preparedness, land-use planning, and sustainable flood mitigation strategies, thereby supporting informed decision-making and risk reduction efforts in the state.

***Keywords*:** Flood hazard risk map, Multi-Criteria Assessment, GIS; Remote Sensing, Weighted Overlay Analysis, SRTM.

1. INTRODUCTION

Flooding is one of the most destructive natural hazards worldwide, causing severe socio-economic and environmental consequences. Flood events frequently lead to loss of life, damage to infrastructure, displacement of populations, agricultural losses, and long-term ecosystem degradation. Increasing climate variability, extreme precipitation, rapid urbanization, and land-use changes have further intensified flood frequency and magnitude, particularly in developing regions (Joshi et al., 2025). Consequently, accurate flood susceptibility assessment, defined as the likelihood of an area being affected by flooding, is essential for effective disaster risk reduction, land-use planning, and sustainable development. Remote Sensing (RS) and Geographic Information Systems (GIS) have become indispensable tools for flood susceptibility mapping due to their ability to efficiently process large volumes of spatial and temporal data (Bhukya et al., 2025). Remote sensing provides synoptic and repetitive observations that enable the detection of land cover changes, drainage patterns, and flood extents. GIS enhances flood analysis by enabling the integration, management, and visualization of diverse spatial datasets, including topography, soil characteristics, land use/land cover (LULC), rainfall distribution, and hydrological variables. Through spatial overlay and multi-layer analysis, GIS supports the development of flood susceptibility and hazard maps, providing critical information for decision-making and flood mitigation planning (Refice et al., 2022). The integration of RS and GIS has been widely recognized as an effective approach for assessing flood vulnerability and identifying areas at risk (Tomar et al., 2021; Hagos et al., 2022; Diriba et al., 2024). Various methodological approaches have been employed for flood susceptibility mapping, including statistical models, multi-criteria decision analysis (MCDA), machine learning, and physically based hydrological and hydrodynamic models.

Numerous studies have demonstrated the effectiveness of integrated RS-GIS frameworks combined with probabilistic and MCDA approaches for flood susceptibility assessment (TakeWang et al., 2015). In the Kelantan River Basin of Malaysia, statistical flood susceptibility analysis using RADARSAT imagery provided reliable flood extent information and improved hazard assessment. Similar approaches have been applied in different geographical settings, including the Kosi River Basin in India (Kumar and

Jha, 2025), the Vamanapuram River Basin (Ajin et al., 2013), and the Lower Awash Sub-basin in Ethiopia (Wondim, 2016). MCDA techniques such as the Analytical Hierarchy Process (AHP) have also been widely adopted to incorporate expert judgment and relative importance of flood-conditioning factors. GIS-based AHP approaches have been successfully used to map flood susceptibility in various regions (Swain et al., 2020). In recent years, machine learning and deep learning techniques including Random Forest (RF), Support Vector Machine (SVM), Naïve Bayes, boosted-tree algorithms, and neural networks have gained increasing attention due to their ability to model complex nonlinear relationships between flood drivers and flood occurrence (Ahmadlou et al., 2023). Physically based hydrological and hydrodynamic models have been applied to simulate flood processes and inundation dynamics, providing detailed insights into flood depth and extent. Coupled hydrological-hydrodynamic frameworks have been successfully implemented in several Indian river basins, including the Damodar, Brahmani Baitarani, and Bengal Basins, to support flood risk mitigation and infrastructure planning (Malik et al., 2020; Ghosh and Kundu, 2023).

The present study was carried out for the Telangana State, India, which frequently experiences flooding that results in significant economic losses and severe impacts on livelihoods. Rapid urbanization, altered drainage systems, and intense monsoonal rainfall have increased flood vulnerability across the region. Despite this, comprehensive flood hazard and risk mapping studies remain limited. Therefore, the present study aims to delineate flood hazard and risk zones in Telangana State using an integrated RS-GIS framework and weighted overlay analysis (Bhukya et al., 2025). The resulting flood hazard maps are expected to support policymakers and planners in developing effective flood mitigation strategies, early warning systems, and sustainable land-use planning initiatives.

2. MATERIALS AND METHODS

This study employs an integrated Remote Sensing (RS) and Geographic Information System (GIS) based approach to develop a flood susceptibility map for Telangana State. The methodology combines satellite-derived datasets with multiple flood-conditioning factors to assess spatial flood susceptibility across the study area. The overall framework was organized into several sequential steps: (1) delineation of the study area, (2) data collection and pre-processing, (3) generation of the land use/land cover (LULC) map, (4) selection and weighting of flood-conditioning factors, (5) weighted overlay analysis, and (6) flood susceptibility mapping. Each step was systematically executed to ensure accurate representation of the physical and hydrological characteristics influencing flood occurrence. The integration of thematic layers through weighted overlay analysis enabled the classification of flood susceptibility zones based on their relative contribution to flood risk.

2.1. Study Area and Data Collection

The present study was conducted for the Telangana State, India, located on the Deccan Plateau in the central part of the eastern Indian Peninsula. The study area lies between latitudes 15°46′ and 19°47′ N and longitudes 77°16′ and 81°43′ E, covering a total geographical area of approximately 114,840 km² and comprising 33 administrative districts. Telangana is predominantly drained by two major river systems, with nearly 79% of its area falling within the Godavari River basin and about 69% within the Krishna River basin. Despite the presence of these major rivers, large parts of the state remain semi-arid in nature. Physio-graphically, Telangana is divided into two agro-ecological regions: The Deccan Plateau and the Eastern Ghats. The state is characterized by a semi-arid climate with predominantly hot and dry conditions. Summer begins in March and peaks in May, during which maximum temperatures often reach up to 42°C. The southwest

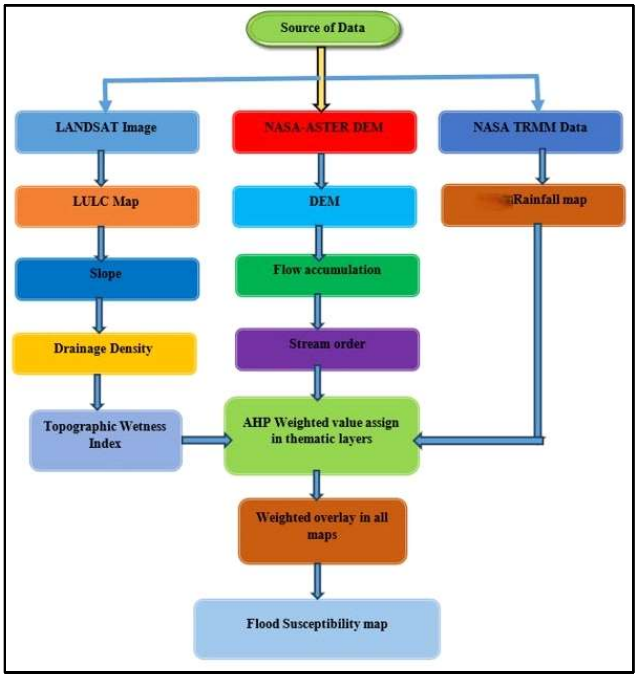

Figure 1. Flow chart of flood susceptibility mapping over the Telangana state

monsoon season extends from June to September and contributes the majority of the annual rainfall. The average annual rainfall varies spatially, ranging from 900-1500 mm in northern Telangana to 700-900 mm in southern Telangana, with nearly 80% of the rainfall occurring during the monsoon period. The winter season, from late November to early February, is generally dry and mild, with average temperatures ranging between 22 and 23°C and relative humidity varying from 25 to 30%.

Flood occurrences in Telangana are primarily driven by prolonged and intense monsoonal rainfall, which overwhelms natural drainage systems and results in surface runoff accumulation. Additional factors influencing flood severity include basin characteristics, drainage density, stream morphology, soil type, rainfall intensity, and anthropogenic obstructions to natural flow paths. In particular, districts such as Khammam are highly vulnerable to monsoon-induced flooding due to their physiographic and hydrological settings. The datasets used in this study, encompassing topographic, hydrological, meteorological, and land use parameters, are summarized in Table 1 and form the basis for flood susceptibility assessment across the state (Bhukya et al., 2025).

2.2. Hillshade

The hillshade layer was generated from the ASTER Digital Elevation Model (DEM) using the Spatial Analyst tools to enhance terrain visualization and support topographic interpretation. The output raster was classified into five categories to represent variations in surface illumination and relief, and subsequently used as an auxiliary layer in flood susceptibility analysis.

2.3. Contour

Contour lines were derived from the ASTER DEM to represent elevation variability across the study area. The generated contour map was used to support the interpretation of terrain configuration and drainage patterns, providing supplementary information for identifying low-lying and flood-prone zones.

2.4. Roughness

Surface roughness was computed from the ASTER DEM to quantify terrain irregularity across Telangana State. The roughness index was classified into multiple classes and incorporated into the analysis to represent spatial variability in surface complexity influencing runoff behaviour.

2.5. Slope

Slope gradients were derived from the ASTER DEM using spatial analysis tool. The slope raster was reclassified into five categories to represent variations in terrain steepness. These classes were incorporated into the flood susceptibility model, considering the influence of slope on runoff velocity and water accumulation (Ahmadlou et al., 2023).

2.6. Elevation

An elevation layer was extracted from the ASTER DEM and classified into five elevation zones using the Spatial Analyst extension. This layer was included in the analysis to account for topographic controls on water movement and flood concentration in low-lying areas (Lappas and Kallioras, 2019; Askar et al., 2022).

2.7. Aspect

The aspect layer was derived from the ASTER DEM at 30 m spatial resolution. Aspect values were classified into directional categories and incorporated into the flood susceptibility analysis to represent terrain orientation effects on surface runoff and drainage behavior (Pham et al., 2021; Askar et al., 2022).

2.8. Flow Accumulation

Flow accumulation was generated from the hydrologically corrected ASTER DEM using standard flow direction and accumulation algorithms. The resulting raster was classified to identify areas of concentrated surface flow, particularly along drainage networks and outlet zones, and

Table 1. Types of data collection and sources

Data type	Sources of data collected	Extracted data
ASTER DEM (Grid) 30 m × 30 m resolution	NASA's official website https://search.earthdata.nasa.gov	Hillshade, Slope, Roughness, Aspect, Elevation, Soil type, Drainage Density, Flow Accumulation and TWI
LULC data (Grid) 10 m× 10 m resolution	ESRI 2020 data, https://livingatlas.arcgis.com/landcover/	Land use/land cover map
Landsat8 Imagery (band5, band4)	USGS official website https://earthexplorer.usgs.gov	NDVI map
Precipitation (TRMM data)	NASA's official website https://giovanni.gsfc.nasa.gov/	Rainfall map

was used as a key indicator of flood-prone locations.

2.9. Drainage Density(D_d)

Drainage density was derived to represent the spatial distribution of stream networks across the study area. Stream order data were extracted from the ASTER Digital Elevation Model (DEM). The drainage density raster was generated using the density tool and subsequently classified into five categories to represent varying degrees of drainage concentration. The computation of drainage density was performed using Eq. (1), and the resulting layer was incorporated into the flood susceptibility analysis.

$$Dd = \sum_{n}^{1} L/A \quad (1)$$

Where drainage density is denoted by D_d, the length of waterways is signified by L, and the total area of the basin is signified with symbol A.

2.10. Topographic Wetness Index (TWI)

The Topographic Wetness Index (TWI) was computed to represent spatial variations in terrain-controlled moisture accumulation. TWI was derived from the ASTER Digital Elevation Model (DEM) using flow accumulation and slope rasters. The index was calculated using the standard TWI formulation (Eq. 2) and subsequently classified into multiple categories to distinguish areas of varying wetness conditions. The resulting TWI layer was incorporated into the flood susceptibility analysis to identify zones with a higher potential for surface saturation and flood occurrence (Samanta et al., 2018; Swain et al., 2020).

$$TWI = \mathrm{In}\, AS/\tan(\beta) \quad (2)$$

Where the upstream contributing area is denoted by AS and the gradient of the slope is denoted by β. The final TWI map was divided into five classes showing very low to very high.

2.11. Land Use/Land Cover (LULC)

The Land Use/Land Cover (LULC) layer was prepared to represent spatial variations in surface characteristics influencing runoff behaviour. LULC data for Telangana State was classified into major thematic classes, and the areal extent of each class was quantified. The LULC layer was subsequently incorporated into the flood susceptibility analysis as one of the key conditioning factors.

2.12. Normalized difference Vegetation Index (NDVI)

The NDVI value typically ranges from -1 to +1 (Habibi et al., 2023). Positive NDVI values indicate active vegetation, such as dense forests, while values close to zero represent barren areas. Negative values are associated with water bodies. To create the NDVI map, satellite data were obtained from the Landsat 8 Collection 1, provided by the USGS, and the NDVI values were calculated using the formula (Eq. 3).

The NDVI formula is expressed as:

$$NDVI = [\mathrm{frac}\{(NIR - Red)\}]/[NIR + Red] \quad (3)$$

Where the NIR represents near-infrared light and the Red is the visible light. The NDVI map of the study area (Telangana State) was categorized into five sub-categories ranging from −0.26 to 0.62 using the natural break tool (Figure 4). NDVI values typically range from -1 to +1, with higher values indicating healthier and denser vegetation. Here's what different ranges of NDVI values generally signify: Values close to +1 indicate dense, healthy vegetation. Values around 0 indicate little to no vegetation. Negative values are rare in natural environments but can occur in water bodies or highly reflective surfaces like clouds and snow.

2.13. Rainfall

Rainfall plays a crucial role in Telangana, influencing agriculture, water resources, and socio-economic development. Understanding rainfall variability is essential for sustainable water management and climate change adaptation, as rainfall is directly correlated with flood hazards. Rainfall data from the Tropical Rainfall Measuring Mission (TRMM) for the year 2023 were used to generate the rainfall map. The study area was classified into five rainfall sub-categories using the IDW method (Fig. 2m). Owing to its satisfactory performance across different climatic zones of Telangana (Habibi et al. 2023), TRMM data were preferred over other gridded precipitation products. Rainfall weights were assigned based on annual rainfall depth (mm), with higher rainfall regions receiving greater weights due to their stronger influence on flooding. Accordingly, areas with higher rainfall exhibit a greater flood risk. The overall study framework is presented in Fig. 1.

3. RESULTS and DISCUSSION

3.1. Terrain and Morphometric Characteristics

The roughness distribution of Telangana State is presented in Fig. 2a, classified into five categories: very low, low, moderate, high, and very high (Table 2). Low roughness dominates much of the study area, whereas higher roughness values correspond to structurally complex terrains influencing runoff routing and localized water accumulation. Slope values, derived from the DEM, range from 0 to 74.2° and were classified into five subclasses (Figure 2b; Table 2). Most of the study area exhibits gentle slopes, which facilitate water accumulation, while steeper slopes accelerate runoff toward adjacent lowlands. The slope distribution emphasizes terrain-controlled variations in flood risk across the state.

Hillshade analysis (Figure 2c) reveals five classes along with a composite mean hillshade map (Table 2). The classification highlights variations in illumination, slope, and elevation, which indirectly reflect geomorphological controls on surface water flow, erosion, and flood susceptibility. Aspect distribution (Figure 2d) categorizes slope orientation into five classes (Table 2). Spatial variability in slope direction affects runoff pathways, soil moisture retention, and localized hydrological responses, with similar aspect orientations showing clustered flood-prone zones.

Contour maps revealed elevation gradients ranging from 300 to over 800 m above mean sea level (Figure 2e). Low-lying areas coincide with major drainage channels and flood-prone plains, whereas elevated zones act as watershed divides, controlling flow convergence and inundation patterns.

3.2. Vegetation and Land Cover

NDVI classification highlights spatial variability in vegetation density (Figure 2g). Areas with high and very high NDVI correspond to dense forests and croplands, whereas low and very low NDVI values are associated with sparse vegetation and built-up surfaces. The NDVI distribution influences runoff patterns, with denser vegetation promoting infiltration and reducing local flood vulnerability. The 2023 LULC map (Figure 2h, Table 3) indicates that trees cover 62.53% of the area, followed by bare land (18.62%), crops (9.40%), built-up areas (5.56%), flooded vegetation (4.41%), and water bodies (3.70%). Built-up and barren areas exhibit higher flood susceptibility due to lower infiltration and higher surface runoff potential.

Table 2. Details of thematic layers used in the study

Roughness	**Class**	**Score**
0-3.9	Very low	5
3.9-10.9	Low	4
10.9-22.6	Moderate	3
22.8-38.7	High	2
38.7-253	Very high	1
Slope	**Class**	**Score**
0-2.3	Very low	5
2.3-61	Low	4
6.1-12.5	Moderate	3
12.5-21.5	High	2
21.5-74.2	Very high	1
Hill shade	**Class**	**Score**
1-53	Very low	5
53.001-114	Low	4
114.01-161	Moderate	3
161.01-204	High	2
204.01-255	Very high	1
Aspect	**Class**	**Score**
0-67.7	Very low	5
67.7-139.7	Low	4
139.7-210.2	Moderate	3
210.2-282.2	High	2
282.2-359.8	Very high	1

3.3. Soil and Hydrological Parameters

Soil distribution includes eight major types: Black Vertisols (BV), Inceptisols (I), Laterite (LC), Lithosols (LP), Natrustalfs (Na), Vertic Clays (Vc), Vertic Paleosols (Vp), and Water-Related Soils (WR) (Fig. 5a). Clay-rich and poorly drained soils correspond to higher flood-prone zones due to reduced infiltration capacity. Flow accumulation values (Figure 2i) are highest along major drainage channels and lowlands, indicating areas prone to surface water convergence and flooding.

Drainage density varies between 21 and 107 km/km² and was classified into five classes (Figure 2j). Lower drainage density is associated with higher flood susceptibility, whereas higher drainage density facilitates efficient runoff, reducing flood risk. The Topographic Wetness Index (TWI) map (Figure 2l) shows regions with persistent moisture accumulation, with higher TWI values corresponding to low-lying and flood-prone zones. The classification into five categories highlights areas of enhanced flood vulnerability across the state.

3.4. Rainfall Distribution

Annual rainfall in Telangana ranges from 615 mm to 1964 mm, with the highest precipitation concentrated in specific regions (Figure 2m; Table 3). Rainfall was classified into five categories: no drought, slight, moderate, severe,

Table 3. Details regarding LULC and rainfall used in the study

LULC class	**Percentage (%)**	**Rainfall (mm/year)**	**Class**
Water	3.70	615-969	Very severe drought
Crops	9.40	969-1140	Severe drought
Trees	62.53	1140-1287	Moderate drought
Built area	5.56	1287-1459	Slight drought
Flooded Vegetation	4.41	1459-1964	No drought
Base ground	18.62		

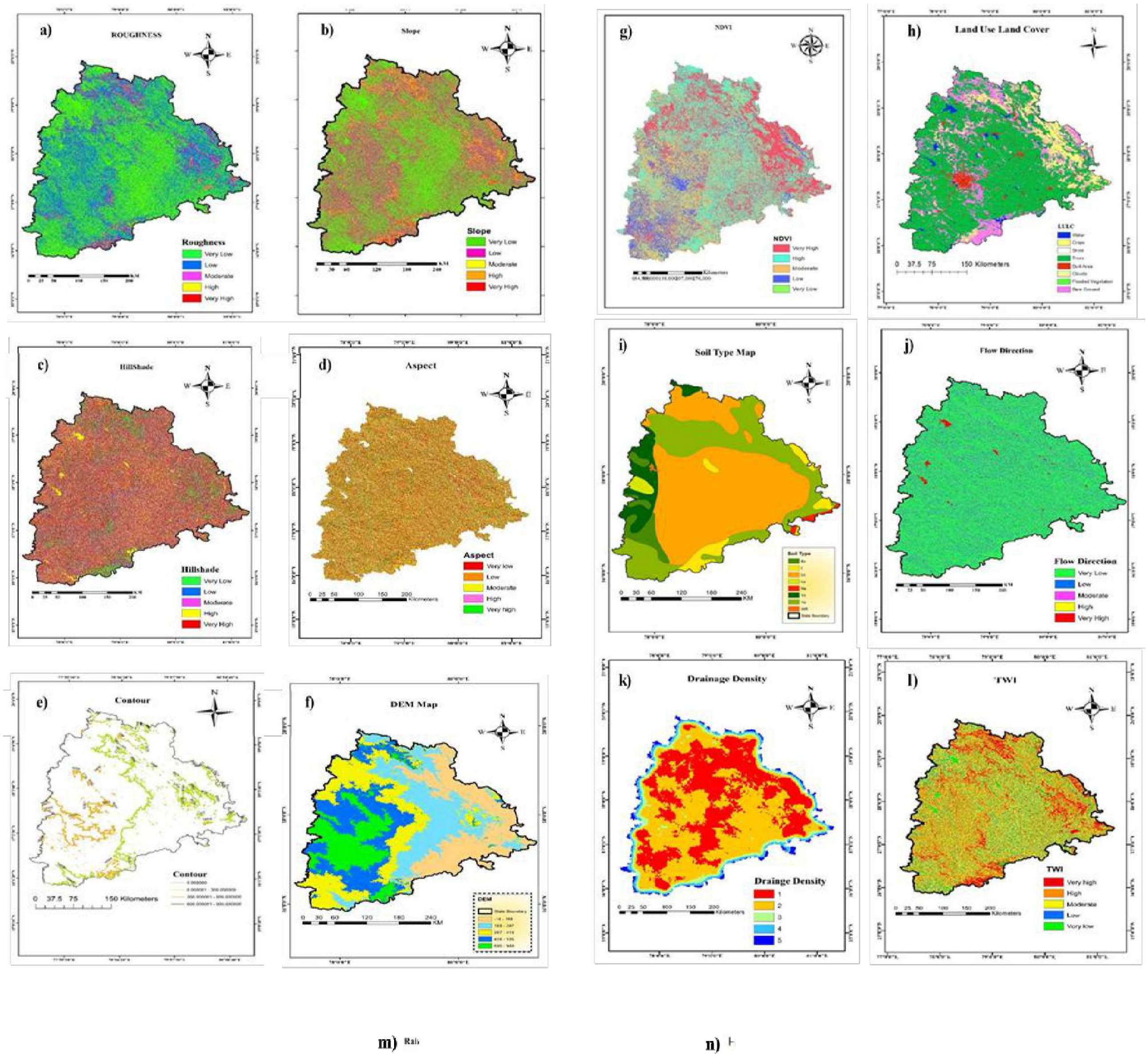

Figure 2. Generated thematic layers a) Roughness b) Slope c) Hillshade d) Aspect e) Contour f) DEM g) NDVI h) LULC i) Soil type j) Flow direction k) Drainage density l) TWI m) Rainfall n) Flood risk map of the study area

and very severe. High rainfall zones correspond to increased flood susceptibility, whereas low rainfall areas exhibit reduced flood risk.

3.5. Influence of Flood-Conditioning Factors

The normalized weights derived as shown in Table 4 showed rainfall as the most influential factor, accounting for 24.56% of the total weight. This is followed by soil type (14.68%) and drainage density (9.56%). Slope (9.52%) also played a significant role, while land use/land cover contributed 7.34%. Hydrological and surface condition indicators such as the Topographic Wetness Index (6.53%), NDVI (5.32%), hill shade (5.24%), and flow direction (5.14%) exhibit moderate influence. Elevation (4.60%) and aspect (4.35%) contributed comparatively less. Overall, these results underscore the integrated influence of topographic, hydrological, and land surface characteristics in shaping flood risk patterns across Telangana.

3.6. Flood Susceptibility Zonation

The integrated flood susceptibility map categorizes Telangana into five classes-very high, high, moderate, low, and very low (Figure 2n; Table 5). Areas identified as having high to very high flood susceptibility represents zones of

Table 4. Normalized weights of different thematic layers used in the study

Factor	Scale	Classes	Weight (%)	Factor	Scale	Classes	Weight (%)
Slope	5	Very low	9.52	LULC	5	Very low	7.34
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	
Roughness	5	Very low	3.16	Drainage density	5	Very low	9.56
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	
Elevation	5	Very low	4.60	Flow direction	5	Very low	5.14
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	
Hillshade	5	Very low	5.24	Soil type	5	Very low	14.68
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	
Aspect	5	Very low	4.35	TWI	5	Very low	6.53
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	
Rainfall	5	Very low	24.56	NDVI	5	Very low	5.32
	4	Low			4	Low	
	3	Moderate			3	Moderate	
	2	High			2	High	
	1	Very high			1	Very high	

Table 5. Details of flood risk class of the study area

Flood risk class	Area (km^2)	(%)	Remarks
Very low	535	14.25	Least affected by flooding
Low	416	25.58	Occasional flooding
Moderate	297	19.65	Mostly in south-western, north-central, and eastern areas
High	213.7	22.42	Adjacent to very high flooded regions areas
Very high	188	18.10	Concentrated in western, north-western, and some eastern regions

elevated risk and therefore require priority mitigation and flood management measures. In contrast, regions classified as low to very low susceptibility indicate relatively stable conditions, where the implementation of sustainable land and water management practices can help maintain long-term resilience.

4. CONCLUSION

This study demonstrated the effectiveness of a remote sensing and GIS based approach for identifying flood susceptibility zones in Telangana. Key findings revealed that terrain, hydrology, soil characteristics, vegetation cover, and rainfall are the primary factors influencing flood risk. The integrated analysis highlights areas that are highly vulnerable to flooding and emphasizes the spatial variability of flood susceptibility across the state.

Based on these findings, it is recommended to implement targeted flood mitigation strategies in high-risk areas and adopt sustainable land-use practices in less vulnerable regions to enhance overall resilience. Additionally, incorporating community-based adaptation and early warning systems can further strengthen flood preparedness and management.

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Data Availability Statement

All data used in this study are publicly available from the respective data providers as cited in Table 1.

Authors' Contributions

Bhukya Srinivas: Conceptualization, Data curation, Formal analysis, Writing-original draft; Bhupendra Joshi, Ashok Amgoth: Writing-review and editing

Conflict of Interest

Author's declare no conflict of interest.

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