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Today's Students, Tomorrow's Bioeconomy Professionals

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***Abstract*:** This paper reflects on the outcomes of a student-centered workshop held during the Indian Ecological Society International Conference, held at Punjab Agricultural University, Ludhiana, Punjab, 12 – 15 November 2024.The students' workshop was a follow-up on the topics developed in the Symposium on "Agriculture, Climate Changes, and Energy Transitions: Building Capacity to Maintain Yields of Innovative Agrifood Systems" held during the conference. Its major purpose was to encourage discussions among conference participants about specific research agendas and potential teaching plans. Students worked in groups of four to five to provide ideas for improving teaching and learning and ideas for future research. They consistently mentioned a need to grasp the practical application of their classroom studies. Most of the research ideas focused on conservation or enhancement of resources used in agriculture and many involved cross-disciplinary topics, such as the expansion of data analysis technologies to promote resilience for agricultural communities. The results parallel the principles outlined as key to a bioeconomy, outlined by the Food and Agriculture Organization of the United Nations.

Keywords: Agriculture, Agrifood systems, Bioeconomy, Climate change, Ecology, Education, Farmers, Knowledge, Sustainability

Consider: According to the United Nations, globally, nearly 1 in 10 people went hungry in 2022 and 2.4 billion people (almost 30% of the population) experienced moderate to severe food insecurity (United Nations 2024).

Consider: Despite food insecurity in parts of the world, the average per capita consumption has increased steadily since the 1960s, a trend that is expected to continue in Asia and Africa (Pomeroy et al., 2023).

Consider: Modern agrifood systems emit roughly one-third of anthropogenic greenhouse gas emissions (Crippa et al., 2021). How can we make the transition to a clean energy future while preserving agricultural livelihoods and maintaining agricultural yields?

Consider: About 60% of the inputs to the global economy could, in theory, be produced biologically by substituting biologically based materials for fossil fuels in the production of energy, chemicals, and the like (Chui et al., 2020, p. vi).

Facts like these have led researchers, policymakers, educators, business-people, farmers, and other stakeholders to propose replacing current economic and agricultural systems with a sustainable, circular "bioeconomy." According to the Food and Agriculture Organization of the United Nations (FAO), the bioeconomy can be thought of as "the production, utilization, conservation, and regeneration of biological resources, including related knowledge, science, technology, and innovation, to provide sustainable solutions (information, products, processes and services) within and across all economic sectors and enable a transformation to a sustainable economy" (FAO 2022, p. 1). The incorporation of traditional and local knowledge along-side that of scholars can help generate new ideas in the realms of plant and animal health, food production, and the environment.

According to the FAO, the first principle of a bioeconomy is that it should support food and nutrition security (FAO 2021). At the same time, natural resources must be conserved and enhanced (Principle 3). Appropriate, efficient, and effective use of resources will then strengthen local communities and promote environmental resilience (Principles 4, 5, 8 and 9).

We must then ask the question: Are today's university-level students being adequately prepared to participate in a bioeconomy?

MATERIAL AND METHODS

We had the opportunity to get a glimpse into the readiness of students during a workshop at the Indian Ecology Society International Conference held in Ludhiana, Punjab, on 13 November 2024. This year's conference focused on the intersection of climate change, energy use, and agriculture. The students attending the symposium on "Agriculture, Climate Changes, and Energy Transitions: Building Capacity to Maintain Yields of Innovative Agrifood Systems" were invited to participate in the workshop. Fifty-one of the

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undergraduate and graduate level students in attendance responded to an invitation to a discussion of ideas for future research as well as suggestions for improving teaching and learning in the agricultural education in India. The students worked in groups of 4 – 5 to list at least two ideas for research and at least two for teaching. A spokesperson for each group shared the findings with all workshop attendees at the end of the session. We gathered the worksheets filled out by the groups and have summarized the information below.

RESULTS AND DISCUSSION

Teaching and Learning

We created a word cloud using wordcloud.com and the list of ideas for teaching and education in agriculture generated by students during the afternoon workshop. The word cloud provides a visualization of the relative weighting of different topics—those mentioned more often appear in larger font than those mentioned only once or twice (Fig. 1).

Aside from agriculture and agricultural, the words that stand out in this array are practical, farmers, knowledge, climate, researchers, and models. Students consistently mentioned the need to understand the practical applications of what they were learning in the classroom, to incorporate practice with the theory. Students encouraged faculty to integrate farmers and their knowledge, field trips to farms, and hands-on experience at farms into the curriculum. Students also underscored the need for greater use of technology for modeling (the impacts of climate change on the agricultural sector, for example), to augment lectures, and to share information among academics, people working at research centers, students and farmers in their fields. While some students may appreciate work undertaken to advance agricultural sciences, or on what might benefit the sciences in the future, most seemed focused on those things that would allow them to contribute tangible benefits in the present.

These suggestions align the concept of a bioeconomy and with the conception of agriculture as part of a larger value chain (Freer 2015). Because they may be involved with aspects of agriculture that span from selecting the proper varieties for use in remote fields to advising governments on agricultural policy, graduates of agricultural programs need a wide variety of skills ranging from the sciences of plants and animal husbandry, production and processing/post-harvest handling, food safety, agro-economics and logistics, to marketing and supervision. Freer also stressed the importance of soft skills need to "work with rural farmers and adapt information based on literacy level, geography, resources, and farming systems (Freer 2015).

In their analysis of curricula of American post-secondary food system programs, Valley, Wittman, Jordan, Ahmed, and Galt (2017), argued that traditional programs that taught

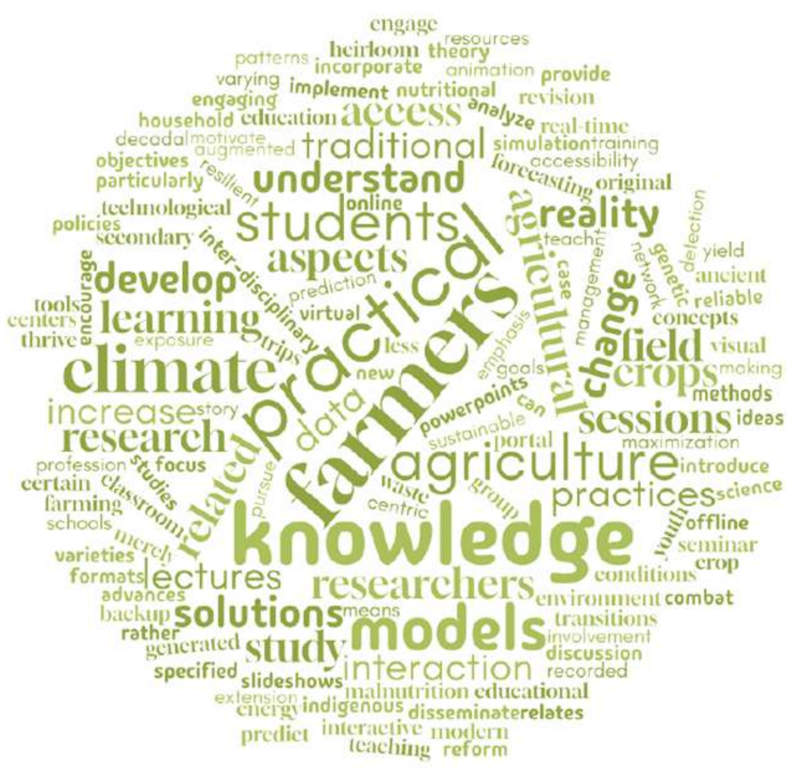

Fig. 1. Ideas for teaching and learning

students in narrowly defined disciplines, such as food science, agronomy, or animal biology, did not adequately prepare them for the complex economic, social, and environmental aspects of agriculture. They advocated a more holistic approach and the incorporation of interdisciplinary experiential learning that helps students better grasp the many facets of agricultural issues.

One part of a more holistic approach to agriculture would be the incorporation of local and Traditional Ecological Knowledge (TEK or TK). Like its more academic counterparts, TEK relies on careful observation of phenomena. But unlike biology or chemistry, TEK employs qualitative rather than quantitative methods, often involves an observer immersed in the very subject of observation, and may occur over long periods of time, perhaps even generations (Kimmerer 2002). Agricultural TEK would incorporate the history and culture of a place along with the science.

While in countries like the United States, less than 2% of the population engage in farming or ranching (Farm Bureau, n.d.), a 2023 - 2024 Economic Survey of India reported agriculture supported over 42% of the population (Ministry of Finance 2024). In those countries and elsewhere, the average age of the farmer has risen to almost 60 years (Robinson 2024). Such facts underscore the importance of the workshop attendees' suggestion that schools introduce programs to motivate students to pursue farming as a profession. This echoes the results of a survey of South African youth-- when youth are exposed to agricultural studies at secondary and tertiary levels of education, they are more likely to have plans to participate in occupations related to agriculture and businesses related to it (Magagula and Tsvakirai 2020). The growing world population will need the food they will provide.

Ideas for Research

We used a similar approach to capture the ideas suggested for future research.

We then categorized the research suggestions according to the guiding principles of the bioeconomy as outlined by the FAO (FAO 2021). The number in parentheses indicates how many of the suggestions we classified under that particular principle; note that suggestions could be classified under more than one principle (Fig. 2). Examples of specific project ideas follow each of the listings.

Sustainable Bioeconomy Development Should . . .

Principle 1: Support Food Security at all Levels (4)

- Explore nutritional aspects of agriculture to combat malnutrition
- Incorporate millets into complex foods to increase their nutritional value
- Develop insect farming as a protein substitute

Principle 2: Ensure That Natural Resources are Conserved, Protected and Enhanced (23)

- Understand the impact on farmers of reduced varietal diversity

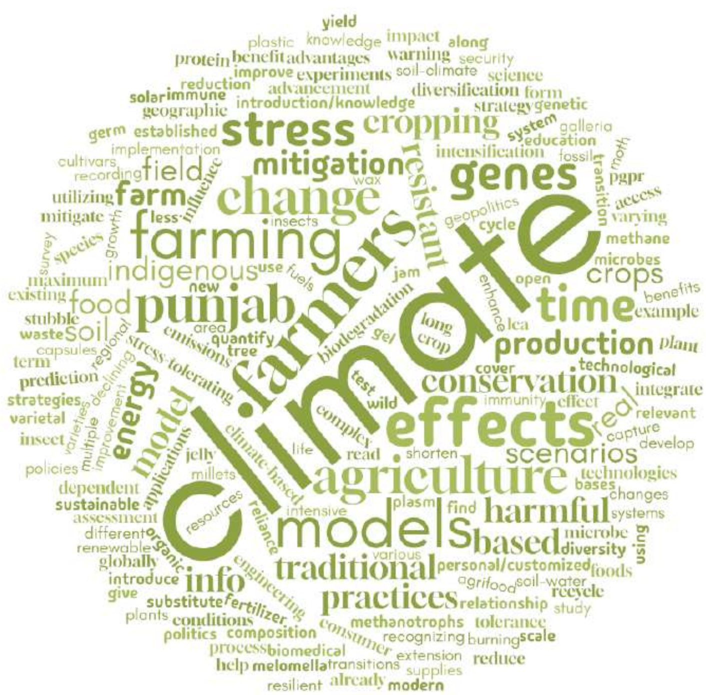

Fig. 2. Ideas for future research

- Model crop growth under climate stress
- Probe the soil-water relationship at field scale under different cropping systems
- Map genetics of wild and traditional varieties
- Introduce climate-resistant genes into already established crops
- Improve stress tolerance using plant-growth-promoting rhizobacteria (PGPR) microbes

Principle 4: Make Communities Healthier, More Sustainable, and Harness Social and Ecosystem Resilience (4)

- Explore conservation practices for various geographic, climate, and soil combination scenarios
- Understand the impact of declining tree cover on agricultural regions

Principle 5: Rely on Improved Efficiency in the Use of Resources and Biomass (3)

- Recycle food and agricultural waste
- Utilize methanotrophs in paddies to reduce emissions of methane

Principle 7: Make Good Use of Existing Relevant Knowledge and Proven Sound Technologies and Good Practices and, Where Appropriate, Promote Research and Innovations (9)

- Integrate modern, local, and traditional ecological knowledge as a basis for climate mitigation
- Harness understanding of geography and traditional farming practices for sustainable food production

Principle 10: Promote Cooperation, Collaboration and Sharing Between Interested and Concerned Stakeholders in all Relevant Domains and at all Relevant Levels (12)

- Adapt technologies to capture real-time farm conditions and climate data

Most ideas for research underscored the need for the conservation, protection, and enhancement of agricultural resources in the face of climate change. Even when not called out specifically, the research topics suggested cross-disciplinary approaches, such as combing computer science modeling with plant and soil sciences or understanding the impact of carbon emissions on agricultural practices**.** Students suggested the need for developing agricultural practices that also contribute to the resilience of the rural economy in the face of a changing climate (combining Principle 3: Support Competitive and Inclusive Economic Growth, and Principle 4: Make Communities Healthier and More Sustainable). Students also stressed the need to incorporate both modern and traditional knowledge systems. As with the teaching and learning ideas, the topics reflected a growing awareness that we must appreciate agriculture as part of a large, complex system—we cannot isolate agriculture, its inputs or its impacts, from the world around it. It is an integral part of the bioeconomy.

Concluding Thoughts

When the organizers of this workshop first conceived of this session as part of a larger conference, we did not know how student participants would react. Would they be interested? Concerned? Would they participate?

We discovered they were indeed interested and concerned. These students would soon be entering the workforce of trained professionals supporting farmers and agricultural production and they wanted to ensure that they and their fellow students had the skills necessary to meet the challenges ahead. The political, economic, and environmental landscape will be very different from that of even one decade ago.

To revisit the question that prompted this paper: Are today's university-level students being adequately prepared to participate in a bioeconomy? They will be if we listen to them. Two good places to start would be to: 1. Incorporate more hands-on experience, field trips, and interactions with farmers in the areas of agricultural institutions, and 2. Expand interdisciplinary approaches to agricultural education and research.

We are reminded of the words of Jawaharlal Nehru:

A University stands for humanism, for tolerance, for reason, for the adventure of ideas and for the search of truth. It stands for the onward march of the human race towards ever higher objectives.

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Assessing Socio-Economic Vulnerability to Climate Change in Forest-Fringe Communities: Evidence from Sindh Forest Division, India

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Abstract: The Indian Himalayan Region (IHR) is highly vulnerable to the impacts and variability of climate change. The present study examines fifteen forest blocks across three ranges Sindh, Manasbal, and Harran-Shallabugh within the Sindh Forest Division, with particular consideration of the associated socioeconomic profiles. Six socioeconomic indicators were used to determine socioeconomic vulnerability: population density (km^2), literacy rate (%), percentage of BPL households, percentage of fuel wood consumption, percentage of main workers, and percentage of Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) beneficiaries. The multistage sampling approach was employed to select blocks and ranges, and primary data was collected through field surveys and interactions, using a structured interview schedule. The weight was assigned using a pairwise comparison method (PCM) for the construction of the vulnerability index (VI). Dagapora forest block was the most vulnerable, with VI of 0.774 (1st rank) having socio-economic vulnerability under the present scenario and the Lar forest block had the lowest VI (0.143) indicating that was the least susceptible. Range-wise, the Harran Shallabugh was the most vulnerable with a VI of 0.667 (Ist rank) followed by the Sindh range with a VI of 0.401 (2nd rank), whereas the Manasbal range was the least vulnerable with a VI of 0.344 (3rd). The major drivers of vulnerability across blocks and ranges were high BPL households, fuelwood dependency, rapid population growth, low literacy rates, and a lack of alternative livelihood opportunities, contributing to increased vulnerability. The study highlights the need for targeted adaptation measures, sustainable land management practices, livelihood diversification, and strengthening adaptive capacities to reduce vulnerability and protect forest resources in the region

Keywords: Climate change, Socioeconomic, Forests, vulnerability, Drivers, Adaptation

Climate change has gained significant importance in the global development agenda, owing to its multidimensional impact on economies and human well-being (Musakwa and Odhiambo 2022, Islam 2025, Sharma et al., 2025). Developing nations, whose populations are the most fragile and least likely to adapt readily, would face some of the most severe consequences of climate change (Brenton and Chemutai 2021). These implications jeopardise the capacity to fulfil the 2030 Agenda for Sustainable Development Goals (SDGs) (Singh et al., 2019, Ingutia 2021). According to the World Bank (2007), 25% of the world's poor population rely on forests for their livelihoods, either directly or indirectly. Rural households and communities that depend on forests have close relationships with them, and as a result, derive their economic viability and frequently, their cultural and spiritual identity (Byron and Arnold 2014, Roux et al., 2022). Himalayan forest ecosystems are significant because of the diversity of their flora, fauna, human communities, and cultural practices (Negi 2022). Apart from its significance, it is one of the world's most fragile ecosystems that experience higher levels of climate change impact, with severe consequences for socioeconomic systems (Chakraborty et al., 2010). Climate change has significant negative impacts on mountain ecosystems, particularly in the Indian Himalayan forest ecosystems. Rising temperatures, earlier snowmelt, and changing precipitation patterns alter forest dynamics (Basharat et al., 2016, Hamid et al., 2020). Forest cover decreases with increasing meadow and shrubland cover, which affects biodiversity (Yao et al., 2019). These changes pose significant challenges to biodiversity conservation and the sustainable management of mountain resources in the region (Negi et al., 2017, Wambulwa et al., 2021).

Vulnerability is defined as a function of exposure, sensitivity, and adaptive capability (McDowell and Hess 2012, Segnon et al., 2020). Vulnerability assessments of forests under climate change at the local level are highly relevant for sustainable development (Ordonez and Duinker 2014, Lecina-Diaz et al., 2020). These evaluations provide critical insights into the potential impacts of climate change on forest ecosystems and the communities that depend on them. Such information supports the formulation of adaptive management strategies and informed policy decisions (Brandt et al., 2014, Boulanger et al., 2023, UNDP 2020, Nunes, 2023). This localized approach facilitates the design of targeted adaptation measures that are responsive to specific environmental and socio-economic conditions (Seidl et al., 2011, Sharma et al., 2017a). However, the effectiveness of these assessments in guiding local decision-making can vary, depending on the nature of the data produced and the strength of institutional linkages to policy processes (Greiving et al., 2015). The limited long-term observational data and region-specific assessment frameworks in developing countries like India pose significant challenges to accurately predicting climate vulnerability (Kumar et al., 2018). Assessing the socioeconomic vulnerability of forest fringe communities is a critical priority for informed policy and sustainable forest management (Patt et al., 2012, Reed et al., 2013, Weibhuhn et al., 2018, Darabi et al., 2018). Various approaches have been used to examine the relationships between the socioeconomic factors that influence climate vulnerability throughout the nation (Gopalakrishnan et al., 2021, Kathirvelpandian et al., 2024, Gopalakrishnan et al., 2025). On a regional basis, limited literature is available on the vulnerability assessment of socioeconomic status to climate change. In light of this, the present study aimed to address existing gaps by conducting a realistic assessment of socioeconomic vulnerability in the Sindh Forest Division, with the goal of establishing baseline information on the status of forest fringe communities.

MATERIAL AND METHODS

Study area: The current study was conducted in the Sindh Forest Division, located in the Union Territory of Jammu and Kashmir. This division lies on the geographical coordinates of 34°07'00" to 34°28'00" North Latitude and 74°24'00" and 75°26'00" East Longitude with an area of 37,901 ha (Fig. 1). It comprises three ranges Sindh, Manasbal, and Harran-Shallabugh and fifteen blocks viz; Akhal I, Akhal II, Ganiwan, Gund, Kullan, Kangan Town, Lar, Chattergul, Wangat, Branabugh, Gultibagh, Harran I, Harran II, Dangarpora, and Shallabugh. The altitudinal range varies from 1828.8 meters AMSL near the village of Harran to 5248 m AMSL at the Harmukh peak (Khan et al., 2019). The Sindh Forest Division, is densely populated, primarily by rural communities. The area experiences a mix of temperate and sub-alpine climatic conditions and receives substantial annual precipitation, averaging around 700 mm. Temperature fluctuations typically range from 5°C to 20°C throughout the year. The forests are predominantly composed of Kail (*Pinus wallichiana*), with Fir (*Abies pindrow*) occurring in mixed stands on more exposed sites and Deodar (Cedrus deodara) found in scattered, isolated areas (Banday et al., 2019).

Socioeconomically, the forest-fringe communities of the Sindh Forest Division are predominantly dependent on subsistence agriculture, livestock rearing, wage labour, and forest-based resources such as fuelwood, fodder, medicinal plants, and Non-Timber Forest Products (NTFPs) for their livelihoods. The majority of these households belong to economically marginalised groups with limited access to formal employment, modern agricultural practices, financial services, and healthcare, making them highly vulnerable to external factors. Infrastructure deficits including poor road connectivity, unreliable electricity supply, and inadequate

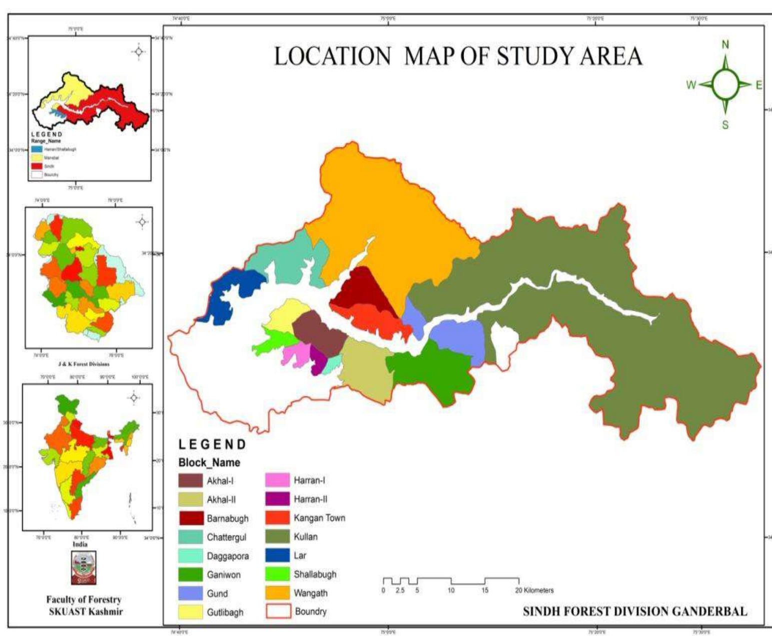

Fig. 1. Location of study area

irrigation facilities further compound their hardship, limiting adaptive capacity and resilience (Hussain 2012, Banday et al., 2021). The region has witnessed a notable rise in temperature an increase of 1.8°C in recent decades (Lone et al., 2022) which has led to erratic precipitation patterns, early snowmelt, and more frequent extreme weather events. These changes have directly affected agricultural productivity, livestock health, water availability, and forest regeneration, thereby undermining traditional livelihoods and increasing dependency on forest resources (Mir et al., 2021). Seasonal migration for employment is also on the rise due to declining on-farm income and resource scarcity (Hussain 2017). In addition, the absence of secure land tenure, limited participation in decision-making, and inadequate implementation of welfare schemes exacerbate the socio-economic fragility of these communities.

Sampling and data collection: The socio-economic vulnerability of forest fringe communities in the Sindh Forest Division was evaluated using an indicator-based approach comprising six key indicators: population density (km^{-2}), literacy rate (%), number of BPL households, percentage of fuel wood consumption, percentage of main workers, and percentage of Mahatma Gandhi National Rural Employment Guarantee Act beneficiaries (MGNREGA) (Table 1). Through a preliminary survey, purposive multistage sampling was conducted, and block- and range-wise samples were collected from the whole forest division. The present study employed a combination of qualitative and quantitative research methods to ensure a comprehensive understanding of the subject. Systematic interview schedules designed for in-depth and direct observations, field surveys and interactions were conducted to collect primary data and Secondary data. Primary data collection involved structured interviews with selected respondents and non-participant observations, following the methodology outlined by (Bernard et al., 2017). These data were collected at both the individual/household and village levels. Meanwhile, secondary data were compiled at multiple levels, including block, village, and household/individual levels, to support the analysis and provide context. Secondary data were obtained from a wide range of sources, including academic journals, research reports, forest department records, village records, internet-based resources, previous studies, annual reports, and other relevant documents from governmental and non-governmental organizations The datasets used in this study are publicly available for download from the following links https://jkforest.gov.in, https://censusindia.gov.in/census.website/, https://fsi.nic.in/forest-report-2023, https://jkfcsca.gov.in/Schemes.html, https://nregastrep.nic.in/ and https:// bhuvan-panchayat3.nrsc.gov.in/. The interview schedule characteristics were derived from the scales designed by (Sharma et al., 2018).

Assigning weights: Each indicator is assigned a weight according to its significance in identifying the vulnerability of the system. The nature and significance of each indicator

Table 1. Indicators, their description, and rationale for their selection for the block and range level analysis

Indicator	Description	Category	Rationale for selection	Function	Weight
Population density (km^{-2})	The number of people living per unit area of land in a forest block or range.	Sensitivity	High population density puts pressure on limited resources, reducing their availability per person. It also increases exposure to environmental risks and disasters.	(+)	0.368
Literacy rate (%)	The percentage of people (aged 7 and above) who can read and write with comprehension	Adaptive capacity	Literacy equips individuals with knowledge and skills to respond to crises effectively, improving disaster preparedness and recovery.	(-)	0.227
Percentage of BPL households	The percentage of households categorized as Below the Poverty Line (BPL).	Sensitivity	A higher share of BPL households indicates economic hardship, making it difficult for communities to adapt to environmental changes.	(+)	0.117
Percentage of fuel wood consumption	The proportion of households primarily dependent on fuelwood for cooking and heating.	Sensitivity	Reliance on fuelwood suggests a lack of access to modern energy sources, often associated with rural communities that have lower resilience.	(+)	0.085
Percentage of main workers	The percentage of people engaged in stable employment for at least six months annually.	Adaptive capacity	A higher employment rate reflects economic stability, ensuring better preparedness and financial security against environmental hazards.	(-)	0.117
Percentage of MNGREGA beneficiaries	The average number of workdays provided per household under the MGNREGA scheme.	Adaptive capacity	MGNREGA offers a financial safety net by providing employment, helping communities sustain their livelihoods during economic hardships.	(-)	0.085

require careful consideration and expert assistance, and the Pairwise Comparison Method (PCM) was used to assign weights to the chosen indicators (Saaty et al., 2008) to ensure that the proportion or weight given to each indication equals one.

Normalization: Normalization is based on a linear maximum and minimum scaling procedure that considers the functional link between indications and vulnerability. Equation (1) has been used to positively connect indicators, that is, those in which vulnerability increases as the indicator's value increases. Equation (2) was employed to establish an inverse relationship between the indicators, specifically in cases where an indicator's increasing value corresponded to a decrease in vulnerability.

$$X_{ij}^{P} = \frac{Xij - Mini\{Xij.}{Maxi\{Xij\} - Min\,i\{Xij\}} \quad (1)$$

$$X_{ij}^{N} = \frac{Maxi\{Xij\} - Xij.}{Maxi\{Xij\} - Mini\,\{Xij\}} \quad (2)$$

Where

Xij represents the value of the jth indicator for the ith block/range. That is, Mini{Xij}, which represents the minimum value for the jth indicator of all blocks/ranges, and Maxi{Xij}

Vulnerability index: After multiplying the weight by the normalized indicator value, the results were combined. The following formula was used to aggregate the normalized and weighted values of the indicators to create the overall vulnerability index:

$$VI = \sum_{j} (x_{ij} \times w_j)$$

Where,

VI is the Vulnerability Index. xij are normalized values, and Wj are the assigned weights.

RESULTS AND DISCUSSION

Population density (km^{-2}): Population increase has resulted in high pressure on natural resources. The highest population density of 1387 people per sq. km² was in the Harran I block, indicating that the block places strain on natural resources. Among all forest blocks, Gund had the lowest population density of 90 people per sq. km² (Table 2). The Sindh forest range had the lowest population density among the three ranges, at 241 people per sq. km² (Table 3). Acceleration of population growth has been linked to various forms of environmental degradation, such as the destruction of forests, excessive grazing, deterioration of soil, and depletion of soil nutrients, yielding results comparable to those reported by (Malik et al., 2016, Singh and Yadav 2024).

Table 2. Indicators values of blocks of Sindh forest division

Indicators/Blocks	Population density (km^{-2})	Literacy rate (%)	Percentage of BPL households	Percentage of fuel wood consumption	Percentage of main workers	Percentage of MNGREGA beneficiaries
Akhal 1	164	60.1	0.04	32	6.35	6.93
Akhal II	153	54.18	0.02	33	7.5	6.88
Ganiwon	248	48.97	0.09	39	7.33	6.94
Gund	90	56.85	0.07	42	7.42	7.29
Kullan	160	48.75	0.07	58	7.52	8.09
Kangan Town	636	43.94	0.11	21	8.35	7.98
Lar	143	73.77	0.03	35	7.48	8.51
Chattergul	772	54.91	0.1	31	6.03	7.52
Wangat	201	50.98	0.09	37	7.06	6.35
Branabugh	220	55.1	0.03	29	6.37	6.75
Gutlibagh	455	54.11	0.09	35	6.18	6.68
Harran I	1387	40.61	0.04	15	6.79	6.61
Harran II	1320	40.61	0.04	14	3.98	5.12
Shallabugh	988	52.08	0.03	12	7.31	6.24
Dagapora	1140	38.4	0.1	11	4.21	2.04
Average	538.46	51.55	0.06	29.6	6.65	6.66
Minimum	90	38.4	0.02	11	3.98	2.04
Maximum	1387	73.77	0.11	58	8.35	8.51
Range	1297	35.37	0.09	47	4.37	6.47

Regarding forest commercialization, timber harvesting, and deforestation in Uttaranchal, demonstrating that wood demand increased alongside population growth (Singh et al., 2019). The most landscapes dominated by tropical forests are vulnerable to human-induced disturbances and face potential degradation and loss due to factors unrelated to climate change (Siyum 2020). Sustainable land management practices and increased awareness among populations are crucial for safeguarding resources for future generations (Wairiu 2016, Gupta 2019).

Literacy rate (%): Lar had the highest literacy rate (73.77%), indicating that the block had strong adaptive potential (Table 2), and Dagapora had the lowest percentage (38.4%) (Table 2). The highest literacy rate (57.77%) was in the Manasbal range, indicating that the block has a strong adaptive potential. Among all the ranges, the Harran–Shallabugh range had the lowest percentage (42.94%) (Table 3). Piper et al. (2015) and Seymour and Busch (2016) concluded that regenerated and restored forests with more biodiversity and a multi-tiered forest canopy structure are more resilient to external pressures and improve literacy rates and that lowering poverty rates among families reduces their susceptibility to climate change. (Negi et al., 2019, Mawad et al., 2022) also found a link between extensive forest cover in the IHR, commonly attributed to numerous local governing organizations and schools. Singh et al., (2016) Dulay et al. (2019) also observed that low literacy might be caused by poor socioeconomic situations among parents as well as a lack of educational opportunities. (Leichenko and Silva 2014 , Adeniyi et al., 2018) observed that climate change impacts were unequally felt along gender lines, with illiterate women and, in particular, the elderly being the most vulnerable groups to climate change due to the community's gendered discrimination roles and responsibilities.

Percentage of BPL households: The higher proportion of BPL households suggests a poorer ability to adapt. The maximum number of BPL households was in Block Kangan Town and the Sindh forest range, with 1892 and 6834, respectively (Tables 2, 3), demonstrating that the population has limited adaptive potential. Hahn et al. (2009) observed that, in Mozambique, poor households' livelihood alternatives impact their sensitivity to climate change. (Fisher et al., 2010) also observed that Malawi and low-income families live near forests and are less educated and especially reliant on forests, resulting in lower adaptive ability for forest management. Study conducted in southern Mozambique and South Africa, reported that the vulnerability stems primarily from physical, financial, and social capital, with factors such as food shortages, weak social networks, and a lack of financial resources due to unemployment all influencing households reduced adaptive capacity (Osbahr et al., 2010, Zacarias 2018). This finding emphasizes the importance of adopting livelihood diversification and strengthening adaptive capabilities when developing climate change adaptation strategies.

Percentage of fuel wood consumption: Maximum fuelwood consumption of 58% was in Kullan (Table 3), indicating that the block places a strain on natural resources. Among all the forest blocks, Dagapora had the lowest fuelwood consumption of 11% (Table 2). The Sindh range had the highest value of 37.5%, indicating that natural resources were under severe strain. The Harran Shallabugh forest range had the lowest fuelwood consumption of the three ranges (12.7%) (Table 3). Firewood is the cheapest source of energy in Tanzania, and approximately 90% of the population uses firewood and charcoal for cooking and heating, which negatively affects the forest ecosystems (Felix and Gheewala 2011). Msoffe (2017) reported that the energy balance in Tanzania is dominated by biomass-based fuels, particularly wood fuel (firewood and charcoal), which accounts for more than 90% of the primary energy supply. However, this has led to increased pressure on forest resources, resulting in deforestation and desertification (Mwampamba 2018). Firewood is primarily used at the

Table 3. Indicators values of ranges of Sindh forest division

Indicators/Ranges	Population density (km^{-2})	Literacy rate (%)	Percentage of BPL households	Percentage of fuel wood consumption	Percentage main workers	Percentage of MNGREGA beneficiaries
Sindh	241	52.13	0.42	37.5	7.41	7.35
Manasbal	298	57.77	0.35	33.4	6.62	7.16
Harran	1208	42.94	0.22	12.7	5.57	5
Average	582.33	50.94	0.33	27.86	6.53	6.50
Minimum	241	42.94	0.22	12.7	5.57	5
Maximum	1208	57.77	0.42	37.5	7.41	7.35
Range	967	14.83	0.2	24.8	1.84	2.35

subsistence level, and charcoal production has become more commercialized, potentially posing a greater threat to forest sustainability (Butz 2012). Various approaches have been suggested to address the environmental issues associated with biomass energy consumption. These strategies include enhancing the techniques for producing firewood and charcoal, advocating for the adoption of fuel-efficient stoves, and promoting the utilization of alternative energy resources such as liquefied petroleum gas (LPG) and biogas (Grimsby et al., 2016). Additionally, offering a variety of improved cooking stoves (ICS) and flexible payment mechanisms could increase adoption rates and help reduce fuelwood consumption (Kulindwa et al., 2018). However, it is crucial to consider complex socioeconomic factors and local preferences when implementing these solutions to ensure their effectiveness in conserving forest resources, while meeting the energy needs of the population.

Percentage of main workers: The highest percentage of workers (8.35%) was in Kangan, indicating that the block was easily available for manpower. Among all forest blocks, Harran II had the lowest number of main workers (3.98%) (Table 2). The Sindh range had a maximum (7.41%). The Harran–Shallabugh forest range had the lowest (5.57%) among the three ranges (Table 3). Forest cover in Indian provinces appears to be affected by the peripheral workforce. These activities, along with firewood gathering, may negatively affect the forest cover over time. Consequently, a strategy to reduce forest dependency and expand resources outside of these natural areas could help preserve forest cover and maintain the evolutionary potential of the species. The government has launched Joint Forest Management to boost forest regeneration and rural income. This program could be expanded to address various local issues related to overall village development, potentially enhancing forest cover changes and native species regeneration (Murali et al., 2002). Hofflinger et al. (2021) study in southern Chile demonstrates that the expansion of the forestry industries has not reduced unemployment or improved incomes for indigenous or non-indigenous groups. Instead, they exacerbate the poverty and inequality among these populations. This suggests that the link between forestry employment and socioeconomic outcomes is intricate and may differ based on context (Adams et al., 2016, Malkamaki et al., 2018).

Mahatma Gandhi National Rural Employment Guarantee Act Beneficiaries (MGNREGA): The implementation of MGNREGA suggests a scarcity of alternative employment options, resulting in a reduced adaptive capacity. Significant variations exist among the states during the execution of MGNREGA within the IHR. The highest MNREGA of 8.51% was observed for Lar (Table 2), indicating that the block places a strain on natural resources. Among all the forest blocks, Dagapora had the lowest MNREGA (2.04%) (Table 2), and the Sindh range had the highest MNREGA of 7.35% people per square kilometer, indicating that natural resources were under severe strain. The Harran-Shallabugh

Table 4. Normalized value vulnerability indices (VIs) and corresponding ranks of blocks of Sindh forest division

Indicators/Blocks	Population density(km^{-2})	Literacy rate (%)	Percentage of BPL households	Percentage of fuel wood consumption	Percentage of main workers	Percentage of MNGREGA beneficiaries	VI	Rank
Dagapora	0.81	1.00	0.89	0.00	0.95	1	0.774	1
Harran II	0.95	0.94	0.22	0.06	1.00	0.52	0.615	2
Chattergul	0.53	0.53	0.89	0.43	0.53	0.15	0.509	3
Gutlibagh	0.28	0.56	0.78	0.51	0.50	0.28	0.484	4
Harran I	1.00	0.94	0.22	0.09	0.36	0.29	0.482	5
Wangat	0.09	0.64	0.78	0.55	0.30	0.33	0.448	6
Ganiwon	0.12	0.70	0.78	0.60	0.23	0.24	0.445	7
Kullan	0.05	0.71	0.56	1.00	0.19	0.06	0.428	8
Kangan Town	0.42	0.84	1.00	0.21	0.00	0.08	0.426	9
Gund	0.00	0.48	0.56	0.66	0.21	0.19	0.349	10
Shallabugh	0.69	0.61	0.11	0.02	0.24	0.35	0.337	11
Branabugh	0.10	0.53	0.11	0.38	0.45	0.27	0.307	12
Akhal 1	0.06	0.39	0.22	0.45	0.46	0.24	0.302	13
Akhal II	0.05	0.55	0.00	0.47	0.19	0.25	0.252	14
Lar	0.04	0.00	0.11	0.51	0.20	0.00	0.143	15

forest range had the lowest population density among the three ranges at 5% (Table 3). These findings are related to the diminishment or absence of woodland resources (indicated by forest coverage), which heightens vulnerability through reduced ecological and biophysical adaptive abilities (Lawson et al., 2020). In certain instances, lack of forest cover is an unchangeable geographical feature. Another crucial factor contributing to decreased adaptive capacity, particularly in rural areas, is the prevalence of poverty, which is also a key driver of vulnerability (Wester et al., 2019). In the Hindu Kush Himalaya (HKH), the lack of employment opportunities coupled with other factors has serious implications for the overall development of the region (Mishra et al., 2019). The absence of alternative employment opportunities exacerbates this situation as people are forced to rely on forest resources for their survival, leading to increased deforestation and land degradation (Qasim et al., 2010). In study from the Philippines, irrigation development in lowland areas created new employment options, which subsequently led to reduced forest-clearing rates in upland regions (Shively and Pagiola 2004). This suggests that promoting alternative livelihoods and economic diversification in the HKH region could alleviate forest encroachment while supporting sustainable development. The lack of alternative employment opportunities in the HKH region has significant implications for forest development and conservation. Addressing this issue through integrated approaches that consider economic, social, and environmental factors is crucial for achieving sustainable regional development (Rasul et al., 2017). Policymakers should focus on creating diverse livelihood options, enhancing human capital, and implementing proper and gender-responsive adaptation strategies to reduce vulnerability and protect forest resources in the HKH region (Dilshad et al., 2018).

Vulnerability indices (VIs) of forest blocks and ranges: Composite vulnerability indices lie between 0 and 1, with 0 being the lowest possible vulnerability index and 1 being the highest (Table 5). The Dagapora block had the highest vulnerability index (0.774 (1st rank)), followed by Harran II (0.615) (2nd rank)), indicating that these blocks are more vulnerable. The Lar Forest block had the lowest vulnerability index value of 0.143, indicating that it was less vulnerable, with a vulnerability rank of 15th (Table 4, Fig. 2). The range-wise Harran-Shallabugh range had the highest vulnerability index of 0.667 (Ist rank), followed by the Sindh range of 0.401 (2nd rank), indicating that these are more vulnerable ranges. The Manasbal range had the lowest composite vulnerability index value of 0.344, indicating that it was less vulnerable than the (3rd rank) vulnerability rank (Table 5, Fig. 3). Once sufficient indicators are identified, the vulnerability of each ecosystem can be assessed. Gupta et al. (2019) and Pokhriyal et al. (2020) used various indicators to measure susceptibility throughout the IHR. Sharma et al. (2017b) performed a nationwide vulnerability assessment of India using four vulnerability indicators: biological diversity, disturbance index, forest canopy cover density, and slope. This indicator-based evaluation encompassed the entire country. The same indicators were utilized by (Upgupta et al., 2015) to estimate the vulnerability to the HP condition of the IWH. The current findings are consistent with those of Sharma et al. (2013), investigated the inherent vulnerability of the Aduvalli Forest in the Western Ghats of South India by computing vulnerability indices to evaluate inherent susceptibility and design adaptation strategies.

Major drivers of vulnerability at the block and range levels: The main drivers of vulnerability are identified as indicators with normalized values greater than 0.7 for each block and range, typically resulting in approximately 3-4 primary drivers for each block and range. The major drivers of vulnerability across blocks and ranges were high BPL households, fuelwood dependency, rapid population growth, low literacy rates, and a lack of alternative livelihood opportunities, contributing to increased vulnerability (Figs. 4, 5). Chauhan et al. (2018) also identified climate change-induced disasters, forest fires, overgrazing, over-extraction, and encroachment as primary causes of forest degradation in Uttarakhand. Rotich and Ojwang (2021) concluded that the main factors driving climate change in Kenya's Cherangany Hills forest ecosystem include conversion of forests to croplands and grasslands, grazing, encroachment, illegal logging, firewood harvesting, charcoal production, forest fires, and population growth. Similarly, Debebe et al. (2023) reported that forest cover decline in Northwest Ethiopia was

Table 5. Normalized value of ranges, vulnerability indices (VIs) and corresponding ranks of ranges of Sindh forest division

Indicators/Blocks	Population density(km^{-2})	Literacy rate (%)	Percentage of BPL households	Percentage of fuel wood consumption	Percentage of main workers	Percentage of MNGREGA beneficiaries	VI	Rank
Harran Shallabugh	1	1	0	0	1	1	0.667	1
Sindh	0	0.380	1	1	0	0	0.401	2
Manasbal	0.058	0	0.65	0.834	0.429	0.080	0.344	3

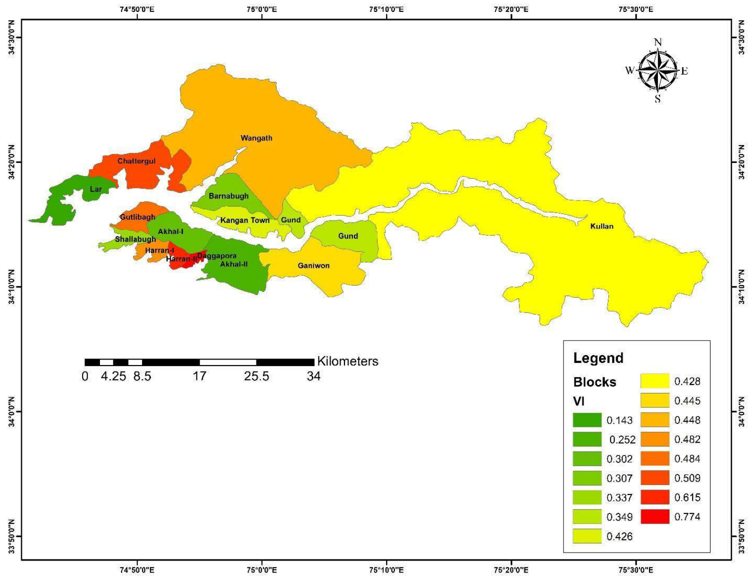

Fig. 2. Block level vulnerability map of Sindh Forest Division

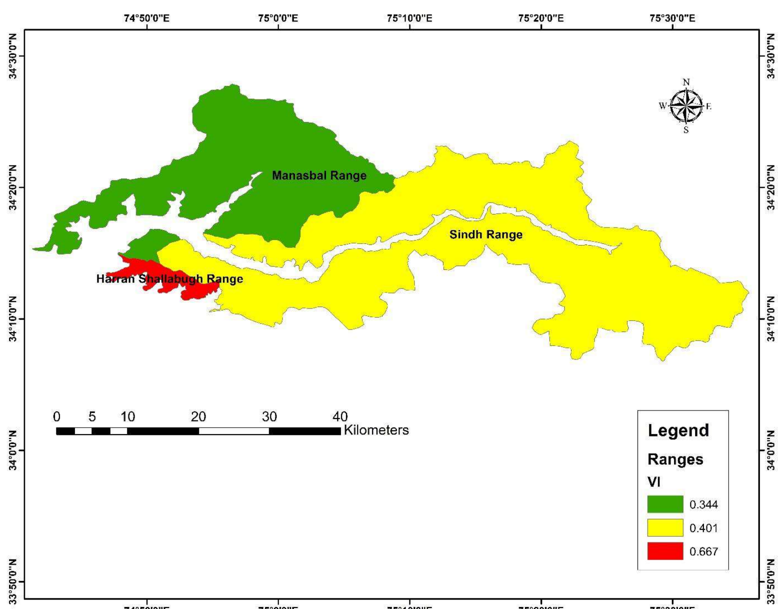

Fig. 3. Range level vulnerability map of Sindh Forest Division

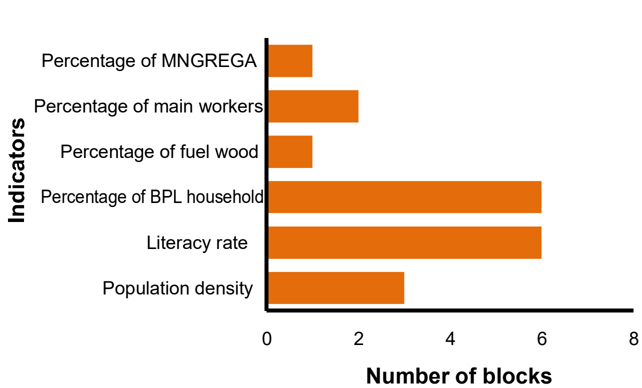

Fig. 4. Key drivers of forest blocks of Sindh Forest Division

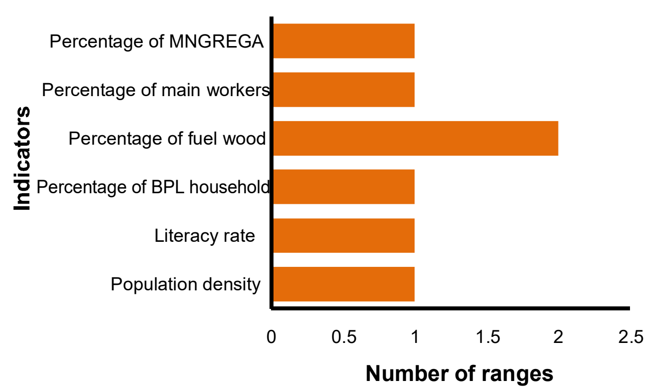

Fig. 5. Key drivers of forest ranges of Sindh Forest Division

driven by agricultural expansion, population growth, growing demand for fuelwood, livestock pressure, and forest fires. Local adaptation strategies have often been overlooked in predicting forest species vulnerability, because they play a more significant role in reducing forest vulnerability under changing climate scenarios (Razgour et al., 2019). The vulnerability of global forest ecoregions to future climate change threatens their biodiversity. Conservation decision making and management may help mitigate climate change-induced forest vulnerability (Wang et al., 2019). Bourgoin et al., (2020) observed the ecological vulnerability of forested landscapes in Vietnam's central highlands and found that multidate satellite data offers promising monitoring capabilities for agricultural and forested landscapes.

CONCLUSION

This study underscores the socioeconomic vulnerability of forest firing communities of Sindh forest division to climate change using six socioeconomic indicators: population density, literacy rate, BPL households, fuelwood consumption, main workers, and MGNREGA participation. The Dagapora forest block was most vulnerable while the Lar forest block was the least vulnerable,. Among the ranges, the Harran Shallabugh was the most vulnerable followed by the Sindh range and the Manasbal range was the least vulnerable . The major drivers of vulnerability across blocks and ranges were high BPL households, fuelwood dependency, rapid population growth, low literacy rates, and lack of alternative livelihood opportunities. Strategies should include afforestation with deep-rooted native species, development of alternative livelihoods for marginalized groups, and integration of poverty alleviation with forest conservation. Promoting income through eco-tourism and Non-Timber Forest Products (NTFP), along with watershed and soil conservation measures, is essential. An integrated resilience plan involving government bodies, civil society, and local communities is vital. Building long-term community resilience calls for a holistic approach that includes progressive policies, legal frameworks, and targeted government schemes. Livelihood diversification through MGNREGA and skill development can reduce forest dependency. Improved food security via the Targeted Public Distribution System (TPDS), along with investments in healthcare and education, supports community stability. Legal frameworks such as the Forest Rights Act (2006) enable forest dwellers to secure traditional rights and benefit from conservation-linked livelihoods. The National Forest Policy (1988) and Joint Forest Management (JFM) promote community participation and equitable benefit-sharing. Selective thinning and fast-growing afforestation for fuelwood help manage forest pressure.

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Machine Learning-Driven Land Use Land Cover Classification using Different Algorithms on Sentinel-1 and Sentinel-2 Imagery

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Abstract: Land Use Land Cover (LULC) classification is crucial for understanding and managing the planet's resources. The present study examines LULC classification using machine learning (ML) algorithms and geospatial data in the Karulai region of Kerala. Sentinel-1 and Sentinel-2A satellites provided Multispectral and radar imagery, offering high-resolution (10 m), frequent data. Three ML models Random Forest (RF), Classification and Regression Trees (CART), and K-Nearest Neighbors (KNN) were evaluated for classification accuracy. RF achieved the highest accuracy (93.87%) and Kappa coefficient (0.916), outperforming CART and KNN in complex land cover types, particularly forest and built-up areas. RF accurately detected 101.7 ha of water and 19,692.67 ha of forest, while CART and KNN showed variability in urban and plantation areas. Producer and user accuracy metrics further validated RF's reliability, with 98% producer accuracy for teak plantations. Challenges emerged in classifying rubber plantations and built-up areas, but RF remained the most robust model. These findings highlight the importance of ML in LULC mapping, with applications in urban planning, forest monitoring, and agricultural management. This study improves LULC classification accuracy, aiding sustainable land management and planning.

Keywords: Land use land cover, Machine learning, Random forest, Classification, Regression trees, K-nearest neighbors

Land, as a fundamental resource, undergoes significant changes due to human activities, shaping landscapes and impacting natural systems (Geri et al., 2010, Plieninger et al., 2015 and Wassie 2020). Land use land cover (LULC) classification has emerged as a critical aspect of environmental management (Wang et al., 2022) and urban planning (Gaur and Singh 2023), owing to its significance in understanding and managing natural resources (Olorunfemi et al., 2020). Land use involves managing and transforming natural environments into urban areas, agricultural lands, and infrastructure (Mitchell, 2023). Land cover is the observed physical and biological cover on the Earth's surface, including vegetation types, built environments, and water bodies (Wang et al., 2023, Mosca et al., 2024). These categories help in assessing the ecological balance, monitoring environmental changes, and planning sustainable development. Earlier LULC classification was primarily conducted using ground surveys and aerial photography, which were labour-intensive and time-consuming (Santosh et al., 2019). Over the past decades, the concept and methodology of LULC have evolved significantly, driven by advancements in remote sensing technologies and computational techniques. With the advent of satellite imagery, particularly with the launch of the Landsat program in the 1970s, the field experienced a paradigm shift (Khan et al., 2018). Satellite images provide a broader and more frequent view of the Earth's surface, enabling more efficient and comprehensive LULC analysis (Li and Stein 2020). The launch of Sentinel-1 and Sentinel-2 satellites by the European Space Agency (ESA) has further revolutionized the field by providing high-resolution radar imagery and multispectral imagery with a revisit time of six days (Languille et al., 2017, Phiri et al., 2020, Upadhyay et al., 2022). This advancement has opened new avenues for precise and timely LULC classification. Accurate Land Use Land Cover (LULC) data is vital for various applications, including urban planning, agriculture management, forest monitoring, and disaster management (Moayedi et al., 2020, Vandansambuu et al., 2020). Understanding urban expansion patterns aids in infrastructure development. Monitoring deforestation is essential for biodiversity conservation and climate change mitigation. In agriculture, LULC data assists in crop monitoring and yield prediction, enhancing food security. In the context of climate change, LULC studies help understand carbon sequestration patterns and formulate mitigation strategies (Sohl et al., 2012, Rajbanshi and Das 2021). In forestry LULC classification is essential for forest management, conservation, and policy-making, offering vital data on ecological dynamics and environmental changes (Junaid et al., 2023).

Machine learning (ML), a key area of artificial intelligence, develops algorithms that allow computers to learn from data without explicit programming, with broad applications across many fields (Munde 2024). Recently, the combination of machine learning (ML) techniques with satellite data for ecosystem monitoring has gathered considerable attention (Sharma and Chishty 2019,Masolele et al., 2021, Morais et al., 2021, Veeramani et al., 2024, Markuna and Dumka 2024). Machine Learning (ML) has emerged as a powerful tool in the realm of LULC classification (Rawat et al., 2024) and outperform traditional statistical methods by assessing complex non-linear relationships (Garg and Tai 2013) between reflectance and ecosystem structure, without requiring prior assumptions about the underlying processes or data distribution (Ghosh and Behera 2018). ML algorithms can automatically detect patterns and relationships in large datasets (Oprea and Bâra 2021). This capability makes ML particularly suited for handling the complex and high-dimensional data obtained from satellite imagery. The role of ML in LULC classification is multifaceted (Tassi and Vizzari 2020). ML algorithms can handle the vast amounts of data generated by modern satellites (Bhattacharyya et al., 2023), processing them efficiently to produce accurate classifications. ML can also integrate various types of data, such as spectral, spatial, and temporal information, to improve classification accuracy (Du et al., 2020). These algorithms can adapt to changes in data patterns (Naqa and Murphy 2015), making them robust against varying environmental conditions and seasonal changes (Huntingford et al., 2019, Balogun et al., 2021).

The importance of this study lies in its potential to enhance understanding and management of land resources through advanced LULC classification techniques. By leveraging ML algorithms on Sentinel-1 and Sentinel-2 imagery this study aims to improve LULC classification accuracy and emphasizes the broader implications for sustainable development and environmental management, by systematically exploring the intersection of ML and LULC classification.

MATERIAL AND METHODS

Study area: The study was conducted in the Karulai forest of Nilambur South division in Malappuram district, Kerala, located between 76°18'19.65"- 76°32'51.88"E longitude and 11°23'14.05"- 11°13'42.70"N latitude (Fig. 2). This area spans altitudes from 50 to 2600 meters MSL covers a total area of 26,285 ha and includes tropical evergreen, semi-evergreen, moist deciduous forests, and teak plantations. The climate is hot and humid, with temperatures ranging from 20 to 30°C and an average annual rainfall of 2900 mm. Malappuram consist of 3 natural divisions, lowland, midland and highland. The district has dry season from December to February, hot season from March to May, the south west monsoon from October to November and usually very heavy and nearly 75 percent of the annual rains are received during this season. The climate is generally hot and humid (Haani and Murari 2022).

Satellite data: This study used open-source European Space Agency (ESA's) Sentinel-1 (S1) and Sentinel-2 (S2) satellite data. S1 satellites operate at 693 km (431 mi) altitude, with 3-axis altitude stabilization. provides dual-polarized (VV and VH) C-band which provides a collection of data in all-weather conditions at a spatial resolution of 10 m, and the sensor operates at a central frequency of 5.405 GHz. Over the land S1 acquires images with interferometric wide (IW, 250 km) swath mode. S2 provides multispectral optical data with 13 bands ranging from 443 to 2190 nm wavelength and a spatial resolution of 10–60 m and 290 km field of view. Both S1 and S2 data were regularly available over the test site with an average temporal resolution of six days. Sentinel-1A (S1A) ground resolution detected (GRD) level-1 product consists of multi-view (10 m × 10 m) and ground range projected images using ellipsoid model WGS84. Sentinel- 2A (S2A) Level-2A orthorectified atmospherically corrected surface reflectance data were used.

Modelling and calibration: The study was conducted using the Google Earth Engine. Filed visits are conducted for sample point data collection. To enhance the dataset, three indices were calculated and added as bands: the normalized difference vegetation index (NDVI), normalized difference water index (NDWI), and enhanced vegetation index (EVI). This data was median-composited and merged with the Sentinel data image stack. A total of 3886 sample points representing water, built-up, bare land, rubber plantation, teak plantation, grassland, and forest classes were merged, forming a labelled dataset for supervised classification. LULC class water body include river and pond in the study area. Using the collected bands, samples were generated from the study region and split into training (70%) and testing (30%) subsets. A CART, RF, and KNN classifier were trained and applied to the image stack, producing a land use land cover (LULC) classification. Model performance evaluated using producer's accuracy user's accuracy, kappa coefficient and overall accuracy and its formulas are:

$$Producer's\ Accuracy == \frac{True\ Positives\ (for\ a\ class) \times 100}{Total\ Actual\ Pixels\ (for\ that\ class)}$$

$$User's\ Accuracy = \frac{True\ Positives\ (for\ a\ class)}{Total\ Predicted\ Pixels\ (for\ that\ class)} \times 100$$

$$Overall\ Accuracy = \frac{\sum True\ Positives}{Total\ Pixels} \times 100$$

$$Kappa\ Coefficient = \frac{po - pe}{1 - pe}$$

po = observed accuracy (Overall Accuracy),

pe = expected accuracy (probability of random agreement).

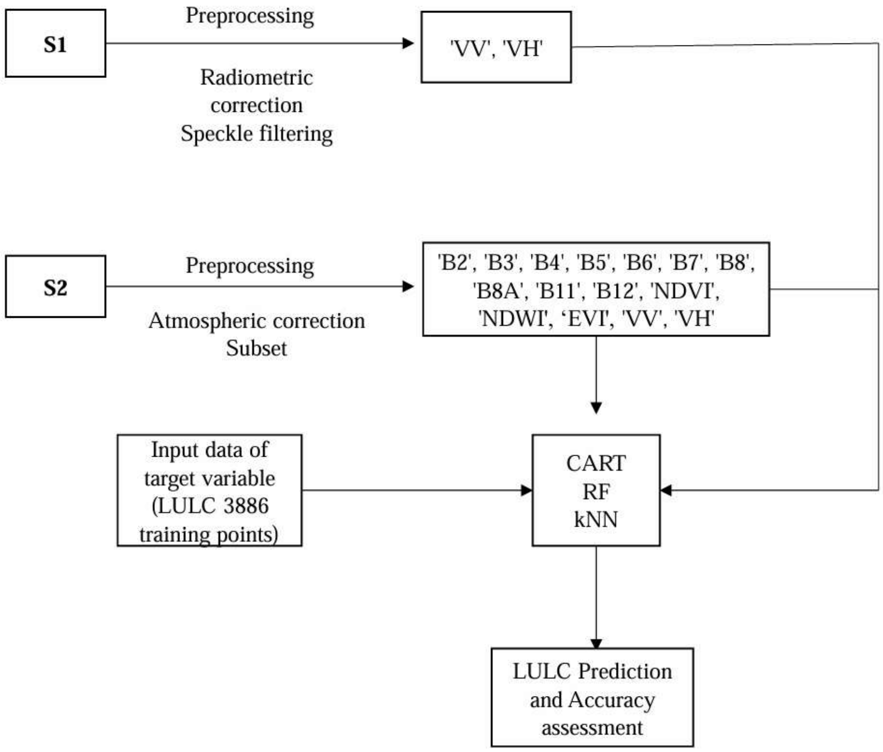

Fig. 1. Flow chart of the methodology for LULC

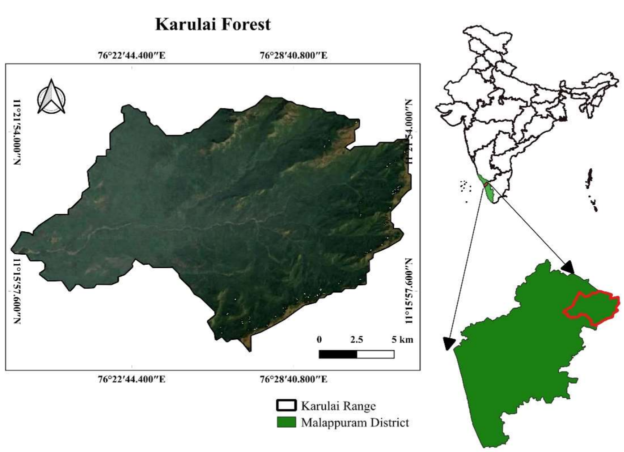

Fig. 2. Map of the study area

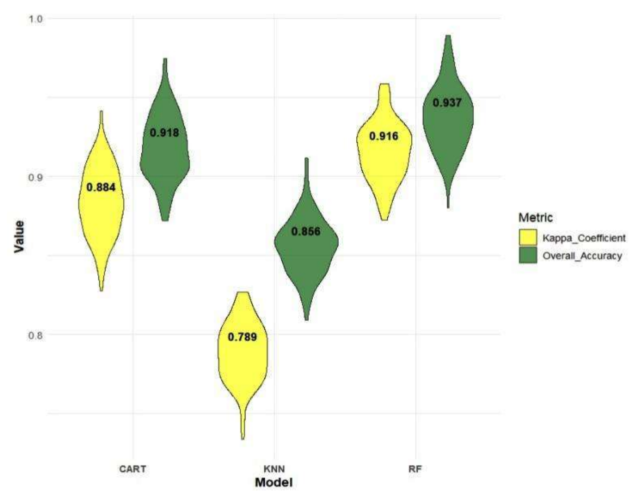

Fig. 3. Overall accuracy and Kappa Coefficient of CART, RF and KNN model

RESULTS AND DISCUSSION

Model performance and LULC mapping: The Land use land cover (LULC) models were predicted using three machine learning algorithms: classification and regression trees (CART), random forest (RF) and k-nearest neighbors (KNN) (Fig. 4). All models achieved an overall accuracy exceeding 85% and a Kappa coefficient of 78% (Fig.3), indicating strong agreement between predicted and actual classifications. The model accuracies were further evaluated using the producer's accuracy, user's accuracy, and Kappa values (Fig. 3, Fig. 6). Among the models, the random forest algorithm exhibited the highest overall performance, with an accuracy of 0.937, outperforming both CART and KNN. The Kappa coefficient was utilized as a robust metric to assess the classification accuracy of various machine learning models. It indicates the model's agreement with actual classifications, adjusted for the possibility of random chance. RF model exhibited the highest Kappa value of 0.916, indicating substantial agreement beyond random chance, followed by CART with a Kappa value of 0.884, KNN model exhibited the lowest Kappa score at 0.789 (Fig. 3), suggesting relatively lower classification reliability in comparison to the other models. The findings confirm Kappa as a valuable complement to overall accuracy in evaluating model performance.

Quantification of LULC classification: The results of the area under different land use land cover classifications using different machine learning algorithms exhibited significant differences between the algorithms (*i.e.,* RF, CART, and KNN) in their performance. RF demonstrated the most consistent and accurate results across various land cover types. RF identified 101.7 ha for the water area, while CART and KNN detected significantly different values of 116.2 ha and 162.3 ha, respectively. In built-up areas, RF's classification of 4.03 ha starkly contrasted with CART's 100.9 ha and KNN's 28.51 ha, showing major discrepancies in urban area detection. RF also displayed superior accuracy in forest area classification, capturing 19,692.67 ha, compared to CART's 19,090.68 ha and KNN's 15,950.64 ha.

Minor deviations were observed in bare land detection, with RF, CART, and KNN identifying 1358.9, 1308.5 and 1387.9 ha, respectively. However, significant variations arose in rubber and teak plantation classifications. For rubber plantations, RF classified 176.8 ha, while CART and KNN overestimated the area at 383.9 ha and 368.3 ha. Similarly, teak plantations showed stark contrasts, with RF identifying 4286.6 ha, CART 4318.1 ha, and KNN an inflated 7375.4 ha.

Grassland classification also reflected algorithmic variability, with RF detecting 664.2 ha, compared to CART's 966.6 ha and KNN's 1011.8 ha. These results underscore RF's superior performance in land cover classification, particularly in forest and built-up areas, where the other algorithms showed significant limitations. RF provided the most consistent and accurate results, making it a robust tool for capturing the complex landscape of Karulai. These findings suggest RF's reliability in handling diverse land cover types, outperforming CART and KNN.

RF model showed the highest producer accuracy (PA) for teak plantation (0.98) indicating that out of 10000 pixels of teak plantation in the ground, 9803 pixels are correctly labelled as Teak plantation in the RF model. KNN model showed the lowest (0.4355) representing that if the 10000 pixels are built up areas in the ground only 4355 pixels are labelled as Builtup area in the model. RF model built up area showed highest users' accuracy (1) indicating that all the pixels are labelled as built up area by the model belongs to Builtup area in ground truth data. lowest UA (0.6136) in built up area class of KNN model indicating that when classifier model labelled 10000 pixels as built up area but only 6136 pixels are built up area in ground truth data.

The random forest (RF) model demonstrated superior performance in LULC classification compared to CART and KNN. RF achieved the highest overall accuracy and Kappa coefficient, indicating strong agreement with reference data and minimal random chance. This superior performance of RF is attributed to ensemble learning nature (Mienye and

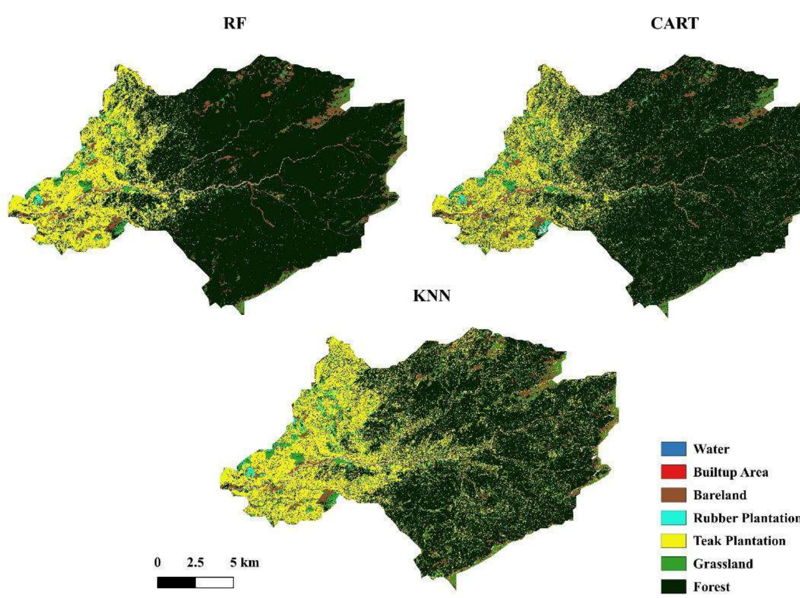

Fig. 4. LULC maps of the study area using RF, CART and KNN machine learning algorithm

Sun 2022) i.e., combining multiple decision trees, handle complex spatial patterns (Mariano and Monica 2021), reduce overfitting (Ali et al., 2012 and Belgiu and Drăguţ 2016), and improve generalization (Fawagreh et al., 2014). RF's ability to assess feature importance enables it to focus on the most relevant information (Hapfelmeier et al., 2014 and Gregorutti et al., 2017), leading to more accurate classifications. CART, a single decision tree algorithm, is susceptible to overfitting (Zhao et al., 2021), especially when the tree depth is too large . KNN, which relies on distance-based similarity measures, can struggle with high-dimensional data and noisy samples (Halder et al., 2024). Since our model is trained with 15-

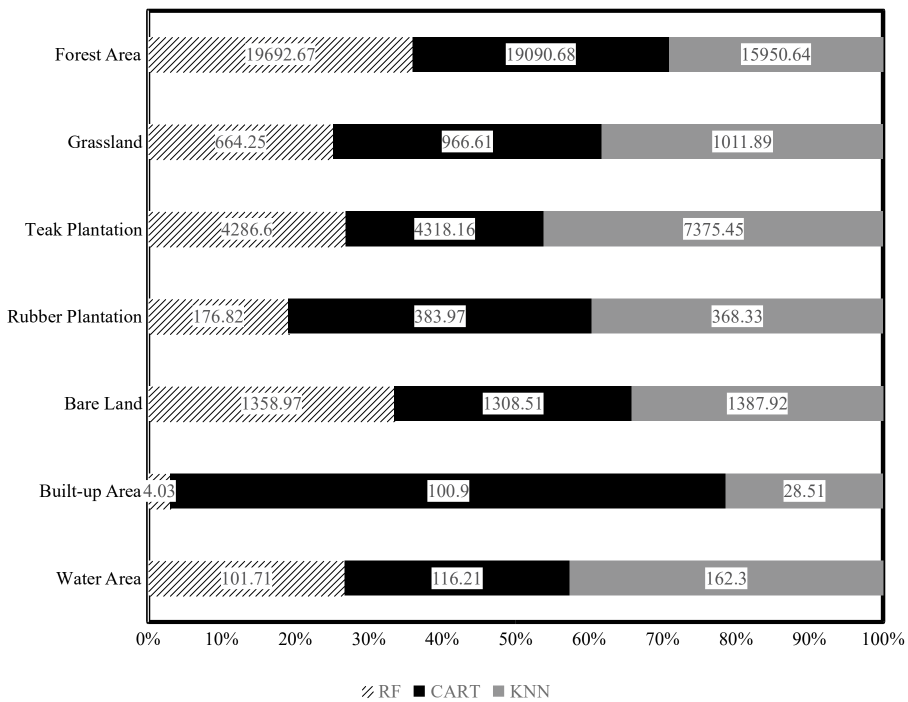

Fig. 5. Area under different LULC class as predicted by RF, CART and KNN model

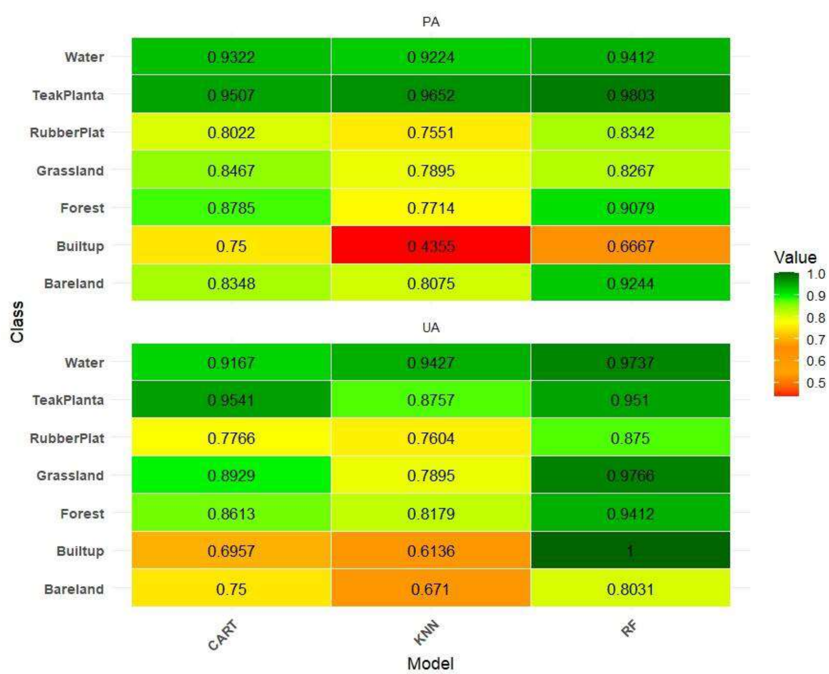

Fig. 6. Producers and users' accuracy heatmap of CART, RF and KNN model

dimensional data, it may become a hurdle to handle by the KNN algorithm. Hence the performance of CART and KNN algorithms was not as high as RF. The results highlight the importance of model selection in LULC mapping. RF's robustness and versatility make it a valuable tool for accurate and reliable land cover assessments, particularly in diverse landscapes like Karulai.

CONCLUSION

The study reveals significant insights into environmental modeling through a combination of ground truth, geospatial data, and machine learning. Machine learning algorithms enhance the accuracy of LULC classification significantly compared to traditional methods. Among the evaluated models, random forest achieved the highest overall accuracy of 93.87% and a Kappa coefficient of 0.916. RF demonstrated superior performance in identifying complex land cover types. Producer accuracy for teak plantations reached 98%, indicating RF's reliability in classifying specific vegetation types. Challenges persisted in classifying rubber plantations and built-up areas. Integrating multiple data types, including spectral and temporal information, improved classification outcomes. The study emphasizes the transformative role of advanced technologies in understanding and managing earth's resources effectively.

AUTHOR'S CONTRIBUTION

This study was conducted as part of the Master's research project under Kerala Agricultural University. Co-author K.S. Aneesh contributed to the conceptualization of the study and provided theoretical and academic inputs. K.T. Shanid assisted with field visits and communication in the local language. K.V. Murali contributed to the development of JavaScript and R scripts. K. Mahathwa, C. Gayathri, and N.J. Meenakshi supported the interpretation of the results.

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Quantification of Phytomass and Carbon Storage of Woody Vegetation in Logone Valley (Far North, Cameroon)

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***Abstract*:** The degradation of the vegetation through anthropogenic or natural phenomena leads to the release of more carbon into the atmosphere than that occurs during photosynthesis. This constitutes a great burden to world. In order to assess the contribution of the Logone Valley vegetation to climate change mitigation, the present study aims to estimate the phytoomass and carbon stock. Woody carbon was quantified using the non-destructive Brown equation in 500 x 20 m transects for each vegetation formation. The species and families with the highest phytomass were *Balanites aegyptiaca* (3 16.35 t/ha) and Balanitaceae family (316.35 t/ha). The quantities of carbon sequestered in the plant formations were 373.06 tC/ha in the woody savannah, 237.23 tC/ha in the shrub savannah, 45.82 tC/ha in the grassy savannah and 71.31 tC/ha in the cultivated zone. Hence, this study add knowledge of the contribution of each plant formation to carbon sequestration. Actions to strengthen the woody potential are required to minimise the long-term degradation of this site and to make it a sustainable source of carbon sequestration.

Keywords: Phytomass, Carbon stock, Climate change, Logone Valley, Cameroon

Woody flora play a crucial role in the well-being of the world population by providing various goods and services, including biodiversity conservation, economic climate regulation, air and water purification and recreation (Ezekiel and Bhoke 2024). It also produces fruits, seeds, tubers, flowers, sap and other edible products that are used for human and animal consumption, traditional medicine, handicrafts and to heat timber, moreover, contributes directly to the diet (Popoola 2001). Despite its importance, the management of the woody vegetation is highly leading to degradation. However, the degradation of the vegetation through anthropogenic or natural phenomena, leads to the release of more carbon into the atmosphere than that occurs during photosynthesis (Brown 2002). The deforestation disrupts the natural carbon cycle by reducing greenhouse gas emissions (Swarnkar et al., 2025). The vegetation plays a key role in mitigating climate change by absorbing and storing atmospheric carbon (Arianasari et al., 2021). The loss of plant cover reduces carbon reserves, resulting in the accumulation of carbon in the atmosphere. The carbon absorbed by the plant is converted into organic matter and stored in the phytomass (Fadillah et al., 2023). In Cameroon, and more specifically in the Sudano-Sahelian zone, climate change, the result of deforestation and the degradation of vegetation cover is increasing at an alarming rate (Agbanou et al., 2018). Given the recognised importance of vegetation in the climate change process, this degradation is continuing and poses a real threat to the populations living in the Logone Valley, Far North region, Cameroon. The present study seeks to quantity carbon stock in woody vegetation of the Logone Valley, addressing a critical data gap in the region. More specifically, our study aimed to assess the living above-ground and below-ground phytomass in order to deduce the quantity of sequestered carbon.

MATERIAL AND METHODS

Study area: The study area is located in Logone et Chari Sub Division from Far North Region of Cameroon between 12° North latitude, 15° East longitude. The present study was carried out in four (04) divisions namely Kousseri, Goulfey, Waza and Logone-Birni (Fig. 1). The site was chosen because of the pressure of human activities, due to the advancing desert, global warming, the drop in rainfall, the population's exposure to poverty, the anarchic and illicit exploitation of non-timber forest products and timber forest products by the population to meet their needs and the expansion of agriculture, as well as the agropastoral conflict which oppose regularly the Arabs and Musgoum tribes.

Floristic inventory: The sampling technique used to carry out the floristic inventories mentioned by Tchobsala (2011). In each subdivision, according to the orientation of the local population, we chose the place where plant formations exist with a dominance of anthropogenic activities. The experimental design is a split-plot with two factors. The first factor is the different subdivisions (main treatments), the second factor is the types of plant formations (secondary treatments) and the transects chosen in each type of plant formation constitute the repetitions. The inventory of plant species was carried out on a transect of 10,000 m^2 (500m x 20m), 5 layons 4 metres wide and 500m long. In each transect, all the trees were surveyed and the following dendrometric parameters were measured for all individuals with a height of one and a half metres (1.30 m): height, crown diameter and circumference at the base of the trunk at 1.30 cm from the ground (Arbonnier 2000). Samples of species not identified in the field were taken and identified at the Plant Biology Laboratory of the University of Maroua and through the identification key for sahelian woody plants.

Floristic surveys were carried out in four (04) districts, taking into account four (04) plant formations (cultivated area, shrub savannah, tree savannah and herbaceous savannah), so that twelve (12) surveys per district with four (04) per plant formation. A total of 48 transects were placed throughout the sub division.

Quantification of carbon stock: This is carried out in the 500m x 20m transect. The diameters at breast height (1.30 m from the ground) and the heights of all the trees in the transects are measured using a tape measure and a graduated pole respectively. This phytomass is estimated by the indirect method, using a mathematical model that takes into account the diameter at breast height and the height of the trees. Brown's (1997) and Tchobsala (2014), mentod was used. Coefficient of determination is highly significant (R^2= 0.987). It was also developed in the Sahelian climate.

$Ba = \exp[-3.114 + 0.9719 \mathrm{Ln}(D2H)]$

where Ba is the above-ground phytomass of the tree in kg, **DBH** is the diameter at breast height in m and H is the height of the tree in m.

Root phytomass: Measuring roots is difficult and expensive, but root phytomass can be estimated indirectly and accurately using an equation used by Cairns et al. (1997).

$Br\,(kg) = \exp[-1.0587 + 0.8836 \times \ln(Ba)]$

Where Br = root phytomass, Bt= total phytomass above ground from the total above-ground phytomass of each type of plant formation, the root phytomass was calculated. It was also expressed in tonnes per hectare.

Estimating the amount of carbon in above-ground phytomass: The carbon in the various components is generally assessed by evaluating the phytomass present in the transects. Following the recommendations of the IPCC (2003), the majority of studies use an average carbon concentration value of 50% for vegetation when more precise data is not available. This consists of assessing the quantity of carbon from the phytomass present in several components (above and below ground). The carbon quantities were obtained as per method of Ibrahima et al. (2002) and Tchobsala et al. (2014).

$QCv = Ba \times Cv,$

Where: QCv = vegetation carbon (t/ha), Ba = above-ground phytomass (t/ha), Cv = vegetation carbon concentration (0.5)

Estimating the amount of carbon in root phytomass: To determine the final carbon values, the root phytomass below the soil surface was added. The amount of carbon is obtained as per Ibrahima et al. (2002) and Saïdou et al. (2012).

$QCr = Br \times Cv$

With: QCr = root carbon (tC/ha), Br = root biomass (t/ha), Cv = vegetation carbon concentration (0.5)

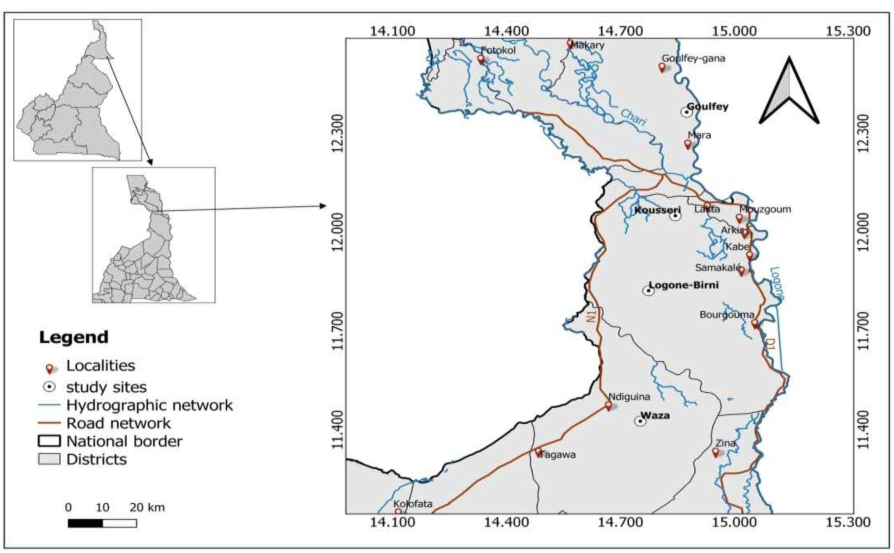

Fig. 1. Study site

Estimation of the total quantity of carbon in each type of plant formation: This was simply obtained by summing the quantities of carbon in all the components (aerial and root) of each type of plant formation. In other words, it is the sum of aerial carbon and root carbon.

QC total = QC aerien + QC racinaire

Calculating CO_2 emissions

The quantity of carbon dioxide (CO_2) that is emitted into the atmosphere was calculated as per Woods Hole Research Center (2011) in Mali.

C02 = AGBP* PMC02/PMC where:

AGBP is the total above-ground biomass of the sample plot;
PWC02 is the molecular weight of carbon dioxide (44);
PMC is the molecular weight of carbon (12).

Statistical analysis of the data: The data collected during this study were recorded in an Excel sheet and analysed using i-Xlstat where anova one-way was used to bring out the difference between plant formations and boroughs.

RESULTS AND DISCUSSION

Total biomass by plant formations and sites: Phytomass increases with higher DBH and species density. This wooded savannah has the highest phytomass production with 234.78 t, 207.19 , 177.35 and 174.47 t/ha respectively in Waza, Goulfey, Kousseri, and Logone-Birni (Table I). The lowest phytomass yields were in the grassy savannah (51.30 and, 21.45 t/ha) and the cultivated zone (39.65, 33.68, 27.78 and 17.51 t/ha) in the subdivisions of Waza, Goulfey, Kousseri and Logone-Birni respectively.

Phytomass distribution by species: The estimated phytomass of each species as a function of the diameter at breast height and the density of the species in the study area (Table 2). The species with the highest phytomass are *Sclerocarya birrea* (75.20 t/ha) followed by *Combretum glutinosum* (61.63 t/ha) in the Waza site. In the Logone-Birni district, *Acacia nilotica* (82.87 t/ha) followed by *Balanites aegyptiaca* (64.64 t/ha). The species *Balanites aegyptiaca* (104.82 t/ha) and Acacia seyal (61.82 t/ha) have a high phytomass rate in the Kousseri site. In the Goulfey area, *Acacia seyal* (96.60 t/ha) and *Balanites aegyptiaca* (95.63 t/ha) sequester more phytomass than the other species on the site. the species *Balanites aegyptiaca* (316.35 t/ha), followed by *Acacia nilotica* (227.85 t/ha) and *Acacia seyal* (224.38 t/ha) sequester more phytomass than the other species on the site.

Contribution to phytomass by plant family : Mimosaceae families (187.93, 172.06 and 147.18 t/ha), followed by the Balanitaceae family (95.63, 64.64, and 104.43 t/ha) in the arrondissements of Goulfey, Logone-Birni and Kousseri respectively; In the arrondissement of Waza, the Combretaceae (94.99 t/ha) and Anacardiaceae (75.20 t/ha) families had the highest phytomass at this site. For the site as a whole, the Mimosaceae family (577.56 t/ha), followed by the Balanitaceae (316.35 t/ha) and Rhamnaceae (108.96 t/ha) have more phytomass than the other families on the site.

Carbon stock estimation: The largest carbon stocks per hectare are located in wooded savannahs with carbon stocks of 110.34, 97.37, 82.35 and 82.00 tC/ha respectively in Waza, Goulfey, Kousseri and Logone-Birni; followed by shrub savannas at 65.43 tC/ha in Waza, 62.91 tC/ha in Goulfey, 56.37 tC/ha in Logone-Birni and 52.52 tC/ha in Kousseri. Low carbon sequestration in the cultivation zone of 23.8 tC/ha in Logone-Birni, 18.63 tC/ha in Waza, 15.83 tC/ha in Goulfey and 13.05 tC/ha in Kousseri (Table 4).

Potential CO_2 equivalent: This table shows that wooded savannahs release the greatest quantities of carbon into the environment, maximun 404.60 tCO_2/ha in Waza, and minimum 20.50 tCO_2/ha to Logone-Birni (Table 5).

The phytomass results obtained from the sites and plant formations are similar to those obtained in the peri-urban savannahs of Ngaoundéré by Tchobsala et al. (2014), who showed that tree savannahs sequester much more carbon than other plant formations. The low phytomass production in the grassy savannah can be justified by the fact that it is devoid of woody species, and in the cultivated zone this can be explained by the strong human action observed in these formations. Uncontrolled felling by the local population for a variety of reasons (fodder, firewood, food, NTFPs) leads to a reduction in phytomass. The low phytomass in the cultivation zone is due to low densities, which can be explained by the spacing of trees chosen by local people for cultivation. Phytomass production at different sites shows that Waza has the highest phytomass. This phytomass performance in this locality is justified by the existence of a park consisting of denser vegetation where anthropogenic activity is controlled. These results do not corroborate those of Zapfack (2005) in the Yaoundé forest region, where the cultivated areas showed low phytomass production (1.91 t/ha/). This

Table 1. Phytomass in the different plant formations and sites (t/ha)

Arrondissement/PF	SA	SAR	SH	ZC
Waza	234.78^{k}	139.22^{h}	51.30^{e}	39.65^{de}
Log-Birni	174.47^{i}	119.94^{fg}	11.90^{a}	17.51^{ab}
Goulfey	207.19^{i}	133.85^{gh}	21.45^{abc}	3.68^{cd}
Kousseri	177.35^{i}	111.72^{f}	12.87^{a}	27.78^{bcd}

Numbers with the same letters are not significantly different at the 5% level. P= 0.05, n= 03.
SA= Wooded Savannah, SAR= Shrubby savannah, SH= Grassy savannah, ZC= Growing area, PF= Plant Formation

Table 2. Estimated species phytomass (t/ha)

Names of species	Waza	Log-Birni	Kousseri	Goulfey	PT
Acacia albida	9.55	15.04	2.75	9.94	37.28
Acacia ataxacantha	/	0.41	/	/	0.41
Acacia gerrardii	/	0.55	/	/	0.55
Acacia hockii	0.59	3.95	6.14	/	10.68
Acacia nilotica	24.62	82.87	57.94	62.42	227.85
Acacia polyacantha	0.49	2.92	2.13	/	5.54
Acacia senegal	/	1,68	/	/	1,68
Acacia seyal	23.37	42.58	61.82	96.60	224.38
Acacia sieberiana	11.23	22.07	16.39	18.96	68.66
Annona senegalensis	1.18	/	/	/	1.18
Anogeissus leiocarpus	5.35	/	0.71	/	6.06
Azadirachta indica	4.87	16.56	6.10	1.13	28.66
Balanites aegyptiaca	51.65	64.64	104.43	95.63	316.35
Bauhinia rufescens	2.99	3.10	2.98	8.30	17.37
Borassus aethiopum	/	1.40	0,.4	/	1.84
Boscia angustifolia	5.66	1.77	20.04	42.74	70.21
Calotropis procera	47.78	6.73	8.87	18.08	81.45
Celtis integrigolia	2.32	/	/	/	2.32
Combretum aculeatum	/	0.95	/	0.35	1.30
Combretum adenogonium	0.87	/	/	/	0.87
Combretum glutinosum	61.63	/	/	/	61.63
Commiphora africana	0.86	/	/	/	0.86
Dalbergia melanoxylon	1.35	0.32	/	/	1.67
Dichrostachys cinerea	0.54	/	/	/	0.54
Diospyros mespiliformis	/	2.44	/	/	2.44
Gardenia aqualla	0.73	/	/	/	0.73
Guiera senegalensis	27.14	/	/	/	27.14
Hyphaene thebaica	/	12.35	13.97	/	26.32
Khaya senegalensis	/	8.27	/	/	8.27
Mangifera indica	/	1.04	/	/	1.04
Mitragyna inermis	8.68	/	3.97	7.92	20.57
Moringa oleifera	/	/	2.95	/	2.95
Parkinsonia aculeata	/	/	0.99	/	0.99
Phoenix dactylifera	/	/	2.31	/	2.31
Piliostigma thonningii	10.13	5.51	/	/	15.65
Sclerocarya birrea	75.20	/	/	/	75.20
Stereospermum kunthianum	4.77	0.41	/	/	5.17
Strychnos innocua	/	/	0.70	/	0.70
Tamarindus indica	5.35	1.89	2.95	8.63	18.82
Ziziphus mauritiana	47.97	21.65	8.65	25.46	103.73
Ziziphus mucronata	/	2.74	2.49	/	5.24

PT= Total Phytomass, /= No data

difference is due to the ecological environments, the survey methodology used and the density of species in the different sites and plant formations. The statistical test showed a highly significant difference between the biomass values of the different plant formations and sites at the 5% threshold ($P<0.001$).

With regard to the biomass of the species, those that have a significant quantity of biomass, have a strong power of very rapid regeneration, their densities are high and have a characteristic affinity for the zone and are recognised in the region for their multiple use value. These results are superior to those obtained by Il-Mataî *et al.* (2020) on vegetation dynamics in reforestation sites in the Sudan-Sahelian zone of Cameroon, where they found the highest values of total phytomass of species observed in Balanites aegyptiaca (108.64 t/ha), Azadirachta indica (121.02 t/ha) and Acacia nilotica (95.17 t/ha). The lower phytomass observed may be explained by the rarity of certain species in the area.

The large amount of phytomass following certain families can be explained by the fact that these families have a very rapid capacity to adapt in the study area. These results differ from those of Aboubakar, (2022) who found high phytomasses of Combretaceae in the southern part of the Benoué in northern Cameroon. This difference is due to the density of species in the different sites and plant formations, the methodology applied and anthropic action.

The carbon stock is proportional to the quantity of phytomass produced. These differences in results are

Table 4. Quantity of carbon sequestered by plant formations (tc/ha)

Sites/PF	SA	SAR	SH	ZC
Waza	110.348^{k}	65.43^{h}	24.11^{e}	18.63^{de}
Logone-Birni	82.00^{i}	56.37^{fg}	5.59^{a}	23.8^{ab}
Goulfey	97.37^{j}	62.9^{gh}	10.08^{abc}	15.83^{cd}
Kousseri	83.35^{i}	52.52^{f}	6.04^{a}	13.05^{bcd}

Numbers with the same letters are not significantly different at the 5% level. P= 0.05, n= 03.
SA= Wooded Savannah, SAR= Shrubby savannah, SH= Grassy savannah, ZC= Growing area and PF = Plant formatios.

Table 5. Potential CO_2 equivalent by plant formations and site (tCO_2/ha)

Sites/PF	SA	SAR	SH	ZC
Waza	404.60^{k}	239.92^{h}	88.41^{e}	68.33^{de}
Logone-Birni	300.67^{i}	206.70^{fg}	20.50^{a}	30.18^{ab}
Goulfey	357.05^{i}	230.66^{gh}	36.96^{abc}	58.04^{abc}
Kousseri	305.63^{i}	192.53^{f}	22.18^{a}	47.87^{bcd}

Numbers with the same letters are not significantly different at the 5% level. P= 0.05, n= 03.
SA= Wooded Savannah, SAR= Shrubby savannah, SH= Grassy savannah, ZC= Growing area and PF = Plant formatios.

Table 3. Estimated phytomass of families

Families	Waza	Log-Birni	Kousseri	Goulfey	PT
Anacardiaceae	75.20	1.04	/	/	76.24
Annonaceae	1.18	/	/	/	1.18
Arecaceae	/	13.75	16.71	/	30.46
Asclepiadaceae	47.78	6.73	8.87	18.08	81.45
Balanitaceae	51.65	64.64	104.43	95.63	316.35
Bignoniaceae	4.77	/	/	/	5.17
Burseraceae	0.86	/	/	/	0.86
Caesalpiniaceae	18.48	10.50	6.92	16.93	52.83
Capparaceae	5.66	1.77	20.04	42.74	70.21
Combretaceae	94.99	0.95	0.71	0.35	96.98
Ebenaceae	/	2.44	/	/	2.44
Fabaceae	1.35	0.32	/	/	1.67
Loganiaceae	/	/	0.70	/	0.70
Meliaceae	4.87	24.83	6.10	1.13	36.93
Mimosaceae	70.39	172.06	147.18	187.93	577.56
Moringaceae	/	/	2.95	/	2.95
Rhamnaceae	47.97	24.39	11.14	25.46	108.96
Rubiaceae	9.40	/	3.97	7.92	21.29
Ulmaceae	2.32	/	/	/	2.32

PB= Total phytomass, /= No data

justified by the fact that the capacity of a savannah to store carbon depends mainly on the species composition of the flora and the diameter classes of the trees present. The methodological approach for estimating carbon stock also differs from one study to another. These results are similar to those of Tsoumou et al. (2016) in the Dimonika model forest in the south-east of the Republic of Congo, where they found a value of 129 tC/ha. The statistical test shows that there is a highly significant difference between the sequestered carbon values of the different plant formations and sites at the 5% threshold (P<0.001).

Potential CO_2 equivalent in the wooded savannah can be explained by the high phytomass production observed in wooded savannahs. These results differ from those obtained by Firdaus et al. (2010), who found a similar average carbon rate between formations. The differences observed between the studies can be explained by the varying climatic conditions and anthropogenic activities between the study areas, on the one hand, and by the existence of a park at Waza, a reserve at Kousseri and a protected area at Goulfey, on the other. The statistical test showed a highly significant difference between the values of the different plant formations and the sites at the 5% threshold (P<0.001).

CONCLUSION

The present study showed that the phytomass per hectare by species varied between sites and the highest was Sclerocarya birrea (Waza), Acacia nilotica, (Logone-Birni), Balanites aegyptiaca (Kousseri) and Acacia seyal (Goulfey) and also depends on the plant formations while, the tree savannah had the highest phytomass. The greatest amount of carbon sequestration was recorded in the tree savannah. These differences were also significant. The results showed that the tree savannah has a higher carbon sequestration potential than the other plant formations. The results of these findings can serve as a baseline for possible future payments for ecosystem services. Based on studies it is recommend avoid late fire, limit abusive cutting of fresh wood, Encourage local populations to reforest with endogenous species, promote improved stoves and other energy sources to reduce demand for wood.

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Morphological Assessment and Land Use Land Cover Change Detection of Hindon River Basin on Geospatial Platform

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Abstract: The present study is carried out the morphometrical analysis of River Hindon basin by linear, areal and relief aspects and land use land cover analysis of the basin by supervised classification technique. The basin occupies 6940 km^2 geographical area and it's boundary perimeter is 1081.88 km. The minimum and maximum elevation of the basin was 190 m and 868 m and average elevation of entire basin is 528.5 m from mean sea level. The land use land cover by supervised classification, indicated that total 4047.93 km^2 area about 58.32 % of total area of basin used for agriculture purpose and covered by vegetation, 953.81 km^2i.e. 1.37 % of basin area is covered by water bodies like river, streams, ponds, canals etc., 647.43 km^2 i.e. 9.32 % of basin area is barren land and 2149.94 km^2 i.e. 30.97 % of basin area is covered by build-up. There are also carried out the analysis of NDVI and NDVI ranges of the basin during 1990 was -0.13 to + 0.47 and in 2022 was -0.10 to +0.48.

Keywords: Morphometry, GIS & Remote Sensing, LANDSAT, SRTM Digital Elevation Model, LULC, NDVI

Morpho-metrical analysis is important for any watershed to address water erosion prone areas, flood prone areas, highly affected sediment transport areas and sediment accumulated areas of river (Haokip et al., 2022). It is important to know about morpho-metrical parameters of the basin in terms of linear, areal and relief aspects for watershed management planning and hydrological studies of the basin (Avijit Mahala 2020). The watershed management projects can be designed, such as establishment of gully control and flood control structures like spillways, grassed waterways, reservoirs, barrages, dams, soil and water conservation structures like bunds, terraces etc., water supply and disposal units like canal distributaries and watercourses, culverts, surface and sub-surface drainage systems and can also address water logging affected areas in the basin through morphometrical analysis (Sinha and Eldho 2021). The morphometric analysis of watershed is useful to investigate the erosion status of basin, flood prone areas and critical area suffering to soil erosion within drainage basin (Bajirao et al., 2019). The morphological analysis is generally performed for planning and implementing soil and water conservation and quality measures, groundwater development and management and erosion control measures (Agrawal et al., 2022). Hydrological and geomorphic processes occur within the watershed, and morphometric characterization at the watershed scale reveals information about formation and development of land surface processes and thus provides a holistic insight into the hydrological behaviour of a watershed (Shekhar et al., 2022). Morphological parameters affect the various hydrological characteristics of the basin such as runoff, peak discharge, time of concentration, depression storage, flow velocity etc (Anand et al.,2020). The parameters required for the morphometrical analysis of the basin from linear, areal and relief aspects are area, perimeter, stream order, stream length, number of streams, maximum length of the main channel of the basin, maximum and minimum elevation from mean sea level and slope etc (Annayat et al., 2022). Using morphological parameters, different hotspot locations which may be expected to have extreme events such as drought, flood etc. can be identified (Joy et al., 2023). This will helps in selection of particular site for the prevention of the area by constructing different structures such as spillways, grassed waterways, artificial water harvesting structures and dams. It also helps to address the erosion affected areas and for prevention of that adopted various erosion control temporary and permanent structures.

Most of the industrial and municipal waste are dissolving in the river therefore degree of contamination of the river is increasing day by day and their adverse effect on groundwater quality and groundwater contamination is also increasing in Hindon river basin due to dissolving toxic elements (Sharma et al., 2021). Most of the farmers nearby the villages of Hindon river use that polluted water for irrigation purpose for their agricultural land, which also affects the crop yield and productivity. For the rejuvenation of river Hindon, it is important that the study of morphometrical parameters for the quantitative and qualitative assessment of the basin must be carried out. The national mission for clean Ganga (NMCG) approved 4 projects worth rupees 407 crore in April 2023 to rejuvenate river Hindon in the district Shamil of Uttar Pradesh state (https://pib.gov.in/PressRelease Page). Prabhakar et al. (2019) and Gupta et. al. (2023) highlighted the morphometric analysis with land use and land cover (LULC) changes and hydrologic performance over the watershed. This study is carried out for the morphometrical analysis in linear, areal and relief aspects and temporal variation of land use land cover in river Hindon basin and their applications in the basin for management & planning of watershed.

MATERIAL AND METHODS

Site location: Hindon river is an important tributary of river Yamuna. It originates from Shivalik hills of Saharanpur district and joins to river Yamuna near village Tilwada of Gautam Buddha Nagar district in Uttar Pradesh. It travels the total distance from origin to confluence point of 312 km and many of streams, drains and rivers joins the path of Hindon river main stream. The extent of the basin is from 28° 24' 9" N to 30°17'18" N latitude and 77° 12' 30" E to 77° 55' 4" E longitude. The minimum and maximum elevation of the basin from mean sea level is 190 m and 868 m respectively and average elevation of the basin is 528.5 m. The Kali River, Krishni River, Nagdev nala, Dhamola Nala, budhana drain, dasna drain, star paper mill drain, Jonney escape are the important tributaries of river Hindon, passing through 6 districts of UttarPradesh, Saharanpur, Muzaffarnagar, Bagpat, Ghaziabad, Meerut and Gautam Buddha Nagar and some area of the basin is occupied by Haridwar District Uttarakhand and Union Territory Delhi (Fig. 1).

Data-sets and methodology: All morphometrical parameters of Hindon river basin has been evaluated and secondly the change detection of land use land cover analysis in the gap of 30 years by supervised classification techniques as well as NDVI analysis has been performed. Shuttle Radar Topography Mission (SRTM) Digital elevation model (DEM) data downloaded from USGS earth explorer and thereafter the elevation map, flow direction, flow accumulation, stream order, aspect, and slope map were prepared. LANDSAT-8-9 C2 L2 and LANDSAT 4-5 C2 L2 all 7 bands (30 m resolution) were downloaded from USGS earth explorer for the land use land cover analysis of Hindon river basin. For the preparation of NDVI maps LANDSAT 4-5 (for 1990) and LANDSAT 8-9 (for 2022) 30 m resolution data is acquired from USGS earth explorer. The Arc GIS 10.3 software used for preparation, analysis and representation of data through maps. The flowchart of the methodology has been depicted in the Figure 2-a & 2-b.

RESULTS AND DISCUSSION

Linear Aspects

Stream order (U): Stream ordering is the first step to be performed for the quantitative study of the river basin (Rai et al., 2019). According to Strahler Stream Ordering System, smallest stream designated as 1st order stream and confluence of two 1st order streams derive 2nd order stream.

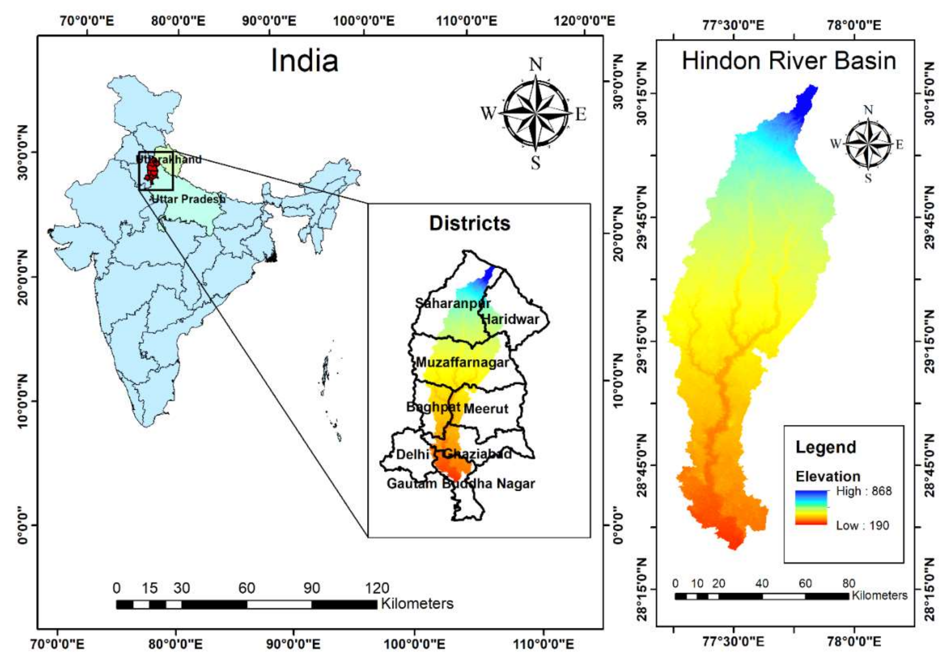

Fig. 1. Location map of Hindon River Basin

Similarly the confluence of two 2nd order stream makes 3rd order stream and so on till the end of stream outlet. Stream order map of the basin is created by SRTM DEM of 30 m resolution. Hindon river basin was have maximum 5th order stream.

Stream number (Nu): Stream number is the count of individual streams in the basin of each order. There are total 758 streams found in Hindon river basin. Out of these, 658 streams of 1st order, 84 streamsof 2nd order, 13 streams of 3rd order, 2 streams of 4th order and 1 stream of 5th order were determined by calculation.

Stream length (Lu): Total sum of all stream length of Hindon river basin was 2047 km in which 1124 km length of 1st order, 471 km length of 2nd order, 156 km length of 3rd order, 155 km of 4th order and 141 km of 5th order stream werefound.

Bifurcation ratio (Rb): In Hindon river basin, the mean bifurcation ratio is found to be 5.69, which indicates that there are very less number of stream branches in Hindon basin (Table 1). Lower the mean bifurcation ratio, greater the branching in the stream network within a watershed and vice-versa (Prabhakaran and Jawahar Raj 2017).

Stream length ratio (R_L): The R_L values between streams of different order in the basin reveal that there are variations in slope and topography (Waikar and Nilawar 2014).

Areal Aspects

Form factor (R_f): The present study of Hindon river basin, basin area (A) was calculated 6950 km^2 and length of main stream (L) was as 312 km so the form factor will be 0.065 thus confirm that either visually or numerically Hindon basin is an elongated basin.The form factor less than 0.78 indicates an

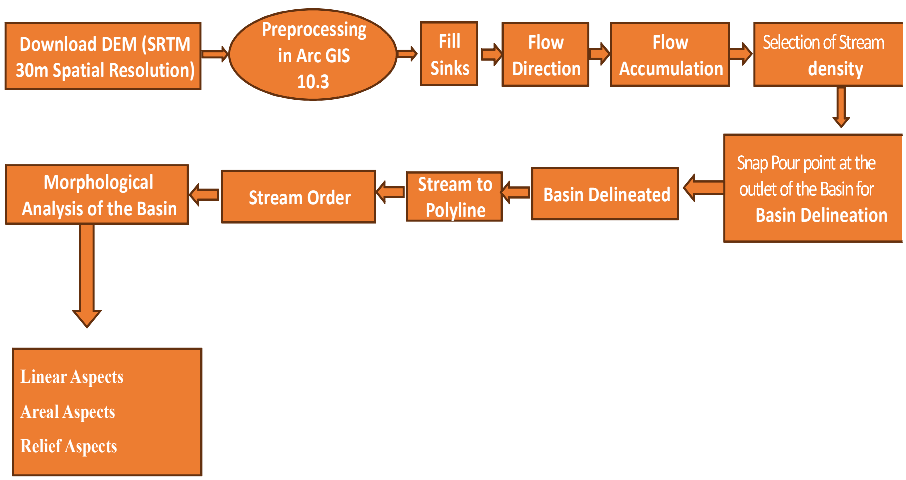

Fig. 2a. Methodology of flowchart for linear, aerial & relief aspect analysis of Basin

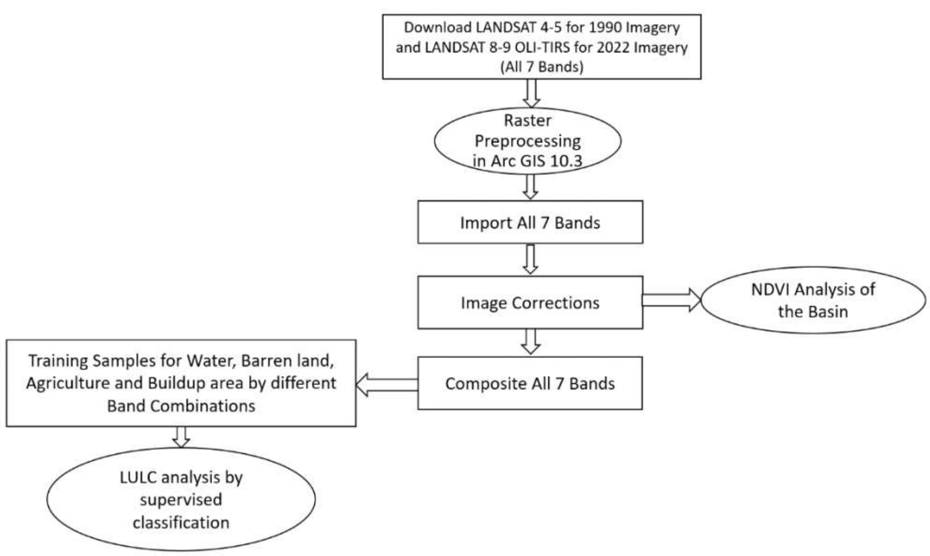

Fig. 2b. Methodology of flowchart for land use land cover & NDVI analysis

elongated basin, and greater than 0.78 indicates a circular basin (Farhan et al., 2016).

Shape factor (S_f): The shape factor of Hindon river basin was 14.The greater the circular character of the basin, the greater is the rapid response of the watershed after a storm event (Altaf et. al., 2013).

Compactness coefficient (Cc): The compactness coefficient value is independent of size of watershed and dependent only on the slope (Rai et. al., 2017). The compactness coefficient of Hindon river basin was 3.66.

Elongation ratio (R_l): In Hindon river basin, the elongation ratio was obtained as 0.301and states that the basin is much elongated.The varying slopes of watershed can be classified with the help of the index of elongation ratio, i.e. circular (0.9-0.10), oval (0.8-0.9), less elongated (0.7-0.8), elongated (0.5-0.7), and more elongated (< 0.5) (Pareta and Pareta, 2011).

Circulatory ratio (Rc): The circulatory ratio was 0.074.Circulatory ratio (Rc) is influenced by the length and frequency of the stream, geological structures, land use/land cover (LULC), climatic variability, relief and slope of the sub-watersheds (Patel et al. 2013). The high value of circularity ratio shows the late maturity stage of topography.

Drainage density (D_d):. In Hindon river basin, the drainage density was found to be as 0.29 km per km^2.It indicates that how many streams are there per unit area of the basin generally represented as km/km^2

Stream frequency (S_f): The stream frequency of Hindon river basin was 0.119 per km^2. The higher stream number indicates the higher stream density in the basin so probability of extreme events by runoff accumulation like a flood on a particular site of the basin may be increased.

Constant of channel maintenance (C): The constant of channel maintenance value of Hindon river basin was 3.395.The constant of channel maintenance indicates the relative size of landform units in a drainage basin and has a specific genetic connotation

Relief Aspects

Relief ratio (R_r): The relief ratio of the Hindon river basin was 3.2285 and maximum relief of the basin is 684 m. In general, the relief ratio (R_r) indicates the overall slope of watershed surface (Prabhakar et. al., 2019). The rate of potential energy to kinetic energy of flowing water is dependent on relief ratio due to slope and elevation characteristics of the basin.

Ruggedness number (R_n): The ruggedness number (R_n) of Hindon river basin was 0.201 which indicates that smoother topography of the basin. Most of the area of Hindon basin are flatter or less slopy, only some parts of the basin area are having a steep slope like Shivalik hills where Hindon originates, other areas of the basin are not having a steep slope. The ruggedness number is the product of relief and drainage density and both parameters should be in same units. It indicates that the structural complexity of the terrain in association with the relief and drainage density (Altaf et al., 2013). The extremely high values of ruggedness number occur when slopes of the basin are not only steeper but also longer (Umrikar 2016). The values range from 0 to 1. The values close to 0 show relatively smoother topography, and ruggedness number (R_n) close to 1 shows rough topographical characteristics (Nath et. al., 2022).

Land use land cover (LULC), change detection and NDVI analysis of basin: Land use land cover analysis of Hindon river basin is carried out by supervised classification

Table 2. Areal aspects of Hindon river basin

Areal aspects	Value
Total area of the basin (sq. km)	6950
Form factor	0.07
Shape factor	14.00
Compactness ratio	7.32
Drainage density	0.29
Circulatory ratio	0.07
Elongation ratio	0.30
Constant of channel maintenance	3.39
Stream frequency	0.10

Table 3. Relief aspects of Hindon river basin

Relief aspects	Value
Maximum relief	678
Relief ratio	3.22
Ruggedness number	0.20

Table 1. Linear aspects of Hindon River Basin

Stream order	Stream number (N_u)	Total no. of streams	Stream length (L_u)	Total stream length (KM)	Stream length ratio (R_L)	Bifurcation ratio (R_b)	Mean bifurcation ratio (R_{bm})	Length of overland flow
I	658		1124			7.84		
II	84		471		0.91	6.46		
III	13	758	156	2047	0.99	6.5	5.69	1.55
IV	2		155		0.33	2		
V	1		141		0.42			

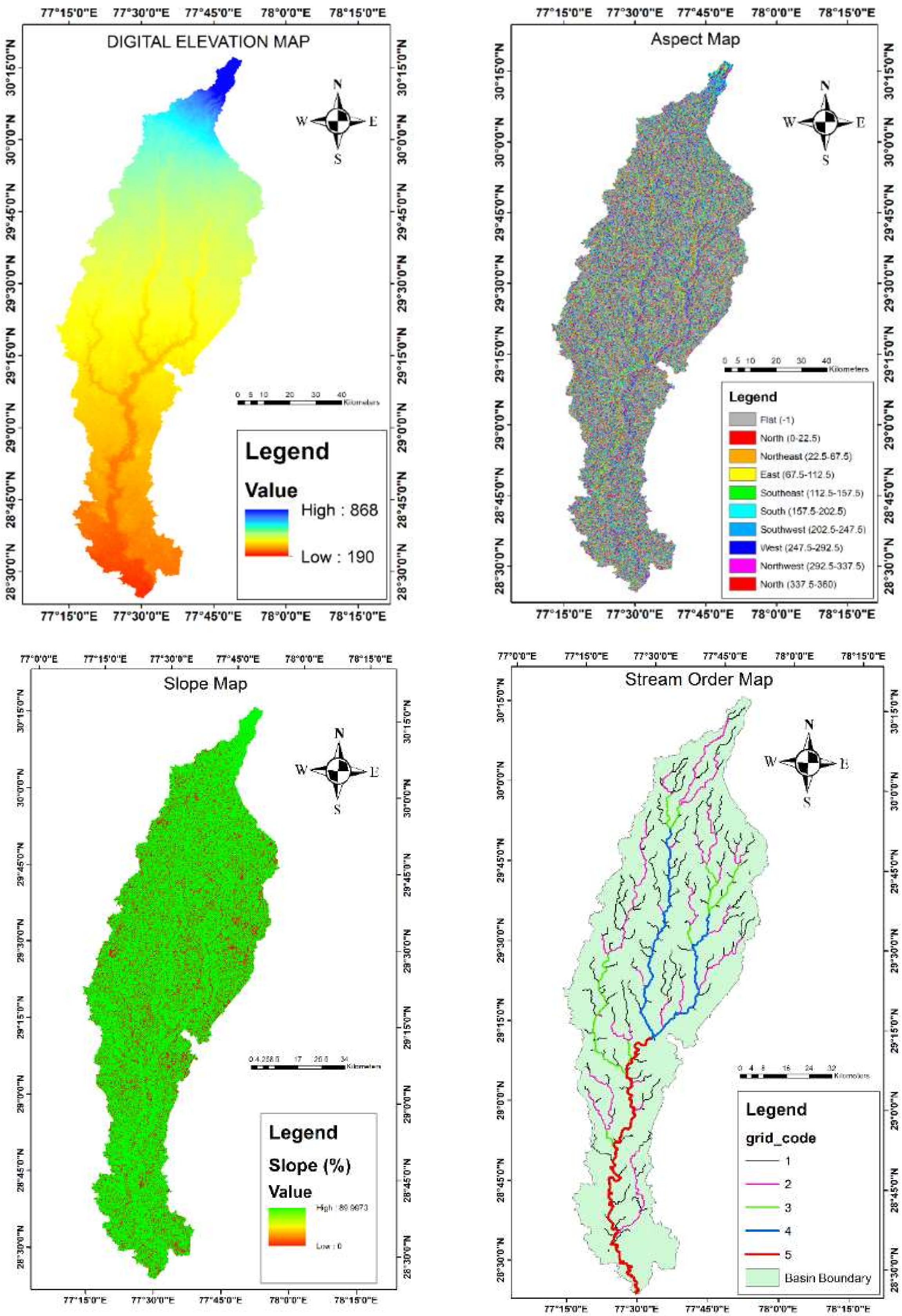

Fig. 3. Hindon basin digital elevation, aspect, slope and stream order maps

technique. The large water bodies in the entire basin are extended in the area of about 953.81 km^2 i.e., approximately 1.37 % of the total area and the largest area of the basin accounts to be as 4047.93 km^2,approximately 58.32% of total area, covered by agriculture land and vegetation (Table 6). The LULC map for the years 1990 and 2022 has been shown in Figure4 and the NDVI maps in Figure 5. Figure 4 and Figure 5, indicate land use land cover changes decade wise. Before the 1990 most of the area of basin was agricultural and pastoral land and during the 1990 to 2022 urbanisation and industrialisation started and it is increasing continuously with respect to time. About 30.97 % of total basin area has been converted into built-up in 2022.This indicates the continuous encroachment of the rivers by built-up in the basin. Most of the area in 1990's that lay nearby the river Hindon and their tributaries was barren land and after three decades we can see the significant changes in the basin and barren land is converted into agriculture and pastoral land during the past three decades due to green revolution and climate change (Mishra et al., 2018). Total built-up area in the basin was 15.72 % during 1990 and now total built-up area is 30.97 % increased about 15.25 % whereas total barren land 21.93% during 1990 which is now only 9.32% and decreased about 12.61%. Similarly water bodies like stream channels, canals, rivers, ponds has increased from 0.24 % to 1.37 % due to changes in several meteorological and climatic conditions. The study of normalized difference vegetation index (NDVI) of the basin was carried out for understanding land cover characteristics and their changes in the basin during 1990 and 2022. The ranges of NDVI in 1990 varies from - 0.13 to 0.47 whereas in 2022 it ranges from -0.10 to 0.48. Negative NDVI values indicates that clouds and water bodies of the basin and those values which are positive and close to zero up to 0.1 indicates the exposed surface such as

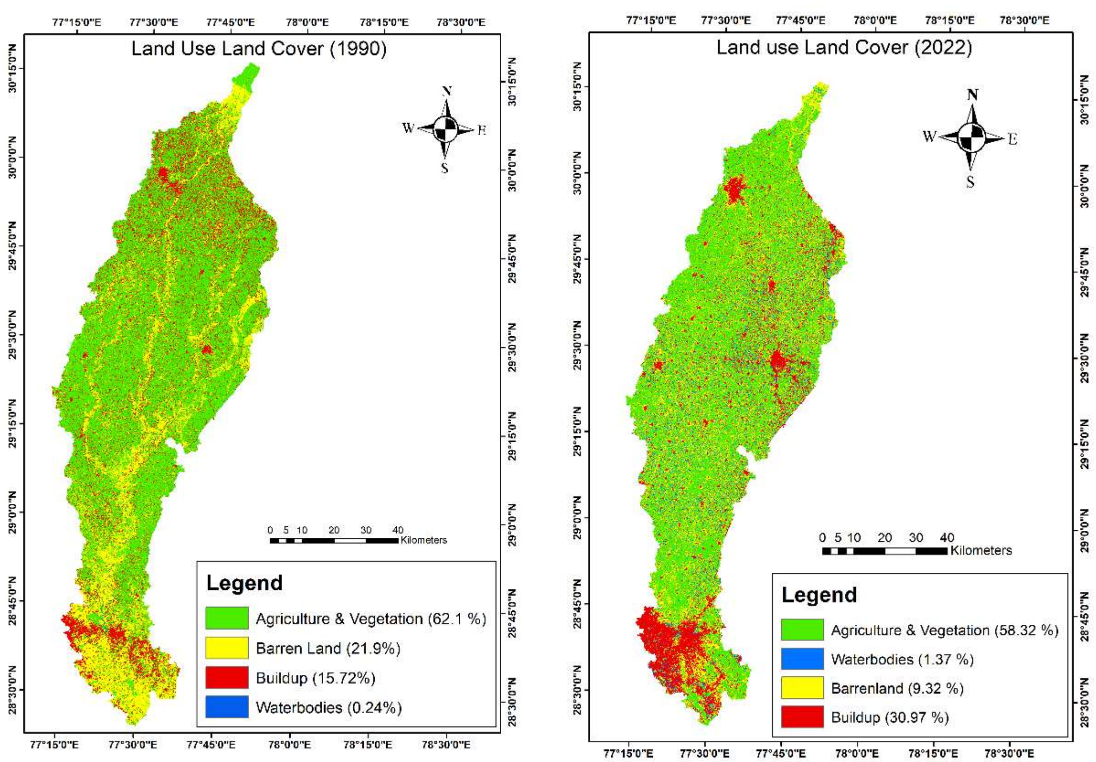

Fig. 4. Temporal changes of land use land cover by supervised classification technique

Table 4. Land use land cover classification of the basin (1990 and 2022)

Class	Area (1990)	Percentage (1990)	Area (2022)	Percentage (2022)
Agriculture land and vegetation	4309.74	62.10	4047.93	58.32
Build up	1090.96	15.72	2149.94	30.97
Barren land	1521.94	21.93	647.43	9.32
Water	16.65	0.24	95.38	1.37
Total			6940	100

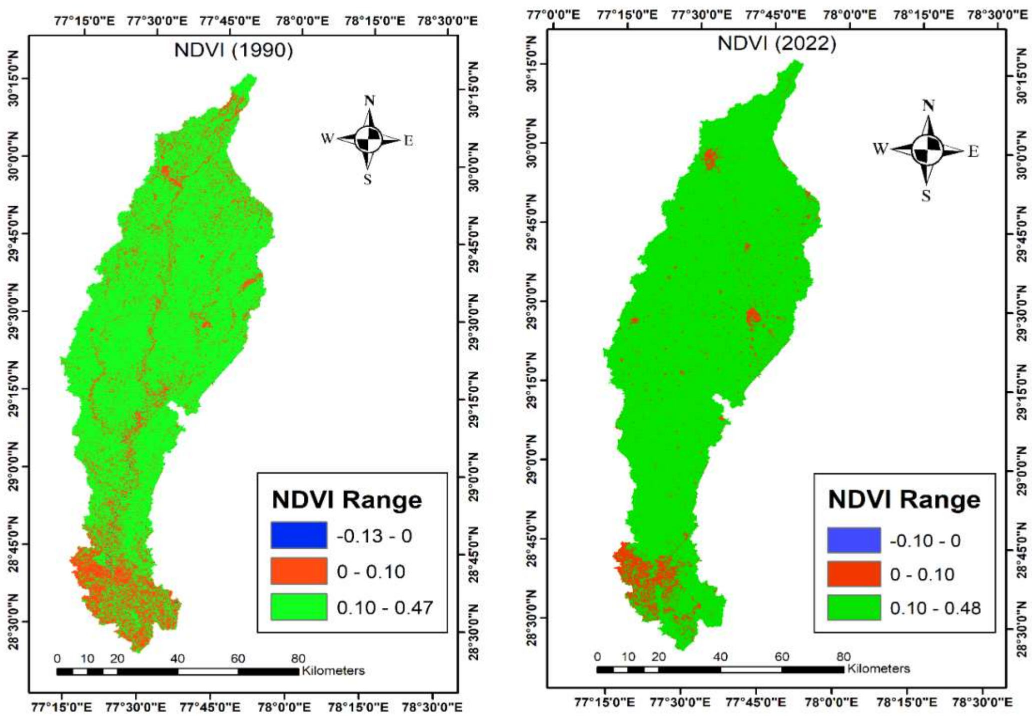

Fig. 5. Temporal variation in normalized difference vegetation index

bare soil, over grazed land, barren land etc. as well as built-up area and the value of NDVI from 0.1 to 0.5 indicates sparse vegetation over the basin (Jeevalakshmi et. al., 2016). The barren land is transformed into agricultural and pastoral land. During 1990 most of the area of the basin was barren land, after 3 decades now most of the area is converted into either built-up or agricultural and pastoral land. After the study of NDVI it is also concluded that basin does not contains dense forest and large water bodies such as reservoirs and lakes.

CONCLUSION

The morphometric analysis is essential for overall watershed investigation and management. It also plays an important role in hydrological investigations of a river basin or watershed. The geographical area of the basin is 6940 km^2, perimeter is 488 km, consisting the total of 758 streams (including small and large streams) of 2047 km length and the length of main stream channel which originates from Shivalik hills, Saharanpur to its outlet at Tilwada, is 312 km. The basin is drained by the highest 5^{th} order stream. The elevation of the basin varies from 190 m to 868 m withan average elevation of 528.5 m.The built-up area and water bodies increased 15.25 and 1.13% respectively whereas agriculture and vegetative area and barren land decreased to 3.78 and 12.61 % respectively from 1990 to 2022 andthe NDVI analysis also concludes the same results.

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AUTHORS CONTRIBUTION

All authors contributed significantly to the development of this workShivam Kumar Dwivedi conceptualized the study design and supervised the research process. Vikash Singhcollected and processed the data. Mukesh Kumar Sharmaperformed the data analysis and interpretation. Anjali Bhagwat contributed to the literature review, analysing results and writing of the final manuscript draft. All authors reviewed, edited, and approved the final version of the manuscript.

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Seasonal Variability in Physico-Chemical Attributes of Spring Water in Nagaland, India

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Abstract: This study evaluated the seasonal variability of spring water quality across four districts of Nagaland, *viz*., Dimapur, Phek, Mokokchung, and Noklak. Forty sampling stations were investigated in rainy, summer, and winter seasons for key physico-chemical parameters such as pH, free CO_2, total alkalinity, chloride, conductivity and total dissolved solids (TDS). Results revealed distinct spatio-temporal differences. Mean pH values indicated higher alkalinity during rainy and summer months (up to 6.6 in Noklak) and lower in winter (5.2 in Mokokchung). Free CO_2 concentrations were consistently elevated in Noklak and Phek during winter (17. mg/l and 12.1 mg/l, respectively), reflecting increased solubility at low temperatures. Total alkalinity reached maximum levels in Mokokchung during the rainy season (53.8 mg/l) but declined sharply in winter (13.1 mg/l). Chloride concentrations peaked in Dimapur and Phek during the rainy season (>65 mg/l), while Noklak exhibited strong seasonal contrasts. Conductivity was highest in Mokokchung (125.0 µS/cm, rainy season), and TDS values were most elevated in Noklak (85.8 mg/l, rainy season). Across all districts, the general sequence was rainy > summer > winter for solute parameters, whereas free CO_2 peaked in winter. Despite these variations, all parameters except pH remained within BIS/ICMR/WHO permissible limits. These findings provide baseline data on spring water parameters in the hilly regions of Northeast India and emphasize the need for extended monitoring of additional physico-chemical, biological parameters and heavy metals contaminants to ensure long-term drinking water safety.

Keywords: Water quality, Physico-chemical parameters, Seasonal variation, Nagaland, Springs

Springs with their natural water quality are highly sensitive to pollution from human activities and changes in climate affecting surface run-off that replenishes groundwater (Saad et al., 2011). Additionally, the quality of these bodies of water can greatly differ based on their location and various environmental factors. Nagaland, situated in North-East India, faces significant challenges in water resource management due to its steep topography and heavy monsoonal rainfall. The state's rugged terrain contributes to rapid runoff, preventing effective rainwater harvesting and limiting water retention in the soil. Consequently, much of the rainwater is lost, resulting in minimal groundwater recharge (Lalmalsawmzauva et al., 2021). In addition to these natural challenges, human activities such as shifting cultivation which involves clearing forested areas for agricultural purposes, urban expansion, and infrastructure projects like road construction exacerbate the depletion of groundwater reserves. These factors have led to the drying up of natural springs, which are a crucial water source for many rural and urban communities in Nagaland (Lkr et al., 2020, Temjen et al., 2021) and this has become an pressing issue, particularly during the dry winter months when water shortages are most acute. Springs serve as lifelines for many communities, providing the primary source of drinking water and supporting agricultural activities. However, recent studies indicate a decline in spring discharge rates, further intensifying water scarcity in the region (Dass et al., 2021). This uneven distribution poses challenges for water resource management, especially in rural areas where communities depend on natural springs and streams for domestic and agricultural use (Semy et al., 2022). Despite the critical reliance on these natural water sources, there has been limited research on the quality of spring water consumed by the local population. Most people rely on this water without any form of treatment, raising concerns about potential health risks, as poor water quality can lead to a host of waterborne diseases. Given the importance of springs for local communities, there is a critical need to understand the physical and chemical properties of spring water and assess whether it meets the standard permissible limits for safe drinking water. This study seeks to fill this gap by analyzing spring water from four districts in Nagaland, comparing its quality with permissible standard limits. The findings will help in rejuvenation of spring water and highlight the need for interventions, such as filtration and treatment systems, to ensure the safe consumption of spring water by local populations.

MATERIAL AND METHODS

Altogether 40 stations of springs were inventorized from the four districts of Nagaland (Table 1). Ten sites were selected from each district. The sites selected from each districts were represented as D1 to D10 from Dimapur, P1 to P10 from Phek, M1 to M10 from Mokokchung and N1 to N10 from Noklak district. These districts were chosen to represent a diverse range of geographical and hydrological conditions within the state. Each sampling station was strategically located to capture water from critical springs and streams that are relied upon by the local population. This regional representation allows for a comprehensive assessment of water quality across different environments within Nagaland. The water quality parameters examined in this study include free CO_2, total alkalinity, chloride, conductivity and total dissolved solids (TDS), which are key indicators of water chemistry and suitability for consumption. These parameters were analyzed using standardized methods proposed by Trivedy and Goel (1986) and the American Public Health Association (APHA, 2005). The data obtained were compared to the drinking water quality standards (Table 2) set by the Bureau of Indian Standards (BIS 2003), the Indian Council of Medical Research (ICMR 1975) and World Health Organisation (WHO 1995). The water samples were collected from the selected springs during three distinct seasons: rainy season, spring and winter between 2021 and 2022. This seasonal approach was critical for capturing the variations in water quality that result from changing hydrological conditions throughout the year. All analysis was performed in triplicate for each water sample and the results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The seasonal variation in few selected physico-chemical parameters of spring water from 4 (four) districts of Nagaland is shown in Table 2. Results of each parameters are described as under.

pH: Spring water pH exhibited seasonal and spatial variability across the four districts. Lowest pH of 5.2 was during winter from the springs of Mokokchung district and highest of 6.6 in summer from the spring waters of Noklak district. The overall seasonal pattern indicated higher pH during summer and rainy months, while winter waters were nearly acidic, reflecting reduced buffering capacity under cooler conditions. Shah et al. (2019) also observed that in Himalayan springs where seasonal photosynthetic activity and dilution by monsoonal rainfall raised pH, whereas cooler winter conditions promoted acidification but were below the permissible limit .

Free CO_2: Free CO_2 concentrations demonstrated pronounced district-level differences. Maximum free CO_2 of 17.10 mg/l was in winter from the spring water samples of Noklak district and minimum from the Dimapur district during summer (4.30 mg/l). The district-wise pattern followed Noklak > Phek > Mokokchung > Dimapur, highlighting Noklak's distinct CO_2 enrichment. The values of Free CO_2 were within the permissible limits (Table 2). Elevated winter CO_2 concentrations can be attributed to enhanced solubility under low temperatures, reduced photosynthetic uptake in colder months, and restricted gas exchange in covered spring channels. Conversely, lower concentrations in summer reflect greater photosynthetic drawdown and increased atmospheric exchange. This seasonal solubility-driven pattern is consistent with hydrological observations in cold-water spring systems (Orr et al., 2022).

Total alkalinity: Both the highest and lowest values of the Alkalinity of spring water samples from various sites was in the district of Mokokchung. Highest value of 53.80 mg/l was during rainy season and lowest value of 13.10 mg/l was reported from the water samples during winter month. All values were below the permissible limit of 120 mg/l set by BIS (2003), ICMR (1975) and WHO (1995). The consistent reduction in winter alkalinity across districts reflects lower biological activity and decreased carbonate input, while rainy-season enrichment is linked to monsoonal runoff and organic matter leaching. Such monsoonal enhancement of bicarbonate has been well-documented in hilly watersheds (Li et al., 2020).

Chloride: Chloride concentrations fluctuated considerably both spatially and temporally. Maximum chloride content of 66.70 mg/l was during rainy season from the spring water samples of Dimapur district and minimum reported from the spring water samples of Noklak district (23.60 mg/l). but were within the permissible limit (Table 2). Overall, chloride followed the sequence rainy > summer > winter across all districts, reflecting increased leaching and surface runoff during the monsoon. The consistently higher values in Dimapur and Phek may also indicate anthropogenic influence from agriculture and residential activities. These results align with earlier studies that highlighted rainfall and human-induced runoff as key drivers of chloride variability in spring-fed systems (Haake et al., 2019).

Conductivity: Higher value of conductivity was from the spring water of Mokokchung district during rainy season (125.0 µS/cm) whereas lower was during winter from water samples of spring from various sites of Dimapur district (59.90 µS/cm). Overall district wise average values confirmed the overall pattern in the trend of rainy > summer > winter. All the values were fall within the permissible limit. Elevated rainy conductivity reflects enhanced ion leaching, soil erosion, and domestic inputs, particularly in steep terrains of Mokokchung district. Similar seasonal enrichment of conductivity due to monsoonal hydrology was observed in hilly watersheds (Longchar et al., 2023).

Total Dissolved Solids (TDS): TDS showed strong spatial and seasonal differences. Maximum was recorded during rainy season from the spring water samples of Noklak (85.0

Table 1. Study sites with their geo-coordinates

Sampling station	Latitude	Longitude	Altitude (above msl)
Station (D1): Chumukedima 1	25° 47' 7.8924"N	93°48' 13.0032"E	178m
Station (D 2): Chumukedima 2	5°46'53.79531"N	93°48'5.83856"E	468m
Station (D 3): Medziphema 1	25° 45' 3.6788"N	93° 52' 53.2596"E	438m
Station (D 4): Medziphema 2	25° 45' 42.174"N	93° 53' 9.9024"E	440m
Station (D 5): Niuland Town 1	25°56'3.15412"N	93°58'39.81943'E'	179m
Station (D 6): Niuland Town 2	5°56'13.98312"N	94°0'21.12033"E	273m
Station (D 7): Hovishe 1	25°56'3.08925"N	93°58'39.81430"E	184m
Station (D 8): Hovishe 2	25°56'0.76126"N	93°59'54.43218"E	192m
Station (D 9): Ghotovi 1	25°56'3.19024"N	94°0'4.98154"E	273m
Station (D 10): Ghotovi 2	25°55'59.34457"N	93°59'50.39632"E	220 m
Station (P1): Pfutsero 1	25°34'5.8764"N	94°17'42.0972"E	1941m
Station (P2): Pfutsero 2	25°34'11.5572"N	94°17'40.686"E	1942m
Station (P3): Kami 1	25°31'40.6164"N	94°16'10.5132"E	1742m
Station (P4): Kami 2	25°31'58.6056"N	94°16'11.676"E	1813m
Station (P5): Zapami 1	25°31'26.8284"N	94°15'25.668"E	1703m
Station (P6): Zapami 2	25°31'20.2476"N	94°15'51.534"E	1686m
Station (P7): Lasumi 1	25°32'2.382"N	94°14'23.0244"E	1696m
Station (P8): Lasumi 2	25°32'3.6492"N	94°14'19.1904"E	1666m
Station (P9): Leshemi 1	25°32'19.986"N	94°14'3.3612"E	1665m
Station (P10): Leshemi 2	25°32'11.7024"N	94°13'57.6804"E	1638m
Station (N1): Shim pokdom	26° 12'33.7"N	95° 59'43.4"E	1565m
Station (N2): Timpulang 1	26° 12'41.0"N	95° 00'00.9"E	1563m
Station (N3): Timpulang 2	26° 12'39.1"N	94° 59'59.3"E	1616m
Station (N4): Longshitso	26° 13'07.0"N	95° 00'18.6"E	1649m
Station (N5): Laahkhiam	26° 12'36.4"N	95° 01'57.5"E	1235m
Station (N6): Kutongshae	26° 12'57.0"N	95° 01'32.6"E	1524m
Station (N7): Jin jit	26° 12'53.1"N	94° 59'43.4"E	1514m
Station (N8): Thangkhiam	26° 12'55.1"N	95° 00'37.1"E	1552m
Station (N9): Lunghing	26° 12'51.0"N	95° 01'30.8"E	1505m
Station (N10): Laapha	26° 13'10.9"N	95° 01'34.5"E	1638m
Station (M1):Kinunger 1	26°36'51.242"N	4°41'81.705"E	563m
Station (M2): Kinunger 2	26°36'24.932"N	94°38'86.382"E	404m
Station (M3): Longkhum 1	26°40'65.639"N	94°40'55.104"E	1436m
Station (M4): Longkhum 2	26°26'12.858"N	94°40'64.125"E	1489m
Station (M5): Ungma 1	26°29'41.139"N	94°50'55.722"E	1265m
Station (M6): Ungma 2	26°30'21.648"N	94°50'71.824"E	1133m
Station (M7): Mokokchung Village 1	26°33'31.756"N	94°53'97.194"E	1316m
Station (M8): Mokokchung Village 2	26°32'90.318"N	94°53'10.195"E	1322m
Station (M9): Chuchuyimpang 1	26°33'40.256"N	94°54'77.762"E	1394m
Station(M10):Chuchuyimpang 2	26°33'15.637"N	94°55'27.695"E	1387m

D = Dimapur, P = Phek, M = Mokokchung, N = Noklak

Table 2. Seasonal variation in few selected physico-chemical parameters of spring water of Nagaland

Parameters	Sites	Rainy	Winter	Summer	BIS/ICMR/WHO
pH	D1 to D10	6.2 ± 0.7	5.7 ± 0.8	6.0 ± 0.6	6.5-8.5
	P1 to P10	6.2 ± 0.2	5.7 ± 0.3	6.3 ± 0.4	
	N1 to N10	6.3 ± 0.5	5.9 ± 0.5	6.6 ± 0.5	
	M1 to M10	5.7 ± 0.6	5.2 ± 0.2	6.4 ± 0.8	
Free CO_2 (mg/l)	D1 to D10	4.3 ± 1.4	5.4 ± 1.5	4.3 ± 1.4	22 mg/l
	P1 to P10	10.2 ± 3.9	12.1 ± 4.6	9.1 ± 3.4	
	N1 to N10	12.6 ± 5.3	17.1 ± 5.3	12.1 ± 4.9	
	M1 to M10	5.4 ± 1.9	9.4 ± 3.6	5.1 ± 2.6	
Total alkalinity (mg/l)	D1 to D10	44.8 ± 11.9	26.5 ± 13.4	32.3 ± 11.6	120 mg/l
	P1 to P10	39.0 ± 13.4	22.6 ± 5.0	40.8 ± 13.4	
	N1 to N10	39.3 ± 12.7	23.0 ± 5.0	34.5 ± 18.7	
	M1 to M10	53.8 ± 16.3	13.1 ± 3.5	31.2 ± 10.7	
Chloride (mg/l)	D1 to D10	66.7 ± 13.2	45.6 ± 14.9	47.3 ± 10.2	250 mg/l
	P1 to P10	65.8 ± 9.3	42.9 ± 15.8	48.9 ± 9.7	
	N1 to N10	52.5 ± 6.0	23.6 ± 7.7	31.3 ± 9.5	
	M1 to M10	56.3 ± 9.3	42.8 ± 8.8	46.4 ± 9.2	
Conductivity (µS/cm)	D1 to D10	96.6 ± 30.7	59.9 ± 21.0	70.8 ± 18.1	300 µS/cm
	P1 to P10	98.9 ± 24.7	77.6 ± 13.8	90.5 ± 19.5	
	N1 to N10	100.8 ± 29.0	82.6 ± 22.0	88.8 ± 21.7	
	M1 to M10	125.0 ± 38.3	78.9 ± 39.1	116.0 ± 40.5	
Total dissolved solid (mg/l)	D1 to D10	52.7 ± 13.9	32.3 ± 5.1	32.3 ± 5.1	500 mg/l
	P1 to P10	65.2 ± 21.4	45.7 ± 18.1	77.5 ± 75.4	
	N1 to N10	85.8 ± 20.1	56.9 ± 19.0	68.5 ± 20.0	
	M1 to M10	54.1 ± 13.6	31.8 ± 14.0	44.7 ± 17.7	

All values are expressed as mean ± SD of all the sites from each district

mg/l) whereas the minimum was from Mokokchung district during winter month (31.80 mg/l). All the values lie far below the permissible limit of 500 mg/l (Table 2). The general seasonal sequence was rainy > summer > winter, corresponding to enhanced erosion and runoff during monsoon periods. Khadka et al. (2023) also reported monsoon-driven increases in TDS in spring systems due to soil and agricultural runoff.

CONCLUSION

This study highlights significant seasonal variability in the physico-chemical attributes of spring water across four districts of Nagaland. Although all measured parameters except pH were within the permissible limits of BIS/ICMR/WHO standards. During the rainy season, pH, alkalinity, chloride, conductivity and TDS increased, largely due to surface runoff, soil leaching, and anthropogenic inputs. In contrast, winter samples showed elevated free CO_2 and reduced alkalinity, reflecting the influence of cooler water temperatures and diminished biological buffering. Spatial variation was also apparent in Noklak and Phek districts consistently exhibited higher CO_2 and TDS concentrations, while Mokokchung district displayed the widest seasonal fluctuations. These findings provide a valuable baseline data for assessing spring water quality in Nagaland and emphasize the need for further analysis of others remaining physico-chemical parameters along with biological parameters and minerals composition in order to check the suitability of spring water benefit for human health.

AUTHORS CONTRIBUTION

Wati Temjen, Zhulika Aye, Mewkhro, Jeolam and Imli Jamir prepared the initial manuscript draft, assisted in the collection of water samples, laboratory analysis, and compilation of manuscript. All authors contributed to data entry, statistical analysis and figure preparation. Maibam Romeo Singh supervised the overall research work, conceptualized the study design, provided guidance in methodology and interpretation, and revised the manuscript for intellectual content..

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Diversity of Alien Invasive Plants Species by Random Sampling Method and Impact on Local Flora at Kokrajhar, Assam

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***Abstract*:** Studies recorded 43 invasive alien species (IAS) belonging to 19 different families from four different sub areas of Kokrajhar town. Frequency class was studied from selected area and results were compared with Raunkiaer's frequency class to find homogeneity among the species. Random sampling methods shows highest density value with 1.2, 1.4, 2.3 and 2.4 in *Ageratum conyzoides* with least values as seen in *Cleome gynandra* (0.1), *Crotalaria pallida, Oxalis corniculata* (0.2) and *Solanum viarum, Ipomea carnea, Eichhornia crassipes* (0.3). Distributional pattern shows that majority of these invasive species are native of Tropical America. Thus, IAS recorded from the study area are found to be highly disturbed and it does not follow Raunkier's frequency class. Ethnomedicinal usage for the recorded species shows that most of them are used externally for cuts, burns and wounds with some of them also being consumed internally by the ethnic tribes residing in Kokrajhar town.

Keywords: Raunkier's frequency class, Ethnomedicine, Invasive plants

Globally invasive species have been a threat to native plants due to their allelopathic effects. Invasive species are defined as those plant species that were intentionally or unintentionally introduced to an area beyond their native habitat due to human involvement causing negative economic and ecological impacts (Gawad et al., 2021). These species due to their high reproductive rates can spread rapidly in vast areas within a very short period causing the death of many important native plants levelling them as unwanted weeds. All over the world, these invasive plants can be found in aquatic, semi-aquatic, terrestrial, and even remote and hostile ecosystems. Some of the invasive plants that are native to India have been translocated outside their natural habitats or were introduced in India from other countries or regions causing negative impacts to the entire ecosystems (Hiremath 2013).

Invasive Alien Species (IAS) have a huge negative impact mainly on forest regeneration, production of agriculture, livestock grazing and human health, and its native vegetation and ecosystems due to their allelopathic effects. Often some of these invasive alien species may provide food, fuel, or fodder to the local communities (Kull et al*.,* 2007, Roder et al., 2007) and hence are also sometimes cultivated. However, their excessive growth and rapid expansion, not only cause infestation damage to vast tracts of agricultural and forest land in India but also threaten many faunal diversities present in native ecosystems including wetlands and drylands (Kalita et al,. 2019). Initially, invasive plants were distributed all over the world by trading and transportation systems of various kinds. The world's ecosystems already thrived in their role before humankind started to crisscross around the globe. The scale of the impacts of these invasive plants frequently increased because of modern technological systems. Moreover, modern intensive agriculture created the circumstances for invasive plants leading to changes in land habitat fragmentation and also increasing the persistent organic pollutants (POPs) level, ultimately linked directly or indirectly to biological invasions resulting in great biodiversity loss, and also community structure, composition and functions (Rai 2015). Various natural ecosystems, both aquatic and terrestrial, are impacted by these invasive species (Sharma et al*.,* 2005, Dawson et al*.,* 2017). In India, Reddy (2008) was the first to compile about invasive alien species reporting a total of 1599 species providing information on their diversity and distribution in different families and genera, native ranges, and also their status of invasion (Khuroo et al*.,* 2012a, b).

In Assam, aquatic ecosystems or wetlands which are locally known as "pukhuris", "beels", and "hola", etc. are resourceful ecosystems providing many resources of faunal diversity including fishes and waterfowls to the nearby villages (Kalita et al*.,* 2019). The cover of invasive alien species on high mountain ranges is said to have a very low risk of invasion (Pauchard et al., 2009). But most invasive plants are distributed on the low land terrestrial ecosystems and well distributed on the roadside. However, invasive aquatic plants consequently change the structure of the ecosystem negatively impacting water quality and aquatic biodiversity (Chamier et al., 2012, Brundu 2015). From time to time many researchers in Assam have significantly made contributions and reported diversity about aquatic as well as terrestrial invasive plants and their ecological impacts are also studied macrophytically (Verma 1971, Malakar 1995, Baruah et al., 1997, Kar et al., 2000, Kalita 2009). Various kinds of weeds worstly invade Assam. Management of these weeds is quite challenging to remove or avoid because of their allelochemicals present in those invasive plants entering into soil employing exudation of roots, also during tissue decomposition or leaf leachates of the plants influences the germination, growth and survival of weed plants (Inderjit and Duke 2003). Kokrajhar district of Assam is one of the regions that is greatly diversified with different plant kinds. Various kinds of invasive alien species have been established in the district spreading in massive form and colonizing its native species. The survey was undertaken at Kokrajhar town to study diversity of invasive weeds in different drylands including roadside, etc., and in different wetlands and also to identify the most widespread weed invaders.

MATERIAL AND METHODS

Study area: Kokrajhar district of Assam covers a total area of 3,169.22 km^2. The district is located on the north bank of the Brahmaputra river and shares an international boundary with Bhutan on the north and the Indian state West Bengal on the west, district Dhubri on the south, and Bongaigaon district (now known as Chirang) on the east. The district lies between 26.19" N to 26.54" N latitudes and 89.46' E to 90.38' E longitudes (Narzary G. S., 2013). The district has vast land of forest area constituting a major part of the region and the region is mainly inhabited by local tribes such as *Bodos* and *Adivasis* and some other migratory people (Nath and Mwchahary, 2012). Kokrajhar district has a humid subtropical climate with high humidity and rainfall, hot summer, wet monsoons, and dry winter climate and typically experiences about 111.34 millimetres of precipitation and the district also has average rainfall which varies between 2400 – 3000 mm annually. The district has a heterogeneous soil condition type. The northern part of the district is composed of clayey and loamy soil type, loamy and sandy type soil is present in the middle part whereas soil of southern part of the district has sandy soil. Diverse aquatic plants and ecotone plant species as well as terrestrial plant species are being harboured within the district. However, these native plants are invaded by the alien invasive plants impacting negatively.

Methodology: The result of the present study was prepared mostly based on extensive fieldwork within the study area. A total of four sub-areas within Kokrajhar town were selected *viz.,* North West, North East, South East and South West to estimate the diversity of invasive alien species. Field surveys included swamp areas, roadsides, ponds and some terrestrial habitats where these invasive alien species were dominant in habitat. The fieldwork comprises plant collection, ethnobotanical investigations, face to face interaction with local people and photographing the specimens. The survey was carried out during the mid-month of March 2022 to May 2023 at different locations of the study area site following firm procedures embraced for collection as well as characterization of plants and analysing data as per objectives. Specimens were collected and herbariums were prepared following standard protocols following the methods of Jain and Rao (1976). Plants for herbarium were collected along with additional reproductive structures such as twigs, leaves, flowers, cones etc. Voucher specimens were prepared for each plant species which were carefully numbered and deposited in the Department of Botany, Bodoland University. Further authenticity, nomenclature and

Source: www.googlemap.com/assam/kokrajhar

Fig. 1. Location of study area (Kokrajhar town), Assam

identification of the collected plant specimens were made by consulting various websites like www.bsi.gov.in, www.tropicos.org and http://www.invasiveplantsofindia.com. Several steps were made while preparing herbarium techniques (Baruah A., 2013). To achieve good durability of xerophytes and invasive succulent submerged plants, specimens were specially treated to remove a high percentage of water before drying and were placed below or mounted well in blotting paper or newspapers which is then pressed rapidly with a hot iron. To remove bulky and fleshy parts of the specimens, they were often treated with a combination of diluted acetic acid (CH_3COOH) or strong ethyl alcohol (C_2H_5OH) or 1.5-part formaldehyde (HCHO) and 1.5-part water before mounting on blotting papers to avoid decaying and fungal infections. (Baruah A., 2013). Photographs were taken on the spot where invasive plants are found. Further protection of herbarium sheets with moth balls naphthalene and dichloro-diphenyl-trichloroethene (DDT) were used to control and repel insects.

Since frequency refers to the degree of dispersion in terms of percentage occurrence and frequency distributions, frequency observation was done by random sampling methods following Raunkiaer's law (1934). The sampling procedure was carried out by sampling on stratified random places under selected areas. A quadrant size of 5 m × 5 m was laid at different places. Determination of density was done to estimate the numerical strength of a species with a definite unit space as follows -

$$\text{Population Density} = \frac{\text{Total no. of individual of a species in all the quadrant studied}}{\text{Total no. of quadrant studied}}$$

Similarly, Percentage of frequency is determined as follows –

$$\text{Percentage Frequency} = \frac{\text{Total no. of quadrants in which the species occurred}}{\text{Total no. of quadrant studied}} \times 100$$

For each family, the frequency classes were done based on Raunkiaer's method (1934). The frequency class provides an idea regarding the distribution of a species throughout a community. Therefore, frequency classes were- A: 20% (1-20), B: 40% (21-40), C: 60% (41-60), D: 80% (61-80) and E: 100% (81-100) where high frequency indicates the community was homogenous.

RESULTS AND DISCUSSION

A total of 43 invasive alien plant species belonging to 19 different families were recorded from the undisturbed areas, roadsides, wetlands, low-lying areas and crop land areas. These invasive recorded plants belong to different life forms, viz. aquatic herbs, herbs, shrubs and a few climbers. On the North-West side of the study site, a total number of 15 families were recorded and dominant family was Asteraceae and Fabaceae consisting of 5 species, followed by Amaranthaceae, Cyperaceae, Onagraceae, Polygonaceae and Malvaceae. Families like Capparidaceae, Pontederiaceae, Euphorbiaceae, Convolvulaceae, Verbenaceae, Lamiaceae, Araceae and Solanaceae, consisting of only a single species. In the North-East area, a total number of 27 invasive alien plant species were belonging to a total of 13 families were observed. The dominant family was Asteraceae consisting of a total of 6 species, followed by Fabaceae consisting of 5 total species, and Solanaceae consisting of 4 species, while Amaranthaceae and Poaceae had 2 species and the rest families had the least species consisting of only 1 species each. In the South-East area, a total number of 28 invasive plant species were belonging to 13 different families were observed . Among them, the dominant family was Asteraceae which consisted of 7 different species which is followed by Fabaceae consisting of 4 different species and Amaranthaceae had 3 species. Under South-West, a total number of 26 invasive plant species belonging to 14 different families were recorded. Asteraceae was dominant consisting of 5 species, followed by Fabaceae which consist of 4 species, and Amaranthaceae 3 species, while Convolvulaceae, Polygonaceae and Euphorbiaceae had 2 species each and the rest families had only 1 species.

Raunkiaer (1934) suggested that the number of species in frequency class A is greater than that of B, B is greater than in class C, class C is greater or lesser than class D, and D is lesser than class E. Hence, according to Raunkiaer's method (1934),

A > B > C = D < E

In the North-West area *Ageratum conyzoides* L. shows the highest density 2.4 and the least was in *Cleome gynandra* L. (0.1). The frequency class of the invasive alien species in the North-West having maximum, comparing these values with Raunkiaer's frequency class as A>B>C>D=E. Similarly, frequency class of the North-east area shows frequency class with class A (8) followed by class B (16) and class C (3) while classes D and E lack any species indicating that the maximum dominant species was in class B, hence Raunkiaer's frequency class becomes as follows A<B>C>D=E and highest density value was 1.2 of *Ageratum conyzoides* and the least was seen in *Solanum viarum* with 0.2 value. Results from frequency class of the South-East area show class A (11) followed by class B (15) and class C (2) while classes D and E show no species indicating the maximum dominant species was in class B, i.e., A<B>C>D=E and with 1.4 value *Ageratum conyzoides* shows highest density and *Crotalaria pallida* shows the least density with 1.2 value. Again, in the South-West area, we find the following frequency class A>B>C>D=E, where maximum frequency was found in A (12) followed by B (11), class C has 3 value while classes D and E lacks any species and *Ageratum conyzoides* shows highest density with 2.3 value and with 0.2 value *Oxalis corniculata* shows the least density.

Table 1. Density and frequency of IAS of North-West, Kokrajhar Town

Name of the species	Family	S	N	D	F%
Ageratum conyzoides L.	Asteraceae	24	6	2.4	60
Alternanthera tenella Colla.	Amaranthaceae	12	4	1.2	40
Amaranthus spinosus L.	Amaranthaceae	4	3	1.4	30
A. viridis L.	Amaranthaceae	5	3	0.5	30
Chromolaena odorata (L.) King & Robinson	Asteraceae	3	3	0.3	30
Cleome gynandra L.	Capparidaceae	1	1	0.1	10
Crotalaria pallida Aiton.	Fabaceae	2	1	0.2	10
Cyperus rotundus L.	Cyperaceae	6	2	0.6	20
Cyperus haspan L.	Cyperaceae	3	2	0.3	20
Eichhornia crassipes (C. Martius) Solms-Loud.	Pontederiaceae	4	2	0.4	20
Euphorbia hirta L.	Euphorbiaceae	9	2	0.9	20
Gynura crepidioides (Benth.) S. Moore	Asteraceae	2	1	0.2	10
Ipomoea carnea Jacq.	Convolvulaceae	6	2	0.6	20
Lantana camara L.	Verbenaceae	10	5	1.0	50
Ludwigia erecta (L.) H. Hara	Onagraceae	6	2	0.6	20
Ludwigia octovalvis (Jacq.) Raven	Onagraceae	5	2	0.5	20
Mesosphaerum suaveolens (L.) Kuntze	Lamiaceae	6	3	0.6	30
Mikania micrantha (Willd.) Kunth.	Asteraceae	6	4	0.6	40
Mimosa pudica L.	Fabaceae	5	3	0.5	30
Parthenium hysterophorus L.	Asteraceae	8	3	0.8	30
Persicaria hydropiper L.	Polygonaceae	8	3	0.8	30
Persicaria lapathifolia L.	Polygonaceae	4	2	0.4	20
Pistia stratiotes L.	Araceae	6	2	0.6	20
Senna alata L.	Fabaceae	3	2	0.3	20
Senna occidentalis (L.) Link.	Fabaceae	2	1	0.2	10
Senna tora L.	Fabaceae	2	1	0.2	10
Sida acuta Burm. F.	Malvaceae	13	5	1.3	50
Solanum nigrum L.	Solanaceae	4	3	0.4	30
Urena lobata L.	Malvaceae	5	3	0.5	30

Total number of individuals=S; Total number of quadrants in which species occurred = N; Total number of quadrants studied = Q; Density (D) = S/Q; Percentage frequency (F) %= N/Q×100

Since Raunkiaer's frequency law states that species with low-frequency values are higher in number than the species with higher frequency values in most natural communities, thus comparing results with Raunkiaer's frequency class, it can be assumed that the invasive alien species studied in the study site are comparatively disturbed and it does not follow Raunkiaer's frequency class. The root cause for these may be over exploitation of some invasive species due to its medicinal usage, over grazing, soil erosion, pollution or ongoing road side and building constructions in the study area.

Table 2. Density and frequency of IAS of North-East, Kokrajhar Town

Name of the species	Family	S	N	D	F%
Acanthospermum hispidum DC.	Asteraceae	10	4	1.0	40
Ageratum conyzoides L.	Asteraceae	12	6	1.2	60
Alternanthera tenella Colla	Amaranthaceae	6	3	0.6	30
Amaranthus spinosus L.	Amaranthaceae	8	4	0.8	40
Chromolaena odorata (L.) King & Robinson	Asteraceae	6	3	0.6	30
Cleome gynandra L.	Capparidaceae	4	3	0.4	30
Crotalaria pallida Aiton	Fabaceae	6	3	0.6	30
Eichhornia crassipes (C. Martius) Solms-Loud	Pontederiaceae	3	1	0.3	10
Gynura crepidioides (Benth.) S. Moore	Asteraceae	4	3	0.4	30
Imperata cylindrica (L.) Raeusch	Poaceae	8	3	0.8	30
Ipomoea carnea Jacq.	Convolvulaceae	3	2	0.3	20
Lantana camara L.	Verbenaceae	5	3	0.5	30
Melastoma malabathricum L.	Melastomaceae	5	2	0.5	20
Mesosphaerum suaveolens (L.) Kuntze	Lamiaceae	8	5	0.8	50
Mikania micrantha (Willd.) Kunth.	Asteraceae	6	3	0.6	20
Mimosa diplotricha C. Wright	Fabaceae	10	3	1.0	30
*Mimosa pudica*L.	Fabaceae	6	4	0.6	40
Parthenium hysterophorus L.	Asteraceae	11	5	1.1	50
Persicaria hydropiper L.	Polygonaceae	5	4	0.5	40
Saccharum sponteneum L.	Poaceae	11	3	1.1	30
Senna alata L.	Fabaceae	6	2	0.6	20
Senna occidentalis (L.) Link	Fabaceae	4	2	0.4	20
Solanum nigrum L.	Solanaceae	5	3	0.5	30
Solanum sisymbriifolium Lam.	Solanaceae	6	3	0.6	30
Solanum torvum Sw.	Solanaceae	4	3	0.4	30
Solanum viarum Dunal.	Solanaceae	2	2	0.3	20
Urena lobata L.	Malvaceae	4	2	0.4	20

See Table 1 for details

The maximum species surveyed were herbs, followed by shrubs and a few are climbers. Some invasive alien plant species like *Ageratum conyzoides* L., *Alternanthera tenella* Colla., *Amaranthus spinosus* L., *Chromolaena odorata* (L.) King & Robinson, *Cleome gynandra* L., *Lantana camara* L., *Mikania micrantha* (Willd.) Kunth., *Mimosa pudica* L., *Parthenium hysterophorus* L., *Persicaria hydropiper* L., *Pistia stratiotes* L., *Senna occidentalis* (L.) Link., *Senna tora* L. is having herb life forms. The nativity of the recorded plant species mainly belonged to Tropical America with a maximum number of 14 and only a few belonged to the Mediterranean, Tropical Madagascar, Tropical West Asia and Europe with only a single species each.

From times memorial it has been observed that Invasive Alien plant species has caused economic or environmental harm that has offset their benefits from employing them for their cultivation. In many parts of the world like China and Taiwan a number of invasive species have been intentionally introduced for biological control of disease, weeds and insect pest. Since the general public of Kokrajhar District is not aware of the harmful effects caused by these alien species and its future potential rate to the local ecosystem, a preliminary was done to study the growth forms and its distribution in an around different part of Kokrajhar town area.

Table 3. Density and frequency of IAS of South-East, Kokrajhar Town

Name of the species	Family	S	N	D	F%
Acanthospermum hispidum DC.	Asteraceae	7	3	0.7	30
Achyranthes aspera L.	Amaranthaceae	7	3	0.7	30
Ageratum conyzoides L.	Asteraceae	14	6	1.4	60
Amaranthus spinosus L.	Amaranthaceae	9	4	0.9	40
Amaranthus viridis L.	Amaranthaceae	8	4	0.8	40
Chromolaena odorata (L.) King & Robinson	Asteraceae	8	4	0.8	40
Clerodendrum infortunatum L.	Lamiaceae	4	2	0.4	20
Crotalaria pallida Aiton	Fabaceae	2	2	0.2	20
Cuscuta chinensis Lam.	Convolvulaceae	9	1	0.9	10
Eichhornia crassipes (C. Martius) Solms-Loud	Pontederiaceae	3	1	0.3	10
Euphorbia hirta L.	Euphorbiaceae	6	4	0.7	40
Grangea maderaspatana (L.) Poir.	Asteraceae	5	2	0.5	20
Gynura crepidioides (Benth.) S. Moore	Asteraceae	3	2	0.3	20
Imperata cylindrica (L.) Raeusch.	Poaceae	20	6	2.0	60
Ipomoea carnea Jacq.	Convolvulaceae	3	2	0.3	20
Lantana camara L.	Verbenaceae	7	4	0.7	40
Melastoma malabatricum L.	Melastomaceae	5	2	0.5	20
Mesosphaerum suaveolens (L.) Kuntze	Lamiaceae	8	4	0.8	40
Mikania micrantha (Willd.) Kunth.	Asteraceae	5	3	0.5	30
Mimosa pudica L.	Fabaceae	4	3	0.4	30
Oxalis corniculate L.	Oxalidaceae	8	2	0.8	20
Parthenium hysterophorus L.	Asteraceae	9	4	0.9	40
Persicariahydropiper L.	Polygonaceae	6	3	0.6	30
Ricinus communis L.	Euphorbiaceae	5	2	0.5	20
Senna alata L.	Fabaceae	6	4	0.6	40
*Senna tora*L.	Fabaceae	6	3	0.6	30
Solanum nigrum L.	Solanaceae	6	4	0.6	40
Solanum viarum Dunal.	Solanaceae	5	2	0.5	20

See Table 1 for details

Table 4. Density and frequency of IAS of South-West, Kokrajhar Town

Name of the species	Family	S	N	D	F%
Ageratum conyzoides L.	Asteraceae	23	6	2.3	60
Alternanthera tenella Colla	Amaranthaceae	5	3	0.5	30
Amaranthus spinosus L.	Amaranthaceae	13	4	1.3	40
Amaranthus viridis L.	Amaranthaceae	6	3	0.6	30
Clerodendrum infortunatum L.	Lamiaceae	13	6	1.3	60
Chromolaena odorata (L.) King & Robinson	Asteraceae	10	3	1.0	30
Cleome gynandra L.	Capparidaceae	4	2	0.4	20
Cuscuta chinensis Lam.	Convolvulaceae	6	2	0.6	20
Cyperus haspan L.	Cyperaceae	9	2	0.9	20
Eichhornia crassipes (C. Martius) Solms-Loud	Pontederiaceae	8	2	0.8	20
Euphorbia hirta L.	Euphorbiaceae	7	3	0.7	30
Gynura crepidioides (Benth.) S. Moore	Asteraceae	5	2	0.5	20
Ipomoea carnea Jacq.	Convolvulaceae	5	2	0.5	20
Lantana camara L.	Verbenaceae	6	3	0.6	30
Mikania micrantha (Willd.) Kunth.	Asteraceae	11	3	1.1	30
Mimosa diplotricha C. Wright	Fabaceae	6	2	0.6	20
Mimosa pudica L.	Fabaceae	4	2	0.4	20
Oxalis corniculate L.	Oxalidaceae	2	2	0.2	20
Parthenium hysterophorus L.	Asteraceae	20	4	2.0	40
Persicaria hydropiper L.	Polygonaceae	13	5	1.3	50
Persicaria lapathifolia L.	Polygonaceae	7	2	0.7	20
Phyllanthus urinaria L.	Phyllanthaceae	6	3	0.6	30
Ricinus communis L.	Euphorbiaceae	6	4	0.6	40
Senna alata L.	Fabaceae	4	2	0.4	20
Senna occidentalis (L.) Link	Fabaceae	4	2	0.4	20
Solanum nigrum L.	Solanaceae	7	3	0.7	30

See Table 1 for details

A total of 43 invasive alien plant species belonging to 19 different families were recorded from the undisturbed areas, roadsides, wetlands, low-lying areas and crop fields of study sites of Kokrajhar town. These invasive recorded plants belong to different life forms, viz. aquatic herbs, herbs, shrubs and few as climbers. Among these species many of them were have medicinal values and are used by different ethnic communities as ethnomedicines. Since these species are obnoxious weeds in several crop fields, they have harmful effects including reduction of crop production, effecting grazing of livestock, allelopathic and toxic impacts on various native plants like common crops, blocks drainage system and have also great negative impacts on human health and livestock. *Ageratum conyzoides, Alternanthera tenella*, *Mikania micrantha*. *Amaranthus spinosus, Amaranthus viridis, Lantana camara,* weeds are common in the crop field which reduces production of crops. Species like *Parthenium hysterophorus* causes asthma and bronchitis in man as well as livestock. *Senna occidentalis* and *Senna tora* effects on seed germination and growth of mustard due to the presence of allelopathic in the plant. *Eichhornia crassipes* not only reduces crop production and blocks drainage system but also induce itching when eaten due the presence of alkaloid, hydrogen cyanide, triterpenoid in the plant.

Besides these species have negative impacts on environment, have also various medicinal and other uses. Juice extract from *Ageratum conyzoides* is used in jaundice and leaf juice can be applied on wounds and cuts to prevent from bleeding (Das et al*.,* 2013, Wagh and Jain 2018). Leaf paste from *Alternanthera tenella* is medicinally useful in common weakness and in fever, leaf juice of *Amaranthus*

Table 5. Frequency class of invasive alien plant species of study site

Class frequency	North- West area
A (0 – 20)	15
B (21 – 40)	11
C (41 – 60)	3
D (61 – 80)	0
E (81 – 100)	0
Class frequency	North-East area
A (0 – 20)	8
B (21 – 40)	16
C (41 – 60)	3
D (61 – 80)	0
E (81 – 100)	0
Class frequency	South-East area
A (0 – 20)	11
B (21 – 40)	15
C (41 – 60)	2
D (61 – 80)	0
E (81 – 100)	0
Class frequency	South-West area
A (0 – 20)	12
B (21 – 40)	11
C (41 – 60)	3
D (61 – 80)	0
E (81 – 100)	0

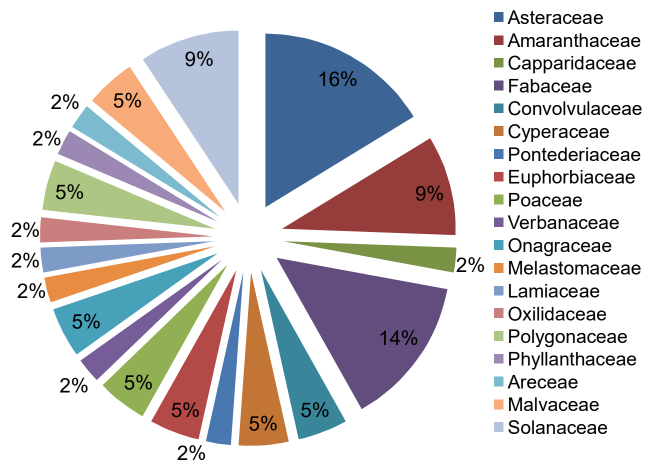


Fig. 2. Distributional pattern of total recorded plants into different families

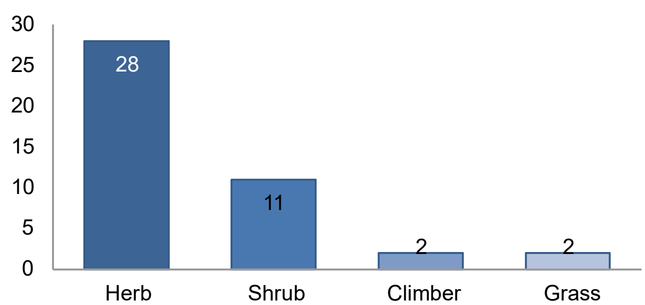


Fig. 3. Distributional pattern of total recorded plants into different habits

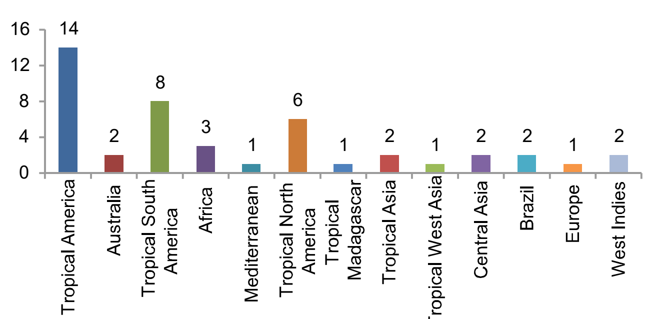


Fig. 4. Distributional pattern of different nativity of species

Table 6. Invasive plant species recorded from study site with their nativity, harmful effects and uses

Name of the species	Growth habit	Nativity	Harmful effects	Uses
Acanthospermum hispidum DC.	Herb	Tropical America	It is a problem weed for almost 24 crops.	Treats various diseases including malaria, abdominal pain, stomachache, snake bite, jaundice, eruptive fever, etc. (Chakraborty et al., 2011).
Achyranthes aspera L.	Herb	Australia	It is a common weed that eliminates native plants.	Traditionally used to treat many ailments like wound, snake bite, diabetes, kidney stone, piles, rabies, toothache, asthma etc. (Dey 2011).
Ageratum conyzoides L.	Herb	Tropical America	This weed is common in the crop field which reduces crop production	Juice extract is used in jaundice and leaf juice can be applied on wounds and cuts to prevent from bleeding (Das et al., 2013, Wagh and Jain 2018).
Alternanthera tenella Colla	Herb	Tropical America	Reduces crop production in the crop field.	Leaf is medicinally useful in common weakness and in fever (Das et al., 2013).
Amaranthus spinosus L.	Herb	Tropical South America	It is a nuisance weed of row crops and vegetables occurring around livestock holding areas and roadside edges.	Plant leaf juice can be applied on scabies (Wagh and Jain 2018). Also consumed as vegetable.
Amaranthus viridis L.	Herb	Tropical America	It is a serious weed in virtually any crop.	This plant is utilized in various aspects such as pharmaceutical, agricultural and also in food applications (Kumari et al., 2018).
Chromolaena odorata (L.) King & Robinson	Herb	Tropical America	The presence of alkaloids in the flowers causes death to livestock (Sajise *et al.* 1974; McFadyen, 2004). This weed is allelopathic to tomato (Onwugbuta 2011)	Local people use leaf paste in cut and wounds.
Cleome gynandra L.	Herb	Tropical America	Grows spontaneously in paddy fields and harms the paddy (Mishra et al., 2011).	When fresh leaf of plant is macerated with goat milk can be apply on the whole body of a person suffering from jaundice (Wagh and Jain 2018).
Crotalaria pallida Aiton.	Shrub	Africa	Harmful to native vegetation.	Plants used as fodder for cattles.
Cuscuta chinensis Lam.	Climber	Mediterranean	These parasitic plants penetrate the host stem via haustoria by winding around plants ultimately.	Decoction of plant is used in jaundice.
Cyperus haspan L.	Herb	Tropical North America	Harmful to native vegetation and block drainage system.	Plants are used as fodder for domestic cattles.
Cyperus rotundus L.	Herb	Africa	Harmful to native vegetation and block drainage system	These plants are used as fodder for cattles.
Eichhornia crassipes (C. Martius) Solms-Loud	Aquatic Herb	Tropical America	It is one the most obnoxious weed that reduces crop production and blocks drainage system. The presence of alkaloid, hydrogen cyanide, triterpenoid in the plant may induce itching when eaten (Perry, 1980).	Relive backache when massaged with root paste with sesame oil on affected parts. The stem fiber is utilized in making ropes.
Euphorbia hirta L.	Herb	Tropical America	Nill	Local people used this plant to prevents worn infestations in children and pimples. Used as traditional remedies for skin ailments, asthma as well as hypertension (Wong et al., 2013).
Grangeama maderaspatana (L.) Poir	Herb	Tropical South America	Nill	In India, leaves are a valuable stomachic and antispasmodic properties and also roots are chewed after meal for 15 days for dyspepsia. It is a popular medicinal plant used for knee joint rheumatism and muscle pain (Huang, at el., 2016).
Gynura crepidioides Benth.	Herb	Tropical Madagascar	Reduces crop production.	Leaves are traditionally used to treat indigestion, stomachache and wound (Can and Tao, 2020).

Cont...

Table 6. Invasive plant species recorded from study site with their nativity, harmful effects and uses

Name of the species	Growth habit	Nativity	Harmful effects	Uses
Imperata cylindrica (L.) Raeusch.	Grass	Tropical Asia	Affects farmer practicing dry agriculture (Holm et al., 1977).	Extracted leaf effects as anti-cancer (Keshava et al., 2016).
Ipomoea carnea Jacq.	Shrub	Tropical America	Due to their dense growth habit blocks drainage system.	Shoots of plant are used by local people as vegetable.
Lantana camara L.	Shrub	Tropical South America	Greatly effects on livestock grazing, and also reduce production of crop. Seeds are toxic.	Leaves are useful in snakebite. The plant is also used as hedge and live fencing (Das et al., 2013).
Ludwigia erecta (L.) H. Hara	Herb	North America	Eliminates other native aquatic plants (Zheng 2018).	Local people consume young leaves as vegetable, used as fodder and traditionally used as medicine to treat malaria fever.
Ludwigia octovalvis (Jacq.) P.H. Raven	Herb	Central America	Eliminates other native aquatic plants (Zheng 2018).	Local people consume young leaves as vegetable, used as fodder and traditionally used as medicine to treat malaria fever.
Melastoma malabatricum L.	Shrub	Australia	Effects the crop production and reduces and eliminates its native vegetation.	Treats various type of ailments and diseases like- diarrhea, dysentery, cuts and wounds, infection during confinement, toothache and stomachache (Joffry et al., 2011).
Mesosphaerum suaveolens (L.) Kuntze	Herb	Tropical America	Colonize areas and dominates over native species (Arzoo et al., 2016).	This plant contains antibacterial, antifungal and insecticidal properties and traditionally used as treatment of various ailments (José et al., 2022).
Mikania micrantha (Willd.) Kunth.	Climber	Tropical south America	Reduces production of crops and eliminates native vegetation.	Juice is use in insect bite. Extraction of leaf is given to domestic animals to prevent from diarrhea (Wagh and Jain, 2018).
Mimosa diplotricha C. Wright	Shrub	Brazil, North America	Aggressively decline native biodiversity and negatively effect to agricultural production (Uy O, 2020).	It controls soil erosion. It fixes nitrogen and also this species is used as a fodder to cattle.
Mimosa pudica L.	Herb	Brazil	Effects greatly on livestock grazing, reduces production of crops. Eliminates native vegetation.	A decoction of root parts can be used in urinary disorders and root paste is useful in stopping in bleeding piles (Wagh and Jain, 2018). Decoction of root is applied in toothache (Das et al., 2013).
Oxalis corniculate L.	Herb	Europe	Nill	Local people of Assam use this species in preventing or may be to heal conjunctivitis of eyes (Arpita, 2019).
Parthenium hysterophorus L.	Herb	Tropical North America	It is an obnoxious weed threating to the biodiversity globally. It causes asthma and bronchitis in man as well as livestock.	Root decoction is use to prevent dysentery and juice of leaf is applied externally to prevent skin disorders. Extraction of whole are used as insecticide (Das et al., 2013; Wagh and Jain, 2018).
Persicaria hydropiper L.	Herb	Tropical America	Due to their allelopathic properties it can widespread in the crop field and effects the production and also eliminates the native plants.	In food preparations, it is used as spice and beside it is used also as medicines for snake bite (Ahmad et al., 2021).
Persicaria lapathifolia (L.) Delarbre	Herb	Tropical North America	Due to their allelopathic properties it can widespread in the crop field and effects the production and also eliminates the native plants.	Traditionally used as medicine for antiviral, anti-fungal, antiseptic, burns, dysentery, etc. (Mahanta et al. 2023).
Phyllanthus urinaria L.	Herb	Tropical Asia	Native species are removed or eliminated by the invasion of this plant.	The leaves are used as a fodder to cattle. Local used this species as oriental medicine in treating liver disease (Du et al., 2018).
Pistia stratiotes L.	Aquatic Herb	Tropical North America	This plant blocks the river current and drainage system.	The cooked leaves are used as soups, and the plant is used to treat swellings and urinary tract infections (Tripathi and Shukla, 2007).

Cont...

Table 6. Invasive plant species recorded from study site with their nativity, harmful effects and uses

Name of the species	Growth habit	Nativity	Harmful effects	Uses
Ricinus communis L.	Herb	South America, North Eastern Africa	Castor plant contain ricin that produce symptoms like fever, nausea, respiratory distress when inhaled (Worbs et al., 2011).	A vegetable oil, known as castor oil is prepared from the seed of the castor plant (Subramaniyan, 2020). Local use the leaves to feed silkworm.
Saccharum sponteneum L.	Grass	Tropical West Asia	Forest ecosystem is disturbed and it is serious weed of many agricultural fields. It's hard to get rid of, since natural regeneration is promoted by rhizomes propagation and seeds spreaded over large area that invades new land (Ganguly et al., 2019).	It prevents soil erosion. Tribal people make mats, brooms, etc. from this plant. Besides these uses it has many medicinal uses such as sexual weakness, burning sensations, mental illness, urinary tract infections (Lamba et al., 2023).
Senna alata L.	Shrub	West Indies	This plant effects crop field and reduce the production of crop as well as its native vegetation.	Leaf juice prevents ringworm infection (Thakur et al.,2024)
Senna occidentalis (L.) Link.	Herb	Tropical South America	Due to the presence of allelopathic in the plant effects on seed germination and growth of mustard. Reduces production of crops as well as native vegetation (Das et al., 2013).	Leaf paste is helpful in preventing skin diseases and decoction of root is used as anti-dote in snakebite (Das et al., 2013).
Senna tora L.	Herb	Tropical South America	Due to the presence of allelopathic in the plant effects on seed germination and growth of mustard (Sarkar et al., 2012).	Leaf juice can be applied to prevent ringworm, eczema and scabies (Das et al., 2013).
Sida acuta Burm. F.	Shrub	Tropical America	Due to the presence of allelopathic chemical it is difficult to remove this species from vegetation, moreover, this species eliminates its native plants.	Applying juice of plant cools wounds and cuts (Purkayastha & Nath, 2006).
Solanum nigrum L.	Herb	Tropical America	Vomiting, headache and other side effects when consumed.	Plant leaves and stem are used in constipation.
Solanum sisymbriifolium Lam.	Shrub	Central and South America	Aggressively invade farm lands, roadside, etc. (Julissa 2020).	Whole plant parts are used to treat diarrhea, infections in urinary tract and respiratory problems (Chidambaram et al.,2022).
Solanum torvum Sw.	Shrub	West Indies	Effects native vegetation and eliminates.	The fruit is eaten as curry by the local people (Tripathi 2013).
Solanum viarum Dunal.	Shrub	Tropical America	Consumption of fruit in excess may result in gastrointestinal problems like constipation (Chidambaram et al., 2022).	Sometimes fruit can be consumed raw directly. They are also cooked and consumed (Singh, 2023).
Urena lobata L.	Shrub	Tropical America	Plant contains allelopathic chemical which invades land plants eliminating native species.	This plant has a vast potential in curing and healing many diseases and disorders. Moreover, it also used in making ropes, mats, etc. (Islam and Uddin 2017).

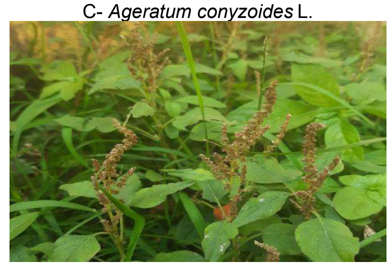

A- *Acanthospermum hispidum* DC.


B- *Achyranthes aspera* L


C- *Ageratum conyzoides* L.


D- *Alternanthera tenella* Colla


E- *Amaranthus spinosus* L


F- *Amaranthus viridis* L.


G- *Chromolaena odorata* (L.) King & Robinson


H- *Cleome gynandra* L

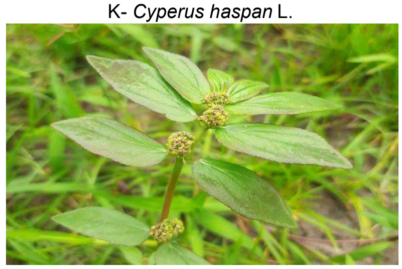

I- *Crotalaria palida* Aiton.

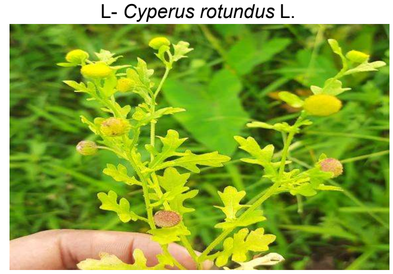

J- *Cuscuta chinensis* Lam.


K- *Cyperus haspan* L.


L- *Cyperus rotundus* L.


M- *Eichhornia crassipes* (C. Martius) Solms-Loud.

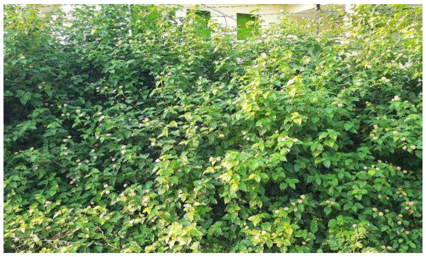

Q-*Imperata cylindrical* (L.) Raeusch.


R-*Ipomoea carnea* L.


P-*Gynura crepidioides* (Benth.) S.


Q-*Imperata cylindrical* (L.) Raeusch.


R-*Ipomoea carnea* L.


S- *Lantana camara* L.


T- *Ludwigia erecta* (L.) H. Hara.


U-Ludwigia octovalvis(Jacq.)


V-Melastoma malabathricum L.


W- *Mesosphaerum suaveolens* (L.) Kuntze


X-*Mikania micrantha* (Wild.) Kunth.

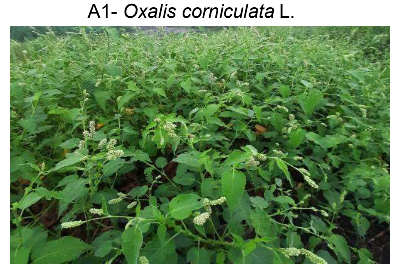

Y- *Mimosa diplotricha* C. Wright

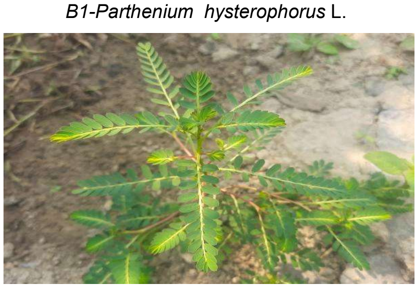

Z- *Mimosa pudica* L.


A1- *Oxalis corniculata* L.


B1-Parthenium hysterophorus L.


C1-Persicariahydropiper L.


D1-*Persicaria lafathifolia* (L) Delarbre.


E1-*Phyllanthus urinaria* L.


F1-*Pistia stratiotes* L.


G1-*Ricinus communis* L.


H1-*Saccharum sponteneum* L.


I1-*Senna alata* L.


J1-*Senna occidentalis* (L.) Link.


K1-*Senna tora*L.


L1-*Sida acuta* Burm. F.


M1-*Solanum nigrum* L.


N1-*Solanum sisymbriifolium* Lam.


O1-*Solanum torvum* Sw.


P1-*Solanum viarum* Dunal.


Q1-*Urena lobata* L.

Fig. 5. Alien invasive species recorded from the study area

spinosus can be applied on scabies, decoction of *Cuscuta chinensis* is used in jaundice and fresh leaves of *Cleome gynandra* plant is macerated with goat milk and applied on the whole body of a person suffering from jaundice (Wagh and Jain 2018). Local people use leaf paste of *Chromolaena odorata* in cut and wounds. Root paste of *Eichhornia crassipes* relive backache when massaged with sesame oil on affected parts, the stem fiber is utilized in making ropes. Shoots of *Ipomoea carnea* are used by local people as vegetable. *Lantana camara* are useful in snakebite and as hedge for live fencing (Das et al., 2013). *Mikania micrantha* juice is use in insect bite and extraction of leaf is given to domestic animals to prevent from diarrhea (Wagh and Jain, 2018). The decoction of *Mimosa pudica* root parts can be used in urinary disorders and root paste is useful in stopping in bleeding piles and in toothache (Das et al., 2013). Root decoction of *Parthenium hysterophorus* is use to prevent dysentery and juice of leaf is applied externally to prevent skin disorders and extraction of whole plant are used as insecticide (Das et al., 2013, Wagh and Jain 2018). Leaf juice of *Senna alata* prevents ringworm infection, leaf juice of *Senna tora* can be applied to prevent ringworm, eczema and scabies (Das et al., 2013). Leaf paste *Senna occidentalis* is helpful in preventing skin diseases and decoction of root is used as anti-dote in snakebite. Many local people still rely on these species on medicinal purposes and some ethnobotanically purposes. Moreover, few species are used as ornamentally in many local households.

CONCLUSION

There is a lack of general understanding about the impacts of invasive species on ecosystem functioning, local people should be made aware of the outcompeting native species and damaging ecosystem caused by these alien species. The critical need for public awareness and education is required among the local people. The balanced approach should be conducted to provide knowledge and guidance about the threats, benefits and harmful effects of these invasive species. Knowledge gained on invasive species and control in one country can be valuable to other countries in making decisions on prevention and control of the same species. Hence invasive species should be put on India's

National Agenda immediately and action should be taken with appropriate exposure to cover substantial economic loss and damage to endemic flora and rich biological heritage. Thus, can conclude that it is particularly important to strengthen international communication and co-operation in sharing, linking and integrating invasive species databases and information systems should be strengthened and research should be conducted to support effective prevention and control strategies about these invasive species.

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Induction of Mutation in Chrysanthemum (*Dandranthema grandiflora* L.) cv. Yellow Stone through Gamma Radiations and Ethyl Methyl Sulphonate

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Abstract: This study investigates the effects of gamma rays and ethyl methyl sulphonate (EMS) on chrysanthemum variety yellow stone which is renowned for resistance to disease to enhance its growth and floral characteristics. Four EMS concentrations (0.10, 0.15, 0.20, and 0.25%) and four gamma-ray doses (10, 20, 30, and 40 Gray) were applied to rooted cuttings in order to take advantage of variability and assess heritable impacts on a number of characteristics, including survival rate, vegetative growth, and blooming. Reduction in survival rate and various morphological parameters was observed with chemical mutagens and higher doses of gamma rays. Gamma rays (10 Gy) significantly improved most of the vegetative and flowering parameters by recording maximum plant height, leaf length, leaf width, number of branches per plant, stem thickness, diameter of flower head, flower weight, pedicel length, number of flowers per plant, flower yield per plant, minimum days taken to appearance of first flower bud and opening of first flower bud as compared to control. More morphological variations in vegetative and floral characters were observed with EMS treatments exhibiting three flower mutants at 0.10% EMS. One leaf mutant was produced from gamma rays applied at 30 Gy. LD_{50} dose for gamma rays and EMS was 25 Gy and 0.112 per cent, respectively.

Keywords: Morphological variations, Gamma rays, Ethyl methyl sulphonate, Mutation

Chrysanthemums (*Dendranthema grandiflora* L.), is a perennial herb plant belonging to the family Asteraceae. It is referred to as the "Queen of the East" and the "Autumn Queen" because of its vivid blossoms, which make it a beloved flower, especially in November. The chrysanthemum is a deeply rooted cultural institution and a symbol of monarchy in Japan. As one of the most exquisite and maybe the oldest flowering plants, chrysanthemums have a long history of cultural interchange and are grown commercially all over the world. Several cultivars that rank among the top 10 cut, potted, and garden plants in the world have been created during the years of breeding (Kishi-Kaboshi et al., 2017, Shahrajabian et al., 2019, Din et al., 2020). Two common techniques used in chrysanthemum breeding programs are crossbreeding and mutation breeding. Many cultivars have long been based on crossbreeding and naturally occurring sexual and asexual reproduction. The selection of superior hybrid progenies is hampered by the complex genetic variables and self-incompatibility of chrysanthemums (Anderson et al., 2017, Zhang et al., 2018, Kumari et al., 2019, Baghele 2021).Su et al. (2019) and Suprasanna and Jain (2022) observed that mutation breeding prove to be a successful breeding strategy for generating genetic variety in ornamental plants, such as chrysanthemums. Mutations can be caused spontaneously or artificially by chemical mutagens like EMS or physical agents like X-rays or gamma-rays (Kharkwall, 2017). Chrysanthemums have benefited greatly from mutation breeding (Patil et al., 2017, Kumari et al., 2019, Datta 2020), especially when paired with *in vitro* culture methods, which are occasionally the only means of enhancing an existing cultivar in vegetatively propagated species (Kumari et al., 2019). Gamma rays are electromagnetic radiation with a high energy level, deep penetration, and no particles (Anne and Lim 2020). They are essential for mutant breeding programs. EMS has proven to be highly successful in causing point mutations in plant genomes. EMS is a useful tool in the chrysanthemum mutation program because, in addition to producing significant levels of gene mutations and also induces low rates of chromosomal abnormalities during mutagenesis (Jankowicz-Cieslak et al., 2012, Luan et al., 2006).

There are 3377 authorised mutant varieties, of which 285 are mutant chrysanthemum varieties and 728 are ornamental plant mutants, according to the mutant variety database (IAEA 2022). 400 mutants were dispersed among vegetatively generated plants, with a small number of fruit trees and the bulk being floricultural plants (Kumari et al., 2019, Melsen et al., 2021). This demonstrates how well mutant breeding has worked in horticulture, particularly in floriculture. Chrysanthemum research in recent years has concentrated on in vitro methods for producing new varieties. Purente et al. (2020) created leaf and stem mutants with variable lignin and cellulose content, leaf size, and plant height in C. *indicum* var. *Aromaticum* using different EMS doses. Chrysanthemums have also been successfully mutated by physical irradiation; the ideal dosage varies from 1.0 to 3.0 Krads, depending on the genotype (Dilta et al., 2003). Cultivars with their original yellow flower colour are thought to be resistant to mutation including both radio and chemo mutagens, however chrysanthemum mutants have been successfully created (Schum 2003, Miler et al., 2020). Therefore, the creation of mutants with unique inflorescence shapes and colours may result from the efficient mutation of yellow-colored chrysanthemum types. Mutation breeding is already widely accepted for producing changed phenotypes because it creates a permanent heritable change in the structure of the genetic material, making it a cost-effective and time-efficient method for generating flower harvests (Rego and Faria 2001). Consequently, the goal of the current study was to determine the LD_{50} dose of mutagens and standardise the ideal amount of mutagens that would cause variation in the chrysanthemum cultivar Yellow Stone growth and floral features.

MATERIAL AND METHODS

The research was carried out with the Chrysanthemum (*Dendranthema x grandiflora* L.) spray-cut yellow flower cultivar Yellow Stone, during the 2020–21 growing season, the rooted cuttings of the chrysanthemum cultivar Yellow Stone were procured from Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology (K), Shalimar campus. A set of rooted cuttings was exposed to four doses of gamma rays (10, 20, 30 and 40Gy) at Baba Atomic Research Institute, Zakura Srinagar, Jammu and Kashmir. Another set was treated with four dosages of EMS (0.10, 0.15, 0.20, and 0.25%) over duration of three hours. Following the treatments, the rooted cuttings were immersed in a 0.3% solution of sodium thio-sulphate (STS) for fifteen minutes in order to alleviate any solution-related stress on the plant sections. The rooted cuttings were ringed for an hour-long under running water to get rid of any remaining particles of chemical residue. One set of rooted cuttings that was not treated with any mutagen served as control. Both treated and control plants were planted in raised beds prepared in the unheated polyethylene-covered greenhouse in July. Cultural operations like weeding, irrigation, and pest and diseases management, were performed well in time according to recommended package of practices except pinching and disbudding. Beds were drenched with 0.1% Bavistin 10 days before planting to prevent soil borne disease like root rot. Staking was also done in plants to avoid lodging. Observations on vegetative growth and flower characters were recorded on five randomly selected plants from each treatment. Desirable variants with change in plant morphology and flower colour was also recorded as mutation spectrum (Table 1). The ratio of variegated useful plants to irradiated plants was called the mutation frequency (MF).

LD_{50} value: Sensitivity tests were conducted to determine LD_{50} which is the safe dose at which half of the planting materials survive with maximum recovery of viable plant materials. LD_{50} values were determined with the help of probit analysis based on the survival rate of the rooted cuttings after treatment with varying doses of gamma rays and EMS, compared with untreated control, to minimize loss of experimental material. Thirty days after planting, the rooted cuttings were counted, and the survival rate for both gamma irradiation and EMS treatments was estimated.

Statistical analysis: The system software (SASs) V. 9.1 (June 2006), SAS Institute, was used to statistically assess the data of all characters that were collected

RESULTS AND DISCUSSION

Mutagenic effect on vegetative parameters: The dose-dependent negative linear relationship between applied doses of EMS and gamma rays and survival percent of plants

Table 1. Effect of different mutagens on vegetative growth parameters of chrysanthemum cultivar yellow stone

Mutagenic treatment	Survival (%)	Plant height (cm)	Plant spread (cm)	Leaf length (cm)	Leaf width (cm)	Number of branches per plant	Branch length (cm)	Stem thickness (mm)
EMS (0.10%)	66.66 (53.81)	41.53	28.09	5.56	4.42	33.04	34.66	4.14
EMS (0.15%)	56.66 (48.86)	39.31	21.20	5.32	4.33	31.89	30.45	4.04
Gamma rays (10 Gy)	0.00 (0.00)	53.18	23.68	6.06	4.87	39.25	31.64	4.40
Gamma rays (20 Gy)	0.00 (0.00)	50.91	22.07	5.70	4.63	34.81	30.95	4.16
Gamma rays (30 Gy)	76.66 (61.25)	42.45	21.23	5.50	4.52	33.51	30.14	4.04
Gamma rays (40 Gy)	56.66 (48.86)	39.79	20.22	5.23	4.36	30.96	29.43	3.92
Control	43.33 (41.17)	47.56	23.92	5.87	4.73	35.25	32.98	4.29
CD (p-0.05)	(0.73)	1.78	1.47	0.20	0.18	2.21	2.15	0.17

(Table 1). The survival rate of the plants decreased consistently with increasing doses of both mutagens. The chrysanthemum cultivar Yellow Stone responded differently to different doses of EMS with survival rate of 53.33% at 0.10% EMS concentration. The concentration of 0.15% EMS reduced the survival rate, with none of the plants survived at concentrations beyond this concentration. Similarly gamma rays treated plants showed a decreasing trend of survival percentage with increasing dosage. Untreated rooted cuttings had the highest survival rate of 81.66%, followed by a survival rate of 71.66% at 10 Gys of gamma rays. Kiran Kumari et al. (2013) observed significant decrease in survival following gamma ray exposure. Poor establishment and survival following gamma-ray exposure were attributed to auxin depletion and/or inactivation, which impair cell division (Gordon 1957, Mahure et al., 2010) or lethal effect of gamma rays caused due to chromosomal aberration (Datta and Banerji 1993). Dilta et al. (2003) concluded that higher concentrations of EMS reduced the plant survival per cent in chrysanthemum. Lower doses of gamma rays (10 Gy) has been proved to significantly improve most of the vegetative parameters by recording maximum plant height (53.18 cm), leaf length (6.06cm), leaf width (4.87 cm), number of branches per plant (39.25), stem thickness (4.40 mm) as compared to control. Plants exposed to gamma rays may exhibit a decrease in vegetative characteristics depending on the type and degree of chromosomal damage or irradiation-induced physiological, morphological, and cytological disruption (Banerji and Datta 2002). EMS treated plants showed drastic reduction as compared to gamma radiation. All doses of gamma rays resulted in reduction of both plant spread as well as branch length as compared to control. The lower dose of EMS (0.10%) resulted in increase of plant spread and branch length as compared to control. However higher doses significantly reduced both of these parameters.

Mutagenic effect on flowering and yield parameter: Lower doses of gamma rays (10 Gy) significantly shortened the time for the first flower bud to appearance (56.59 days) and first flower to open (86.99 days) in comparison to the control (Table 2). Higher doses, on the other hand, delayed the bud formation. Dobanda (2004) also observed that gamma ray at lower doses induce earliness with respect to opening of first floret. The initiation of flowering may be affected by mutagenic treatments due to alterations in various biosynthetic pathways, which are believed to be directly or indirectly associated with the flowering physiology (Mahure et al., 2010, Ismael and Mohmoud 2015). Lower doses of gamma rays (10 Gy) recorded maximum flower head diameter (6.50 cm), flower weight (4.19 g), pedicel length (6.41 cm), number of flowers per plant (48.40), and flower yield per plant (98.47 g) in comparison to the control. However, all of these characteristics exhibited a declining trend with increasing gamma rays doses. Conversely, EMS caused significant reduction in flowering and yield parameters compared to control. These results are in conformity with Kapadiya et al (2014). Mahure et al. (2010)

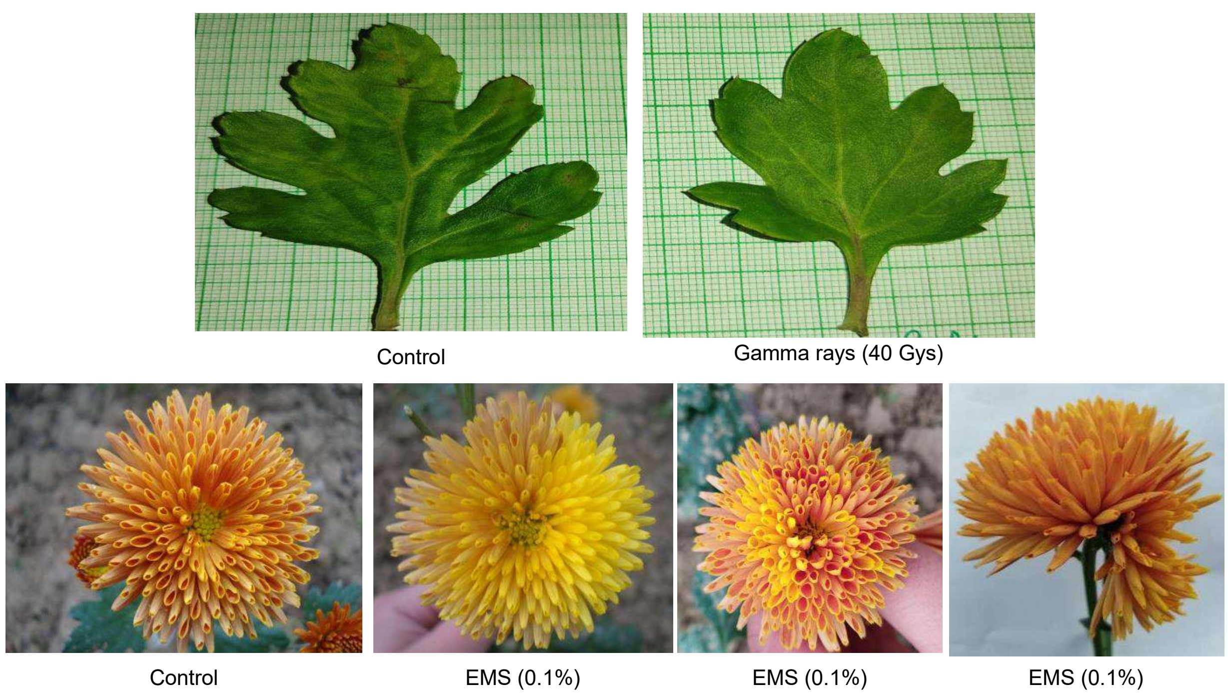

Fig. 1. Mutants in chrysanthemum cv. yellow stone

Table 2. Effect of different mutagens on flowering parameters of chrysanthemum cultivar yellow stone

Mutagenic treatment	Days to appearance of first flower bud	Days to opening of first flower bud	Diameter of flower head (cm)	Number of ray florets per head	Weight of flower (g)	Length of pedicel (cm)	Number of flowers per plant	Flower yield per plant (g)
EMS (0.10%)	61.83	92.32	6.25	241.58	3.42	6.12	42.13	67.80
EMS (0.15%)	64.98	101.62	6.16	230.86	3.32	6.05	41.20	60.74
Gamma rays (`10 Gy)	56.59	86.99	6.50	247.80	4.19	6.41	48.40	98.47
Gamma rays (20 Gy)	60.78	94.00	6.20	241.79	3.64	6.22	42.20	69.55
Gamma rays (30 Gy)	68.72	94.99	6.18	230.60	3.58	5.79	41.53	59.36
Gamma rays (40 Gy)	71.72	102.19	6.10	224.62	3.31	5.61	40.40	53.26
Control	59.87	91.66	6.42	248.47	3.88	6.28	43.93	82.02
CD (p-0.05)	0.06	0.61	0.12	0.40	0.07	0.05	2.23	6.46

Table 3. LD_{50} for gamma rays and EMS on 30th day after planting

Treatment	Survival (%)	LD 50 value
EMS (0.1%)	53.33	0.112%
EMS (0.15%)	40.00	
EMS (0.20%)	0,00	
EMS (025%)	0,00	
Gamma Rays (10Gy)	66.66	25 Gys
Gamma Rays (20Gy)	56.66	
Gamma Rays (30Gy)	40.00	
Gamma Rays (40Gy)	30.00	

also observed that in cultivar Red Gold flower size was smaller in plants treated with EMS and DES compared to gamma ray treated plants. Yellow Stone variety exhibited three flower mutants with 0.1 per cent EMS and only one foliage mutant with 40 Gy gamma rays of mutation frequency of 5.62 and 3.33 per cent, respectively.

LD_{50} value: LD_{50} for EMS was 0.112 percent, and the LD_{50} for gamma rays 25 Gy on the basis probit analysis (Table 3). Because gamma rays are more intense and penetrating in nature, they may have caused damage to cells undergoing meiotic division in the bud region.

CONCLUSION

Most of the floral and vegetative characteristics improved with lower dose of gamma radiation (10Gy). The number of morphological mutants decreased with increase in dosage of EMS but vice versa in gamma rays. Maximum morphological mutant were observed in EMS as compared to gamma rays.

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Molecular Diversity of Fern Species of Agumbe Ghats of Karnataka using ISSR Markers

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Abstract: Survey of the fern flora of the Agumbe Ghats in Karnataka was undertaken to assess the genetic diversity of ferns. Nine ISSR primers were selected to study the genetic diversity among 12 fern species. The annealing temperature for all ISSR primers was standardized using gradient PCR, where the annealing temperatures ranged from 42 °C to 56°C. ISSR fragments generated 32 to 66 bands per primer. A total of 389 polymorphic bands exhibited 100 per cent polymorphism per primer. The similarity coefficient between the species was within the range of 4 to 35 per cent. The dendrogram generated by ISSR markers revealed two major clusters, indicating that fern species have distributed based on frond shape, frond type, rhizome, habitat, stipe colour and texture. Based on molecular data, the highest genetic similarity of 35 % was observed between *Pteris confusa* and *Pteris biaurita.* In comparison, *Tectaria coadunate* and *Adiantum philippense* showed the least genetic similarity index of 4 per cent.

Keywords: Genetic diversity, Dendrogram, Similarity coefficient, ISSR markers, Fern species

India is one of the mega biodiversity countries among seventeen countries. It ranks 10th among the plant-rich countries of the world with the significant distribution of fern and fern allies observed in the Himalayas, western and eastern ghats (Myers et al., 2000). About 1289 pteridophytes are distributed in different geographical regions of India out of 12,000 species in the world. The eastern Himalayas and northeast India with about 845 taxa in 179 genera, representing approximately 67% of the pteridophytes known from the country. It is followed by South India, with the eastern and the Western Ghats, with 345 taxa in 117 genera and northern India, including the western Himalaya, with 340 taxa in 101 genera. About 17% of the total pteridophytic species are endemic to India (https://bsi.gov.in).

Pteridophyta (*Pteron*=Feather, *Phyton*=Plant) are the earliest vascular plants, often known as 'vascular-cryptogams. They are the world's first vascular plants to emerge from the ground (Qiu and Palmer 1999). They are a critical evolutionary feature of the Earth's vegetation because they reflect the evolution of the vascular system and represent the development of seed habitat in the plants (Dixit 2000). The foliage of fern is highly valued in the international florist greenery market because of its long post-harvest life, low cost, year-round availability and versatile design qualities in form, texture and colour (Safeena 2013). Because of their delicate beauty and elegance, ferns are grown as ornamentals either indoors in households or outdoors in botanical gardens. Charaka Samhita describes *Adiantum capillus-veneris* and *Marsileaminuta* as medicinal plants. At the same time, the rhizome of *Polystichum squamosum*, also known as 'Nirviri' in India, is used against scorpion and bug stings (Dixit 2000). Phytoecdysones are produced by ferns, which inhibit insect development and induce deformities (Balasubramanian et al., 2008). Ferns also play an essential part in wastewater bioremediation. Chinese bracken fern, *Pteris vittata* L., is hyper-accumulator of the hazardous arsenic metal (Ma et al., 2001).

Genetic diversity is the primary constituent that enables long-term survival and adaptation of a species, particularly under varying environmental conditions. The genetic structure of populations is often a reflection of various biological processes and interactions, including mutation, genetic drift, mating system, gene flow (Chung et al., 2023, Salgothra et al., 2023). Genetic diversity measurements are crucial when it comes to the conservation of some species. An organism's capacity to react to natural selection may be harmed by a loss in genetic variability, reducing its evolutionary potential.

Morphological characters which are used for the description are not highly reliable. Most morphological characters are controlled by the environment or change with the plant's growth, and isozymes are limited due to low polymorphism levels (Asha et al., 2006). As a result, DNA-based molecular marker techniques such as RAPD, ISSR and micro-satellites, or simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSRs), are effective in assessing plant genetic diversity because they provide an infinite number of potential markers to reveal differences at the molecular level (Ghafoor et al., 2007). The fundamental limitations of these approaches are the limited reproducibility of RAPD, the high cost of AFLP and the necessity to know the flanking sequences to generate species-specific primers for SSR polymorphism. The ISSR marker is such a DNA-based marker system that could screen genetic variability. Changes in DNA sequences and single-base substitutions, including DNA conformation changes, can be detected as shifts in electrophoretic mobility. The technique is useful in fields like genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology across a wide range of crop species. The primary objective of this study was to assess the genetic diversity and relationships among 12 fern species collected from the Agumbe Ghats in Karnataka. This evaluation was conducted using ISSR markers to generate molecular data for species identification, phylogenetic classification, and the development of conservation strategies.

MATERIAL AND METHODS

Survey of fern flora of Agumbe Ghats of Karnataka was undertaken to assess the genetic diversity of ferns, and 12 fern species were collected (Table 1). The experiment was conducted at the Horticulture Research Station, Department of Horticulture and Department of Plant Biotechnology, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru-560065. The leaf samples were collected from the 12 different fern species and were stored at -80°C until DNA extraction. Genomic DNA was isolated from young leaves using the Cetyl Trimethyl-Ammonium Bromide (CTAB) method (Doyle and Doyle 1987) with some modifications. The purity of DNA was analysed by running in 0.8 % agarose gel and the quantification of DNA was done using a UV spectrophotometer.

Twenty µl of PCR reaction mixture was prepared containing Takara mixture (master mix) 10.0µL, ISSR primer 2.0 µL from 5 pM stock, DNA template1.0 µL, and Double distilled water 7.0 µL. PCR reaction was performed using 9 ISSR primers (Eurofins Genomics India Pvt. Ltd.). The list of ISSR markers primers used and annealing temperature used for the study is presented in Table 2. The PCR was performed by initial denaturation at 95°C for 4 min. Followed by 35 cycles of denaturation at 94°C for one minute, annealing at 42-56 °C for one minute (depending on primer), and extension at 72°C for 1.3 minutes and final elongation at 72°C for 20 min. The PCR products were resolved on 1.5 % Agarose gel prepared in 1x TAE buffer containing 0.5 ug/ml of the ethidium bromide at 85 V for 2 hours. PCR amplification was done for twelve fern species for different primers. Consistent and well-resolved amplified bands were manually

Table 2. ISSR primers used for diversity analysis with standardized annealing temperature

Primer	Sequence	Tm (°C)
ISSR-1	GAGAGAGAGAGACC	44.0
ISSR-2	CTCTCTCTCTCTCTCTAC	48.0
ISSR-3	CACACACACACAAG	50.0
ISSR-4	CAGCACACACACACACA	42.0
ISSR-6	CTCTCTCTCTCTCTCTGC	54.0
ISSR-807	AGAGAGAGAGAGAGAGTG	56.0
ISSR-835	AGAGAGAGAGAGAGAGCC	56.0
ISSR-844	CTCTCTCTCTCTGC	42.0
ISSR-850	GTGTGTGTGTGTGTGTCC	56.0

Table 1. Ferns species collected from Agumbe Ghats of Karnataka

Botanical name	Latitude (E)	Longitude (N)	Elevation (m)
Bolbitis semicordata	75°05.283'	13°29.995'	663
Cyathea gigantea	75°05.283'	13°29.472'	670
Christella parasitica	75°05.270'	13°29.290'	666
Angiopteris evecta	75°05.279'	13°29.989'	673
Pteris confusa	75°05.279'	13°29.993'	667
Tectaria coadunata	75°04.931'	13°29.838'	615
Athyrium falcatum	75°04.875'	13°29.878'	619
Adiantum philippense	75°04.962'	13°29.864'	638
Odontosoria chinensis	75°04.947'	13°29.828'	625
Pteris biaurita	75°04.917'	13°29.861'	623
Selaginella delicatula	75°04.978'	13°29.840'	700
Nephrolepis biserrata	75°04.947'	13°29.852'	630

scored. Scoring of bands was done based on their presence ('1') or absence ('0') in the gel. The genetic associations were evaluated by calculating the Jaccard's Co-efficient (J) for pair-wise comparisons based on the proportion of shared bands produced by the primers. The pooled ISSR binary data were utilized for cluster analysis using Paleontological Statistics software (PAST; version 4.07) developed by Hammer *et al*. (2001).

RESULTS AND DISCUSSION

In the present study, 12 different fern species were collected from the Agumbe Ghats of Karnataka and their genetic diversity was studied using ISSR markers. Nine ISSR primers were used to study the relatedness within the species based on their geographical distribution. The ISSR fingerprint for 12 fern species using nine primers revealed 389 scorable bands. The number of bands scored for each primer varied from 32 to 66 bands (Table 3), with an average of 43.22 bands per primer. Primer ISSR1 scored a maximum number of bands (66) followed by ISSR 850, ISSR 6, ISSR 3, and ISSR 844., The minimum number of bands was n ISSR 4 and ISSR 2 (32 and 33 bands, respectively). Total of 389 bands were observed with 100% polymorphism from all primers used in the study .For illustration, this vast range in percent polymorphism obtained in the present study proved the discriminatory power of ISSR molecular markers for distinguishing different cultivars reported in earlier literature Abirami et al. (2018) in *Asplenium nidus*, Chelliah et al. (2014) in Adiantum incisum Forssk, Vidyashree et al. (2019) in fern species and Dong *et al*. (2007) in *Ceratopteris pteridoides*.

Table 3. Polymorphism scoring of twelve fern species in relation with nine ISSR primers

Name of the primer	No. of polymorphic bands	Total No. of bands produced	Percentage polymorphism
ISSR 1	66	66	100
ISSR 2	33	33	100
ISSR 3	42	42	100
ISSR 4	32	32	100
ISSR 6	49	49	100
ISSR 807	38	38	100
ISSR 835	39	39	100
ISSR 844	40	40	100
ISSR 850	50	50	100
Total	389	389	100
Mean	43.22	43.22	

Using the UPGMA cluster analysis method, a dendrogram was generated based on Jaccard's similarity coefficient differentiated all the 12 fern species. All species were grouped into two major clusters. These were further divided into sub-clusters (Fig. 2). The first significant cluster constituted eight species. The second cluster constituted four species. The major clusters I in the dendrogram (Fig. 2) is further divided into two sub-clusters. Sub-cluster I consist of two groups; Group I and Group II. Group I consist of *Tectaria coadunata* and *Bolbitis semicordata*. Group II includes *Odontosoria chinensis* and *Nephrolepis biserrate.* Subcluster II includes two groups: group I and group II. Group I include single specie *Adiantum philippense*. Group II is further divided into two subgroups: subgroup I include two species, *Christella parasitica, Selaginella delicatula,* and subgroup II consists of single species *Cyathea gigantea*. The major cluster II is subdivided into two sub-clusters. Subcluster I includes two groups, group I consists of two species, *Pteris confusa* and *Pteris biaurita,* group II includes a single specie *Athyrium falcatum,* Sub-cluster II two consists of single species *Angiopteris evecta*.

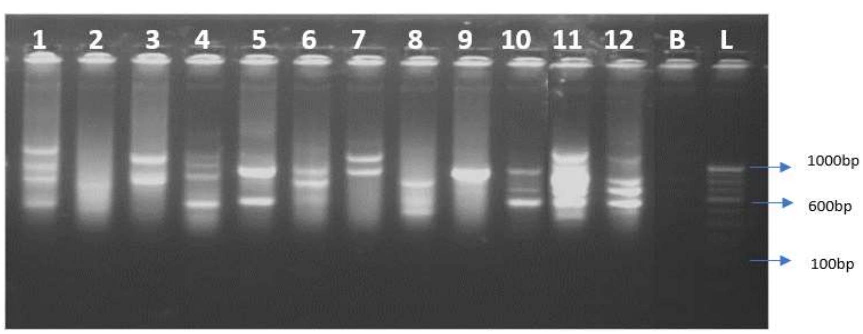

Fig. 1. Agarose gel electrophoresis separation of PCR products using ISSR 4 primer for 12 fern species from 1 to 12 1) *Bolbitis semicordata* 2) *Cyathea gigantea* 3) *Christella parasitica* 4) *Angiopteris evecta* 5) *Pteris confusa* 6) *Tectaria coadunata* 7) *Athyrium falcatum* 8) *Adiantum philippense* 9) *Odontosoria chinensis* 10) *Pteris biaurita* 11) *Selaginella delicatula* 12) *Nephrolepis biserrata,* L- ladder, B- blank

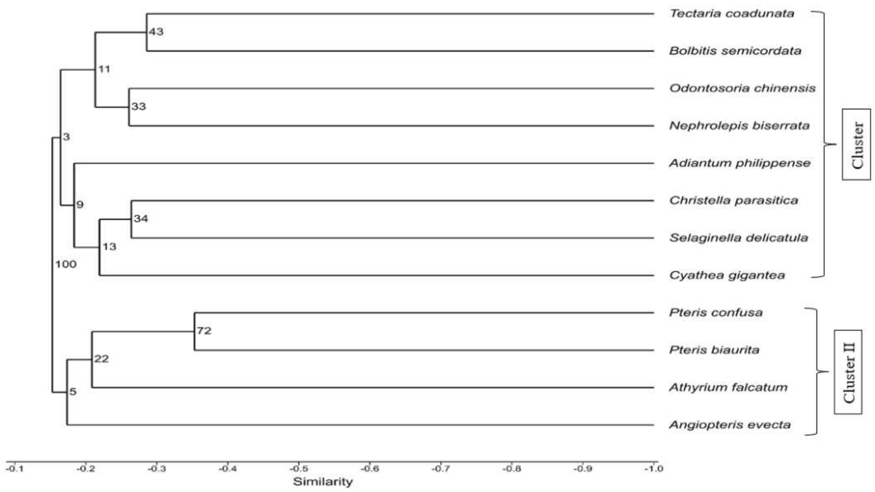

Fig. 2. Dendrogram of genetic relationship among 12 ferns based on ISSR marker

Table 4. Similarity coefficient matrix of 12 fern species by using ISSR marker

Botanical name	1	2	3	4	5	6	7	8	9	10	11	12
Bolbitis semicordata	1											
Cyathea gigantea	0.21	1										
Christella parasitica	0.25	0.23	1									
Angiopteris evecta	0.23	0.10	0.18	1								
Pteris confusa	0.20	0.20	0.21	0.19	1							
Tectaria coadunata	0.29	0.10	0.20	0.08	0.15	1						
Athyrium falcatum	0.16	0.10	0.15	0.20	0.19	0.21	1					
Adiantum philippense	0.12	0.20	0.18	0.12	0.16	0.04	0.09	1				
Odontosoria chinensis	0.23	0.20	0.17	0.12	0.22	0.24	0.23	0.16	1			
Pteris biaurita	0.13	0.07	0.15	0.13	0.35	0.09	0.23	0.14	0.16	1		
Selaginella delicatula	0.23	0.21	0.26	0.25	0.17	0.09	0.09	0.17	0.17	0.09	1	
Nephrolepis biserrata	0.22	0.18	0.16	0.16	0.28	0.16	0.10	0.19	0.26	0.12	0.16	1

The similarity matrix coefficient ranged from 4 to 35 per cent suggesting a higher to low genetic variation within fern species (Table 4). The highest genetic similarity of 35 per cent was observed between *Pteris confusa* and *Pteris biaurita*, followed by *Christella parasitica and Selaginella delicatula, Odontosoria chinensis, Nephrolepis biserrata* had 26 per cent genetic similarity. In comparison, *Tectaria coadunate* and *Adiantum philippense* showed the least genetic similarity index of 4 per cent. The variation in similarity among the species may be attributed to species diversity and their inherent genetic makeup. Chelliah et al. (2014) reported that genetic identity among five Adiantum incisum Forssk. individuals ranged from 0.46 to 0.75, while genetic distances varied from 0.29 to 0.78, as measured by the ISSR marker system. Similar findings were reported by Perrie et al. (2003) in New Zealand fern species, Lalitha et al. (2014a) in chrysanthemums, Lalitha et al. (2014b) in tuberose, and Vidyashree et al. (2019) in other fern species. The present molecular characterization study using ISSR markers would be an effective tool for genetic diversity analysis and its relationships within species and the conservation of this potential fern species.

CONCLUSION

The study assessed the genetic diversity of 12 fern species from the Agumbe Ghats of Karnataka using ISSR markers. Nine primers generated 389 scorable bands, with an average of 43.22 bands per primer, and revealed 100% polymorphism, confirming the high discriminatory potential of ISSRs. Among the primers, ISSR1 produced the maximum bands (66), while ISSR4 yielded the least (32). Cluster analysis based on UPGMA grouped all the species into two major clusters with several sub-clusters, clearly reflecting their genetic relatedness and divergence. The similarity coefficient ranged from 4 to 35%, indicating substantial variation among the species. The closest genetic relationship was observed between *Pteris confusa* and *Pteris biaurita* while the lowest similarity was between *Tectaria coadunata* and *Adiantum philippense*. These findings highlight considerable genetic variation, likely due to inherent genetic makeup and ecological adaptations. The study establishes ISSR markers as a reliable and robust tool for evaluating genetic diversity, species identification, and conservation planning in ferns of the Western Ghats biodiversity hotspot.

AUTHORS CONTRIBUTION

Suneetha C was responsible for the initiation and conceptualization of the study. Nagesha N supervised the molecular work and designed the ISSR primers. Yuvaraj S wrote and edited the manuscript. Nataraj A, Kavita Kandpal, and Vinayaka Muttu contributed to the collection, identification, herbarium preparation of ferns, and laboratory work. Sunitha Hegde carried out the identification and classification of the collected ferns and critically reviewed the manuscript.

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Response of Medicinal and Aromatic Plants under Prevailing Air Quality in Subhumid Mid Hills Zone of Himachal Pradesh, India

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Abstract: The increasing atmospheric pollution have become a global problem threatening both human and ecosystems health. Therefore, the air pollution tolerance index (APTI) and the anticipated performance index (API) are evaluated in order to determine both the degree of tolerance or sensitivity of medicinal and aromatic plants to pollutants in mid hill subhumid zone of Himachal Pradesh. The commonly occurring thirty medicinal and aromatic plants comprised of seventeen herbs, six shrubs, three climbers and four trees were selected for the study. All the herbs, shrubs and trees under study behaved similar, whereas significant variation was observed in climbers for the APTI. The highest APTI was in *Spilanthes acmella* (10.40), *Murraya koenigii* (7.735), *Tinospora cordifolia* (8.530) and *Terminalia arjuna* (5.770) with respect to herbs, shrubs, climbers and trees. The anticipated performance index of the selected medicinal and aromatic plants ranged from very poor to very good. Among the herbs, *Spilanthes acmella* with the highest tolerance index and *Cymbopogon citratus* with very good and *Acorus calamus* and *Valeriana jatamansi* with good performance score and among the shrubs, *Murraya koenigii* with the highest tolerance index and *Vitex negundo* with a moderate performance score and among the climbers, *Tinospora cordifolia* with the highest tolerance index and moderate performance score and among the trees, *Terminalia arjuna* with the highest tolerance index and moderate performance score can be suggested for plantations to manage the harmful effect of air pollution in the mid hill sub humid zone in particular and in Himachal Pradesh in general.

Keywords: Air pollution, APTI, API, Tolerance, Performance, Plants

Himachal Pradesh is a state in the northern part of India, situated in the Western Himalayas. According to Census Population Estimate report & UIAI for 2021 estimates, Himachal Pradesh has a population growth rate of 8.56% during 2011-2021. Its estimated population in 2021 is 74.81 Lakh an increase of around 6.36 lakhs from 2011. The population density rose to 133/km^2 from 109/km^2 in 2001. This shows a clearcut population pressure over fragile economy of the state this implies rapid urbanization processes, which is a key factor in the alteration and degradation of states' spatial structure, environment, and ecology (Kumara 2018), because of the growing demand for food, fuel, energy, water, and industrial raw materials, among others. Presence of forests and trees, however, has been recognized as beneficial since their presence has been correlated with atmospheric pollutant sinks and removal and climate change mitigation (Nowak et al., 2014, FAO 2018). Due to air quality problems, anatomical, morphological, physiological, and biochemical disturbances have been observed in different plants (Gulia et al., 2015, Uka et al., 2017). Particulate matter (PM) and other gases can cause negative effects and have a high potential of affecting forests and trees (Kumar 2012 and Rai 2016). These effects depend on PM concentrations and vegetation characteristics, due to the deposition of particles on leaves, results in reduction of incident light, stomatal closure, interference with gaseous exchange, thus altering the thermal balance of the plant (Singh and Verma 2007). The deposition of pollutant on leaves transform the nitrates and sulphates into acid and increased the level of trace and heavy metals such as lead (Grantz et al., 2003). So, it is fundamental to evaluate the tolerance and sensitivity of different plants to estimate atmospheric pollutants stress on vegetation by exposure to atmospheric pollutants (Lodenius 2013).

Among the different methodologies used to evaluate the tolerance and sensitivity of plant species to atmospheric pollutants is the air pollution tolerance index (Singh et al., 1991), which incorporates the biochemical parameters of a tree to determine the degree of impact to its organism (Kaur and Nagpal 2017). Additionally, the anticipated performance index supplements the APTI with biological and socioeconomic parameters of the species in order to determine its performance (Govindaraju et al., 2012). Both indexes have been widely used to characterize different plant species according to their tolerance and performance (Skrynetska et al., 2018, Qiu et al., 2019, Alotaibi et al., 2020, Javanmard et al., 2020, Banerjee et al., 2021 and Watson et al., 2021). In India these indexes have been successfully used to report plant that are key in the development and management of the ecosystem due to their tolerance and performance as well as their high capacity to filtrate and retain PM in the atmosphere (Achakzai et al., 2017 and Skrynetska et al., 2018). These mentioned reports have shown consensus regarding the classification of the trees using the APTI and API indexes, without a finding in conflicting reports between the studies consulted.

Because of the consistently rising levels of air pollutants (mostly PM) that have been observed, the mid-hill subhumid zone of Himachal Pradesh has drawn attention from locals, visitors, public administration, and other segments of society in recent years. In view of this context, the current study assessed the APTI and API indices of thirty species of medicinal and aromatic plants that are found in mid hill subhumid zone of Himachal Pradesh to determine the species' susceptibility or tolerance to air pollution.

MATERIAL AND METHODS

Study area: The present study was carried out at Dr. YS Parmar University of Horticulture and Forestry, Nauni in Solan district located in mid hill region of Himachal Pradesh. The Nauni area is at the geometrical centre (30˚52'0" N, 77˚11'30" E with 1275 m amsl. Himachal Pradesh lies in the Indian Himalayan Region (IHR), one of the richest reservoirs of biological diversity in the world. The state is endowed with a high diversity of flora around 3500 plant species including more than 1000 species of medicinal and aromatic plants (Chauhan 2003). Because of increasing productivity, innovation, and the attraction of human capital to establish various educational institutions, this region has witnessed better economic growth.

Its climate is characterized by a bimodal rainfall pattern: western disturbances during winter and south west monsoon during summer season. This rainfall pattern is associated with the dynamics of the Intertropical Convergence Zone (ITCZ), which determines two seasons (dry and humid). The area receives the mean annual rainfall of 1450 mm with average of 64 rainy days with a mean annual temperature of 18.4°C, fluctuates from 2.1°C to 40°C for winter season to summer season.

Selection of species and sample analysis: Preliminary identification of different medicinal and aromatic plant species present in the campus was done, out of which 17 herbs, 6 shrubs, 3 climbers and 4 trees were selected randomly for the study (Table 1).

Leaf samples from the selected plant species were collected in accordance with a standard procedure in order to analyze a number of anatomical and biochemical parameters. The leaf samples were brought in an ice box to the lab, where they were first cleaned with regular water, then with 0.1 N HCL, and lastly with distilled water.

Physiological and biological parameters determination: The physiological and biochemical parameters of the selected plants were determined by using triplicates to ensure quality control of the data.

Relative water content (R): Thirty sample of leaves were selected. After taking the fresh weight of leaves, the leaves were immersed in water over night, blotted dried and then weighted to obtain turgid weight. The leaves were dried overnight at 70 ºC in oven and reweighed for dry weight. Relative water content (RWC) of the samples was estimated by using the method proposed by Singh (1977).

$$\text{RWC (\%)} = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Where

FW = Fresh weight, DW = Dry weight, TW = Turgid weight

pH of leaf extract (P): The fresh leaves (5g) were homogenized in 10 ml deionized water and supernatant obtained after centrifugation and collected for determination of pH and leaf pH was measured (Prasad and Rao 1982). Determination of pH was done by using pH meter (Model – ESICO 1013) with buffer solution of pH 4 and 9 (Barrs and Weatherly 1962).

Total chlorophyll content (T): The leaf chlorophyll content was estimated by using Hiscox and Israeistam, (1979) method. The fresh leaves were chopped to fine pieces under subdued light and 100 mg of chopped leaf sample was placed in vial containing 7 ml of Dimethyl sulphoxide. Then vials were incubated at 65º C for 30 minutes. The extract was then transferred to graduate test tube and the final volume was made to 10 ml with Dimethyl sulphoxide. The optical density values of the above extract were recorded on spectrophotometer (Model- spectronic-20) at 645 and 663nm wavelength against dimethyl sulphoxide blank.

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{20.2A645 + 8.02A663}{a \times 100 \times w} \times v$$

Where, v = volume of extract made, a = length of light path in a cell (usually 1cm)

w = weight of the sample taken, A645 = absorbance at 645 nm

A663 = absorbance at 663 nm

Ascorbic acid content (A): The ascorbic acid content was estimated by AOAC (1980) method. Fresh leaves (10 g) were homogenized in metaphosphoric acid solution and volume was made up to 100 ml. This solution was titrated against indophenols dye. Appearance of rosy pink colour was taken as the end point. The amount of ascorbic acid in milligrams per grams was calculated.

$$\text{Amount of ascorbic acid (mg g}^{-1}\text{)} = \frac{\text{Dye factor} \times \text{Titre reading} \times \text{Volume made}}{\text{Weight of leaves taken} \times \text{Volume taken for estimation}}$$

Air pollution tolerance index (APTI) and anticipated performance index (API)

Air pollution tolerance index (APTI): The four biochemical parameters of the plant species; ascorbic acid, total chlorophyll content, pH of the leaf extract, and relative water content were used to determine the APTI score in order to

Table 1. Description of selected medicinal and aromatic plants

Plant species	Common name	Family	Habit	Leaf shape
Herbs				
Acorus calamus	Sweet flag	Acoraceae	Perennial herb	Sword-shaped
Bryophyllum pinnatum	Patharchatta	Crassulaceae	Perennial herb	Broad and flattened
Cymbopogon citratus	Lemon grass	Poaceae	Perennial herb	Linear in shape
Lavandula angustifolia	Lavender	Lamiaceae	Perennial herb	Erect
Matricaria chamomilla	Babuna	Asteraceae	Annual herb	Long and narrow
Melissa officinalis	Balm	Lamiaceae	Perennial herb	Ovate
Mentha piperita	Peppermint	Lamiaceae	Perennial herb	Ovate
Ocimum basilicum	Bhavri	Lamiaceae	Perennial herb	Oval
Ocimum sanctum	Holy basil	Lamiaceae	Perennial herb	Oval
Origanum vulgare	Oregano	Lamiaceae	Perennial herb	Oval or round
Pelargonium graveolens	Scented geranium	Geraniaceae	Annual herb	Lobed or toothed
Rosemarinus officinalis	Rosemary	Lamiaceae	Perennial herb	Needle-shaped
Silybum marianum	Milk thistle	Asteraceae	Annual herb	Oblong to lanceolate
Spilanthes acmella	Akarkara	Asteraceae	Perennial herb	Ovate
Tagetes minuta	Wild marigold	Asteraceae	Annual herb	Linear to narrowly lanceolate
Thymus vulgaris	Thyme	Lamiaceae	Perennial herb	Linear or diamond shaped
Valeriana jatamansi	Muskwala	Valerianaceae	Perennial herb	Cordate-ovate
Shrubs				
Asparagus racemosus	Satavari	Asparagaceae	Perennial shrub	Pine needle like leaves
Justicia adhatoda	Basuti	Acanthaceae	Perennial shrub	Lance-shaped
Murraya koenigii	Curry leaf	Rutaceae	Perennial shrub	Lance-shaped
Vitex negundo	Banna	Lamiaceae	Perennial shrub	Toothed or serrated
Withania somnifera	Ashwagandha	Solanaceae	Annual shrub	Ovate
Zanthoxylum armatum	Timar	Rutaceae	Perennial shrub	Lanceolate, obovate or elliptic
Climbers				
Celastrus paniculatus	Mal-kangni	Celastraceae	Perennial climber	Ovate or elliptic
Tinospora cordifolia	Giloy	Menispermaceae	Perennial climber	Heart shaped
Tylophora indica	Damabuti	Apocynaceae	Perennial, climber	Obviate-oblong to elliptic-oblong
Trees				
Phyllanthus emblica	Amla	Phyllanthaceae	Deciduous tree	Oblong
Terminalia arjuna	Arjun	Combretaceae	Deciduous tree	Oblong, conical
Terminalia bellirica	Bahera	Combretaceae	Deciduous tree	Simple, alternate, clustered at the tip
Terminalia chebula	Harad	Combretaceae	Deciduous tree	Ovate or elliptic obovate

classify the species as either tolerant (when the APTI score is high) or sensitive (when the APTI score is low). These parameters together provide a basis for classifying species as tolerant or sensitive. These biochemical parameters were estimated by using experimental design CRD with 3, 4, 5 and 4 replications for herbs, shrubs, climbers and trees.

The deterioration of chlorophyll in leaves was correlated with pH values because of stress brought on by air pollution. The ascorbic acid content plays a crucial function in protecting chlorophyll against hydrogen peroxide (H_2O_2) damage, which starts at pH>3 when superoxide dismutase (SOD) dismutates superoxide radicals (H_2O_2). Ascorbic acid levels in plant organisms promote the integration of chlorophyll (a and b), which helps in the development of resistance to air pollution The relative water content in the equation taken into consideration the ability of cells to remain intact when exposed to air contaminants. The whole term of the equation, comprising the four biochemical parameters, is divided into 10 in order to obtain a manageable value (Singh et al., 1991; Uka et al., 2017) as:

$$APTI = \frac{A\,(T+P)+R}{10}$$

Where,

A = Ascorbic acid content (mg mg^{-1})

T = Total chlorophyll content (mg mg^{-1})

R = Relative water content (%)

P = pH of leaf

Anticipated performance index (API): As per the process described by Mondal et al. (2011), the resulting APTI values were combined with biological and socioeconomic parameters such as plant height, canopy structure, plant size, texture, hardness, and economic value (Anonymous 2008). Different grades (+ or -) were assigned to the plants and plants that scored differently according to their grades were categorized (Table 2). Based on the plant species ability to thrive under stressful conditions, such as air pollution, the API was determined (Table 3).

RESULTS AND DISCUSSION

Biochemical Characteristics

Ascorbic acid (A): There was a significant variation in ascorbic acid content (mg g^{-1}) among 30 individual plant species under analysis ranged from 0.220 to 2.040 mg g^{-1} (Table 4).

Among the 17 herbs, significantly higher content of ascorbic acid (2.040 mg g^{-1}) was in *Valeriana jatamansi* followed by *Tagetes minuta* which was statistically at par with *Spilanthes acmella* and *Acorus calamus* and minimum (0.220 mg g^{-1}) was in *Lavandula angustifolia*, which was statistically at par with Ocimum basilicum. In shrubs ranged between 0.60-1.34 with maximum content in *Vitex negundo* preceded by *Murraya koenigii* whereas minimum was in *Asparagus racemosus*, which was statistically at par with *Withania somnifera* Similarly in climbers the maximum amount of ascorbic acid was observed in *Tylophora indica* (1.23 mg g^{-1}) followed by *Celastrus paniculatus* and minimum in *Tinospora cordifolia* (0.61 mg g^{-1}). The descending order of four selected trees in term of ascorbic acid was *Terminalia chebula* > *Phyllanthus emblica* >*Terminalia bellirica* >*Terminalia arjuna*, however, it was statistically at par in *Terminalia arjuna* and *Terminalia bellirica* (Table 4). There

Table 2. Plant classification based on APTI, biological parameters and socio-economic parameters

Grading character	Pattern of assessment	Grade allotted *
a) Tolerance		
APTI	3.1-4.5	+
	4.6-6.0	++
	6.1-7.5	+++
	7.6-9.0	++++
	9.1-10.5	+++++
b) Biological and socioeconomic		
Plant habit (Ph)	Small	-
	Medium	+
	Large	++
Canopy structure (Cs)	Sparse/irregular/globular	-
	Spreading crown/open/semi-dense	+
	Spreading dense	++
Type of plant (Tp)	Deciduous	-
	Evergreen	+
c) Laminar structure		
Size (Ls)	Small	-
	Medium	+
	Large	++
Texture (Tx)	Smooth	-
	Coriaceous	+
Hardiness (H)	Delineate	-
	Hardy	+
Economic value (Ev)	Less than three uses	-
	Three or four uses	+
	Five or more uses	++

* Maximum score that can be attained is 16, which corresponds to 100%

Table 3. Anticipated performance index (API) of plant species

Grade	Score (%)	Assessment category
0	Up to 30	Not recommended
1	31–40	Very poor
2	41–50	Poor
3	51–60	Moderate
4	61–70	Good
5	71–80	Very good
6	81–90	Excellent
7	91-100	Best

was a significant variation of ascorbic acid content in different plant species, which might be due to their different genetic makeup. Being an antioxidant higher the ascorbic acid content in plant species more will be its pollution tolerance ability (Tambussi et al., 2000) and vice-versa which was observed in *Valeriana jatamansi* and *Lavandula angustifolia,* respectively.

Total chlorophyll content (T): Plant species that are

Table 4. Air pollution tolerance index (APTI)

Plant species	A (mg g^{-1})	T (mg g^{-1})	P	R (%)	APTI
Herbs					
Acorus calamus (AC)	1.61	0.79	7.51	73.39	8.67
Bryophyllum pinnatum (BP)	0.31	0.47	4.67	80.15	8.17
Cymbopogon citrates (CC)	0.67	0.35	6.77	83.12	8.79
Lavandula angustifolia (LA)	0.22	0.47	7.48	66.43	6.82
Matricaria chamomilla (MC)	0.31	0.65	7.03	50.75	5.31
Melissa officinalis (MO)	0.96	1.08	7.42	45.88	5.41
Mentha piperita (MP)	1.22	0.93	7.19	39.02	4.89
Ocimum basilicum (OB)	0.29	0.71	5.03	78.13	7.98
Ocimum sanctum (OS)	0.47	0.52	7.63	59.37	6.32
Origanum vulgare (OV)	0.38	0.89	7.78	77.86	8.12
Pelargonium graveolens (PG)	1.20	0.47	4.45	78.96	8.49
Rosemarinus officinalis (RO)	0.63	0.59	7.47	85.21	9.02
Silybum marianum (SM)	0.38	0.34	4.31	65.57	6.73
Spilanthes acmella (SA)	1.64	1.15	7.32	90.12	10.41
Tagetes minuta (TM)	1.64	0.51	6.61	42.16	5.38
Thymus vulgaris (TV)	0.98	0.43	7.59	91.22	9.91
Valeriana jatamansi (VJ)	2.04	0.86	6.97	68.65	8.46
Mean	0.88	0.66	6.66	69.18	7.58
Shrubs					
Asparagus racemosus (AR)	0.60	0.19	8.07	59.36	6.43
Justicia adhatoda (JA)	0.81	0.75	8.61	57.41	6.49
Murraya koenigii (MK)	1.20	0.63	5.63	69.84	7.73
Vitex negundo (VN)	1.34	0.75	6.97	57.35	6.77
Withania somnifera (WS)	0.64	0.62	7.42	57.16	6.23
Zanthoxylum armatum (ZA)	1.21	0.60	7.23	47.69	5.71
Mean	0.97	0.59	7.32	58.14	6.56
Climbers					
Celastrus paniculatus (CP)	1.22	1.17	7.12	43.86	5.45
Tinospora cordifolia (TC)	0.61	1.26	7.64	79.84	8.53
Tylophora indica (TI)	1.23	0.21	7.42	57.94	6.74
Mean	1.02	0.88	7.39	60.55	6.91
Trees					
Phyllanthus emblica (PE)	0.77	0.31	4.36	36.41	3.99
Terminalia arjuna (TA)	0.41	1.77	7.30	54.07	5.77
Terminalia bellirica (TB)	0.44	0.42	5.64	41.72	4.44
Terminalia chebula (TC)	1.22	0.63	5.74	36.38	4.42
Mean	0.71	0.78	5.76	42.15	4.66

APTI ≤ 11, sensitive species; 11–16, intermediate species; ≥ 16, tolerant species (Singh and Verma 2007) susceptible to air pollution may exhibit reduced photosynthetic activity, which causes their leaves to lose chlorophyll and carotenoids. (Rai 2016, Uka et al., 2017). There was a significant variation in the total chlorophyll content of selected herbs, shrubs, climbers and trees individually. The T values for the 30 plant species ranged

Table 5. Anticipated performance index (API) score of 30 plant species

Plant species	APTI	Ph	Cs	Tp	Ls	Tx	H	Ev	Total	Score (%)	API Category
Herbs											
Acorus calamus	++++	-	-	+	++	-	+	++	10	62.5	Good
Bryophyllum pinnatum	++++	-	-	+	+	-	+	-	7	43.7	Poor
Cymbopogon citratus	++++	+	-	+	++	+	+	++	12	75.0	Very good
Lavandula angustifolia	+++	-	-	+	-	+	+	+	7	43.7	Poor
Matricaria chamomilla	++	-	-	-	-	+	+	++	6	37.5	Very poor
Melissa officinalis	++	-	-	+	-	+	+	+	6	37.5	Very poor
Mentha piperita	++	-	-	+	-	+	+	+	6	37.5	Very poor
Ocimum basilicum	++++	-	-	+	-	-	+	++	8	50.0	Poor
Ocimum sanctum	+++	-	-	+	-	-	-	++	6	37.5	Very poor
Origanum vulgare	++++	-	-	+	-	+	+	-	7	43.7	Poor
Pelargonium graveolens	++++	-	-	-	-	+	+	-	6	37.5	Very poor
Rosemarinus officinalis	++++	-	-	+	-	-	+	+	7	43.7	Poor
Silybum marianum	+++	-	-	-	+	+	+	-	6	37.5	Very poor
Spilanthes acmella	+++++	-	-	+	-	+	+	-	8	50.0	Poor
Tagetes minuta	++	-	-	-	-	+	+	+	5	31.2	Very poor
Thymus vulgaris	+++++	-	-	+	-	+	-	++	9	56.2	Moderate
Valeriana jatamansi	++++	-	-	+	+	+	+	++	10	62.5	Good
Mean	7.4	46.3	Poor								
Shrubs											
Asparagus racemosus	+++	+	-	+	-	+	+	-	7	43.7	Poor
Justicia adhatoda	+++	+	-	+	+	-	+	-	7	43.7	Poor
Murraya koenigii	++++	-	+	+	-	-	+	+	8	50.0	Poor
Vitex negundo	+++	++	++	+	-	+	-	-	9	56.2	Moderate
Withania somnifera	+++	+	+	-	+	+	+	-	8	50.0	Poor
Zanthoxylum armatum	++	+	-	+	-	-	+	-	5	31.2	Very poor
Asparagus racemosus	+++	+	-	+	-	+	+	-	7	43.7	Poor
Mean	7.3	45.5	Poor								
Climbers											
Celastrus paniculatus	++	++	-	+	+	+	+	-	8	50.0	Poor
Tinospora cordifolia	++++	++	-	+	+	-	+	-	9	56.2	Moderate
Tylophora indica	+++	++	-	+	+	-	+	-	8	50.0	Poor
Mean	8.3	52.1	Moderate								
Trees											
Phyllanthus emblica	+	++	++	-	-	+	+	-	7	43.7	Poor
Terminalia arjuna	++	++	++	-	+	+	+	-	9	56.2	Moderate
Terminalia bellirica	+	++	++	-	+	+	+	-	8	50.0	Poor
Terminalia chebula	+	++	++	-	+	+	+	-	8	50.0	Poor
Mean	8.1	50.1	Moderate								

between 0.19 to 1.77 mg g (Table 4).. Chlorophyll content in leaves of different herbs ranged from 0.34 to 1.15 mg g^{-1}, in shrubs from 0.19 to 0.75 mg g, in climbers from 0.21 to 1.26 mg g and in trees from 0.31 to 1.77 mg g (Table 4). Begum and Harikrishna (2010) reported that chlorophyll content varied from species to species. Tripathi and Gautam (2007) explained that a decrease in the total chlorophyll content in plant leaves subjected to air pollution stress may be the result of air pollutants like SPM attacking the chloroplast.

Leaf extracts pH (P): There was a significant variation in leaf extract pH ranged between 4.31 and 8.61 (Table 4). For herbs 4.31 to 7.78, shrubs 5.63 to 8.61, climbers 7.12 to 7.64 and trees 4.36 to 5.76. The highest (most basic) value of was observed in climbers and the lowest (most acidic) in trees (see the order: climbers > shrubs > herbs > trees). The pH of approximately 7 is essential as it creates optimal conditions for photosynthesis in tolerant tree species, while less tolerant plants usually have lower stomatal sensitivity and photosynthetic activity (Rai 2016).

Relative water content (R): *R* values for the 30 plants range between 36.38 and 91.22 % (Table 4). In herbs ranged between 39.02 and 91.22%, shrubs 47.69 and 69.84 %, climbers 43.86 and 79.84 % and in trees 36.38 and 54.07 %. The average relative water content was observed highest in shrub (69.18 %), followed by climbers (60.55%), shrubs (58.14 %) and Trees (42.15 %). The non-significant variation in *R* value was observed in herbs, shrubs, trees but, significant in climbers (Table 4). Nwadinigwe (2014) noted that the relative water content varied with different plant species. The ability of a plant species to withstand pollution may be the reason for its maximum relative water content. The relative water content was characterized by means of turgor, growth, stomatal conductance, transpiration, photosynthesis, and respiration; hence, higher the *R* more will be the tolerance to the stressful environmental conditions (Rai 2016).

APTI: The non-significant variation in APTI was recorded which varied from 3.99 to 10.4. As per the criteria of Singh and Verma (2007) out of 30 plants 26.8 % were observed as very poor, 50% as poor, 13.3% as moderate 6.6% as good and 3.3% as very good. No plant was observed under excellent and best category. The mean APTI value of observed highest for herbs (7.58) followed by climbers, shrubs and lowest for trees (4.66). Among the herbs highest APTI was in *Spilanthes acmella* (10.41) followed by *Thymus vulgaris* and lowest in *Mentha piperita* (4.892), in climbers highest in *Tinospora cordifolia* (8.53) and lowest in *Celastrus paniculatus* (5.45), in shrubs the highest was for *Murraya koenigii* (7.74) and lowest for *Zanthoxylum armatum* (5.71) similarly for tree it was highest for *Terminalia* arjuna (5.770) and lowest (3.99) was for *Phyllanthus emblica* (Table 4). Gholami et al. (2016) investigated that air pollution tolerance vary from species to species depending on the plant's ability to tolerate the impacts of air pollution.

API: Among the selected herbs *Cymbopogon citratus* stand under very good category, *Acorus calamus* and *Valeriana jatamansi* under good category and *Thymus vulgaris* under moderate category and remaining under poor and very poor categories (Table 5). Lowest API of *Bryophyllum pinnatum*, *Lavandula angustifolia*, Ocimum *basilicum*, *Origanum vulgare*, *Rosemarinus officinalis*, *Spilanthes acmella* was due to low APTI values, sparse canopy structure and low economic value.

Among the selected shrubs high API of *Vitex negundo* was attributed to large plant habit, spreading dense canopy structure along with better laminar characteristics. Low API of Asparagus *racemosus, Justicia adhatoda, Murraya koenigii* and *Withania somnifera* was probably due to their sparse canopy structure, laminar characteristics as well as low economic value. The climbers *Tinospora cordifolia* stand in moderate category, whereas *Celastrus paniculatus* and *Tylophora indica* fell under poor category respectively. The tree *Terminalia arjuna* fell under moderate category, whereas *Phyllanthus emblica*, *Terminalia bellirica* and *Terminalia chebula* fell under poor category, respectively (Table 5). Bahadoran et al. (2019) reported that a combination APTI and API indices could be quite useful for assessing plant responses to a variety of pollutants for green belt purposes. In addition, the plant's socio-economic relevance played a significant role in determining its API (Table 5).

CONCLUSION

The study indicated that in the prevailing air conditions *Ocimum basilicum, Spilanthes acmella, Murraya koenigii, Withania somnifera, Celastrus paniculatus, Tylophora indica, Terminalia bellirica* and *Terminalia chebula* should be grown in mid hills zone of Himachal Pradesh which possessed good performance index. These species can also be included when the objective is to use them as bioindicators of air quality, due to their high susceptibility to atmospheric pollutants and poor performance in urban environments.

AUTHOR'S CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shilpa Sharma, Mohinder Brahmi, Satish Bhardwaj, and Bhupender Dutt. The first draft of the manuscript was written by Shilpa Sharma and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Impact of River Erosion on Agricultural Practices in Bangladesh: Review of Secondary Data

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Abstract: River erosion is a significant environmental challenge in Bangladesh, leading to substantial land loss, reduced crop yields, and socio-economic instability, particularly for populations who depend on agriculture. This study analyzes the multifaceted impacts of river erosion on agricultural productivity, focusing on crop yield variability, land use changes, and socio-economic consequences. Data from governmental and non-governmental sources were analyzed using R programming to identify patterns, causes, and effects of river erosion on agricultural land and crop production. The findings show that erosion-induced land loss threatens food security by reducing arable land, particularly for crops like rice and jute, and displacing communities, which exacerbates poverty and migration. The study also highlights the role of both natural processes, including seasonal monsoon variations, and anthropogenic activities, such as unplanned embankment construction, in worsening river erosion. Mitigation strategies, including vegetative stabilization and crop diversification, have shown promise in reducing erosion impacts, with vegetative stabilization identified as the most effective for long-term resilience. The study highlights the need for integrated river management strategies and sustainable agricultural practices. It suggests the adoption of policy frameworks that combine effective erosion control measures with community-based resilience approaches to safeguard agricultural productivity and livelihoods in erosion-prone areas.

Keywords: River, Erosion, Agriculture, Socio-economic, Bangladesh

Bangladesh riverbank erosion presents a multifaceted challenge, significantly impacting agricultural land, displacing communities and disrupting agricultural activities (Billah et al., 2023). The dynamic nature of the Bengal Delta, characterized by shifting river channels and the formation and erosion of char lands, exacerbates these issues (Haque and Jakariya 2023). This instability leads to the displacement of settlers and a decline in their socioeconomic status, highlighting the need for adaptive management and planning approaches (Haque and Jakariya 2023, Islam et al., 2023). The geographical location of Bangladesh, bordered by the Ganges, Brahmaputra and Meghna River systems, indeed makes it highly susceptible to changing river courses and increased sedimentation, leading to significant land loss (Paszkowski et al., 2021). This susceptibility is exacerbated by the dynamic nature of these river systems, which are prone to frequent erosion and accretion processes (Saleem et al., 2019). The impacts of these changes are profound, affecting land use, agriculture, and the livelihoods of millions of people in the region (Smith and Dawson 2020). The following sections delve into the specific aspects of this issue.

The lower Meghna River has experienced significant erosion, with an average land loss rate of 124.18 ha/year, affecting 43.88% of households as severely vulnerable (Chowdhury et al., 2021). The Jamuna River has also shown substantial erosion, with 3356 ha lost between 1972 and 2013, leading to settlement displacement and increased poverty (Gazi et al., 2020, Alam and Ahamed 2022, Haque et al. 2022). In the Malda district of West Bengal, the Ganga River's erosion has resulted in an average annual land loss of 4.5 km², affecting nearly a million people (Das and Samanta 2022, Haque et al., 2022). Land use and land cover (LULC) changes in Bangladesh have shown a significant decrease in agricultural land by 11.68% due to inundation risks from river systems (Dey et al., 2022). Vulnerability maps indicate that more than 50% of areas in Khulna and Dhaka divisions are highly vulnerable to agricultural land use changes due to potential inundation (Dey et al., 2022). Riverbank erosion in the Meghna River has led to severe impacts on livelihoods, with 91% of affected households losing their homes and 87% borrowing money to cope with the disaster (Gazi et al., 2020, Halima and Maria 2021). The socio-economic scenarios predict increased flood potential and reduced water availability, which could exacerbate the impacts on agriculture and livelihoods (Khan et al., 2022, Gupta and Chembolu 2024). While the geographical location of Bangladesh makes it vulnerable to these natural processes, it is important to consider the role of human activities and climate change in exacerbating these issues (Bandh et al., 2021).

The construction of upstream water transfers and socio-economic changes could further impact water availability and quality, affecting the delta region's ecosystems and livelihoods (Khan et al., 2022). Addressing these challenges requires comprehensive riverbank erosion management policies and sustainable land use planning to mitigate the adverse effects on the population and environment. The goal of this study is to analyze secondary data to quantify the impact of river erosion on agricultural land and crop production in Bangladesh, with the aim of providing actionable insights for policy development and erosion mitigation. This study employs a secondary data analysis approach to explore the complex relationships between river erosion, land-use changes, crop yields and socio-economic consequences, thereby offering a comprehensive assessment of the impacts on agriculture in Bangladesh.

MATERIAL AND METHODS

The study in question utilizes a secondary data analysis approach, integrating datasets from various sources like the Bangladesh Water Development Board (BWDB) and Bangladesh Bureau of Statistics (BBS), along with academic and NGOs reports, provides a robust framework for analyzing these impacts to understand the socio-economic impacts of river erosion in Bangladesh Figure 1. The data were analyzed using descriptive statistics to identify patterns in river erosion, land loss, and agricultural yield variability. Correlation analyses were performed to examine the relationship between erosion intensity and agricultural productivity. R's data manipulation and visualization libraries (e.g., ggplot2, dplyr) were used to create visual representations of erosion trends, land-use changes and socio-economic impacts, facilitating a clearer understanding of the data.

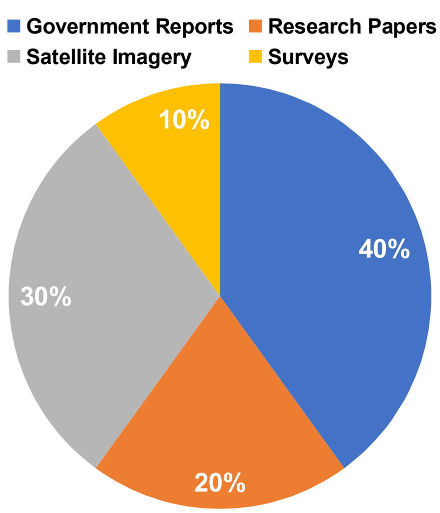


Fig. 1. Distribution of data sources used in the study

Study area: Bangladesh, situated in South Asia, spans a geographic region of 20°34' to 26°38' North Latitude and 88°01' to 92°41' East Longitude (Fig. 2). It is a delta plain created by the Ganges, Brahmaputra, and Meghna rivers, bordered by the Bay of Bengal. The nation shares its borders with India to the west, north, and northeast, and with Myanmar to the southeast (Uddin et al. 2024). This area covers regions most affected by river erosion, particularly in the Ganges-Brahmaputra Delta, where agriculture is integral to the livelihoods of rural communities (Khan and Rahman 2022). The research aims to assess the extent of damage caused by erosion and its ripple effects on agriculture, crop yield, and socio-economic conditions of the affected populations.

RESULTS AND DISCUSSION

River erosion in Bangladesh: Context and causes: Bangladesh's vulnerability to river erosion is significantly influenced by both natural and anthropogenic factors. The country's geographical position at the confluence of the Ganges, Brahmaputra, and Meghna rivers makes it susceptible to natural processes like sediment deposition, river migration, and flooding (Chowdhury et al., 2021, Haque et al., 2022). However, human activities such as

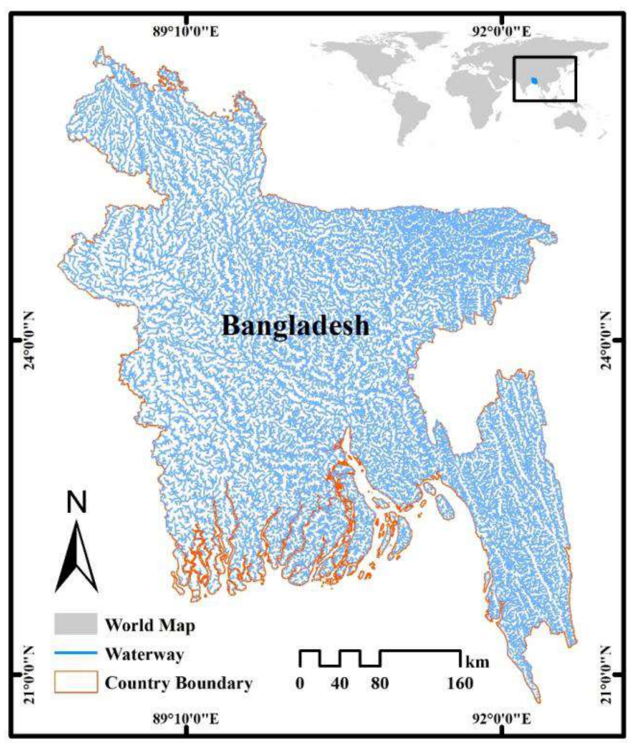


Fig. 2. Map of Bangladesh showing the country's waterways in blue and the national boundary in orange. The inset map provides a global context for the location of Bangladesh

deforestation, unplanned embankment construction, and unsustainable river management practices have exacerbated these natural processes, leading to increased erosion and its associated impacts (Grabowski et al. 2022).

Natural and anthropogenic factors: Bangladesh's location in the deltaic region of the Ganges-Brahmaputra-Meghna River system makes it prone to natural erosion processes due to sediment transport and deposition dynamics (Anzum et al., 2023). Natural drivers of river erosion include seasonal variations in water flow, sediment transport, and hydrological patterns. The annual monsoon rains increase river flow, which in turn accelerates erosion, particularly during flood events. The natural fluvial processes, including riverbank shifting and sediment flux, contribute to the erosion and accretion patterns observed in the region (Anzum et al., 2023). Additionally, the erosion-prone nature of the riverbanks, due to a high sediment load and fluctuating river currents, contributes to the shifting of river channels, further destabilizing the landscape.

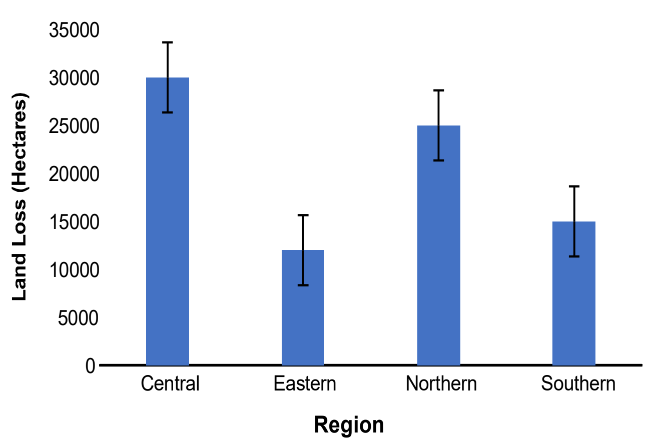
In contrast, human activities have compounded the natural causes of river erosion. Unplanned construction of embankments, as well as illegal sand mining, have disrupted the natural sediment flow and weakened riverbanks (Dey et al., 2022, Haque et al., 2022). Furthermore, the rapid urbanization and expansion of infrastructure in floodplain areas have led to the destruction of natural buffer zones, such as wetlands, which play a crucial role in stabilizing riverbanks (Ali and Ferdos, 2024). Construction activities such as embankments and bridges have been identified as significant contributors to river ecosystem changes. For instance, cross embankments and bridge construction over the Bhairab River have been major factors in altering the river's ecosystem (Islam et al., 2023). Industrial growth has led to increased pollution, which affects river water quality and exacerbates erosion by disrupting the natural aquatic ecosystem (Freihardt and Frey 2023). High-speed water vehicles generate waves that intensify riverbank erosion, as observed in the Kirtankhola River.

While Bangladesh has made strides in disaster risk reduction and anticipatory action, challenges remain in securing livelihoods and properties from erosion-induced damage (Naz et al. 2024). The country's proactive disaster management approach has reduced human casualties, but adaptation failures and the need for improved policies persist (Faisal and Hayakawa 2022). Addressing these issues requires a comprehensive understanding of both natural and human-induced factors contributing to river erosion.

Extent of River erosion and land loss: The Central region experiences the highest erosion, with 30,000 hectares of land lost annually, followed by the Northern region with 25,000 hectares (Fig. 3). The Southern region faces a moderate loss of 15,000 hectares per year, while the Eastern Region has the lowest land loss, at 12,000 hectares annually (Gazi et al., 2020, Chowdhury et al., 2021, Alam and Ahamed 2022, Dey et al., 2022, Haque et al., 2022, Mamun et al., 2022). These figures highlight the significant regional disparities in the impact of river erosion, with the Central region being the most severely affected. The variation in land loss across regions likely reflects differences in river dynamics, sedimentation patterns and geographical factors, which contribute to the extent of erosion.

River erosion significantly impacts agricultural land, particularly in regions like the Brahmaputra and Jamuna rivers, where frequent river course changes and high sediment loads exacerbate the problem. This erosion leads to substantial land loss, affecting the cultivation of essential crops such as rice, jute, and vegetables, thereby threatening food security. The loss of approximately 87,000 hectares of agricultural land annually due to river erosion highlights the severity of this issue (Dey et al., 2022, Mamun et al., 2022). The following sections delve into the extent of river erosion and its implications on land loss and food production. Riverbank erosion is a prevalent issue along major rivers like the Jamuna in Bangladesh, where satellite imagery has been used to track erosion events, revealing significant land loss post-monsoon seasons (Dahal et al., 2023). In the Brahmaputra Valley of Assam, India, riverbank erosion has been linked to socioeconomic challenges, including poverty and migration, due to the loss of cropland and other resources (Mandal et al., 2023).

Food security and poverty: There is a consistent downward trend in rice yields over a five-year period from 2015 to 2019 and in 2015, yield was 3,000 kilograms per hectare, but by 2019, decreased to 2,800 kilograms per



Source: Gazi et al., 2020; Chowdhury et al., 2021; Alam & Ahamed 2022; Dey et al., 2022; Haque et al., 2022; Mamun et al., 2022

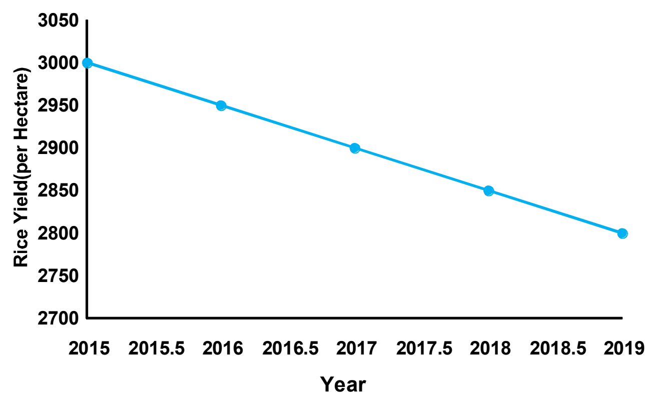
Fig. 3. Annual land loss due to river erosion

hectare (Fig. 4). Each subsequent year saw a slight decline in yield, with reductions of 50 to 100 kilograms per hectare annually (de Pee and Pérez-Escamilla 2022, Upasana 2023). This decline can be attributed to the negative impacts of river erosion, which leads to the loss of fertile agricultural land and soil degradation, resulting in lower crop productivity. The data suggests that the cumulative effects of erosion over time have contributed to a gradual but significant reduction in rice production, with implications for food security and agricultural sustainability in the affected regions. The loss of agricultural land significantly threatens food security by reducing domestic production of staple crops, leading to higher food prices and exacerbating poverty, particularly among low-income households. This situation is further aggravated by factors such as climate change, land degradation, and socio-economic inequalities, which collectively undermine food availability and access.

The decline in crop yields, especially in erosion-prone areas, highlights the urgent need for sustainable agricultural practices and effective land management strategies. The reduction in agricultural land directly affects food availability, as seen in the decline of rice production by 5% between 2015 and 2019 in erosion-prone areas (de Pee and Pérez-Escamilla 2022). Land degradation, including soil nutrient depletion, further diminishes crop yields and farming incomes, posing a significant threat to food security (de Pee and Pérez-Escamilla, 2022). Poverty is intricately linked to food insecurity, as economic inequality limits access to resources necessary for adequate nutrition (Ribotta 2023). The high incidence of poverty and unemployment exacerbates food insecurity, as seen in Nigeria, where unemployment significantly worsens food insecurity (Upasana 2023). While the loss of agricultural land poses a significant threat to food security, it is essential to consider the broader socio-economic and environmental context.

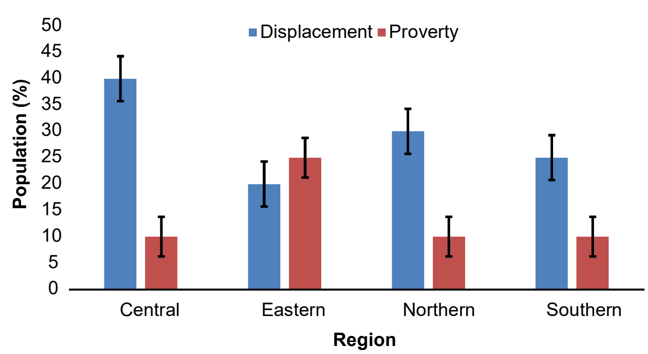
Changes in land use patterns: River erosion in Bangladesh significantly impacts agricultural productivity, leading to the loss of fertile land, reduced crop yields, and forced displacement of farmers. This phenomenon is exacerbated by the country's dense population and socio-economic pressures, which drive changes in land use patterns (Dey et al., 2022). The erosion-induced loss of soil particularly affects crops like rice and jute, which are heavily dependent on fertile soil. The consequences extend beyond agriculture, affecting food security and socio-economic stability. River erosion has led to a significant decrease in agricultural land, with a reduction of 11.68% in agricultural land use across various divisions in Bangladesh (Alam and Ahamed 2022). In the southwestern coastal areas, land use changes due to erosion and other climatic factors have resulted in decreased crop production and increased salinity, further reducing agricultural productivity (Islam et al., 2023). The shift from traditional crops like rice and jute to less soil-dependent crops is a direct consequence of soil erosion and land degradation (Hoque et al., 2022). The expansion of aquaculture, often replacing agricultural lands, has altered the landscape, impacting traditional farming practices and crop yields (Hasan et al., 2023).

Socio-economic impacts: Both the percentage of the population living in poverty and the levels of displacement caused by erosion (Fig. 5). In the Central region has the highest percentage of the population living in poverty, at 50%, followed by the Eastern region and Southern region, Northern region at 45, 40 and 35%. Central region also has the highest displacement rate, with 40% of its population displaced due to river erosion Followed by the Northern region with Southern region and Eastern, at 30, 25 and 20%, respectively. The study demonstrate that river erosion not only exacerbates poverty levels in affected areas but also forces significant portions of the population to migrate, further straining local economies and infrastructure. The socio-economic impact of erosion is particularly severe in the Central and Northern regions, where both poverty and displacement rates are highest.



Source: de Pee & Pérez-Escamilla 2022; Upasana 2023

Fig. 4. Decline in rice yield due to river erosion



Source: Khan et al., 2022; Kaiser 2023; Upasana 2023

Fig. 5. Socio-economic impact of river erosion

The socio-economic impacts of river erosion are profound, affecting both individuals and communities. River erosion leads to the loss of agricultural land, which is a primary source of income for many farmers, resulting in financial instability and poverty (Kaiser 2023). Displacement due to erosion forces communities to migrate, often to urban areas, exacerbating urban overcrowding and straining infrastructure (Tanjeela and Billah 2022, Ali and Ferdos 2024). This displacement disrupts social structures and increases dependency on government aid. Riverbank erosion results in the loss of cropland and property, severely affecting the livelihoods of those dependent on agriculture (Kaiser 2023). In Bangladesh, erosion-induced displacement leads to livelihood uncertainty, contributing to socio-economic instability and poverty. The severity of poverty among riverbank inhabitants is directly related to the degree of erosion (Rahman and Gain 2019, Kaiser 2023).

Correlation between erosion and agricultural productivity: Soil erosion significantly impacts agricultural productivity by degrading soil quality, which is crucial for crop growth (Hossain et al., 2020). This degradation leads to reduced soil fertility, loss of nutrients and diminished water retention capacity, ultimately affecting crop yields and economic stability for farming communities (Chowdhury et al. 2022, Hossain et al., 2020). In Bangladesh, where agriculture is a primary livelihood, these effects are particularly pronounced. Soil erosion reduces soil organic carbon, water holding capacity and nutrient availability, which are vital for crop productivity, severely eroded soils can see a decline in soil organic carbon by up to 81.4% and water holding capacity by 31% (Mandal et al., 2023, Musa et al., 2024). The loss of topsoil and nutrients due to erosion leads to decreased agricultural productivity, as seen in the Rangun watershed of Nepal, where productivity decreased by 0.238 t ha^{-1} yr^{-1} (Bhandari et al., 2021).

Mitigation strategies for river erosion: The review of secondary data highlights the significant negative impact of river erosion on agriculture in Bangladesh including land loss, reduced crop yields and, forced displacement are directly tied to the socio-economic vulnerability of farming communities. To mitigate these impacts, various strategies have been assessed for their effectiveness in reducing land loss due to river erosion. The strategies assessed include embankments, vegetative stabilization, crop diversification, and flood control measures, with each strategy given an impact score based on its projected effectiveness (Fig. 6). Among these, vegetative stabilization is projected to have the highest impact, with an impact score of 75, suggesting it is the most effective strategy to reduce erosion and stabilize riverbanks followed by crop diversification (score of 70).

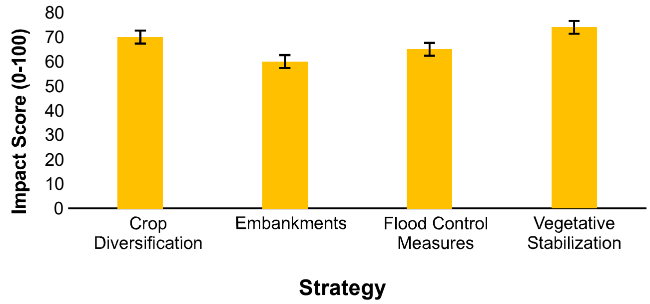


Fig. 6. Projected impact of mitigation strategies on erosion

Flood control measures score 65, highlighting their moderate effectiveness in mitigating erosion, while embankments score the lowest at 60. These findings highlight the significance of integrating multiple strategies for sustainable erosion management and agricultural resilience (Mondal and Patel 2018, Gazi et al., 2020). However, the combined effects of river erosion and its socio-economic repercussions highlights the need for targeted interventions. Effective land management strategies and sustainable agricultural practices are crucial to mitigating the adverse impacts of river erosion and ensuring food security in Bangladesh.

CONCLUSION

This study highlights the significant and ongoing impact of river erosion on agriculture in Bangladesh, emphasizing the vulnerability of agricultural land, which continues to be lost at alarming rates, threatening the sustainability of farming practices. Erosion-induced land degradation has led to decreased crop yields, particularly for staple crops such as rice and jute, which are highly dependent on fertile soils. These environmental changes have resulted in profound socio-economic consequences, including increased poverty and forced displacement, as affected communities migrate to urban areas, further straining local economies, and infrastructure. The changing land use patterns, particularly transition from traditional agriculture to aquaculture, illustrate the broader ecosystem disruptions induced by erosion. The study also indicates that various mitigation strategies, including vegetative stabilization, crop diversification, and flood control measures, have proven effective, with vegetative stabilization emerging as the most promising for sustainable erosion management. Overall, the findings emphasize the urgent need for integrated river management policies and sustainable agricultural practices to address the challenges of river erosion, ensuring the resilience of agricultural livelihoods and securing food security in the region.

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Trend Detection of Temperature, Diurnal Temperature Range and Rainfall in Amritsar by using Mann Kendall Test

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Abstract: The analysis of diurnal temperature range (DTR) for Amritsar from 1970–2023 was done. Daily minimum and maximum temperature were analyzed for additional consideration of DTR trend by using time series. DTR change of the Amritsar and seasonal variations of maximum and minimum temperature, rainfall by separating into *Rabi* and *Kharif* season. Diurnal temperature range (DTR) steadily decreased at the rate of -0.042°C/yr, -0.033°C/yr and -0.046°C/yr during annual, *Kharif* and *Rabi* season in the past 50 years. The yearly maximum averaged temperature indicated marginal decreasing trend but the yearly mean least temperature is increasing more rapidly, 0.028 °C/yr, 0.029 °C/yr and 0.028 °C/yr during annual, *Kharif* and *Rabi* season. The significantly expanding pattern of mean least temperature adds to the reduction of mean DTR. Annual and *Kharif* total rainfall has decreasing trend at the rate of -0.039 mm/yr and -0.223 mm/yr but the total rainfall during *Rabi* showed slightly increasing trend with 0.026 mm/yr.

Keywords: Diurnal temperature, Rainfall, Variability, Amritsar

Climate change studies in Punjab and India have gained significant attention in recent years due to the growing recognition of the region's vulnerability to climate-related changed. Rising temperatures can have adverse effects on agricultural productivity, water resources, and human health. Climate change poses significant risks to agriculture in Punjab and India, which heavily rely on the monsoon season. Studies have assessed the impact on crop yields, changes in pests and diseases, and the need for adaptation strategies to ensure food security. Changes in rainfall patterns and increased temperatures affect water availability, particularly in regions dependent on glacial melt water and monsoon rainfall. Climate change contributes to an increase in extreme weather events like floods, droughts, and heat waves. This includes assessing the feasibility and effectiveness of renewable energy, energy efficiency, sustainable agriculture practices, and resilient infrastructure development. Climate change has socio-economic implications, particularly for vulnerable communities and marginalized groups. Research investigates the differential impacts and explores strategies to enhance resilience and reduce inequalities. Climate change eludes to a significant difference in either the mean condition of the atmosphere or in its changeability, enduring for a completeperiod.The global mean surface temperature is expected to increase by 1.16.4°C by 2100, according to the intergovernmental panel on climate change (IPCC 2007).

Global warming is consequence of climate change that is most commonly acknowledged. The mean temperature has risen by a little over 1°C since 1880, with the majority of hotness occurring after 1975, at an estimated rate of 0.15 to 0.20°C per decade, according to scientists at NASA's Goddard Institute for Space Studies (GISS) (Anonymous 2020a). However, accompanying climate changes and unpredictability in meteorological parameters may result in either an increase or decrease in the net productivity of an ecosystem.

Punjab, the bread basket of the country, is that it is mainly agrarian economy which will be most affected by climate change. Kaur et al. (2016) reported that under A1B scenario, minimum and maximum temperature are expected to boost by 2.9 and 4.9 °C, respectively during 2021-50 and by 5.8 and 7.4 °C, respectively during 2071-2100. Kaur and Hundal (2010) reported gradual increase in minimum temperature at Ludhiana over last 30 years. The aim of this study is to analyze the diurnal temperature range and trends of variation in weather parameters by using Mann Kendall Test.

MATERIAL AND METHODS

Climate and location: Amritsar station is located at latitude of31°38'N and longitude of 74°52'E with an altitude of 234 m above mean sea level, which is located in the Majha region of Punjab under Trans-Gangetic agroclimatic zone of India. The general climatic condition is classified as semi-arid with mean annual rainfall of about 681 mm out of which 75% rainfall is received during monsoon (June to September). The summer temperature exceeds above 38°C and reaches upto 49°C with dry summer spell.

Weather data: The meteorological parameters i.e. daily maximum and minimum temperature and rainfall of Amritsar was obtained from Indian Meteorological Department for 1970-2023 was analyzed for diurnal temperature range. Time series of daily maximum temperature and minimum temperature were also analyzed for further understanding of DTR trend. DTR change were analyzed first, and then impact on seasonal variations of maximum and minimum temperature; rainfall; DTR trends by separating into *Rabi* and *Kharif* season.

Mann-Kendall test: This test is used to determine whether a time series has a monotonic upward or downward trend. It does not require that the data be normally distributed or linear. It does require that there is no auto correlation. The null hypothesis for this test is that there is no trend, and the alternative hypothesis is that there is a trend in the two-sided test or that there is an upward trend (or downward trend) in the one-sided test.

RESULTS AND DISCUSSION

Mann-Kendall test: Most of the time, the time series data show quite strong patterns, with the Mann-Kendall trend either increasing or decreasing (Table 1). H0 is disregarded if the p value is less than the significance level of (alpha) = 0.05. Accepting H0 means no trend was observed whereas rejecting H0 means there is a pattern in the time series. On rejecting the null hypothesis, the result is said to be statistically significant. Null Hypothesis was accepted for maximum temperature and rainfall data over the last 53 years. Similar trend were observed for *annual, rabi* and *kharif* seasons for 1970-2023. Frimpong et al. (2022) analyses temperature indices revealed an increase in warm days and a general rise in the minimum temperature compared to maximum temperatures. Mann Kendall and Sen's slope revealed significant change in the annual and seasonal (dry and wet seasons) in minimum temperature in Ghana.

Temperature: Despite year-to-year changes due to numerous climate causes significant downward trend in diurnal temperature range, with a slope of -0.042 °C/yr. The annual mean maximum temperature declined at -0.013 °C/yr, but the annual mean lowest temperature is rising significantly faster, at 0.028 °C/yr. The significantly increasing trend in the mean lowest temperature adds to a drop in the mean DTR. Because seasonal trends exist in temperature change, time series of seasonal average DTR, Tmax, and Tmin of the Amritsar were analyzed to explore seasonal aspects of DTR change (Figs. 1, 2, Table 2) DTR variation demonstrates

Table 1. Mann-Kendall test statistics on maximum, minimum temperature and rainfall of *rabi, kharif* and annual in Amritsar district of Punjab

Weather parameters	Tau	p value	Alpha	Test interpretation
Annual (1970-2023)				
Maximum temperature	-0.17	0.09	0.05	Accept H0
Minimum temperature	0.21	0.03	0.05	Reject H0
Rainfall	-0.01	0.91	0.05	Accept H0
Kharif season (1970-2023)				
Maximum temperature	-0.12	0.23	0.05	Accept H0
Minimum temperature	0.20	0.04	0.05	Reject H0
Rainfall	-0.003	0.98	0.05	Accept H0
Rabi season (1970-2023)				
Maximum temperature	-0.09	0.37	0.05	Accept H0
Minimum temperature	0.26	0.009	0.05	Reject H0
Rainfall	-0.002	0.99	0.05	Accept H0

Table 2. Regression equations of meteorological parameters of Amritsar

Seasons	Diurnal temperature range	Mean maximum temperature	Mean minimum temperature	Annual rainfall(mm)	Number of rainy days
Annual	y = -0.042x + 99.63 R^2 = 0.421	y = -0.013x + 57.71 R^2 = 0.127	y = 0.028x –41.91 R^2 = 0.264	y = -0.039x + 798.4 R^2 = 7E-06	y = -13.2x + 430.4 R^2 = 0.520
Kharif	y = -0.033x + 60.61 R^2 0.266	y = -0.003x + 42.27 R^2 = 0.005	y = 0.029x –37.03 R^2 = 0.220	y = -0.223x + 1024 R^2 = 0.000	y = -8.6x + 293.6 R^2 = 0.435
Rabi	y = -0.046x + 108.0 R^2 = 285	y = -0.018x + 60.61 R^2 = 0.098	y = 0.028x –47.44 R^2 = 0.219	y = 0.026x + 90.80 R^2 = 2E-05	y = -7x + 145.8 R^2 = 0.147

seasonal patterns. DTR exhibits a declining trend in both seasons, with kharif having the largest decrease rate and Rabi having the lowest decrease rate. Tmax has a slightly decreasing trend of -0.003°C/yr in *Kharif*, but TMIN has the highest increasing rate, 0.029°C/yr than *Rabi*, therefore DTR has the most dramatically declining trend, at -0.033 °C/yr. Similarly, in *Rabi*, Tmax has a slightly decreasing trend of -0.018 °C/yr and TMIN has an increasing trend of 0.028 °C/yr, hence DTR has a slightly decreasing trend of -0.046 °C/yr. A downward trend in mean DTR was observed particularly in recent decades, the DTR has decreased. As the yearly mean most extreme temperature of the Amritsar has a marginally expanding pattern, the yearly mean least temperature is increasing at a lot quicker rate, which might make sense of the lessening of mean DTR. The patterns of normal DTR, greatest temperature and least temperature have huge occasional contrasts. Mehta and Yadav (2019) showed significant increase in temperatures and decreases in monsoon rainfall in most parts of the Rajasthan state and climate in Rajasthan state is growing warmer, especially in summer.

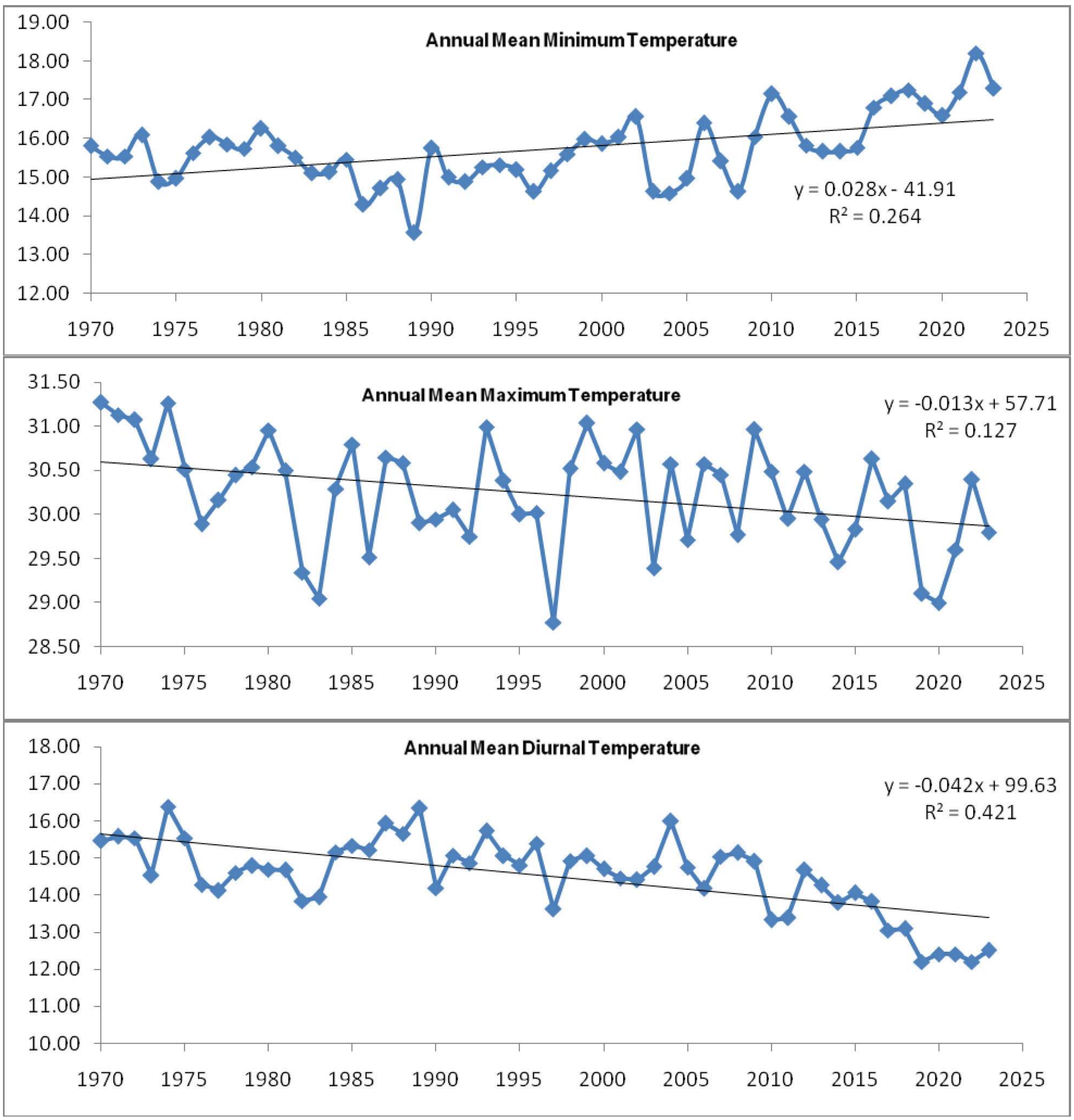

Fig. 1. Annual mean diurnal, maximum and minimum temperature at Amritsar

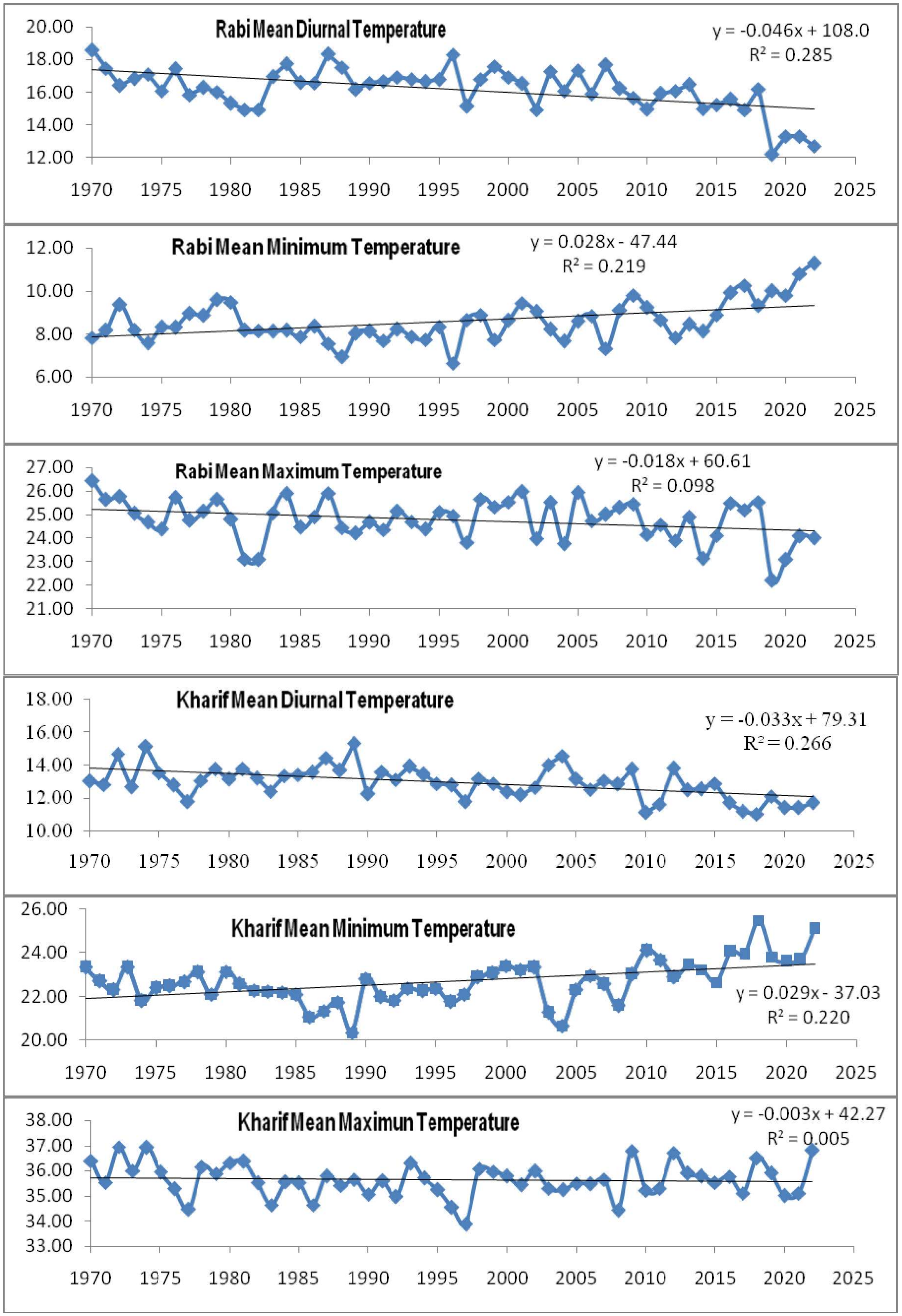

Fig. 2. *Rabi* and *kharif* mean diurnal, maximum and minimum temperature at Amritsar

Rainfall: Along with the temperature rainfall represents the most important parameter of meteorology for any particular region. There was not a significant change. The Annual and *Kharif* total rainfall indicated decreasing trend at the rate of -0.039 mm/yr and -0.223 mm/yr but the total rainfall during *Rabi* has slightly increasing trend with 0.026 mm/yr. During recent decades, the decreasing trend is more significant. The decreasing rate of change in number of rainy days were observed as -13.2 days/yr, -8.6 days/yr and -7 days /yr during annual, *kharif* and *Rabi* season, respectively at Amritsar (Table 2).

Decadal shift: The maximum temperature (T_{max}) decreased from 1970-79 to 2010-18 by -0.46°C, -0.09°C and -0.67°C for annual, *Kharif* and *Rabi* season, respectively. However, the (T_{min}) minimum temperature increased by 0.77°C, 1.02°C and 0.54°C during annual, *kharif* and *Rabi* season, respectively from1970-79 to 2010-18. On contrary , The perusal of diurnal temperature data has significant decreasing shift of -1.4°C, -1.3°C and -0.6°C for annual, *Kharif* and *Rabi* season, respectively from1970-79 to 2010-18. The similar trends of mean maximum and mean minimum temperature were observed by Prabhjyot-Kaur *et al* (2012).

CONCLUSION

The yearly maximum averaged temperature has a marginal decreasing trend but the yearly mean least temperature is increasing more rapidly in the past 53 years. Thus, the significantly expanding pattern of mean least temperature adds to the reduction of mean DTR.

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Agriculture Biochar Production: Applications and Challenges for Crop Improvement

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Abstract: The rapid growth of the global population and increasing environmental stress have intensified the need for sustainable agricultural solutions. Biochar, a carbon-rich material produced through the pyrolysis of biomass under limited oxygen conditions, has shown promising potential in enhancing soil health. Its application improves soil structure, nutrient availability, water retention, and microbial activity, while also serving as a long-term carbon sink. Biochar can limit the mobility of pesticides and heavy metals, reducing environmental risks, and can also be utilized in composting, livestock feed, and greenhouse gas mitigation. The efficiency of biochar depends on factors such as feedstock type, pyrolysis temperature, soil characteristics, and application rate. Prospects highlight biochar's role in sustainable farming and climate change mitigation; however, challenges remain in optimizing production processes, tailoring biochar properties for specific soils, and ensuring economic feasibility for large-scale adoption

Keywords: Rapid pyrolysis, Biomass waste, Microwave pyrolysis, Crop production, Nutrient-rich soils

Promising strategies for ensuring food security for a growing world population include increasing production per unit area and sustainably improving agricultural productivity. Research is being conducted around the world on the potential benefits of using biochar as a solution of food security by enhancing crop productivity (Shackley et al., 2012, Vijay et al., 2015, Narzari et al., 2015, Nair et al., 2017).The application of biochar is coherent with modern green development concepts as it plays critical roles in maintaining ecosystem balance, controlling soil pollution, and the sustainable development of the agricultural environment (Zhang et al., 2021). Burning different kinds of fossil-based resources releases huge amount of carbon pollution to the atmosphere which intensifies climate change. To lessen climate change and avoid devastating consequences of global warming and climate problems, using fossil fuels as fuels needs to be pulled in (Dinca et al., 2018, Safarian 2023). In addition to the urgent requirement to reduce conventional energy resources for environmental issues, the war in Ukraine has much more strengthened effort to substitute a large quantity of conventional fuels with instant effects, for instance by using of biochar produced from biomass (Martinopoulos et al., 2018, Chaudhary et al., 2024). Biochar is a carbon-rich and porous material produced through the thermal decomposition of biomass, such as plant residues, agricultural waste, or wood, under controlled oxygen-limited conditions. This process, known as pyrolysis, involves heating the biomass to high temperatures in the absence or near absence of oxygen, preventing complete combustion. The result is a stable form of charcoal that contains a well-structured network of pores called biochar (Liu et al., 2018, Ali et al., 2023). Biochar is an organic residue that is formed during biomass pyrolysis. Biochar, when added to soil, significantly affects soil fertility by changing the soil's chemical, biological, and physical properties (Awad et al., 2018, Awad et al., 2024). The manufacturing method, soil type and condition, crop type to be planted, and biochar resource could all affect how effective it is. The most common feedstocks used to make biochar are paper goods, animal dung, and agricultural waste. It is crucial to use these wastes to produce biochar since it is an effective method of converting waste into a substance with value (Malghani et al., 2013, Ambaye et at., 2021). The two types of pyrolysis-slow pyrolysis and fast pyrolysis-rely on the heating rate and residence time. Rapid pyrolysis yields more liquids and oils, slow pyrolysis yields more syngas. Slow pyrolysis also produces more charcoal (36%), compared to gasification (12%) and quick pyrolysis (17%) (Makepa et al., 2023). Conventional carbonization, another name for slow pyrolysis, produces biochar by heating biomass to a relatively low temperature over an extended period (days or longer) (Cao et al., 2014). Biochar is made at a higher temperature and has a very short residence period (1 sec) for rapid pyrolysis still. The yields of slow and quick pyrolysis processes are the primary difference between them (Fig. 1). The goal of crop sustainability is to market an environmentally safe alternative to traditional farm inputs, such as chemical fertilizer (Samanta et al., 2025). Pyrolysis is a desirable alternative for using biomass waste. Based on the process parameters and is further classified into three categories that are quick, intermediate and slow. Biochar in solid form and bio-oil in liquid form are the end products of the pyrolysis process. The International Biochar Initiative states that biochar is a carbon-rich solid material obtained from biomass thermochemical conversion under oxygen-free conditions is the most widely recognized and consistent definition of biochar (Begum et al., 2024). The distribution of product yields depends on some factors, including pressure, biomass type, temperature, heating rate, and vapor residence time (Razzak 2024). Lower temperatures and gradual, moderate heating are generally found to promote this technology the biomass volume. In contrast to traditional pyrolysis illustrated that the temperature at the center of the biomass is higher than the material's surface and ambient temperature (Zaker et al., 2019).

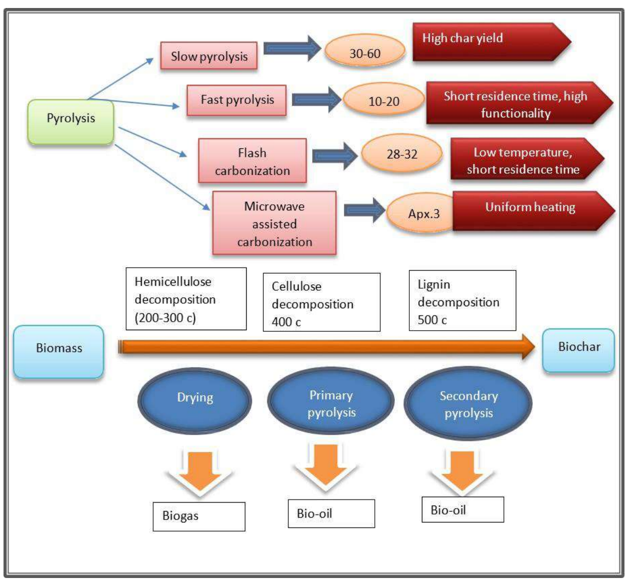

Fig. 1. Pyrolysis & biomass formation

Slow and Fast Pyrolysis

Slow pyrolysis was performed by placing feedstock into a paint-can fitted with a nitrogen purge (1L/min flow rate) and thermocouple for temperature measurement. The sealed can was placed into a muffle furnace and heated at approximately 15°C/min to 500°C. Corn stover (50 g) was held at 500°C for 30 minutes, switchgrass (125 g) was held at 500°C for 2 hours. The char was then cooled under nitrogen flow and stored in sealed glass jars. Mass yield of char was 33.2% and 41.0% for corn stover and switchgrass, respectively (Brewer et al., 2014). Fast pyrolysis was performed on a 5 kg/hour capacity bubbling fluidized bed reactor optimized for bio-oil production (Lehmann et al., 2011). The sand bed was fluidized with nitrogen pre-heated at 500°C. Char was collected using a high-throughput cyclone catch and cooled under nitrogen before being stored in resealable plastic bags. Therefore, phase transition phenomena and heat and mass transfer processes, as well as chemical reaction kinetics, play a vital role. So, it is important to minimize the exposure of biomass particles with the intermediate temperature to favor formation of charcoal (Brewer et al., 2014). By using microwaves in a novel way, the process is comparatively accelerated. pyrolysis of biomass involves the transfer of energy instead of heat. Pyrolysis that occurs slowly low temperatures, a prolonged residence period, and a slow heating rate are used for slow pyrolysis. Past study reported that 1 kilogram of wood processed by slow at a low heating rate at 400–500 °C will typically provide 30–35% biochar, 45–50% bio-oil, and 20–25% producing gas (Jahirul et al., 2012). In general, 70–80% of biochar is fixed carbon. It has been reported that the slow pyrolysis reactor can be divided into three categories: converters, retorts, and kilns (Garcia-Nunez et al., 2017). Industrial-scale reactors known as retorts are able to extract a volatile fraction and biochar (Fig. 2).

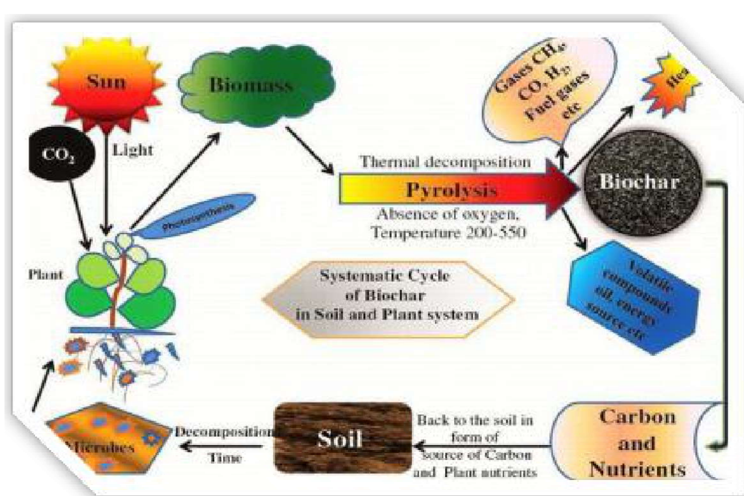

Fig. 2. Biochar production and applied as a soil amendment (Saha et al., 2022)

Microwave Pyrolysis (MP)

Microwave based technology is an alternative heating method and has already been successfully used in biomass pyrolysis for biochar and biofuel production thanks to its fast, volumetric, selective and efficient heating. Previous review mainly focused on production and analysis of bio-oil and gas instead of biochar. The current paper provides a review of microwave-assisted pyrolysis (MP) of biomass and its biochar characteristics, including product distribution and biochar yield, biochar properties, microwave absorbers (MA) and catalysts commonly used in MP, as well as comparison of biochar derived from MP and conventional pyrolysis (Li et al., 2016). It has reported that microwave heating (MH) technique offers several advantages over conventional heating based on previous research, such as more controllable, high-energy efficiency and cost effectiveness (Sun et al., 2016). MH offers a potentially attractive alternative to CP systems owing to its energy transfer rather than heat transfer, non-contact and rapid heating, selective and volumetric heating, high level of safety, as well as quick startup and shutdown. Numerous results have shown that MH is better than conventional. The biochar products can be widely applied in various environmental fields, such as carbon capture and sequestration, soil amendment, adsorption of contaminants in soil, water, and air, and energy production. Nevertheless, challenges remain for these new trends, such as the increase in cost for the installation and operation, the lack of knowledge of the mechanism involved during pyrolysis, the difficulty in scaling up, etc. Further studies are recommended to facilitate the application of these new trends, such as pilot tests or field experiments to evaluate the real effects of biochar products prior to large-scale applications or their long-term risk during use, or prediction of properties of biochar and their impact on environmental applications using modeling or machine learning approaches (Jahirul et al., 20012).

It is a crucial factor to select feedstock selection for biochar affecting soil CH_4 emissions. Biochar from manure or straw has a low C/N ratio and contains many low-molecular-weight organic compounds, leading to increased available C for methanogenesis and ultimately increasing CH4 production (Lehmann et al., 2011, Mallik et al., 2024). In contrast, wood/cellulose-derived biochar decreases CH4 emissions due to the greater porosity resulting from preserving the pore structure during pyrolysis due to lignin's stability (Fungo et al., 2014). Soil aeration can be better by excessive porosity. Biowaste biochar amendments decrease CH_4 emissions have been utilized in the process of biochar production (Fig. 3).

Biochar properties: Biochar porosity and its surface affect metal sorption capacity. When pyrolyzing bio-mass material, micro- pores are formed in biochar because of water loss in the dehydration process (Yin et al., 2016). Biochar has different pore sizes, if the size is below 2.00 nm, then they are micro, if is above 50.00 nm then it is macro-pores and nano if it is less than 0.900 nm, respectively. Its porosity and surface area changes significantly with pyrolysis temperature. Increasing the temperature from 500 to 950°C, the porous structure of biosolids biochar increases between 0.059 and 0.1 cm3/g, the surface area also increases from 25.7 to 68.9 m2/g (Zhou et al., 2017).

Biochar benefits: Biochar significantly reduces nutrient leaching and nitrous oxide (N2O) emissions. Research indicates that N_2O emissions can decrease by approximately 83% following biochar application, which also contributes to reduced gaseous nitrogen losses. These benefits, coupled with its role as a soil conditioner and organic fertilizer, enhance carbon sequestration, soil fertility, microbial activity, pH levels, nutrient recycling, and water-holding capacity, while also mitigating soil contamination (Dong et al., 2017,Nguyen et al., 2019,). Bulk density and porosity are affected by water storage capacity, such soil characteristics are affected by biochar application (Fig. 4). Biochar affects nutrients holding basically for acidic, hydrophilic characteristics. It also increases nutrient retention. It is effective in soil conditioning. Due to agricultural activities, organic carbon merely deteriorates daily (Xia et al., 2017). For the agricultural yields, the presence of carbon is very important. Biochar can be used in removing toxic pollutants from soil. Organic carbon that presents in biochar depends on the source of materials. Carbon sequestration applications are increasing. The ability of biochar to increase

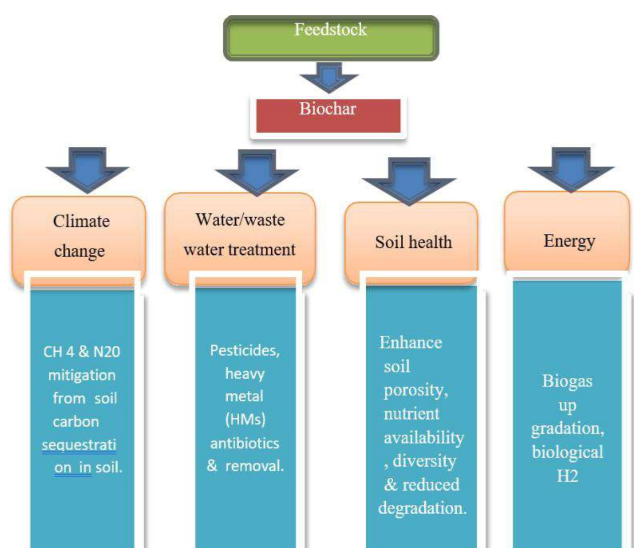

Fig. 3. Factor influences on biochar production

crop yield may decline over time, which may be attributed to nutrients being taken up by plants or leached out of the biochar (Alkharabsheh et al., 2021) and the microporous structure of the biochar becoming rougher and gradually collapsing which may leads to decrease rates of ammonium (NH4+) - nitrogen (N2) fixation and nitrification (Xu et al., 2024). In India most of the work on biochar is concentrated to its application in agricultural sector to improve soil quality and as a sustainable method for waste management (Godwin et al., 2019). Nevertheless, some research has reported effect biochar application in production of catalysts, their activation and their analyses for potential use in removal of toxic elements from soil and water (Jin et al., 2025, Islam et al., 2024). Past study has also reported conversion of a problematic aquatic weed to charcoal by pyrolysis for energy production (Kataki and Kataki, 2022.).

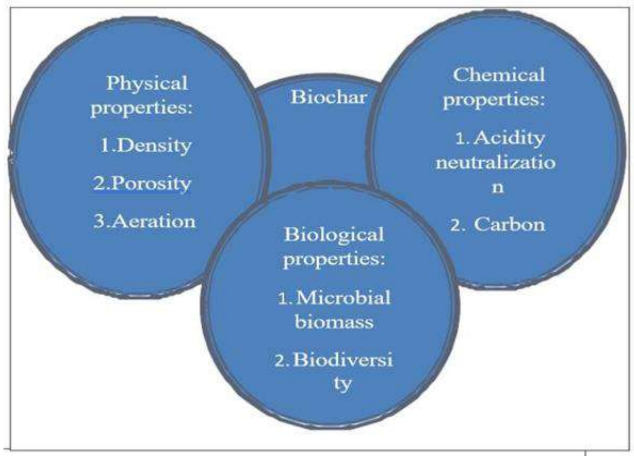

Fig. 4. Properties of biochar

Impact on crop productivity and soil health: Biochar applications may substantially improve soil fertility and crop productivity. For, instance, biochar application (68 t ha^{-1}) increased biomass in rice (Oryza sativa L.) and cowpea (Vigna unguiculata (L.) Walp) by 20 and 50 %, respectively, and at 136.75 t ha^{-1} increased cowpea biomass by 100 % (Adekiya 2022). Biochar addition improved biomass and grain yields in durum wheat (Triticum durum L.) by up to 30 %, but there was no effect on grain N content (Vaccari et al., 2011). Past study also reported that increases of 91% and 44 % in grain and biomass yield, respectively, in maize (*Zea mays* L.) on charcoal-amended soils when compared with adjacent field soils in Ghana (Yeboah et al., 2022). Likewise, in Kenya, maize yield in degraded soils doubled with the addition of Eucalyptus-derived biochar (Borah et al., 2020). Several studies have indicated the strong potential of biochar application for improving crop yields, particularly on nutrient-poor soils (Van-Zwieten et al., 2010, Zhang and Ok 2014). The effect on crop yields, particularly in nutrient-rich soils, remains uncertain. Several other studies have revealed only small improvements or even reductions in grain yield with

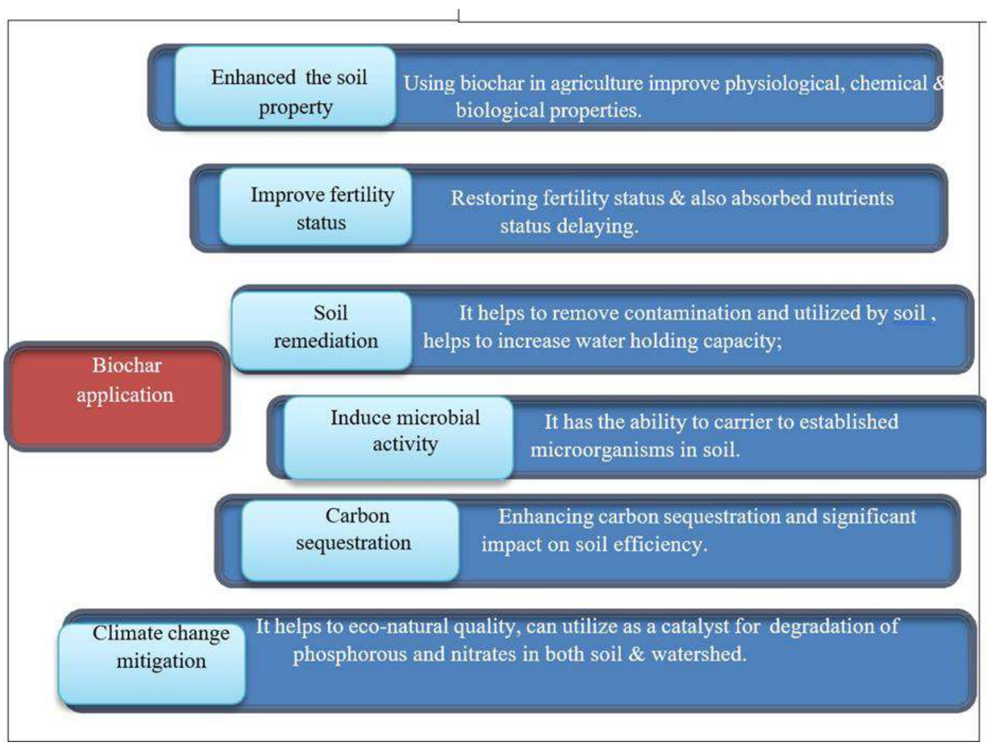

Fig. 5. Different applications of biochar in crop production

biochar application in nutrient-rich soils (Hussain et al., 2017). It has also been reported that there is a linear decrease in grain yield with increasing rates of biochar application (Huang et al., 2019). Biochar is known to improve physical, chemical, and biological properties in soil. The effect of biochar application on soil physicochemical properties, nutrient availability, and soil biota is discussed in the following sections (Fig. 5). Addition of biochar in soils improved soil stability due to improved soil aggregation (Novotny et al., 2015, Rafi et al., 2022). Increased soil carbon stock is the most pronounced effect of biochar soil application (Lehmann et al., 2011, Zhang and Ok 2014). Merging bioenergy production with the application of pyrolysis by-product biochar in soil removes CO2 from the atmosphere, as more carbon is sequestered than emitted (Roberts et al., 2010). It has also been reported that 20 % more CO_2 is captured from the atmosphere and sequestered by biochar soil amendment (Mulabagal et al., 2017). However, certain risks such as the potential source of toxicants, retention of heavy metals, and the suppression of the efficacy of applied pesticides due to retention and ecotoxicology effects on soil microbes are linked with biochar addition to arable land (Alengebawy et al., 2021) As the biochar are in the dust form, they may be dangerous to humans during their application to agricultural soils. For example, rice husk biochar pyrolyzed at higher temperatures may possess toxic crystalline materials, e.g., silica which are very harmful to human health, and they can affect the respiratory system if they enter during the biochar application process in soil.

CONCLUSIONS

In developing countries, crop residue has traditionally been used as animal feed. When not used as animal feed, it becomes a huge surplus biomass, and farmers burning it create a hazy and smoky environment. Conversion of such surplus biomass into biochar circumvents this problem and creates employment and economic opportunities. Quality biochar with high fixed carbon content can be produced by maintaining a reactor temperature between 400 and 600 °C. As a carbon sink, biochar also contributes to atmospheric carbon sequestration, particularly when its oxygen-to-carbon molar ratio is below 0.2, promoting its longevity in the soil. While short-term studies indicate positive impacts on crop yield, the long-term effects on soil health warrant further investigation. Our findings demonstrate biochar's efficacy as a soil amendment in Alentejo, offering a strategy to mitigate environmental concerns within agricultural practices. However, broader adoption and societal benefit necessitate increased efforts to promote its use and capitalize on its positive externalities. The biomass-to-biochar conversion process, encompassing cultivation, collection, transport, and pyrolysis, involves considerable energy and CO2 emissions, with pyrolysis being a major contributor. Enhancing the efficiency of this process, particularly pyrolysis, is crucial for minimizing the carbon footprint associated with biochar production and maximizing its environmental benefits from pyrolysis, so improving its efficiency could notably shrink the carbon footprint.

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Heavy Metal Pollution and Biota-to-soil Accumulation Factor (BSAF) of in Situ Earthworms in Western Zone of Punjab, India

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Abstract: Earthworms are a key species in the terrestrial ecosystem as their responses to contaminated soil reflects the health of ecosystem functioning. The present study was undertaken to ascertain the effect of heavy metal pollution on in situ earthworm communities of Punjab, India. The presence of heavy metals in both soil and earthworm samples collected from all three districts of western zone of Punjab (Fazilka, Ferozepur, Bathinda). The content of heavy metal in soil samples and in earthworms collected from different locations followed the trend: Mn>Zn>Cu>Cr>Ni>Pb>As>Cd and Zn>Mn>Cu>As>Cr>Ni>Pb>Cd respectively. The heavy metal contents were higher in earthworms in comparison to soil samples. The oxidative stress in earthworms was highly significant and positive correlation for enzyme glutathione reductase with cadmium and chromium while negative correlation was observed of glutathione S transferase and glutathione peroxidise with chromium and manganese and copper, respectively. The biota-to-soil accumulation factor (BSAF) followed the trend: Cd>As>Zn>Cu>Pb>Ni>Cr>Mn. The current study is a step forward in the direction of in-situ study of earthworms and to understand the complex relations of various factors that together operate as determinants of bioaccumulation as opposed to the widely prevalent and controlled laboratory studies.

Keywords: Heavy metals, Earthworms, Stress enzyme, Soil, BSAF, Western Zone of Punjab

Soil is a dynamic and vital ecosystem that forms the basis of life on this planet. The soil organisms like earthworms play an important role in the functioning of soil ecosystems (Spurgeon et al., 2003). In this ecosystem earthworms have a special place due to their complex role in the various stages of soil formation as well as production of vermicast and maintaining the dynamics of the soil ecosystem. Although most other soil organisms are protected by the thick cuticle outside their bodies however earthworms are susceptible to soil chemicals as these hazardous substances are ingested and absorbed in their bodies which leads to bioaccumulation which is then passed on to other organisms in the food chain. This bioaccumulation in earthworms may have significant effects on the animal and on other higher level organisms as well (Reinecke and Reinecke 2007).

The rapid industrialization has added new stressors in the ecosystem in the form of pesticides and heavy metals which are the major environmental pollutants released from activities like mining, combustion of fossil fuels disposal of wastewater and sewage sludge on land. The metals like zinc, arsenic, cadmium, chromium etc are harmful to both the environment and the organisms even in minute concentrations (Aulakh et al., 2022, Friis et al., 2004, Mahmood 2020, Yadav et al., 2023). Earthworms are indispensable organisms especially for the study of the degree and effects of various pollutants in the soil primarily due to their edaphic habitat which keeps them in intimate contact with both solid and aquatic phases of soil. The heavy metal contents are accumulated by earthworms inside their tissues during feeding in addition to the uptake of heavy metals by earthworms they also return a portion of these pollutants back to the soil when put into vermicast. Higher concentrations of these pollutants are a serious threat to the survival of these species in the soil ecosystem. When exposed to these pollutants the natural reaction of the body is to release various anti-oxidant defence systems that an organism employs to protect and deactivate the radical toxicity from the exposure to substances. The traces of the metal elements have been implicated with the oxidative stress at cellular level via (Lijun et al., 2005) several enzymes including superoxide dismutase, catalase, and glutathione peroxidase (GPX). The reactive oxygen species (ROS) affects the lipids, proteins, carbohydrates and nucleic acids including the superoxide radical (O^{2}-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These biochemical responses can be early warning signs of environmental pollution and its severity. These metal pollutants accumulate over time resulting in bioaccumulation, studied using the bioaccumulation factor as a measure of quantification of metal bioaccumulation into biota (Melake et al., 2023). Despite the crucial role of earthworms within the soil ecosystem, there is scarcity of studies with a specific focus on the oxidative stress and antioxidant defences in relation to the natural availability of environmental pollutants. The present investigation is an attempt to bridge the gap by studying the effects of heavy metals and their relations to the organism's biochemical defence system through the release of certain enzymes under the in-situ and dynamic conditions of the natural habitat.

MATERIAL AND METHODS

Collection and preparation of sample: The present study was conducted in the Department of Zoology, Punjab Agricultural University, Ludhiana to check the heavy metal residues in western regions of Punjab. The samples of soil and earthworms were collected from the rice fields of three districts in Western zone of Punjab: Fazilka (A), Ferozepur (B), and Bathinda (C). Two villages namely Mansa and Choharian Wali from Fazilka, Pindi and Jiwan Arian from Ferozepur, Pirkot and Chauka from Bathinda were selected for soil sampling. The soil samples up to the depth of 30cm were collected. The soil aggregates were disintegrated while they were still moist in the laboratory and then air dried before sieving. Then these samples were sieved through 2 mm mesh sieve before they were placed in plastic bags for further testing. Earthworms collected from all the three sites were first rinsed with distilled water and placed in petri dishes with Whatman No. 1 filter paper and a few drops of distilled water were put to maintain them. The earthworms were placed alive on moist filter paper with no food source for 24 hours to allow soil to be egested from their gut. The worms were killed by freezing and then oven dried (48 h at 70-80°C) to constant weight. The dry earthworms were processed for heavy metal detection (Bade et al., 2012) by using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

Antioxidant activity in earthworms: After washing the earthworms in distilled water, they were homogenised in 0.02M phosphate buffer (pH 7.5) and centrifuged at 3000 rpm for ten minutes. The homogenate supernatant was used for further enzyme analysis. The enzymes were analysed using standard methods: which include glutathione peroxidase (Hafeman et al., 1974), superoxide (Marklund and Marklund 1974), catalase (Aebi (1983); glutathione reductase (Carlberg and Mannervik 1985) and glutathione-S-transferase (Habig et al., 1974).

Biota-to-Soil accumulation factor : The BSAF is calculated (Cortet et al., 1999).

BSAF = Metal content in biota (earthworm tissue) / Total metal content in soil.

The BSAF was calculated for As, Mn, Zn, Cd, Cr, Ni, Pb and Cu.

RESULTS AND DISCUSSION

The higher contents of heavy metals (As, Mn, Zn, Cd, Cr, Ni, Pb and Cu) were in tissues of earthworm as compared to soil samples from all the three selected sites (Table 1). The metal concentrations in the soil samples collected indicated the trend: Mn> Zn> Cu> Cr> Ni>Pb>As>Cd whereas the content of heavy metal in earthworm species collected from different locations show the trend: Zn>Mn> Cu> As>Cr> Ni>Pb>Cd. This difference in the presence of various heavy metals in soils and their presence as observed in the earthworm tissues are due to the differences in the very nature of these metals. The high value of heavy metals may be attributable to chemical fertilisers which are employed in agricultural activities such as insecticides (Krishna and Govil 2004). The average concentrations of as in the earthworm from all the study locations were in decreasing trend maximum in site B (38.25) followed by site A and C. The concentrations of As in soil was maximum in site B (6.62) followed by A and C. The average concentrations of Cd in earthworms from all the study locations followed the trend, site A(3.4) >site B (3.25) > site C (2.75) while the average concentrations of Cd in soil from all the study locations followed the trend, site B(0.5) >site A (0.44) >site C(0.31). The average concentrations of Ni in earthworms was

Table 1. Heavy metal content in soils and earthworms collected from western zone of Punjab (ppm)

Location	A-Fazilka		B-Ferozepur		C-Bathinda	
Metal	Soil	Earthworm	Soil	Earthworm	Soil	Earthworm
Arsenic	4.68±0.45	21.5±3.59	6.62±1.86	38.25±2.67	4.12±0.78	15.50±2.38
Cadmium	0.44±0.12	3.40±0.58	0.50±0.14	3.25±0.58	0.31±0.08	2.75±0.48
Nickel	17.37±2.74	12.33±1.15	18.94±3.82	18.50±2.45	13.31±2.26	10.75±1.73
Manganese	247.18±40.53	117.5±6.60	295.56±59.74	238.50±35.86	165.21±28.11	127.50±24.72
Zinc	52.37±9.61	254.25±20.51	79.81±19.18	336.50±63.87	67.68±9.41	284.5±17.91
Lead	10.25±1.33	10.50±1.89	11.42±2.39	13.00±1.91	5.68±0.87	7.50±0.58
Chromium	18.56±4.66	10.50±1.91	20.12±2.03	18.50±2.50	15.18±2.86	9.50±1.73
Copper	19.22±2.99	36.85±6.44	26.51±8.28	49.57±4.511	10.27±1.40	26.20±1.61

Values are mean ±S.E

maximum in site B (18.5) followed A and C. The average concentrations of was maximum (18.94) in site B followed closely by site A (17.37). The average concentrations of Mn in earthworms was maximum in site B (238.5) quite low in C (127.5) and A (117.5) The Mn in soil in site B was maximum (295.56) followed by A (247.18) and C (165.21). The average concentrations of Zn in earthworms was maximum at site B(336.5) and was low in C (284.5) and A (254.25). The average concentrations of Zn in soil was maximum in site B (79.81) followed by C (67.68) and A (52.37). The average concentrations of Pb in earthworms from all the study locations followed the trend: B (13) >A (10.5) >C(7.5) while the average concentrations of Pb in soil from all the study locations followed the same trend being maximum in B (11.42) followed by A and C. The average concentrations of Cr in earthworms was high in B (18.5) >A (10.5) >C (9.5) while the average concentrations of Cr in soil from all the study locations followed same the trend, B (20.12) >A (18.56) > C (15.18). The average concentrations of Cu in earthworms was maximum in B (49.57) followed by A (36.85) and C(26.20). The average concentrations of Cu in soil from all the study locations was maximum in site B (26.51) and minimum in C (10.27). Except for Mn, Ni and Cr, the concentration of heavy metals Pb, Cu, As and Zn was higher in earthworms in soil samples collected from different districts.

Analysis of the presence of various enzymes revealed that highest catalase activity in earthworm was at site B (50.32 nmolH_2O_2 consumed/min/mg of protein) followed by C and A (Table 2). The catalase activity in earthworms positive trend for most of the heavy metals. This can be attributed to the increased substrate concentration as a result of heavy metal exposure (Liu *et al* 2011). The present results corroborate with the findings that catalase has a trend of increase and decrease as per the duration of exposure to toxic metals in *E. fetida* (Yadav et al., 2022, Lin et al., 2010). Superoxide dismutase is a metalloenzyme that acts as an antioxidant, highest SOD activity in earthworm was at site B

Table 2. Activity of antioxidative enzymes in earthworms collected from different districts

Oxidative enzymes	A-Fazilka	B-Ferozepur	C-Bathinda
Catalase (nmolH_2O_2consumed/min/mg of protein)	34.33±0.61 [a]	50.32±1.20 [c]	40.24±0.88 [b]
SOD (U /mg of protein)	32.54±0.64 [a]	41.23±0.96 [b]	33.43±0.38 [a]
Glutathione peroxidase (U/mg of protein)	37.87±0.56 [a]	61.76±0.55 [c]	54.22±0.75 [b]
Glutathione S-transferase (nmol/min/mg of protein)	135.33±0.70 [a]	150.44±0.43 [c]	140.44±0.57 [b]
Glutathione reductase (μmol of NADPH conjugate/min/mg of protein)	10.54±0.45 [b]	6.43±0.71 [a]	9.65±0.46 [b]

Values are mean ±S.E
Values with different superscript (a, b and c) are significantly different ($p<0.05$)

Table 3. Correlation coefficient of heavy metal content in earthworms with antioxidant activity

Enzymes	Locations	Heavy metals							
		As	Cd	Pb	Zn	Cr	Ni	Mn	Cu
Catalase	A	+0.76	+0.18	-0.86	-0.59	-0.75	-0.75	-0.92	-0.71
	B	+0.87	+0.69	+0.75	+0.94	-0.14	+0.34	+0.24	+0.65
	C	+0.24	+0.19	-0.03	+0.28	-0.06	+0.01	+0.10	-0.69
Glutathione	A	-0.51	+0.59	+0.11	-0.43	+0.59	-0.01	+0.19	-0.41
Reductase	B	-0.19	+0.91	+0.26	-0.33	+0.95	+0.71	+0.78	+0.05
	C	+0.02	-0.02	-0.13	-0.26	-0.13	-0.12	-0.23	+0.80
SOD	A	-0.45	+0.99	-0.63	-0.87	+0.48	+0.11	-0.005	-0.55
	B	-0.15	-0.10	+0.60	-0.10	+0.43	+0.55	+0.50	+0.83
	C	-0.83	-0.38	+0.56	-0.08	+0.41	+0.40	+0.40	0.27
GPX	A	-0.54	+0.67	+0.03	-0.51	+0.62	+0.01	+0.19	-0.44
	B	-0.16	+0.72	-0.58	-0.03	-0.99	-0.92	-0.95	-0.30
	C	-0.78	-0.05	+0.84	+0.47	+0.28	+0.74	+0.83	-0.42
GST	A	+0.39	-0.51	-0.11	+0.42	-0.47	+0.14	-0.08	0.47
	B	-0.04	+0.81	-0.45	+0.09	-0.99	-0.85	-0.90	-0.18
	C	-0.53	-0.71	+0.09	-0.35	+0.84	-0.10	+0.04	-0.93

A-Fazilka, B-Ferozepur and C-Bathinda

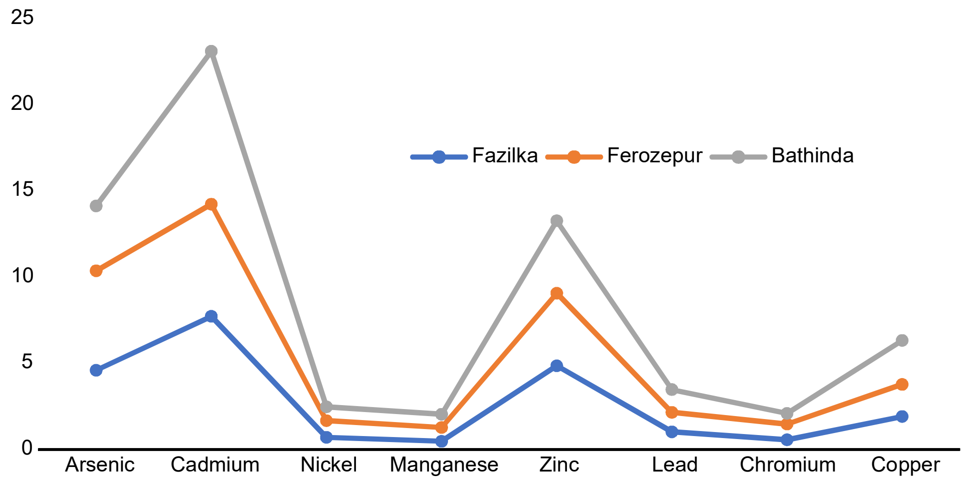

Fig. 1. Biota-to-soil accumulation factor of heavy metals in tissue of earthworm

(41.23 U /mg of protein) followed by site C and site A. The highest GPx activity in earthworm was at site B (61.76 nmol U/mg of protein) followed by site C and site A. GST, a cytosolic enzyme, is involved in the detoxification and biotransformation of a variety of electrophilic chemicals through glutathione consumption. The highest GST activity in earthworm was at site B (150.44nmol H_2O_2 consumed/min/mg of protein) followed by site C and site A. For the enzyme glutathione reductase, highest activity in earthworm was at site A (10.54µmolof NADPH conjugate/min/mg of protein) followed by site C and site B. The negative effect of Cd, As and Zn on the SOD levels can be due to the accumulation of superoxide radicals after prolonged exposure to these heavy metals (El-Demerdash et al., 2009). The activity of enzymes GST, GPx and GR were severely affected by the presence of heavy metals in soils, these findings are in agreement. Laszczyca et al. also (2004) observed that the gradient of heavy metal pollution leads to a gradual increase and subsequent decrease due to hormesis which is a mechanism of balance between the instantaneous production and degradation of specific proteins.

The correlation analysis of heavy metals in earthworm tissues and the activity of various enzymes of site A revealed a highly significant and high positive correlation for the enzyme glutathione reductase and cadmium. The enzyme glutathione reductase has significant high positive correlation for chromium while the enzyme GPX has significantly high negative correlation with chromium and manganese and for the enzyme GST there is a significantly high negative correlation for chromium in samples from site B. At site C, the enzyme GPX has significantly high negative correlation for copper. The Biota-to-soil accumulation factor (BSAF) for the present study follows the trend of Cd>As>Zn>Cu>Pb>Ni>Cr>Mn (Fig. 1) which are in agreement with Dai et al. (2004).

CONCLUSION

The study provides valuable insights into the heavy metal contamination among in situ earthworms in Western zone of Punjab. The Biota-to-soil accumulation factor (BSAF) revealed the trend: Cd>As>Zn>Cu>Pb>Ni>Cr>Mn. There is significant relationship of the presence of oxidative stress enzymes in earthworms with specific heavy metals such as glutathione reductase with cadmium while a significant inverse relation was found with glutathione S transferase with chromium and manganese as well as of glutathione peroxidase with copper. Thereby, the study of various oxidative stress enzymes provides a reliable measure of bioaccumulation of environmental pollutants in the earthworms as well as the severity and penetration of pollutants within the soil ecosystem.

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Effect of Pre- and Post-Emergence Herbicides on Weed Biomass, Nutrient Depletion, and Growth of Soybean in Humid South-Eastern Plains of Rajasthan

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Abstract: Field experiment was conducted during the *kharif* season of 2019 at the Agricultural Research Station, Ummedganj, Kota (Rajasthan), to evaluate the effect of various herbicide treatments on nutrient depletion and growth of soybean (*Glycine max* L. Merrill). The experiment comprised eight treatments, including pre- and post-emergence herbicides and hand weeding, laid out in a randomized block design with three replications. The herbicide application significantly reduced weed density and dry weight, thereby reducing nutrient depletion by weeds and improving crop growth and yield attributes. Maximum nutrient depletion by weeds with losses of 50.2 kg nitrogen, 3.58 kg phosphorus, and 41.4 kg ha^{-1} potassium was under the weedy check, indicating the severity of nutrient loss in untreated plots. In contrast, minimum nutrient removal by weeds under two hand weeding at 20 and 40 days after sowing, followed by the application of sodium acifluorfen + clodinafop-propargyl @ 165 + 80 g *a.i.* ha^{-1} at 20 days after sowing, also resulted in improved crop growth.

Keywords: Soybean, Herbicides, Nutrient depletion, Growth, Sodium acifluorfen + clodinafop-propargyl

Soybean (*Glycine max* L. Merrill) is a major oilseed and legume crop. India ranks fourth globally in soybean area and fifth in production, with 12.14 million hectares of area and 12.99 million tonnes of production in 2022 (SOPA 2023). Weed infestation is a significant challenge in soybean production, causing yield losses of 58–85%, depending on weed species and infestation (Kewat et al., 2000). Weeds also deplete essential soil nutrients up to 21.4 kg N and 3.4 kg P ha^{-1} (Pandya et al., 2005) and reduce yields by up to 55% under unchecked conditions (Malik et al., 2006). Major weed species in soybean fields include *Echinochloa colona*, *Cynodon dactylon*, *Amaranthus viridis*, and *Cyperus rotundus* (Singh and Rajkumar 2008, Sangeetha et al., 2012). Although manual weeding is effective but it is labor-intensive, costly, and often impractical due to labor shortages and unpredictable monsoon rains. In this context, the application of selective pre- and post-emergence herbicides provides a viable and cost-effective alternative for weed management in soybean cultivation.

The study addresses the major constraint of weed infestation in soybean cultivation in the Humid South-Eastern Plains of Rajasthan. Manual weeding is often impractical due to labour scarcity, costly, time consuming and intermittent rainfall during *Kharif* season; therefore, herbicidal weed control is remains the only alternative. The objective was to evaluate the effect of pre- and post-emergence herbicides on weed biomass, nutrient depletion, and soybean growth, and to identify an effective and economically viable herbicidal method of weed control.

MATERIAL AND METHODS

The experimental site is situated at 25°13′N latitude and 75°28′E longitude, with an altitude of 271 meters above mean sea level. The soil of the experimental field was clay loam in texture, with a medium fertility level, and the pH of the soil is 7.6. The experiment was laid out in a randomized block design with three replications. The experiment comprised eight treatments, *viz*., T_1 – pendimethalin 30% EC @ 1.0 kg *a.i.* ha^{-1} as pre-emergence, T_2 – pendimethalin + imazethapyr (premix) @ 960 g *a.i.* ha^{-1} as pre-emergence, T_3 – sodium acifluorfen + clodinafop-propargyl @ 165 + 80 g *a.i.* ha^{-1} at 20 days after sowing, T_4 – quizalofop-ethyl @ 50 g *a.i.* ha^{-1} at 20 days after sowing, T_5 – imazethapyr @ 100 g *a.i.* ha^{-1} days after sowing, T_6 – imazethapyr + propaquizafop premix @ 50 + 75 g *a.i.* ha^{-1} days after sowing, T_7 – hand weeding at 20 and 40 days after sowing, and T_8 – weedy check. The soybean variety RKS-113 was used for experimental material. Each treatment was applied to plots with adequate spacing between rows and replications to avoid spray drift and edge effects. Herbicides were applied by a knapsack sprayer fitted with a flat fan nozzle. Growth parameters*, viz.,* plant height, branches per plant, and dry matter accumulation, were recorded at different growth stages by five randomly selected plants from each plot.

To determine nutrient depletion, weed samples were collected from an area of 1 m^2 selected randomly at three spots in each of the plots within each plot at 30 and 60 DAS. The collected weed samples were initially shade-dried and

Table 1. Effect of pre and post emergence herbicides on dry matter accumulation, branches plant^{-1}, plant height, weed dry weight and nutrient depletion by weeds

Treatments	Dry matter accumulation (g mrl^{-1})	Branches plant^{-1} (no.)	Plant height (cm)	Nutrient depletion (kg ha^{-1})			Dry weight of weeds (g m^{-2})
				N	P	K	
T1	105.34	3.53	67.7	28.1	1.98	23.0	7.09 (49.34)
T2	123.70	3.93	70.3	21.7	1.50	17.7	5.80 (32.63)
T3	138.43	4.13	71.2	19.0	1.31	15.5	5.53 (29.62)
T4	113.54	3.80	68.2	23.8	1.67	19.5	6.35 (39.32)
T5	120.40	3.87	68.9	22.9	1.59	18.6	6.22 (37.75)
T6	131.37	4.07	69.4	20.5	1.46	16.7	5.71 (31.61)
T7	154.13	4.60	75.2	14.5	1.00	11.9	4.55 (19.74)
T8	63.43	3.13	59.8	50.2	3.58	41.4	9.48 (88.83)
CD (p=0.05)	15.00	0.45	7.34	1.93	0.12	1.45	0.29

Data in parenthesis are original values of weed dry weight. **Square root transformed value ($\sqrt{x+1}$) of weed dry weight used for statistical analysis

then dried in an electric oven at 65 ± 5°C till their weight became constant and ground for chemical analysis. Nitrogen content in weed samples was estimated using the Kjeldahl method as outlined by Piper (1966). Phosphorus was determined by the vanadomolybdate yellow color method following the procedure described by Jackson (1973), while potassium was estimated using a flame photometer from the triple acid extract, also as per the method outlined by Jackson (1973). Nutrient depletion by weeds (kg ha^{-1}) was determined by multiplying the nutrient content in weed biomass with the total weed dry matter (kg ha^{-1}) and dividing by 100.

Nutrient depletion by weeds (kg ha^{-1}) = Nutrient content in weeds × Weed dry matter (kg ha^{-1})/100

RESULTS AND DISCUSSION

Effect on growth of crop: Soybean growth parameters responded positively to effective weed management. Plant height and branches in plant showed an increasing trend (almost linear) with advancement in plant age up to 75 DAS and thereafter remained constant till harvest. Plant height (75.2 cm), number of branches per plant (4.60), and dry matter accumulation (154.13 g) were highest under hand weeding twice at 20 and 40 DAS, closely followed by post-emergence application of sodium acifluorfen + clodinafop (premix) 165 + 80 g *a.i.* ha^{-1} at 20 DAS and imazethapyr + propaquizafop (premix) 50 + 75 g *a.i.* ha^{-1} PoE at 20 DAS (Table 1). Minimum were observed in weedy check. Similar results were also reported in earlier studies (Meena et al., 2009, Gupta and Chandrakar, 2014 , Harithavardhini et al., 2016).

Nutrient content in weeds, weed dry matter, and nutrient depletion by weeds: The nutrient content in weed biomass was statistically non-significant across treatments. However, the total nutrient depletion by weeds was directly governed by the amount of weed dry matter accumulated under each treatment. The different herbicides significantly influenced the weed dry matter accumulation, which affected the growth of soybean and nutrient depletion by weeds from the soil (Table 1). The highest weed dry matter in the weedy check, which consequently resulted in the maximum nutrient removal, 50.2 kg ha^{-1} of nitrogen, 3.58 kg ha^{-1} of phosphorus, and 41.4 kg ha^{-1} of potassium. Among the herbicides, sodium acifluorfen + clodinafop-propargyl (premix) 165 + 80 g *a.i.* ha^{-1} PoE at 20 DAS was the most effective in reducing weed dry matter, thereby restricting nutrient depletion to 19.0 kg N, 1.31 kg P, and 15.5 kg K ha^{-1}. This was closely followed by imazethapyr + propaquizafop (premix) 50 + 75 g *a.i.* ha^{-1} PoE at 20 DAS and pendimethalin + imazethapyr (premix) 960 g *a.i.* ha^{-1} as pre-emergence. The lowest dry matter accumulation was observed under two hand weedings at 20 and 40 DAS, which led to minimum nutrient depletion. Similar results were also reported in earlier studies (Meena et al., 2009, Gore et al., 2014, Panda et al., 2015, Elankavi et al., 2019).

CONCLUSION

The effective weed management significantly reduces weed dry matter accumulation and nutrient depletion, thereby improving the growth and productivity of soybeans. Two-hand weeding at 20 and 40 DAS proved to be the most effective in minimizing weed biomass and nutrient loss while simultaneously maximizing crop growth. Sodium acifluorfen + clodinafop-propargyl and imazethapyr + propaquizafop were highly effective post-emergence herbicides that not only suppressed weed growth but also restricted nutrient depletion and enhanced crop growth.

AUTHOR'S CONTRIBUTION

Bharat Lal Meena carried out the field experiment and collected the data, performed statistical analysis, interpreted and drafted the manuscript. Harkesh Meena assisted in statistical analysis, interpretation and drafting of the manuscript. D.S. Meena and R. K. Meena)supervised the work, reviewed it critically and edited the final version. All authors read and approved the final version.

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Musk Melon Cultivation on Reservoir Basin: A Climate Smart and Profitable Farmer Practice in Arid Zone of Rajasthan

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Abstract: Small-holder farmers everywhere have been necessitated to develop location-specific knowledge and adaption techniques in order to maintain their livelihoods in uncertain ecosystems due to climate vulnerability and several stresses. The growing of muskmelon on the reservoir basin has also been made profitable by a climate-smart technique developed by local western Rajasthan farmers. The developed technology is primarily a conservation agriculture technique that includes the use of short-duration varieties, decreased tillage, low fertilizer inputs, no irrigation, and the use of locally accessible mulch to minimize evapotranspiration The findings indicated that musk melon production in the reservoir basin under conserved soil moisture (CMLI) with modest inputs had a minimum days taken to first flowering (29.70 days), first harvesting (82.40 days), high benefit-to-cost ratio (3.64) and net return per day (Rs. 2980 ha^{-1}) than the conventional method (AMHI) of cultivation in which days taken to first flowering (35.40 days), first harvesting (91.10 days), high benefit-to-cost ratio (2.94) and net return per day (Rs. 2646 ha^{-1}), which involved ample irrigation and inputs. In contrast to conventional cultivation practices, the high benefit cost ratio under conservational technology was mostly related to low cultivation costs as well as significant income from early fruit harvest.

Keywords: Semi-arid climate, Conserved moisture, Agroecological knowledge, Opportunistic autonomous adaptation, Muskmelon

Crop production is exceedingly difficult in Rajasthan's arid and semi-arid region because of the harsh climate conditions, scare and poor quality of soil and water resources. In these regions, only hardy crop species with minimal water requirements are produced, mostly in the *kharif* or *rabi* seasons when guaranteed irrigation is available (Singh et al., 2019). Sand dunes, sand plains of varying thickness (some of which are salt-affected), as well as some desolate hills, uplands and gravelly pavements make up the majority of Rajasthan state's (2,11,867 km^2) arid western region, which includes a significant portion of the Thar or the great Indian sand desert (Santra *et al.,* 2013). Over 85% of the region's yearly rainfall falls (June-September) during the south west monsoon, ranging from about 500 mm near the slope of the Aravallis in the east to about 100 mm along the border with Pakistan in the west (Singh et al., 2023). The mean annual potential evapotranspiration is significantly more than precipitation (1400-2000 mm). This region experienced 42 severe droughts during the years 1801 to 2002, which decreased agricultural output (Saxena and Mathur 2019). High biotic pressure on dry areas accelerates the development of desertification and decreases crop productivity, which leads to an exploitation of the area's resources (Gupta and Narain 2003). Crop failure is a common occurrence caused by either insufficient rainfall or a deficiency of soil moisture to meet the crop's water needs during various phenological stages (Barnabas et al., 2008). In addition, the dry region has a number of biotic and abiotic constraints that contribute to its low production. Local farmers used their agro-ecological knowledge in these conditions to shift stress into opportunity with self-adaptation (Faroda et al., 2007). One such evidence is the winter and summer time production of fruits and vegetables in the reservoir basin in Rajasthan's desert region.

Due to the low rainfall, farmers and populations in dry zones were compelled to build reservoirs to collect and store rainwater during the rainy season and use the stored water for irrigation and drinking during the winter and summer (Singh et al., 2019). Due to ongoing water extraction for irrigation, drinking and other purposes as well as evapotranspiration, the water level in this reservoir began to decline throughout the winter and nearly reached an all-time low during the summer. The reservoir then opens up a significant amount for farming (Varghese and Singh 2016). The reservoir basin's soil fertilizes with increased water retaining capacity as finer soil particles are gradually deposited. The small and landless farmers residing in the catchment area of the reservoir realized the potential of the soil of reservoir basin and started to cultivate it for the production of vegetables on residual soil moisture. However, the crop yield and profitability ware poor due to fast depletion of soil moisture in the absence of soil moisture conservation technologies (Singh et al., 2020). A number of indigenous knowledge or methods have gradually developed to improve the preservation of remaining soil moisture as well as the productivity and profitability of the farming in this region (Singh et al., 2023). An example of such an indigenous technique is the cultivation of musk melons using indigenous knowledge that is comparable to the current approach of conservation agriculture in the reservoir basin of the Hemawas Dam, Pali (Rajasthan). Therefore, we assessed and evaluated the socio-economic features of locally evolved conservation agricultural practices for musk melon cultivation in the reservoir basin of the Hemawas dam with traditional musk melon production methods.

MATERIAL AND METHODS

Study area: The two villages of the Pali district of the Rajasthan (India) were selected for the study. The first study location was reservoir basin of the Hemawas dam, in the Hemawas village which was built in 1911, lies on the Bandi river (a tributary of the Luni river) and the catchment area (reservoir basin) of the Dam is about 1,685 km^2. The second location was the Naya Gaon village of the Sojat tehsil in the Pali district of the Rajasthan State, India. The Pali district's climate differs just little from that of the typical arid western Rajasthan. The significant temperature variation is observed due to nearby green and hilly terrain, even though the summer season the temperature rises to 46-47°C (May–June). The temperature falls to 4-5°C in December–January, and winters are moderately cold. The during monsoon (July to October) the average rainfall is 450.7 mm. The soil in the study areas was sandy clay loam, salty nature (brought on by limited rainfall and excessive evaporation) with following characteristics:-

Particulars of soil	Value
Depth of soil	50 to 75 cm
pH	7.73 to 8.20
Organic carbon	0.22 to 0.33%
Nitrogen (N)	231.75 to 277.00 kg/ha
Phosphorus (P_2O_5)	14.33 to 15.00 kg/ha
Potash (K_2O)	210.33 to 214.33 kg/ha

Selection of farmers and data collection: The data was collected from ten musk melons farmers in the Naya Gaon village (25.9238^0 North latitude and 73.6651^0 East longitudes) and Hemawas dam (25.7343^0 North latitude and 73.3620^0 East longitudes) for three-year (2018, 2019 and 2020) on all agronomical approaches, plant growth, development and yield features from twenty five muskmelon plants selected from each location. Data were collected on various parameters, including length of vine (m), number of leaves per vine, days to first flowering, days to first female flowering, days to first harvest, number of fruits per vine, fruit weight (g), fruit yield per vine (kg), fruit yield (t/ha). The economics were calculated based on the cost of cultivation and the price of fruit through at the time. The productivity per day (kg/ha) and total yield per day (Rs/ha) were converted to total yield and total net return per days, respectively. The benefit cost ratio was determined by dividing the absolute net return and cultivation cost.

Statistical analysis: This was carried out using OPSTAT software (Sheoran et al., 1998).

Land availability in the reservoir basin: The Hemawas Dam's reservoir fills up almost completely during the rainy season, making the basin unusable for growing crops from July through September, or the *kharif* season. Following monsoon withdrawal, the water level in the reservoir gradually declines quite quickly as a result of water extraction for drinking, irrigation of *rabi* crops, seepage and evaporation. Due to water receding by October, a significant section (about 65–70%) of the reservoir basin at a higher altitude is open for growing *rabi* crops. The reservoir is nearly empty (approximately 95%) in early December as a result of water extraction, in this basin only *rabi* crops, and cucurbits vegetables are grown in conserved moisture.

Agronomic Practices

Conservational cultivation in the reservoir basin (conserved moisture with low inputs, CMLI): After the availability of land as a consequence of the reservoir's water level reducing (November–December), marginal and small farmers in the area started breaking up hard pans in January. The indigenous short duration variety (locally known as KAJRI) musk melon was sown in last week of January to fortnight of February. The farmer excavated pit holes of 4-6 cm in with 1.80 m difference between rows and 50 to 75 cm between plants. Six to eight treated seeds are sowed and covered with sand in each The farmer covers the seedlings with locally available materials dry plants, such as *Crotalaria burhia* and *Leptadenia pyrotechnica*, to conserve moisture and minimize evaporation after the seeds have germinated within five to six days. The light soil ploughing is done when the plant reaches a height of 10 to 15 cm in order to preserve soil moisture.

Agronomic practice in the conventional cultivation (adequate moisture with high inputs, AMHI): In a conventional farming method, the field is prepared by conventional tillage during bed preparation and seed sowing in early February. Before to final leveling, the recommended dose of fertilizer dosage was applied. Seeds were sown during 2nd week of February on 2.0 m wide bed in two rows at 120 cm apart at 30 cm plant to plant spacing and 1-2 cm soil depth with 1 seed per hill. All recommended practices were followed (Patil et al., 2014).

RESULTS AND DISCUSSION

Crop growth and fruit yield of muskmelon: Perusal of mean data of three years (2018 to 2020) showed that all growth and yield of the musk melon was significantly influenced by both type of the cultivation practice. The growth, development and fruit yield was significantly higher in conventional methods of cultivation as compared to traditional conservational practices (Table 1 and 2). In pool data significantly higher growth length of vine (2.18 m) and number of leaves per vine (80) of plant was recorded under conventional method (AMHI). The better growth and development of plant might be due to intensive cultivation by application of high inputs under conventional method. The recorded mean of minimum number of days taken for first flowering (29.70 days), first female flowering (34.10 days) and first harvesting (82.40 days) from days to sowing was observed in traditional conservational practices (CMLI). This might be due to conserving soil moisture can significantly improve soil quality and health in saline conditions. Moisture conservation can help mitigate the negative impacts of salinity by reducing salt accumulation and improving water availability for plants, excessive irrigation with saline water can worsen the problem. Moisture conservation practices can promote better soil structure, enhancing aeration and drainage, which can help reduce the harmful effects of salinity (Sigh et al., 2020).

Significantly mean data of (Table 1 & 2) more crop duration (132 days), number of fruits per vine (14.7), fruit weight (391.1 g), fruit yield per plant (5.77 kg) and fruits yield per hectare (96.20 t ha^{-1}) of muskmelon was 25.16 % higher observed under conventional method (AMHI) as compared to conservation practices (71.99 t ha^{-1}) over three years of observation. This might be due to beneficial effect of adequate irrigation with high inputs in improving the soil environment which in turn encourages growth resulting in better absorption of moisture, nutrients and thus producing higher biomass. The results are closely associated with those reported by (Ansary and Roy 2005, Arancibia and Motsenbocker 2008, Rani et al., 2012, Anbumani et al., 2017) in melon crops. A perusal of data on gross return and net return (Table 2) revealed that higher was under conventional methods (AMHI) of cultivation (Rs. 5,28,070 ha^{-1} and Rs. 3,48,220 ha^{-1}) while under conservational methods (CMLI) of cultivation it was Rs. 4,68,572 ha^{-1} and Rs. 3,39,788 ha^{-1}. The higher yield and returns under conventional system might be attributed to good plant growth due to combined availability of soil moisture with proper nutrition and plant protection throughout the muskmelon growth period. The above results are close conformity with the various studies in other crops (Johnson et al., 2000, Rani et al., 2012, Anbumani et al., 2017).

Relative economics for musk melon cultivation: The conservational system (CMLI) was more profitable as compared to conventional systems (AMHI) due to significantly higher benefit cost ratio (Table 2). The mean data of B:C ratio (3.64) and net returns per day (Rs 2980 ha^{-1}) was higher under conservational practice, while B:C ratio (2.94) and higher productivity per days (731 kg ha^{-1}) under conventional practices. It was mainly attributed to low input cost and early maturity of the crops under conservational system as compared to conventional system. Early availability of musk melon fruit to the market fetched higher price per unit of fruits as compared to late mature crop under conventional systems resulting into higher B:C ratio

Table 1. Effect of conservational (CMLI) and conventional management (AMHI) on muskmelon production system

Agronomy management practices	Length of vine (m)	Number of leaves per vine	Days to first flowering (DAS)	Days to first female flowering (DAS)	Days to first harvest (DAS)	Crop duration (DAS)	Number of fruits per vine	Fruit weight (g)
CMLI	1.78	69.20	29.70	34.10	82.40	114	12.40	347.40
AMHI	2.18	80.00	35.40	40.00	91.10	132	14.70	391.10
CD (p=0.05)	0.15	4.8	1.4	1.4	2.6	2.2	0.8	17.6
C.V.	7.1	6.3	4.2	3.6	2.9	1.7	5.9	4.6

AMHI: Adequate moisture with high inputs; CMLI: Conserved moisture with low inputs; DAS: Days after sowing

Table 2. Effect of conservational (CMLI) and conventional management (AMHI) on muskmelon production system

Agronomy management practices	Fruit yield per vine (kg)	Fruit yield (t ha^{-1})	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B:C ratio	Productivity per days (kg ha^{-1})	Net returns/day (Rs. ha^{-1})
CMLI	4.32	71.99	468572	339788	3.64	631	2980
AMHI	5.77	96.20	528070	348220	2.94	731	2646
CD (p=0.05)	0.42	6.91	40727	NS	0.27	59	NS
C.V.	8.0	8.0	8.0	11.5	7.9	8.4	12.0

AMHI: Adequate moisture with high inputs; CMLI: Conserved moisture with low inputs; DAS: Days after sowing

Fig. 1. Muskmelon cultivation practices in conserve moisture

combined with low cost of inputs, muskmelon crop was early with regard to days to flowering and also days to produced early marketable fruits under conservational system as compared to conventional systems (Table 1). The increase in growth parameters, delayed flowering and fruiting under conventional systems may be attributed availability of sufficient soil moisture and inputs under irrigated conditions that enhance vegetative growth and delayed flowering (Patil et al., 2014, Anbumani et al., 2017).

CONCLUSION

The cultivation of musk melon with indigenously developed conservational technologies in the reservoir basin of the Hemawas dam provide ensured income and livelihood to the small and marginal farmers of this area. When compared to cultivation on retained moisture in the reservoir basin, muskmelon grown conventionally, with substantial irrigation supply and adequate fertilizer inputs, reported significantly higher growth and production characteristics. This adaptation provides an insight for the formal science about how formal and informal knowledge can be hybridized to co-produce more robust adaptation to convert stressors into opportunity. The farmers having land at scattered site in the Hemawas dam which is used to store runoff water in catchment area of around 260 hectares of land. The farmer very effectively utilized the conserved soil moisture in the Hemawas dam basin for cultivation of muskmelon rather than traditional crop like wheat, barley, mustard and chickpea which have low yield and less return. This site-specific agro-ecological adaptation gives other farmers, who are landless and generally more marginalized, additional influence.

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Evaluation of Spraying Nano Sea Algae Extract on Growth and Yield of *Cucurbita pepo* L.

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Abstract: This study was conducted in Kufa, Najaf province, during the 2019-2020 season to examine the effect of spraying nano sea algae extract (no spraying, 0.5, and 1 ml L^{-1}) on the growth and yield of two cultivars of *Cucurbita pepo* L. c. v. (Domestic and Fadwa). The experiment was set up in a split-plot layout, where the cultivars were the main plots and the concentrations were in subplots with three replicates. Fadwa cultivar excelled the domestic cultivar in vegetative growth, including plant length, total number of leaves, and fresh and dry weight. Spraying with nano sea algae extract at 0.5 ml L^{-1} concentration significant improved vegetative growth for both cultivars compared to the control treatment (spraying with water only). The interaction between cultivars and sea algae extract was also significant, as Fadwa + spraying 0.5 ml L^{-1} of sea algae extract recorded higher vegetative growth and yield traits of *Cucurbita pepo* compared to Domestic + spraying with water only.

Keywords: *Cucurbita pepo*, Cucurbit cultivar, Nano application, Sea algae extract

Summer squash (*Cucurbita pepo* L.) belongs to the Cucurbitaceae family and is commonly cultivated in highlands with mid-altitude agroecosystems ranging from approximately 1,000 to over 2,000 meters in Central and North America, specifically in central Mexico and the southwestern United States (Kates et al., 2017). The Central Organization of Statistics in Iraq (2020) indicate that the production of the area unit of summer squash in 2020 decreased to 9.68 tons per hectare compared to the average production of some of the world's crop-producing countries in the same year (47,322, 58,679, and 66,725 tons per hectare in Spain, Indonesia, and the Netherlands, respectively) (FAO 2020). Hence, there is a need to increase production per unit area, with the use of high-productive cultivars, keeping in increase in demand due to an increase in the population. The plant cultivar is very important in the productivity as genetic characters determine the growth and development of the cultivar, which affects the quantity and quality of the yield. Adaptation to the environment is another factor that determines yield. Moreover, Al- Foliar nutrition uptake also affects the growth and yield (Kostadinov and Kostadinova 2014, Stojanova et al., 2016).

Nanotechnology is a new technology that can help increase the productivity and economic value of crops (Ghormade et al., 2011). Nano Sea algae extract is a new approach for higher productivity. Researchers have found that spraying plants with algae extracts improves metabolism inside the leaves by increasing photosynthesis, as it contains cytokinins, auxins, and many nutrients (Yokoya et al., 2010), and delays plant aging and prevents the fall of leaves, flowers, and fruits. Al-Zubaidy and Al-Hamzawy (2016) mentioned that the spraying of herb extract (Basfolior Kelp) at 6 ml L^{-1} concentration on *Capsicum annuum* increased both the vegetative and yield traits., Al-Aqaeshi and Al-Sahaf (2017) found a significant increase in yield when spraying okra plants two times every 30 days with 3 ml.L^{-1} of sea algae extract. Spraying eggplants with 1.5 3 ml L^{-1} of Agazone and Atonik resulted in the highest average vegetative growth (Taien and Mathkor 2018). The current study aims to examine the effect of spraying nano sea algae extract on the growth and yield of two cultivars of *Cucurbita pepo* L. c.v. (Domestic and Fadwa).

MATERIAL AND METHODS

This experiment was carried out in Kufa, Najaf province (latitude: 32° 03' 4.10" N; longitude: 44° 26' 24.61" E) in the 2019-2020 season. Ten samples of the field soil were taken randomly from 0-30 cm depth before planting and were analyzed using the Walkley and Black method described by Jackson (1958) to measure chemical and physical characteristics (Table 1).

The field was prepared by tilling and levelling, and the area was divided into three lines, each 35 m long and with a row-to-row spacing of 1.5 m. Sowing was performed on September 15, 2018, with a plant-to-plant spacing of 0.5 m. The recommended agronomic practices were followed (Jasim et al 2015). The concentrations of nano sea algae extract provided in Table 2, and the foliar application was repeated three times at 15-day intervals (Alhasnawi et al 2020). The experiment was in a split-plot arrangement in a randomized complete block with three replicates (Montgomery, 2020). Cultivars were set up in the main plots, and concentrations were set up in the subplots. The plot size was 3.75 m^2 (2.5 m length × 1.5 m width) with nine plants per plot. Means were compared using the least significant difference (LSD) at 5% level of significance (P>0.05),. The VSN International GenStat 12th Edition (Payne et al., 2009) was used for the two-way analysis of variance

Vegetative traits: Vegetative traits were estimated from 5 plants randomly selected from each experimental unit at the end of the autumn season on Januray 8, 2020.

Plant length (cm) was measured from the soil surface to the top of the plant, and the means were calculated. The total number of leaves (leaf $plant^{-1}$) was calculated on the main stem and branches. Dry weight of total vegetative (g $plant^{-1}$) was estimated at the end of the season from five plants randomly selected from each experimental unit. After removing the roots, the plants were weighed before drying. Afterward, 100 g of fresh weight was dried in an electric oven at 75^0C until its weight was fixed.

Yield traits: The number of fruits per plant (fruit $plant^{-1}$) was recorded for each experimental unit. 2- Yield per plant (kg $plant^{-1}$) was calculated from the first harvest to the end of the season. (November 6 2019 to January 4, 2020).

RESULTS AND DISCUSSION

Vegetative traits: The vegetative traits of *Cucurbita pepo* L. varied significantly among the different cultivars. The Fadwa cultivar (C2) treatment excelled in all traits, with maximum plant length (129.87 cm), leaf number (21.25 leaves $plant^{-1}$), and dry weight of total vegetative (133.84 g $plant^{-1}$) as compared to the Domestic cultivar (C1) (Table 3). The differences were due to the better adaptation of the Fadwa cultivar (C2) to the environment of Najaf province compared to the domestic cultivar. There was a significant effect of spraying nano sea algae extract on the average vegetative growth (plant length, leaf number, and total dry weight). The 0.5 ml L^{-1} gave the highest plant height (129.13 cm), leaves per plant (19.57), and dry weight (130.40 g $plant^{-1}$), while the lowest average of traits was in E_0 (spraying with water only). Al-Bayati et al. (2020) also observed similar trend. The

Table 2. Treatment of the studied factors

Treatments and their interaction	
Concentrations of nano sea algae extract (ml L^{-1})	Cultivar
E_0 = Distilled water	Domestic cultivar C1
E_1 = 0.5	
E_2 = 1.0	
E_0 = Distilled water	Fadwa cultivar C2
E_1 = 0.5	
E_2 = 1.0	

Table 1. Chemical and physical characteristics of the field soil before planting

Soil texture	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	Organic material (%)	Electric conductivity (dS m^{-1})	Soil pH
Sandy	670	190	140	1.2	4.0	7.2

Table 3. Effect of cultivar and spraying with nano sea algae extract on vegetative and yield traits of *Cucurbita pepo*

Treatments			Plant length (cm)	Leaf number (leaves $plant^{-1}$)	Total dry weight of shoot (g $plant^{-1}$)	Fruit number (fruit $plant^{-1}$)	Total yield (ton h^{-1})
Cultivars		C_1	110.70	10.83	110.93	7.19	8.51
		C_2	129.87	21.25	133.84	12.35	9.73
LSD (p=0.05)			6.51	5.77	3.79	1.02	0.65
Spraying concentrations (ml L^{-1})		E_0	114.01	12.67	116.20	8.16	8.21
		E_1	129.18	19.57	130.40	11.56	10.07
		E_2	117.72	15.87	120.57	9.60	9.08
LSD (p=0.05)			2.44	4.50	2.47	0.79	0.47
Cultivars * Concentrations	C_1	E_0	107.49	8.11	106.63	6.29	7.52
		E_1	113.25	14.78	113.88	8.47	9.33
		E_2	111.36	9.59	112.29	6.81	8.68
	C_2	E_0	120.52	17.23	125.76	10.03	8.90
		E_1	145.00	24.36	146.91	14.65	10.81
		E_2	124.08	22.15	128.85	12.38	9.47
LSD (p=0.05)			12.70	8.07	4.31	1.80	0.92

increase in vegetative growth may occur due to nutrients that form nano sea algae extract, such as nitrogen and phosphorus, which form proteins, enzymes, and nucleic acids (DNA, RNA) (Al-Hurmazy 2011). The interaction between cultivars and spraying concentrations treatments showed a significant effect on vegetative growth (plant length, leaf number, and total dry weight of vegetative) when the treatment C2E1 achieved higher plant height of 145.00 cm, 24.36 leaves plant^{-1}, and 146.91 g plant^{-1}, respectively, compared to the control treatment (Table 3).

Yield and related traits: There were significant differences in the yield traits of the different cultivars. Fadwa gave the highest average of fruit number, and total yield of 12, 35 fruit plant^{-1}, and 9.73-ton h^{-1} respectively, in comparison with the lowest average of 7.19 fruit plant^{-1-} and 8.51-ton h^{-1} in the Domestic cultivar (Table 3). There was significant effect of spraying nano sea algae extract on the yield traits. E1 (0.5 ml L^{-1}) provided the highest fruits per plant (11.56), and yield (10.07 ton h^{-1}) in comparison with the lowest average in E0 (8.16 fruit plant^{-1}), and yield (8.21 ton h^{-1}),. The improvement in yield traits may be due to the nutrients in nano sea algae, which play a significant role in increasing biological processes (Nejatzadeh-Barandozi et al., 2014). There was significant interaction between cultivars and spraying concentrations on yield traits. The treatment C2E1 provided the highest number of fruits (14.65 fruits plant^{-1}) and total yield (10.81 ton h^{-1}), in comparison with the treatment (C1E0), which gave the lowest averages of fruit number (6.29 fruit plant^{-1}) and yield (7.52 ton h^{-1}).

CONCLUSION

Fadwa cultivar with 0.5 ml L^{-1} concentration of nano sea algae extract gave the higher vegetative growth and yield traits of *Cucurbita pepo* plants.

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Optimization of Sowing Methods and Irrigation Levels on Carrot Productivity (*Daucus carota* L.) in Mid Hills of North Western Himalayas, India

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Abstract: The two-year study was conducted from November 2021 to February 2022 and from November 2022 to February 2023 at Dr YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, to evaluate the effect of different irrigation schedules and sowing methods on uptake yield and soil quality under carrot cultivation. The experiment followed a factorial randomized block design with two sowing methods: flat-bed (S_1) and ridge (S_2) and four irrigation schedules based on IW/CPE ratios: 0.6 (I_1), 0.8 (I_2), 1.0 (I_3) and 1.2 (I_4), at 3 cm depth of irrigation. Soil pH remained relatively stable across treatments, while electrical conductivity showed minor variations. Soil organic carbon (SOC) improved significantly with increased irrigation schedules and ridge sowing. The highest biological (478.0 q ha^{-1}) and marketable (342.4 q ha^{-1}) yield were under S_2I_4. Splitting percentage, a key physiological disorder, was lowest under moderate irrigation (I_2) and highest under I_4. Nutrient uptake (N, P, K) was significantly enhanced under ridge sowing and higher irrigation levels, with maximum uptake observed under S_2I_4. The ridge sowing combined with an IW/CPE ratio of 1.2 proved optimal for enhancing productivity and soil health, and is recommended for sustainable carrot cultivation in mid-hill agro-ecological conditions.

Keywords: Carrot, *Daucus carota* L., Irrigation schedule, North Western Himalayas

Carrot (*Daucus carota* L.) is an important biennial herbaceous vegetable belongs to family Apiaceae, extensively cultivated on a large scale in China, India, USA, Russia, Uzbekistan, England, Poland, Malaysia, Philippines, Indonesia, and various regions of Africa. Among different agronomic techniques, optimum irrigation schedule and suitable sowing plays an important role in maximizing a variety's potential since they offer the ideal conditions including aeration, moisture, temperature for proper proliferation root, nutrient availability, productivity throughout the growing season (Philippe et al., 2018).

Water is a key limiting factor and plays critical role in growth and production functions of crop. Optimum irrigation schedule is required to achieve higher yields with efficient water utilization that allows the irrigator/farmer to apply the exact amount of water needed. In-sufficient amount of water supply during the time of active growth period resulted in poor nutrient uptake, increase the wastage of limited water and lowers water use efficiency (WUE) as well as the cost of production (Sharma et al., 2023). Inadequate level of water content during crop growth period decreases not only yield, but it also affects the biochemical parameters in carrot. Proper irrigation scheduling is required to calculate the frequency and exact volume of water application, which is essential for obtaining higher crop and water productivity (Maida et al., 2020). In crop production, preparation of seed bed is also an important function that significantly affects the germination, establishment, growth and development of roots. Common sowing methods are flat bed, ridges and furrows and the crop responses is expected to vary markedly with the methods and amount of water used. It also aids in better aeration, compaction, irrigation, moisture conservation and proper drainage of excess of water from the soil (Yadav et al., 2024). The distribution of nutrients in soil and their uptake by plants are influenced by sowing methods and irrigation schedules, which may significantly affect the yield and quality of crop. However, optimization irrigation schedule with suitable sowing method helps in achieving higher crop productivity without compromising the quality of soil and crop. Keeping this in view, present study was conducted to examine the response of European carrot under irrigation water/cumulative pan-evaporation (IW/CPE) based irrigation scheduling with different sowing methods in mid hills of NW Himalayas.

MATERIAL AND METHODS

The study was conducted during 2021-22 and 2022-23 at Dr YSP University of Horticulture and Forestry, Nauni, Solan (HP). The experimental site is situated at 30°52' N and 77° 11' E, having average altitude of 1175 m above mean sea level with 7-8 per cent average slope. The average annual precipitation in this region is 1100 mm, and there is an annual moisture deficit of around 420 mm, if accounted for yearly PET of 1520 mm. The average maximum and minimum temperatures ranged from 16-27ºC and 3-11ºC, respectively. The soil texture was sandy loam, pH 6.72, EC 0.26 dS/m, organic carbon 1.26 g kg^{-1}, bulk density 1.29 Mg m^{-3}, particle density 2.53 Mg m^{-3}, available N 245.4 kg ha^{-1}, P 26.3 kg ha^{-1}, K 265.5 kg ha^{-1}. The water retention at field capacity (FC) and permanent wilting point (PWP) was 23.9% and 7.2%, respectively.

Experimental design and field management: The current study involved 4 irrigation schedules and two sowing methods with 3 replications under factorial randomized block design. The 4 irrigation schedules included I_1 (IW/CPE = 0.6), I_2(IW/CPE = 0.8), I_3 (IW/CPE = 1.0) and I_4 (IW/CPE = 1.2) at 3 cm depth of irrigation and sowing methods *viz.* flat-bed (S_1) and ridge (S_2). The cumulative pan evaporation (CPE) was measured by summarizing the daily observed evaporation from USWB Class-A pan evaporimeter, which was 0.5 km away from experimental site. Surface irrigation was used to apply measured amounts of irrigation water to the plots through PVC pipes that were equipped with a water flow meter. The study was carried out with *Pusa Yamdagni* variety of carrot (*Daucus carota* L.) in 3 m × 2 m plots of each treatment. There was a 1 m buffer zone between plots and a 3 m buffer zone between replications. Line sowing was done and seeds were sown directly by hands at 30 cm × 10 cm spacing. Standard package of practices were followed and recommended dose of N (50 kg ha^{-1}), P_2O_5 (50 kg ha^{-1}) and K_2O (40 kg ha^{-1}) nutrients with FYM @ 10 t ha^{-1} were uniformly applied. At the time of sowing 1/3rd N and full doses of P, K and FYM were applied and remaining N was administered by top-dressing at earthing up stage and 30 days after earthing up stage.

Nutrient uptake: NPK uptake in root and shoot was calculated by multiplying the corresponding dry matter biomass by the corresponding nutrient concentration.

Statistical analysis: Data were analyzed by OPSTAT.

RESULTS AND DISCUSSION

Soil chemical properties: The effect of different irrigation schedules and sowing methods on soil pH, electrical conductivity and soil organic carbon stock at two different depths were evaluated (Table 1). At the 0–15 cm soil depth, the mean soil pH ranged from 6.65 to 6.76 across different irrigation schedules and sowing methods. The flat-bed method exhibited a slightly lower pH compared to ridge method. Among irrigation schedules, pH showed a marginal decrease in pH with increasing irrigation level. Similarly at 15-30 cm depth, the mean pH values varied from 6.79 – 6.88 with no significant differences observed. EC values showed a minor variation across treatments, with significant effects observed for irrigation schedules and non-significant effect for sowing methods or their interactions. At 0–15 cm depth, EC ranged between 0.24 and 0.26 dS m^{-1}, with the highest value recorded under highest irrigation schedule at IW/CPE 1.2 (0.26 dS m^{-1}). Similar trend was observed at 15–30 cm depth, where EC values increased slightly with irrigation intensity, reaching a maximum of 0.31 dS m^{-1} at IW/CPE 1.2.

Table 1. Effect of different irrigation schedules and sowing methods on available pH, EC and SOC at 0-15 and 15-30 cm soil depth

Sowing methods	Soil depth 0-15 cm														
	pH					EC					SOC				
	Irrigation schedules														
	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean
Flat-bed	6.76	6.73	6.70	6.61	6.70	0.25	0.23	0.24	0.25	0.24	10.65	12.65	14.30	14.82	13.11
Ridge	6.75	6.65	6.68	6.74	6.71	0.24	0.24	0.25	0.26	0.25	11.94	13.80	14.73	14.69	13.79
Mean	6.76	6.69	6.69	6.65		0.25	0.24	0.24	0.26		11.30	13.23	14.52	14.76	
CD (p=0.05)	S: NS I: NS S×I: NS					S: 0.01 I: 0.01 S×I: NS					S: 0.48 I: 0.68 S×I: NS				
Sowing methods	Soil depth 15-30 cm														
	pH					EC					SOC				
	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean
Flat-bed	6.89	6.82	6.80	6.77	6.82	0.28	0.27	0.28	0.31	0.29	8.24	8.94	9.70	10.14	9.26
Ridge	6.86	6.81	6.80	6.81	6.82	0.27	0.28	0.30	0.31	0.29	8.71	9.22	10.47	10.10	9.63
Mean	6.88	6.82	6.80	6.79		0.28	0.28	0.29	0.31		8.48	9.08	10.09	10.12	
CD (p=0.05)	S: NS I: NS S×I: NS					S: NS I: 0.02 S×I: NS					S: 0.23 I: 0.32 S×I: NS				

SOC content exhibited significant variation in response to irrigation schedules and sowing methods at both soil depths (0–15 cm and 15–30 cm). SOC contents were increased with increasing irrigation levels, with the highest SOC (14.76 and 10.12 g kg^{-1}) was at IW/CPE 1.2, which was statistically at par with IW/CPE 1.0 (14.52 and 10.09 g kg^{-1}) at 0-15 and 15-30 cm depths, respectively. Among sowing methods, ridge method of sowing exhibits significantly higher level of SOC (13.79 and 9.63 g kg^{-1}) at 0-15 and 15-30 cm, respectively.

Soil pH remained relatively stable across treatments, indicating that irrigation and sowing methods had minimal influence on soil acidity. Electrical conductivity (EC) increased slightly with higher irrigation level, likely due to enhanced nutrient dissolution and ion mobility. SOC was significantly affected, with higher values under higher irrigation schedule and ridge sowing method which was attributed to improved moisture retention, aeration and microbial activity.

Yield: The biological and marketable yield were significantly influenced by both irrigation schedules and sowing methods (Table 2). Among irrigation schedules, maximum biological (462.0 q ha^{-1}) and marketable yield (324.2 q ha^{-1}) was under I_4 (IW/CPE 1.2), followed by IW/CPE 1.0 (429.5 and 304.0 q ha^{-1}), respectively, indicating positive response to increased irrigation schedules. The ridge sowing outperformed flat-bed method, achieving higher biological (402.5 q ha^{-1}) and marketable yield (279.5 q ha^{-1}) compared to flat-bed method, indicating the superiority of ridge planting in optimizing growth conditions and resource use efficiency. The ridge sowing method under IW/CPE 1.2 produced the highest marketable yield (342.4 q ha^{-1}), followed by ridge at IW/CPE 1.0 irrigation schedule.

The yield attributes were maximum under ridge method which might be due to higher aeration status and moisture availability in ridges that improve soil quality and facilitates better nutrient uptake from soil (Babu et al., 2020, Solanki et al., 2020). For healthy root growth, carrots require loose soil and less compaction that permits taproot development to proceed unhindered and it is made possible under ridge method (Robert et al., 2024). Another possible reason will be ascribed to improved microclimate by switching from flood irrigation to targeted irrigation which leads to better growth of plants (Ciza et al., 2022). Higher frequencies of irrigation reduce soil strength in the root zone and improving nutrient availability which make conducive conditions for proper growth and development of carrot crop (Pal et al., 2020).

Splitting (%): The splitting was observed significantly higher under I_4 (5.50%) and minimum was under I_2 (2.44%) (Table 2). Sowing methods and interaction effect showed a non-significant effect on splitting in carrots. Splitting in carrots is primarily caused by fluctuations in soil moisture at varying irrigation schedules. Excessive irrigation led to rapid uptake of water, causing sudden root expansion and rupture, while irregular watering creates alternating drought and rehydration cycles, increasing mechanical stress and making roots more prone to splitting. Conversely, the lowest splitting was observed under the moderate irrigation schedule (I_2), suggesting that optimal soil moisture conditions reduced mechanical stress on roots, thereby maintaining structural integrity and minimizing splitting (Gutezeit 2001).

NPK uptake: The effect of irrigation schedules, sowing methods and their interaction was significant on the uptake of nitrogen (N), phosphorus (P), and potassium (K) in both roots and shoots of carrot plants (Table 3). The I_4 irrigation schedule (IW/CPE ratio of 1.2) resulted highest nutrient uptake, with significantly greater absorption of N (38.49 kg ha^{-1} in roots and 47.12 kg ha^{-1} in shoots), P (4.39 kg ha^{-1} in roots and 17.07 kg ha^{-1} in shoots), and K (22.14 kg ha^{-1} in roots and 27.29 kg ha^{-1} in shoots). Among sowing methods, ridge method demonstrated superior performance, with maximum uptake of N (30.83 kg ha^{-1} in roots and 39.00 kg ha^{-1} in shoots), P (3.55 kg ha^{-1} in roots and 13.00 kg ha^{-1} in shoots) and K (17.87 kg ha^{-1} in roots and 23.39 kg ha^{-1} in shoots). S_2I_4 combination (ridge sowing under I_4 irrigation) exhibited the highest uptake of all primary nutrients: N uptake of 42.28 kg ha^{-1} in roots and 50.11 kg ha^{-1} in shoots, P uptake of 4.95 kg ha^{-1} in roots and 19.91 kg ha^{-1} in shoots, and K uptake of 24.20 kg ha^{-1} in roots and 29.39 kg ha^{-1} in shoots.

Table 2. Effect of different irrigation schedules and sowing methods on biological yield, marketable yield and splitting

Sowing methods	Biological yield					Marketable yield					Splitting (%)				
	Irrigation schedules														
	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean
Flat-bed	275.2	338.8	409.7	445.9	367.4	168.6	230.3	284.4	306.1	247.3	3.05	2.75	3.37	5.74	3.73
Ridge	307.8	374.9	449.4	478.0	402.5	203.9	247.9	323.6	342.4	279.5	2.83	2.13	3.04	5.26	3.32
Mean	291.5	356.8	429.5	462.0		186.2	239.1	304.0	324.2		2.94	2.44	3.20	5.50	
CD (p=0.05)	S: 3.90 I: 5.52 S×I: NS					S: 2.70 I: 3.82 S×I: 5.41					S: NS I: 0.60 S×I: NS				

Nutrient use is regulated by soil moisture status during the growing season (Maida et al 2020). Higher irrigation schedules (IW/CPE 1.2 and 1.0) led to frequent irrigation, as a result optimum moisture level is maintained during the growing season and it led to higher translocation, mobility and availability of nutrients which might have been efficiently utilized by the crop and resulting in higher nutrient uptake (Kemal 2013). Another reason for higher uptake in I_4 irrigation level might be due to higher vegetative growth in plants (Sharma et al 2023). Maximum yield under ridge method of sowing might be due to higher aeration and moisture availability in ridges which improve soil quality and facilitates better nutrient uptake from soil (Das et al 2020; Singh et al 2021).

Correlation: The correlation matrix was employed to assess the interrelationship among soil physico-chemical properties, microbial populations and yield (Fig. 1). The plot combined pie chart (lower triangle) and elliptical correlation visuals (upper triangle) to effectively communicate both the directions and strength of associations. Intensity of color is directly proportional to correlation coefficient. Red color signifies negative correlation and blue color signifies positive correlation. Circle of pie signifies correlation coefficient, complete circles mean correlation coefficient 1. Yield exhibited very strong positive correlations with microbial population including bacteria, fungi and actinomycetes, suggesting a critical role of soil microbiota in enhancing crop productivity. Similarly, essential nutrients such as soil available nitrogen, phosphorus, potassium, calcium, magnesium, sulphate sulphur and soil organic carbon showed strong positive associations with yield. Conversely, soil pH and EC were exhibited strong negative correlations with yield and soil health indicators, indicating that within the test range, these factors had limited direct impact. These findings highlight the interdependence among chemical and biological soil fertility to carrot yield, offering a robust basis for selecting key indicators in optimizing carrot yield under varying sowing methods and irrigation schedules.

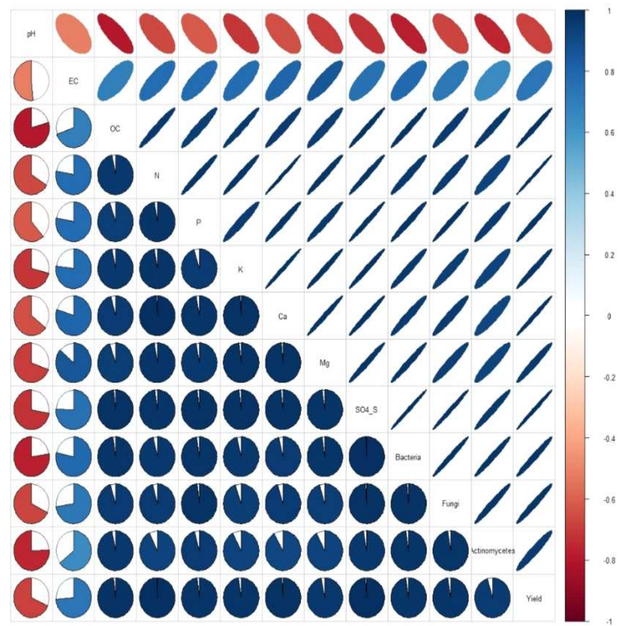

Fig. 1. Correlation matrix of soil properties and carrot yield

Table 3. Effect of different irrigation schedules and sowing methods on NPK uptake (kg ha^{-1}) in roots and shoots

Sowing methods	NPK uptake in roots														
	N					P					K				
	Irrigation schedules														
	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean
Flat-bed	14.99	21.28	30.05	34.69	25.25	1.70	2.39	3.42	3.84	2.84	8.42	12.35	16.80	20.08	14.41
Ridge	19.27	24.22	37.53	42.28	30.83	2.18	2.73	4.34	4.95	3.55	11.48	14.19	21.60	24.20	17.87
Mean	17.13	22.75	33.79	38.49		1.94	2.56	3.88	4.39		9.95	13.27	19.20	22.14	
CD (p=0.05)	S: 0.49 I: 0.69 S×I: 0.97					S: 0.19 I: 0.27 S×I: 0.38					S: 0.44 I: 0.62 S×I: 0.88				
Sowing methods	NPK uptake in shoots														
	N					P					K				
	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean	IW/CPE 0.6	IW/CPE 0.8	IW/CPE 1.0	IW/CPE 1.2	Mean
Flat-bed	23.35	30.42	36.06	44.13	33.49	5.34	8.08	10.53	14.23	9.54	15.40	17.87	21.15	25.18	19.90
Ridge	26.17	32.27	47.46	50.11	39.00	6.11	8.57	17.41	19.91	13.00	16.32	19.90	27.95	29.39	23.39
Mean	24.76	31.35	41.76	47.12		5.72	8.32	13.97	17.07		15.86	18.89	24.55	27.29	
CD (p=0.05)	S: 1.11 I: 1.57 S×I: 2.21					S: 0.37 I: 0.52 S×I: 0.73					S: 0.49 I: 0.70 S×I: 0.98				

CONCLUSION

The irrigation and sowing methods are two important factors for crop production. Ridge sowing combined with higher irrigation schedule (IW/CPE 1.2) significantly enhanced soil organic carbon, nutrient uptake and yield in carrots without adversely affecting pH. Higher irrigation levels improved both yield components but slightly increased splitting percentage. Therefore, it recommended to adopt ridge sowing along with IW/CPE 1.2 for obtaining optimal carrot yield. This combination improved aeration, moisture availability and ensures better soil health, nutrient availability and making it suitable for sustainable crop production.

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Evaluation of Potato Varieties under Various Crop Geometry for Tuber Yield and Quality

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Abstract: The present study was carried out at, Chaudhary Charan Singh Haryana Agricultural University- Hisar, India during *Rabi* season of 2021-22. The field experiment was conducted in split plot design with three potato varieties (Kufri Neelkanth, Kufri Bahar and Kufri Lima) in four different spacing (60×10 cm, 60×15 cm, 60×20 cm with cut tuber and 60×20 cm with whole tuber). The spacing 60×20 cm with whole tuber and varieties Kufri Neelkanth was best for total tuber yield (394.5 q/ha) and biological yield (528.2 q/ha). The maximum foliage dry matter (11.38 %), tuber dry matter content (18.26 %), reducing sugar content (252.2 mg/100g), total sugar (618.4 mg/100g) and total marketable tuber yield (374.4 q/ha) were observed maximum in Kufri Lima, while ascorbic acid content in tuber of Kufri Bahar (27.61 mg/ 100 g). The present studies open up new doors for farmers and researchers to sustainably produce potatoes, which could significantly enhance economic and nutritional benefits.

Keywords: Potato, Crop geometry, *Solanum tuberosum* L

Potato (*Solanum tuberosum* L.) is one of the most important commercial vegetable crops, originated in Andes of Peru and Bolivia and widely grown throughout the world. The world's population is expected to reach 10.5 billion by 2050, increasing food demand by 60% (Alexandratos and Bruinsma 2012). The major problem of potato's low tuber yield are the unavailability and high cost of seed tuber, lack of well-adapted cultivar, and poor agronomic practices (Arega et al., 2018). Maintaining optimal plant population through inter-row and intra-row spacing, as well as other cultural practices, is critical for increasing tuber yield and quality. Cutting seed tuber has been adopted because of the lack of adequate availability of whole seed tuber and also by reducing the seed cost. For normal production, a reasonable size of tuber pieces called as seed tuber should be of about 40 to 50 g. Weather conditions, cultivars, crop geometry, planting of cut tubers, planting date, nutrition and irrigation are only a few of the aspects that influence potato output. Potato plant growth and yield are also determined by the genetic potential of a variety and spacing requirements (Kumar et al., 2009). Soil and time of planting have great influence on potato yield and quality of tubers.

Good quality seed is almost universally considered a requirement for high productivity in all potato production systems. Much of the yield gap currently constraining productivity is attributed to the poor quality of seed. Potato seed sector development is thus a major concern of governments, researchers, development agencies and civil society organizations (Forbes et al., 2020). Cutting seed tuber has been adopted because of the lack of adequate availability of whole seed tuber and also by reducing the seed cost. However, cut surface may be susceptible to attack by soil borne fungi, particularly during the cool and wet conditions. Whole seed tubers of potato (*Solanum tuberosum* L.) have been reported to have some performance advantages over seed pieces produced by cutting whole tubers, even if the cut seed is treated with a fungicide (Kawakami et al., 2003). Small and medium size seed tubers are preferred by farmers engaged in small scale cultivation to reduce seed cost under Bangladesh conditions (Islam et al., 2012). Therefore, this study planned to find out the optimum crop geometry using cut seed tuber of potato and to evaluate the performance of different varieties using cut seed tuber plantation for higher yield.

MATERIAL AND METHODS

The present study was carried out at, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experimental site is situated at 29°10"N latitude and 75°46"E longitude with 215.2 m above mean sea level in north-west part of India coming under Trans- Gangetic Plains agro climatic zone. The treatments comprising of three potato varieties; (V_1):- Kufri Neelkanth, (V_2):- Kufri Bahar and (V_3):- Kufri Lima (V_3) and four different spacing; (S_1):- 60×10 cm with cut tuber, (S_2):- 60×15 cm with cut tuber, (S_3):- 60×20 cm with cut tuber and (S_4):- 60×20 cm in a randomized block design (factorial) with three replications keeping gross plot size 4.8 x 3.6 m^2 net plot size 3.6 x 3.0 m^2. Tubers of 2.5-3.0 cm diameter disease free certified seed tubers were used and recommended package of practices (Singh et al., 2022). The foliage weight and total tuber yield was recorded at the time of harvesting. The tubers above 75 g were counted in 'A' grade, 50-75 g were placed in 'B' grade, 25-50 g were kept in 'C' grade and less than 25 g were noted in 'D' grade.

The mean weekly maximum temperature ranged between 17.1 to 32.0ºC and minimum between 5.2 to 19.6ºC and relative humidity ranged between 88 to 97 per cent. Field soil belongs to inceptisol order and sandy loam texture with 55% sand, 34% silt and 11% clay. Field soil contain organic carbon (0.44 %), available nitrogen (128 kg/ha), medium phosphorus (28.5 kg/ha) and available potassium (378 kg/ha), the fertility status of the experimental area was poor. Field soil is also little bit alkaline having pH value of 7.9. The month wise total precipitation, average maximum & amp; minimum temperature, relative humidity, sunshine hours and evaporation of experimental site of the crop seasons (Table 1). The data were statistically analysed by using OPSTAT-Online Statistical Analysis Tools.

RESULTS AND DISCUSSION

Dry matter foliage and tuber: The dry matter of foliage (12.06 %) and dry matter of tubers (18.04%) was significantly higher in 60×20 cm spacing with whole tuber planting, while lowest dry matter of foliage (10.22 %) and dry matter of tubers (16.56 %) was with 60×10 cm spacing with cut tuber planting (Table 2). The highest dry matter content under wider spacing with whole tuber planting may be due to proper plant stand and receiving proper sunlight which resulted in higher photosynthetic activity and accumulation of higher dry matter (Nayak 2022).

The higher dry matter of foliage (11.38 %) was maximum in Kufri Lima followed by Kufri Neelkanth (11.17 %) and dry matter of tubers in Kufri Lima (18.26 %) which was significantly higher as compared to other two varieties of potato. The higher dry matter in Kufri Lima may be due to the better foliage growth and large size leaves in Kufri Lima variety of potato. Mozumder et al. (2014) found that the maximum dry matter of both haulms and tubers for the cultivar Kufri Shailja and Kufri Himalini as compared to the cultivar Kufri Jyoti under climatic conditions of West Bengal. Nagar *et al.* (2019) recorded significantly higher tuber dry matter for variety Kufri Bahar followed by Kufri Pukhraj, Yadav et al. (2022) also reported higher number of tubers in Kufri Sadabahar as compared to Kufri Khyati under the climatic conditions of Hisar.

Total sugar content, non-reducing sugar content and ascorbic acid in tuber: Maximum reducing sugar, non-reducing sugar and total sugar were recorded (254.6, 348.7 & 603.2 mg/100g) with spacing of 60×20 cm using whole tuber for planting, which was significantly at par with 60×20 cm using cut tuber for planting except non reducing sugar, while minimum sugar content, non-reducing sugar and total sugar (220.3, 322.9 & 543.2 mg/100g) were with 60×10 cm) spacing using cut tuber for planting (Table 3).

Among varieties, the significantly higher reducing sugar content and total sugar were observed in Kufri Lima (252.2 & 618.4 mg/100g) followed by Kufri Neelkanth (237.8 & 575.6 mg/100g) except non reducing sugar content in Kufri bahar

Table 2. Effect of plant spacing using cut seed tuber and potato varieties on dry matter of foliage and dry matter content of tubers (%)

Plant spacing	Dry matter of foliage (%)	Dry matter of tuber (%)
S_1	10.22	16.56
S_2	10.33	16.96
S_3	11.00	17.16
S_4	12.06	18.04
CD (p=0.05)	0.82	0.83
Varieties		
V_1	11.17	17.15
V_2	10.17	16.13
V_3	11.38	18.26
CD (p=0.05)	0.71	0.72

Table 1. Monthly actual weather parameters of the experimental site during 2021-22

Rabi season	T max (°C)	T min (°C)	RH (%)	Sunshine (hours)	Rainfall (mm)	Evaporation (mm)
October	32.0	19.6	88	7.3	3.2	3.2
November	27.9	9.9	90	5.5	0.0	1.8
December	21.3	6.3	95	5.0	0.0	1.3
January	17.1	5.2	97	3.9	10.4	1.0
February	22.7	6.8	93	7.2	10.9	2.1
March	25.9	12.4	92	6.1	95.2	3.1

Tmax- maximum temperature, Tmin- minimum temperature, RH-relative humidity

(286.4 mg/100g) followed by Kufri Lima (366.3 mg/100g). Verma et al. (2022) recorded significant variation in reducing sugar content among the varieties, being maximum with Kufri Gaurav and minimum with AICRP-P-39. The results are in accordance with findings of Kumar et al. (2003), Jatav et al. (2017), Kumar and Ezekiel (2006) and Kaur and Khurana (2017).

The ascorbic acid was observed highest (26.16 mg/100g) with 60×20 cm spacing using whole tuber for planting, however lowest ascorbic acid content (22.82 mg/100g) was in spacing 60×20 cm with cut tuber planting. Significantly maximum (27.61 mg/ 100 g) ascorbic acid was in Kufri Bahar and considerably lowest ascorbic acid (20.91 mg/ 100 g) was recorded in Kufri Lima. Significant difference in ascorbic acid content in potato varieties might be due to the genetic characteristic of the variety. These findings are supported by Brar (2013).

Total number of tubers and different potato grades: The total number of tubers, number of tubers in different grades, *viz.* up to 25, 25-50, 50-75 and >75 g per square meter area recorded in the range of 61.7-75.3, 12.4-21, 17.6-22.5, 14.5-18 and 14.4-17, respectively (Table 4 and 5). The maximum

Table 5. Effect of plant spacing using cut seed tuber of different potato varieties weight of tubers (kg/m^2)

Plant spacing	"A" Grade (>75)	"B" Grade (50-75)	"C" Grade (25-50)	"D" Grade <25)
S_1	2.15	1.08	0.81	0.28
S_2	2.11	1.00	0.78	0.34
S_3	1.94	0.90	0.76	0.38
S_4	2.24	1.07	0.83	0.25
CD (p=0.05)	0.16	0.12	0.06	0.05
Varieties				
V_1	1.99	1.00	0.94	0.45
V_2	1.83	0.90	0.93	0.36
V_3	2.50	1.14	0.52	0.13
CD (p=0.05)	0.14	0.14	0.09	0.04

Table 3. Effect of plant spacing using cut seed tuber of different potato varieties on reducing and non-reducing sugar content in tuber, total sugar content and ascorbic acid in tuber

Plant spacing	Reducing sugar content in tuber	Non-reducing sugar content in tuber	Total sugar in potato tuber	Ascorbic acid in tuber
S_1	220.3	322.9	543.2	25.0
S_2	233.6	326.1	559.7	24.2
S_3	249.1	323.0	571.1	22.8
S_4	254.6	348.7	603.2	26.2
CD (p=0.05)	18.5	21.2	26.4	0.65
Varieties				
V_1	237.8	337.8	575.6	25.1
V_2	228.3	286.4	514.7	27.6
V_3	252.2	366.3	618.4	20.9
CD (p=0.05)	16.0	28.7	22.9	0.5

Table 4. Effect of plant spacing using cut seed tuber of different potato varieties on total number of tubers (kg/m^2)

Plant spacing	Total no of tubers/m^2	"A" Grade (>75)	"B" Grade (50-75)	"C" Grade (25-50)	"D" Grade <25)
S_1	75.3	15.9	17.0	21.4	21.0
S_2	67.7	15.6	16.2	19.5	16.0
S_3	61.7	14.4	14.5	17.6	15.2
S_4	70.3	17.0	18.0	22.5	12.4
CD (p=0.05)	5.9	1.2	1.6	2.6	4.4
Varieties					
V_1	62.4	18.5	17.5	15.8	10.6
V_2	68.0	13.2	14.9	21.7	18.0
V_3	75.9	15.5	16.9	23.7	19.9
CD (p=0.05)	5.1	1.1	1.4	2.4	3.9

number of up to 25 g size tubers per square meter (21.0) was observed with 60×10 cm spacing using cut tuber for planting and minimum was found (12.4) with 60×20 cm spacing using whole tuber for planting.

More number of small size tubers in closer spacing may be due to more competition among the plants resulted lesser accumulation of photosynthate and finally tubers remains small size, while under wider spacing, less number of small size tuber may be due to that plants accumulate more food which stored in tubers ultimately tubers became large size. The number of tubers per square meter in 25-50, 50-75 & >75 g grade under different plant spacing were recorded significantly maximum (22.5, 18.0 & 17.0) with whole tuber planting at 60×20 cm spacing which was significantly at par with cut tuber planting at 60×10 cm (21.4, 17.0 & 15.9). Total number of tubers was recorded significantly maximum (75.3) with 60×10 cm spacing using cut tuber for planting, which was significantly maximum as compared to other plant spacing. This may be due to more plant population per unit area under closer spacing resulted more number of tubers per unit area. Kumar et al. (2009) and Arega et al. (2018) also reported that cut tuber produced significantly higher number of tubers per plant as compared to the whole tubers planted.

Among the varieties, maximum number of tubers up to 25 & 25-50 g grade (19.9 & 23.7) were recorded under Kufri Neelkanth and was closely followed with Kufri Bahar (18.0 & 21.7) and maximum number of 50-75 g grade tubers (17.5/m^2) was recorded in Kufri Lima which was closely followed by Kufri Neelkanth (16.9/m^2). Kufri Lima produced significantly maximum number of tubers (18.5/m^2) of >75 g grade as compared to other varieties (Table 4). Total number of tubers per square meter area (75.9) was recorded significantly maximum in Kufri Neelkanth which was significantly higher as compared to other two varieties. The maximum number of tubers in Kufri Neelkanth may be due to genetic makeup of this variety. The results of the present investigation are supported by findings of Banjare et al. (2014) and Kumar et al. (2009). Yadav et al. (2022) also reported higher number of tubers in Kufri Sadabahar as compared to Kufri Khyati under the climatic conditions of Hisar.

The yield of tubers in different grades viz. up to 25, >25-50, >50-75 and >75 g size affected by different plant spacings was found in the range of 0.25-0.38, 0.76-0.83, 0.90-1.08 and 1.94-2.24 kg/m^2, respectively (Table 5). The maximum yield of up to 25 g size tubers per square meter (0.38 kg) was observed with 60×20 cm spacing using cut tuber for planting which was at par with 60x15 cm spacing using cut tuber for planting. The maximum yield of tuber in >25-50 g grade under different plant spacing was recorded significantly maximum (0.83 kg) with whole tuber planting at 60×20 cm spacing which was significantly at par with cut tuber planting at 60×10 cm (0.81 kg) and also 60×15 cm (0.78 kg). The maximum yield of tuber in >50-75 g grade (1.08 kg/m^2) was noted with plant spacing 60×10 cm using cut tuber for planting, which was significantly at par with 60×20 cm (1.07 kg/m^2) using whole tuber and 60×15 cm (1.0 kg/m^2) using cut tuber for planting. Maximum yield of >75 g grade tubers (2.24 kg/m^2) with 60×20 cm spacing using whole tuber for planting, which was significantly higher than cut tuber planting under same spacing and at par with 60x10 cm spacing (2.15 kg/m^2) as well as 60x15 cm (2.11 kg/m^2) using cut tuber. This may be due to that under wider spacing and whole tuber planting there was a proper growth and development of the plant resulted more yield of large size tubers, while under closer spacing using cut tuber there was higher plant population per unit area which increases the yield of all size tuber. The present findings were also confirmed the results of Malik et al. (2002) and Birhanu et al. (2018) reported that the yield per plant and tuber yield per hectare were higher with whole tubers planting as compared to cut tubers.

Among different potato varieties, significantly maximum yield of small size tubers (up to 25 & 25-50 g) were recorded (0.45 & 0.94 kg/m^2) in Kufri Neelkanth as compared to other two varieties and minimum (0.13 & 0.52 kg/m^2) were in Kufri Lima. Kufri Lima yielded maximum weight of large size tuber (50-75 & >75 g grade), which was significantly higher as compared to other varieties except Kufri Neelkanth in 50-75 & >75 g size and the minimum yield of tubers (0.90 & 1.83 kg/m^2) was in Kufri Bahar. More number of large size tubers in Kufri Lima may be due to early bulking behaviour of this variety because of good foliage spread, more area was exposed to sunlight that increased photosynthesis activity

Table 6. Effect of plant spacing using cut seed tuber of different potato varieties on total tuber yield, marketable tuber yield and biological yield (q/ha)

Plant spacing	Total tuber yield (q/ha)	Marketable tuber yield (q/ha)	Biological yield (q/ha)
S_1	389.0	363.6	568.8
S_2	380.9	349.9	548.8
S_3	357.3	323.4	506.0
S_4	395.8	373.0	583.9
CD (p=0.05)	21.75	19.9	26.25
Varieties			
V_1	394.5	354.3	582.2
V_2	361.5	328.7	520.5
V_3	386.2	374.4	552.8
CD (p=0.05)	18.84	17.3	22.74

Table 7. Effect plant spacing using cut seed tuber of different potato varieties on economics of different treatments

Treatments	Total cost (Rs.)	Gross return (Rs.)	Net return (Rs.)	B:C ratio (ha)
V_1S_1	178354	397975	219621	2.23
V_1S_2	153354	393325	239971	2.56
V_1S_3	128354	378867	250513	2.95
V_1S_4	128354	407842	279488	3.18
V_2S_1	178354	369450	191096	2.07
V_2S_2	153354	359267	205913	2.34
V_2S_3	128354	339775	211421	2.65
V_2S_4	128354	377500	249146	2.94
V_3S_1	178354	399467	221113	2.24
V_3S_2	153354	390033	236679	2.54
V_3S_3	128354	353179	224825	2.75
V_3S_4	128354	401967	273613	3.13

and thus increased starch accumulation, which led to more number of large size tubers. The present findings are in accordance with the results reported by Arega et al. (2018) and Qasim et al. (2013) in potato.

Yield: The data as shown in revealed that different plant spacing's and potato varieties had a remarkable impact on total tuber yield , marketable yield and biological yield (Table 6). The maximum total tuber yield (395.8 q/ha) was with plant spacing 60×20 cm using whole tuber for planting, which was significantly at par with 60×10 cm and 60×15 cm using cut tuber for planting and the maximum total marketable tuber yield (373.0 q/ha) was with plant spacing 60×20 cm using whole tuber for planting, which was significantly higher as compared to other spacing except 60×10 cm spacing using cut tuber for planting (363.6 q/ha).

Significantly, the maximum biological yield (583.9 q/ha) was with plant 60×20 cm spacing using whole tuber for planting, which was significantly at par (568.8 q/ha) with plant 60×10 cm spacing using cut tuber for planting. This may be due to that under wider spacing and whole tuber planting there was a proper growth and development of the plant resulted more yield of large size tubers which ultimately increases the marketable yield, total yield as well as biological yield, while under closer spacing using cut tubers there was higher plant population per unit area which increases the yield of all of size tuber. Malik et al. (2002) and Birhanu et al. (2018) also reported that the tuber yield per hectare were higher with whole tubers planting as compared to cut tubers under same plant spacing.

Among potato varieties, the total tuber yield varied between 361.5 to 394.5 q/ha. Significantly maximum total tuber yield was observed in Kufri Neelkanth, which was statistically at par with Kufri Lima, while Kufri Lima resulted maximum total marketable tuber yield (374.4 q/ha) which was significantly higher as compared to other varieties. The biological yield varied between from 520.5 to 582.2 q/ha. Significantly maximum in Kufri Neelkanth as compared to other varieties. The maximum tuber yield in Kufri Neelkanth may be due to better growth parameters which resulted higher yield. Kufri Lima produced more number of large size tuber because of their genetic behaviour resulted higher marketable and biological yield. The varietal difference in potato varieties with respect to tuber yield, marketable yield as well as biological yield was also observed by Yadav et al. (2022). The present findings are in accordance of results reported by Abrha et al. (2014) and Birhanu et al. (2018). The interaction effect between different plant spacings and potato varieties was found non-significant for tuber yield, marketable yield as well as biological yield.

Economics: The data on economics of different treatments of different spacing's (Table 6), the maximum net income (Rs. 2,79,488/ha) and benefit-cost ratio (3.18) was obtained from Kufri Neelkanth variety of potato planted at a spacing of 60×20 cm with whole tuber.

This may be due to higher total tuber yield under this treatment combination and less cost of cultivation because of less seed rate was used under 60x20 cm spacing as compared to closer spacing's. Yadav et al. (2022) also reported higher net return and benefit cost ratio in the treatment where maximum tuber yield was obtained. The present results are accordance with the findings of Agrawal et al. (2016) and Alam et al. (2016).

CONCLUSION

The study highlights the importance of choosing the right potato variety and spacing to maximize both yield and profitability. Based on above study the planting of 50-60 g size of cut tubers at a spacing of 60 cm × 15 cm was viable option for growing potato for higher yield. The Kufri Neelkanth and Kufri Lima could be used for getting higher returns by the farmers. Potato tubers must be stored properly in order to ensure a consistent supply in the market. This study also suggests the proper monitoring of tuber quality at harvest and storage conditions in storage for maintenance of tuber quality.

AUTHORS CONTRIBUTION

Conceptualization of research (Sandeep Dagar), Designing the experiment & Contribution of experimental materials (V.P.S. Panghal), Execution of field/lab experiments and data collection (Dharmendra K. Janghel), Analysis of data and interpretation (Vijay Daneva) and preparation of the manuscript (Harender Dagar).

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Optimizing Substrate Composition for Enhanced Growth and Corm Development in *Freesia hybrida* under Sub-Tropical Conditions of Punjab

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Abstract: *Freesia hybrida*, a specialty cut flower, is primarily cultivated in India in the cooler climates of the Western Ghats, the Nilgiri Hills, and certain Himalayan regions. However, the sandy soils prevalent in many Indian plains present challenges such as poor water retention and low fertility. This necessitates developing tailored potting media for optimal *Freesia* cultivation. This study investigated the influence of various growing media on the growth, flowering, and corm development of *F. hybrida* under controlled conditions for two consecutive years (2022–2023 and 2023–2024). A total of seven treatments consisting of different combinations of cocopeat, farmyard manure (FYM), perlite, vermicompost, leaf mould, and rice husk were evaluated against control treatment (soil + FYM). Cocopeat-based treatments consistently outperformed the control across all measured parameters. The combination of cocopeat, FYM and perlite yielded the best overall performance, resulting in the tallest plants (31.14 cm), earliest flowering (105.35 days), longest flowering duration (20.85 days), and largest, heaviest corms. The findings emphasize the importance of using improved soilless media, particularly cocopeat enriched with FYM and perlite, for optimizing freesia cultivation in the sub-tropical regions of Punjab.

Keywords: *Freesia hybrida*, Potting media, Cocopeat, Farmyard manure, Perlite

Freesia (*Freesia hybrida*), a valued ornamental plant of the Iridaceae family, is known for its vibrant colours, fragrant blooms, and elegant appearance. Native to South Africa and named after German botanist Friedrich Heinrich Theodor Freese in 1830. Freesia is now cultivated across the globe. In India, it is primarily grown in the cooler regions of the Western Ghats, Nilgiri Hills, and parts of the Himalayas, where favourable climatic conditions support its optimal growth and flowering. The success of freesia cultivation, particularly in containers or non-traditional growing areas, is largely influenced by the quality of potting media, which affects root development, nutrient uptake, moisture retention, and overall plant health. However, sandy soils commonly available in many parts of India, pose significant limitations due to their poor water-holding capacity, low organic matter content and limited nutrient availability (Herawati et al., 2021). To address these challenges, incorporating organic and inorganic amendments into potting media has proven effective in improving soil structure and fertility. Organic composts and soil conditioners enhance water retention, aeration and microbial activity (Thakur et al. (2023), thereby supporting healthier and more vigorous plant growth, and improve soil fertility and structure (Chauhan, 2014). Given these limitations, there is a pressing need to develop customized potting media tailored to the specific requirements of *Freesia hybrida*, especially in sub-tropical regions of Punjab. Hassan et al. (2025) emphasized that the choice of growing media significantly influences freesia's vegetative and reproductive performance. Therefore, this study aims to evaluate the impact of various potting media compositions on the growth, flowering, and corm development of *Freesia hybrida*. The findings will provide valuable insights for optimizing cultivation practices, enhancing flower and corm quality, and promoting sustainable, cost-effective media options suitable for both commercial and small-scale growers.

MATERIAL AND METHODS

Experimental site: The present study on “Optimizing substrate composition for enhanced growth, flowering and corm development in *Freesia hybrida* in sub-tropical region of Punjab” was conducted at Punjab Agricultural University, Ludhiana, during the years 2022-2023 and 2023-2024. Ludhiana, located at 30.91° North latitude and 75.85° East longitude, experiences a subtropical climate with hot, humid summers and cold, frosty winters. The region's soil is predominantly sandy loam, with a slightly alkaline pH, low phosphorus content, but medium to high levels of potassium, nitrogen, and organic carbon, making it suitable for ornamental plant cultivation. The aim of this experiment was to evaluate the impact of various potting media on the growth and flowering characteristics of *Freesia hybrida* under nursery-based growing conditions.

Treatment details: Completely randomized design was employed with seven different potting media treatments, replicated three times. The treatments tested in the study were: T1 – Soil + FYM (1:1) (Control), T2 – Cocopeat + FYM + Perlite (2:1:1), T3 – Cocopeat + Leaf mould + Perlite (2:1:1), T4 – Cocopeat + Vermicompost + Perlite (2:1:1), T5 – Cocopeat + FYM + Rice husk (2:1:1), T6 – Cocopeat + Leaf mould + Rice husk (2:1:1), and T7 – Cocopeat + Vermicompost + Rice husk (2:1:1). The experiment involved planting *Freesia* corms sourced from Delhi, which were placed in 15 cm pots filled with the respective media. The observations were recorded for various growth, flowering and corm parameters. The cocopeat bricks used in the media treatments were soaked in water for one day before being mixed with the other components. Organic amendments such as vermicompost, FYM and leaf mould were collected from the Floriculture Research Farm and prepared using standard composting procedures.

Statistical analysis: Statistical analysis was performed using SAS software version 9.0.

RESULTS AND DISCUSSION

The growth attributes of *Freesia hybrida* were significantly influenced by the various potting media combinations (Table 1). Treatment T2, consisting of cocopeat + FYM + perlite (2:1:1), demonstrated superior performance across all parameters, including the highest plant height (47.5 cm), shortest number of days to flowering (105.35 days), largest flower diameter (5.12 cm), longest flowering duration (20.85 days), highest final corm weight (5.76 g) and the largest final corm diameter (23.59 mm).

In comparison, the control treatment (T1), consisting of soil + FYM (1:1), recorded the lowest values across these parameters. T4 (cocopeat + FYM + perlite) and other combinations like T5, T6, and T7 showed intermediate results, with T4 performing better in most growth and flowering parameters. Overall, T2 consisting of cocopeat + FYM + perlite (2:1:1), proved to be the most effective potting medium, significantly enhancing the growth and flowering attributes of *Freesia hybrida*. The enhanced plant height observed in these media combinations can be attributed to the high nutrient content, optimal porosity and aeration, which allowed better root development and nutrient uptake. This aligns with the previous research findings, which have consistently highlighted the positive impact of cocopeat, especially when combined with organic amendments like vermicompost or FYM, on plant growth (Moghadam et al., 2012).

Higher leaf numbers per plant in T2 and T4 contributed to increased carbohydrate reserves, which promoted quicker transition from vegetative to reproductive growth stages, thus leading to earlier flowering. Seyedi et al. (2012) also observed similar trend on various plant species, including lilium. Both T2 (cocopeat + FYM + perlite) and T4 (cocopeat + vermicompost + perlite) resulted in larger flower diameters, highlighting the significant role of the growing media in determining floral characteristics. Larger flowers are often associated with higher availability of photosynthates and optimal nutrient conditions, both of which were provided by the media combinations containing cocopeat, FYM, and vermicompost. The presence of these organic amendments enriched the media with essential nutrients such as nitrogen, potassium, and phosphorus, which are critical for flower development (Kala et al., 2020).

The duration of flowering was also significantly extended in T2 and T4, aligning with earlier studies that beneficial effects of cocopeat-based media on flower longevity (Cortes et al., 2011, Kameshwari et al., 2014). The increased corm weight in T2 and T4 supports findings on the positive effects of cocopeat and vermicompost on corm development in other bulbous crops (Moghadam et al., 2012, Rajera and Sharma, 2017).

Cocopeat is recognized for its high content of exchangeable and DTPA-extractable ions (Aswath and Pillai 2004). Studies have demonstrated that combining cocopeat with other organic materials, such as vermicompost and

Table 1. Effect of different growing media combinations on vegetative and flowering attributes of *Freesia hybrida* (Pooled data of year 2023-24)

Treatments	Plant height (cm)	Number of days to flowering	Flower diameter (cm)	Flowering duration (days)	Final corm weight (cm)	Final corm diameter (mm)
T1	22.01 e	124.43 d	4.16 b	12.68 f	3.81 c	18.57 e
T2	31.14 a	105.35 a	5.12 a	20.85 a	5.76 a	23.59 a
T3	23.23 b	122.48 d	4.29 b	13.99 de	4.08 c	20.24 cd
T4	28.75 ab	111.60 b	4.87 a	19.56 b	5.19 ab	22.21 b
T5	25.85 cd	116.07 c	4.38 b	15.62 cd	4.02 c	20.81 c
T6	24.99 d	117.56 c	4.18 b	14.98 de	3.58 c	19.05 de
T7	26.71 c	115.22 c	4.44 b	17.84 bc	4.54 bc	21.29 bc

Different letters in column indicate statistical differences at 5 percent farmyard manure, significantly enhances plant growth and flowering in species like croton (Anjana et al., 2017) and tuberose (Nair et al., 2019). Additionally, mixing cocopeat with vermiculite, soil, or perlite has been shown to improve flowering characteristics in Lilium (Chaudhary et al., 2018). The higher nutrient content and better water retention of these media provided an environment conducive to nutrient uptake and storage in the corms, leading to heavier final corm weights. The diameter of the corms was also significantly larger in T2 and T4. The optimal conditions provided by these media likely promoted better corm development, with sufficient nutrients and adequate aeration. Nikrazm et al. (2011), also observed that cocopeat-based media significantly increased bulb size in Lilium.

CONCLUSION

The use of cocopeat-based growing media, particularly when combined with organic amendments like vermicompost and FYM, has a positive impact on several key parameters of plant growth, flowering, and corm development. The combination of cocopeat + FYM + perlite and cocopeat + vermicompost + perlite provided optimal conditions for plant height, early flowering, larger flower size, prolonged flowering duration, and increased corm weight and diameter. These findings underscore the importance of selecting appropriate growing media for optimizing plant growth and yield in bulbous crops.

AUTHORS' CONTRIBUTIONS

R K Dubey and Simrat Singh developed the concept of the experiment. Gururaj executed the trial and collected and analysed the data. Gururaj, R K Dubey, Kritika Pant and Uma Patel prepared the manuscript.

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Efficacy of Plant Defence Activators for Disease Reduction and Growth Promotion in Bell Pepper (*Capsicum annuum* L.) in Sub-Temperate, Semi-Humid Climate Conditions

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Abstract: Bell pepper production is often constrained by diseases such as Cercospora leaf spot, anthracnose, bacterial spot, Phytophthora rot, and viral infections. This study evaluates the efficacy of plant defence activators, including salicylic acid (SA), jasmonic acid (JA), β-aminobutyric acid (BABA), and potassium nitrate (KNO_3), as eco-friendly strategies for disease management. For this, bell pepper seeds were subjected to seed priming with these activators at various concentrations for 4-10 h before sowing. The effects of these treatments on seed quality and mycoflora incidence were assessed to optimize priming protocols for enhanced plant resistance. Seed priming with 75 ppm SA for 8 h increased germination up to 85%, boosted seed vigor index-length (SVI-I) from 856.88 to 1,078.44, and seed vigor index-mass (SVI-II) from 161.25 to 234.39. KNO_3 priming at 1.5% for 10 h enhanced germination up to 87%, SVI-I from 856.88 to 1,108.38, and SVI-II from 161.25 to 229.03, while reducing seed-borne fungal incidence from over 22% in untreated seeds to as low as 2-3%. BABA showed an optimal effect at moderate concentrations, while higher MeJA doses had inhibitory impacts on early seedling growth. These results demonstrate that seed priming treatments can significantly enhance seed quality, vigor, and disease resistance in bell pepper, offering a sustainable and effective alternative to chemical methods.

Keywords: Seed priming, Seed quality, Seed-borne diseases, Sustainable agriculture, Plant defence activators

Bell pepper (*Capsicum annuum* L.) is a globally cultivated solanaceous vegetable known for its rich nutritional value, particularly high in vitamins A and C and antioxidants, moderate in protein, fiber, carbohydrates, fat, and minerals, boosting immunity and heart health (Sanatombi 2023). In India, bell pepper is grown mainly in Himachal Pradesh, Andhra Pradesh, Uttarakhand, and Darjeeling district of West Bengal as a summer crop and in the states of Maharashtra, Karnataka, Uttar Pradesh, and Tamil Nadu as an autumn crop (Sreedhara et al., 2013). However, its cultivation faces significant challenges due to susceptibility to several biotic and abiotic stresses, especially during humid seasons. To tackle these issues sustainably, plant defence elicitors like salicylic acid, jasmonic acid, butyric acid, and potassium nitrate are being used to enhance the plant's innate immunity without harming the environment (Bektas and Eulgem 2015). Among these, salicylic acid, a phytohormone, is a simple phenolic compound that is actively involved in the regulation of diverse metabolism in plants influencing its growth and development involving seed germination, photosynthesis, stomatal regulation, flowering, senescence, yield and defence mechanism against biotic and abiotic stresses (Mishra et al., 2024). It facilitates internal defence signaling and plays a critical role in triggering systemic resistance throughout the plant (AL-surhanee 2022). Seed priming with salicylic acid enhance plant tolerance to biotic stresses by induction of hypersensitive response (HR) via regulation of antioxidant enzymes and to abiotic stresses by activating defence-related genes and transporters, regulating ion balance and boosting stress resilience (Khan et al., 2022). Likewise, jasmonates (jasmonic acid and its derivatives like methyl jasmonate) are plant hormones having major function in regulation of growth and development of plant from germination of seed to maturation and ageing of the plant. Jasmonic acid (JA) is synthesized by the action of several enzymes which get augmented in response to stress and regulate stress tolerance in plants (Mulaudzi et al., 2023). It plays a crucial role in plant defence against necrotrophic pathogens and insect herbivores by activating the COI1-JAZ signaling pathway, which leads to the release of transcription factors that trigger JA-responsive gene expression (Koo et al., 2020). Besides, β-amino butyric acid (BABA) is a naturally occurring plant metabolite which effectively imparts broad spectrum defence in plants. It activates the regulation of oxidative stress through increased antioxidant activity, protects cell membrane against osmotic stress and promotes plant growth (Bhutta et al., 2023), modulates expression of defence genes, and alters several metabolic pathways bolstering the immunity of plants and enhancing their resistance against biotic and abiotic stresses (Catoni et al., 2022). Moreover, potassium nitrate (KNO_3) acts both as a nitrogen source as well as a signaling molecule which helps in enhancing seed germination and plant growth by regulating hormonal balance, specifically gibberellic acid and abscisic acid, activation of aquaporins, enhancing amylase activity and nitric oxide production which helps break dormancy through interactions with phytochrome signaling, ethylene biosynthesis, and reactive oxygen species (Nyandwi et al., 2024). These elicitors are usually applied through seed priming, a pre-sowing treatment involving controlled hydration (without allowing germination) of seeds that activate the metabolic processes to enhance germination, utilizing stored nutrients, increasing enzyme activity and responding more effectively to environmental stress resulting in quicker and more uniform germination (Özkurt and Bektaş 2022). Therefore, in this context, this research aimed to explore eco-friendly disease management strategies for bell pepper using plant defence activators (SA, JA, BABA, KNO_3) and standardize their doses under laboratory conditions.

MATERIAL AND METHODS

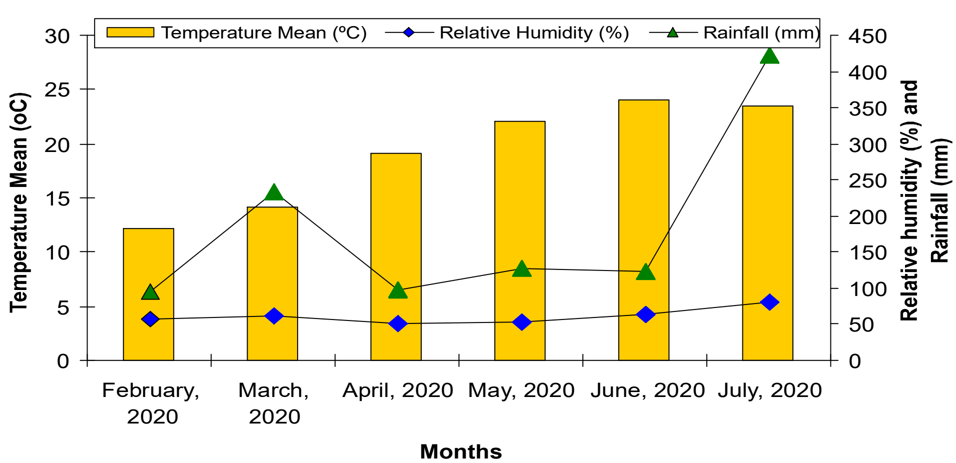
Location and experimental details: The present investigation was carried out during 2019-20 at Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (30.51°N, 77.09°E, Elevation: 1183m) located in the mid-hill zone of Himachal Pradesh features sub-temperate, semi-humid climate conditions with cold winters and hot summers., December and January are the coldest while, May and June are the hottest months (Fig. 1). The study was conducted on bell pepper, cultivar Solan Bharpur. Seeds were surface sterilized with 1% solution of sodium hypochlorite (NaOCl, Central Drug House PVT. Ltd) two minutes, followed by three washings with distilled water to remove the traces of NaOCl. The experiment involved seed priming with four plant defence activators at varying concentrations, each concentration for the durations of for 4, 6, 8, and 10 h: salicylic acid (SA, $C_6H_4(OH)COOH$, Central Drug House Pvt. Ltd.), (25, 50, 75, 100 ppm); methyl jasmonate (MeJA, $C_{13}H_{20}O_3$, Sigma Aldrich), (25, 50, 75, 100 ppm); potassium nitrate (KNO_3, Life Sciences Pvt. Ltd.), (0.5, 1.0, 1.5, 2.0%); and β-Aminobutyric Acid (BABA, $C_4H_9NO_2$, Sigma Aldrich), (25, 50, 75, 100 ppm).

Priming solutions were prepared by measuring required amounts of SA (25, 50, 75, and 100 mg) and mixed with ethanol, subsequently adding drop-wise to distilled water (ethanol/water: 1/1000, v/v) as described by Khandaker et al., (2011). The required amounts of MeJA (25, 50, 75 and 100 µl), BABA (25, 50, 75 and 100 mg) and KNO_3 (0.5, 1, 1.5 and 2 g) were initially dissolved in a small amount of distilled water and were diluted to required final volume with additional distilled water (Kazemi, 2014; Kim et al., 2013; Nego et al., 2015).

The prepared solutions were used for seed priming. Seeds were soaked in the solutions at a volume five times that of the seeds and maintained at room temperature (25ºC) for the required duration. After priming, the seeds were rinsed with distilled water and dried in the shade for 24 h before further use (Shatpathy et al., 2018).

***In vitro* trials**

Rolled paper towel method: The germination and vigor tests were performed using paper towel method as per the ISTA rules in which, over a wax paper, a moist germination



Source: Meteorological Observatory, Department of Environmental Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) 173 230

Fig. 1. Mean monthly meteorological data of Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) for the year 2020 (*w.e.f.* February 2020 to June 2020)

paper was placed and 100 treated seeds were orderly laid on it. Seeds were covered with another moist germination paper and rolled carefully to avoid pressure on the seeds. These rolled towels were kept for incubation in seed germinator for 14 days at 25ºC (Anonymous 1996).

Blotter paper test: The procedure for the standard blotter paper test was followed (ISTA, 2005). One or more layers of blotter paper were placed in the Petri plates after soaking in sterilized water. In aseptic conditions, 50 seeds of bell pepper were placed in the Petri plate equidistantly and kept for incubation for 7 days at 20±2ºC in an incubation room under near UV light/fluorescent light having alternate cycles of 12 hrs light and darkness. Examination of seeds was done on the 7th day of germination for incidence of pathogens. Identification of pathogens was done under the microscope on the basis of the symptoms produced on the germinated seedlings and their incidence were also recorded.

Based on these tests, the observations including germination (%); speed of germination; seedling length (cm); seedling dry weight (mg); seed vigor index-length (SVI-I); seed vigor index-mass (SVI-II) and seed mycoflora (%) were recorded under *in vitro* conditions.

The standard germination procedure of ISTA was followed for testing the germination. The first and final counts were taken on the 7th and 14th day, respectively. Germination was calculated on the basis of number of normal seedlings germinated out of the total seeds used. The number of seedlings that emerged from 1st day to the 14th day were counted, and the speed of germination was calculated.

Speed of gemination = n1/d1 + n2/d2 + n3/d3 +………+ n14/d14

(where, n = number of germinated seeds, d = number of days)

For seedling length and dry weight, ten seedlings were randomly selected and their length was measured from the tip of the root to the tip of the shoot with a scale, and mean values were expressed in centimeters. Seedlings were dried in the oven at 60ºC for 48 h, were weighed and the mean values were expressed in milligrams. The SVI -I & II were calculated (Abdul-Baki and Anderson 1973).

SVI-I = Germination (%) x Seedling length (cm)

SVI-II = Germination (%) x Seedling dry weight (mg)

The standard Petri plate method as per ISTA was followed for observing the seed mycoflora. The numbers of infected seeds were recorded daily. The number of infected seeds was observed, and their per cent incidence was calculated.

Statistical analysis: The statistical analysis was performed using R software (version 4.2.1) according to standard procedures for analyzing variance and treatment effects.

RESULTS AND DISCUSSION

In vitro effect of seed priming in bell pepper on seed quality parameters

Effect of SA: The seed priming with SA at different concentrations significantly influenced the seed quality parameters (Table 1). The germination ranged from 85.00% in seeds primed with 75 ppm SA for 8 h, followed by 100 ppm SA for 4 h and 25ppm SA for 8 h, to 75.00% in unprimed seeds. The seeds primed with 75 ppm SA for 8 h exhibited the highest speed of germination (28.47), followed by 100 ppm SA for 4 h and SA at 75 ppm for 10 h, while the seeds primed with SA at 50 ppm for 8 h had the lowest speed of germination. The maximum seedling length (12.69 cm) and seedling dry weight (2.76 mg) were observed in seeds primed with 75 ppm SA for 8 h, followed closely by SA at 25 ppm for 8 h and 100 ppm for 4 h. The shortest seedlings (11.52 cm) and lowest seedling dry weight (2.24 mg) were recorded in unprimed seeds. Consequently, the seeds primed with 75 ppm SA for 8 h had the highest SVI-I (1,078.44) and SVI-II (234.39), again closely followed by seeds primed with 100 ppm for 4 h and SA at 25 ppm for 10 h. The lowest SVI-II was recorded in unprimed seeds.

Effect of MeJA: Seed priming with MeJA at different concentrations had significant effect on the seed quality parameters. Seed germination was highest (82.50%) in seeds primed with 100 ppm MeJA for 4 h, followed by the seeds primed with 75 ppm for 10 h and 100 ppm for 6 h, while the seeds primed with 50 ppm MeJA for 6 h showed the least germination (70.00%) (Table 2). The speed of germination showed the same trend being maximum (25.27%) in seeds primed with 100 ppm MeJA for 4 h. The unprimed seeds were the slowest to germinate (19.69%). The average seedling length, seedling dry weight, SVI-I and SVI-II were highest in the seeds primed with 100 ppm MeJA for 4 h. (11.93 cm, 2.52 mg, 983.81 and 207.69, respectively). MeJA at 75 ppm for 10 h and 100 ppm for 6 h, closely followed maximum seedling dry weight and SVI-II, while the treatments MeJA at 100 ppm for 6 h and 75 ppm for 10 h were also at par with the highest SVI-I. The minimum average seedling length (11.09 cm), seedling dry weight (2.08 mg), SVI-I (793.45) and SVI-II (150.16) were recorded in the seeds primed with MeJA at 75 ppm for 4 h, 50 ppm for 8 h, 50 ppm for 6 h and unprimed seeds, respectively.

Effect of BABA: Priming the bell pepper seeds with 100 ppm BABA for 4 h significantly enhanced seed quality parameters (Table 3) including germination (84.75%), speed of germination (27.98), average seedling length (12.62 cm), average seedling dry weight (2.66 mg), SVI-1 (1,069.55) and SVI-II (225.01). Seeds primed with 75 ppm BABA for 8h and 6h were at par with the highest germination, while the lowest

Table 1. Effect of seed priming with SA on seed quality parameters in bell pepper

Treatments (SA conc./ duration)	Seed quality parameters					
	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
T1(25 ppm/4hrs)	76.25 (8.79)	24.56	12.35	2.51	941.50	191.01
T2 (25 ppm/6hrs)	77.75 (8.87)	24.93	12.34	2.67	959.05	207.59
T3 (25 ppm/8hrs	82.50 (9.14)	26.17	12.66	2.72	1044.66	214.91
T4 (25 ppm/10hrs)	79.25 (8.96)	20.87	11.70	2.61	927.42	215.56
T5 (50 ppm/4hrs)	78.50 (8.92)	20.97	11.86	2.30	931.01	192.72
T6 (50 ppm/6hrs)	77.25 (8.85)	23.86	12.13	2.43	937.24	191.00
T7 (50 ppm/8hrs)	76.00 (8.78)	19.85	12.08	2.30	917.70	174.80
T8 (50 ppm/10hrs)	77.50 (8.86)	25.75	12.03	2.46	932.52	175.15
T9 (75 ppm/4hrs)	76.00 (8.78)	22.89	12.34	2.55	938.03	193.42
T10 (75 ppm/6hrs)	75.50 (8.75)	25.05	12.39	2.55	935.26	192.53
T11 (75 ppm/8hrs	85.00 (9.27)	28.47	12.69	2.76	1078.44	234.39
T12 (75 ppm/10hrs)	78.50 (8.92)	26.76	12.65	2.61	993.22	204.49
T13 (100 ppm/4hrs)	83.75 (9.21)	27.91	12.61	2.67	1056.30	221.94
T14 (100 ppm/6hrs))	77.50 (8.86)	25.78	12.30	2.29	953.44	177.67
T15 (100 ppm/8hrs)	78.75 (8.93)	22.19	11.91	2.26	938.11	181.13
T16 (100 ppm/10hrs)	76.75 (8.82)	23.22	12.23	2.47	938.84	186.31
T17 (Control)	75.00 (8.72)	20.80	11.56	2.24	866.81	167.81
CD (p=0.05)	(0.19)	2.71	0.08	0.09	58.26	23.76

Parentheses are square root transformed values

Table 2. Effect of seed priming with MeJA on seed quality parameters in bell pepper

Treatments (MeJA)	Seed quality parameters					
	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
T1	72.75 (58.52)	21.00	11.28	2.16	820.26	156.96
T2	75.25 (60.16)	22.14	11.24	2.22	846.00	166.87
T3	77.50 (61.70)	23.54	11.80	2.18	914.11	169.14
T4	76.75 (61.23)	24.82	11.28	2.32	865.93	178.25
T5	72.00 (58.04)	22.47	11.60	2.29	835.20	164.88
T6	70.00 (56.80)	19.95	11.34	2.08	793.45	145.60
T7	75.25 (60.20)	23.21	11.24	2.19	845.62	164.42
T8	71.75 (57.96)	21.18	11.67	2.25	837.32	161.26
T9	74.25 (59.52)	20.50	11.09	2.11	823.62	156.85
T10	73.75 (59.20)	19.69	11.52	2.18	849.42	160.59
T11	78.50 (62.42)	22.57	11.45	2.21	898.83	173.29
T12	80.50 (63.84)	24.01	11.49	2.45	925.15	196.82
T13	82.50 (65.31)	25.27	11.93	2.52	983.81	207.69
T14	79.00 (62.74)	24.58	11.90	2.36	939.71	186.44
T15	74.00 (59.36)	19.85	11.36	2.15	840.64	159.29
T16	76.75 (61.19)	22.07	11.43	2.19	877.06	168.27
T17	74.25 (59.50)	19.69	11.13	2.11	826.03	156.48
CD (p=0.05)	(3.37)	0.38	0.08	0.07	78.64	21.06

Parentheses are angular transformed values
See Table 1 for treatment details

germination (70.50%) was observed in the seeds primed with 25 ppm BABA for 10 h. The unprimed seeds exhibited the slowest germination (19.94). Similarly, the seeds primed with BABA at 75 ppm for 8h and 6h followed the highest seedling length and unprimed seeds showed the minimum average seedling length (11.68 cm). The maximum seedling dry weight was in BABA at 75 ppm for 4 h and 8 h, while the seeds primed with BABA at 25 ppm for 10 h had the minimum seedling dry weight (2.00 mg). The seeds primed with 75 ppm BABA for 8 h and 6 h were significantly close to the maximum SVI-I and SVI-II. The minimum SVI-I (832.96) and SVI-II (140.82) were recorded in the seeds primed with BABA at 100 ppm for 8 h and 25 ppm for 10h.

Effect of KNO_3: The significantly highest germination (87.00%) was recorded in seeds primed with KNO_3 at 1.5% for 10 h, closely followed by the seeds primed with KNO_3 at 2.0% for 10 h and 4 h, while the lowest germination (75.00%) was observed in the unprimed seeds. The seeds primed with KNO_3 at 1.5% for 10 h were the fastest (27.53) to germinate, followed by KNO_3 at 1.5% and 2.0% each for 4 h, whereas the unprimed seeds were the slowest (19.41) to germinate. The maximum average seedling length (12.74 cm) and consequently SVI-I (1,108.38) were also exhibited by the seeds primed with KNO_3 at 1.5% for 10 h followed by the seeds primed with KNO_3 at 2.0% for 10 h and 4 h, while the unprimed seedlings had the lowest length (11.43 cm) and SVI-II (856.88). Seeds primed with KNO_3 at 1.5% for 10 h recorded the maximum seedling dry weight (2.63 mg) and SVI-II (229.03), followed by the treatments KNO_3 at 2.0% for 10 h and1.0% for 8 h, while the unprimed seedlings had the minimum dry weight (2.15 mg) and SVI-II (161.25) (Table 4).

***In vitro* effect of seed priming in bell pepper on seed mycoflora**

Effect of SA: The association of seed mycoflora, including *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp., and *Curvularia* spp., with bell pepper *cv.* Solan Bharpur was observed (Table 5). The highest incidence of all fungal species and total mycoflora (22.50%) was observed in the control. The lowest mycoflora incidence (2.25%) was in the seeds treated with 75 ppm SA for 8 h, followed by priming with SA at 100 ppm for 6 h and 4 h, and 25 ppm for 8 h, recording a total mycoflora incidence of 5.25% ($p \le 0.05$).

Effect of MeJA: *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp., and *Curvularia* spp. associated with bell pepper seeds exhibited the highest total incidence

Table 3. Effect of seed priming with BABA on seed quality parameters in bell pepper

Treatments (BABA)	Seed quality parameters					
	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
T1	72.75 (58.54)	20.55	12.43	2.46	904.46	179.15
T2	72.25 (58.26)	23.60	12.08	2.30	872.78	165.99
T3	73.00 (58.75)	22.90	12.33	2.12	900.09	154.94
T4	70.50 (57.14)	22.52	12.18	2.00	858.51	140.82
T5	76.25 (60.82)	20.85	11.77	2.19	897.08	166.80
T6	73.25 (58.84)	24.62	12.19	2.28	892.92	167.19
T7	72.25 (58.26)	21.91	11.71	2.45	846.23	176.83
T8	75.50 (60.33)	22.87	12.39	2.53	935.63	190.83
T9	77.25 (61.56)	23.93	12.49	2.62	965.43	202.59
T10	80.75 (63.99)	25.67	12.53	2.39	1011.39	193.19
T11	81.50 (64.53)	25.79	12.58	2.56	1025.07	208.64
T12	75.75 (60.52)	24.27	12.46	2.28	943.47	172.52
T13	84.75 (67.00)	27.98	12.62	2.66	1069.55	225.01
T14	73.50 (59.02)	24.41	12.38	2.42	910.11	178.05
T15	70.50 (57.10)	20.74	11.82	2.37	832.96	166.73
T16	75.00 (59.99)	26.13	12.36	2.41	927.00	180.94
T17	73.00 (58.71)	19.94	11.68	2.20	852.46	160.78
CD (p=0.05)	(3.25)	2.63	0.08	0.09	69.92	27.36

Parentheses are angular transformed values
See Table 1 for treatment details

Table 4. Effect of seed priming with KNO_3 on seed quality parameters in bell pepper

Treatments (KNO_3 conc./ duration)	Seed quality parameters					
	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
T1 (0.5%/4hrs)	75.75 (8.76)	21.32	12.06	2.21	913.55	167.029
T2 (0.5%/6hrs)	76.75 (8.82)	20.95	12.42	2.45	953.43	188.23
T3 (0.5%/8hrs)	76.50 (8.80)	21.63	11.74	2.16	897.73	165.81
T4 (0.5%/10hrs)	80.50 (9.03)	23.53	12.40	2.55	998.20	205.28
T5 (1.0%/4hrs)	80.75 (9.04)	22.70	11.82	2.45	954.06	197.43
T6 (1.0%/6hrs)	79.50 (8.97)	24.06	12.73	2.37	1012.23	188.22
T7 (1.0%/8hrs)	78.25 (8.90)	20.95	12.21	2.61	955.43	204.23
T8 (1.0%/10hrs)	81.50 (9.08)	23.73	12.52	2.18	1020.18	177.47
T9 (1.5%/4hrs)	80.75 (9.04)	26.55	11.94	2.26	963.75	182.29
T10 (1.5%/6hrs)	77.50 (8.86)	22.77	12.26	2.33	949.96	180.38
T11 (1.5%/8hrs)	79.00 (8.94)	22.98	12.58	2.51	993.23	198.09
T12 (1.5%/10hrs)	87.00 (9.38)	27.53	12.74	2.63	1108.38	229.03
T13 (2.0%/4hrs)	83.50 (9.19)	25.35	12.68	2.48	1058.99	206.87
T14 (2.0%/6hrs)	78.50 (8.92)	21.57	12.45	2.21	976.93	173.29
T15 (2.0%/8hrs)	76.00 (8.77)	23.63	12.32	2.31	935.94	175.75
T16 (2.0%/10hrs)	83.50 (9.19)	25.19	12.72	2.62	1061.70	218.98
T17 (Control)	75.00 (8.72)	19.41	11.43	2.15	856.88	161.25
CD (p=0.05)	(0.23)	2.53	0.10	0.09	82.15	29.00

Parentheses are square root transformed values

Table 5. Effect of seed priming with SA on seed mycoflora in bell pepper

Treatments (SA)	Seed mycoflora (%)					
	Alternaria spp.	*Penicillium* spp.	*Fusarium* spp.	*Colletotricum* spp.	*Curvularia* spp.	Seed mycoflora (Total)
T1	3.50 (2.10)	1.25 (1.49)	1.50 (1.57)	1.75 (1.64)	0.00	8.00 (2.56)
T2	4.00 (2.24)	1.50 (1.57)	1.75 (1.64)	2.75 (1.87)	0.00	10.00 (3.01)
T3	0.25 (1.10)	2.25 (1.79)	0.75 (1.29)	2.00 (1.73)	0.00	5.25 (2.12)
T4	2.00 (1.68)	2.00 (1.72)	2.50 (1.85)	1.50 (1.57)	0.00	8.00 (2.59)
T5	2.25 (1.74)	1.50 (1.57)	1.50(1.57)	3.00 (1.99)	0.00	8.25 (2.61)
T6	1.00 (1.39)	1.75 (1.64)	3.00 (1.98)	2.25 (1.80)	0.00	8.00 (2.57)
T7	1.75 (1.62)	2.25 (1.79)	2.00 (1.70)	2.00 (1.71)	0.00	8.00 (2.60)
T8	2.50 (1.85)	2.00 (1.71)	2.75 (1.93)	1.75 (1.65)	0.00	9.00 (2.72)
T9	2.00 (1.70)	1.75 (1.64)	2.00 (1.72)	1.50 (1.57)	0.00	7.25 (2.50)
T10	1.00 (1.39)	1.50 (1.57)	1.75 (1.64)	2.25 (1.79)	0.00	6.50 (2.38)
T11	0.00 (1.00)	1.00 (1.39)	0.50 (1.18)	0.75 (1.29)	0.00	2.25 (1.60)
T12	0.75 (1.29)	1.25 (1.47)	1.50 (1.56)	2.50 (1.87)	0.00	6.00 (2.28)
T13	1.25 (1.47)	2.00 (1.72)	1.00 (1.39)	1.00 (1.39)	0.00	5.25 (2.18)
T14	0.50 (1.21)	1.50 (1.57)	0.75 (1.29)	1.50 (1.57)	0.00	4.25 (2.00)
T15	2.50 (1.87)	1.75 (1.64)	2.00 (1.72)	1.75 (1.64)	0.00	8.00 (2.60)
T16	1.00 (1.39)	2.25 (1.79)	1.50 (1.57)	2.00 (1.72)	0.00	6.75 (2.41)
T17	4.75 (2.40)	4.50 (2.33)	4.25 (2.29)	5.00 (2.45)	4.00	22.50 (4.36)
CD (p=0.05)	0.45	0.37	0.41	0.38	NS	1.08

Parentheses are square root transformed values
See table 1for treatment details

(21.25%) in the untreated control (Table 6). The lowest mycoflora incidence (3.25%) was in seeds primed with 100 ppm MeJA for 4 h, followed by MeJA at 100 and 50 ppm for 8 h.

Effect of BABA: The association of *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp., and *Curvularia* spp. in bell pepper seeds was observed (Table 7). The control exhibited the highest mycoflora incidence (23.00%), while the lowest total mycoflora incidence (1.00%) was in seeds treated with 100 ppm BABA for 4 h, followed by priming with 75 ppm for 4 h and 8 h.

Effect of KNO_3: The association of *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp., and *Curvularia* spp. in bell pepper seeds is given in Table 8. The highest total mycoflora incidence (22.25%) was observed in the control and the lowest incidence (3.25%) was in seeds treated with KNO_3 at 1.5% for 10 h, closely followed by KNO_3 at 2.0% and 1.5% for 4 h.

This study provides the first comprehensive evaluation of seed priming across various concentrations of SA, MeJA, BABA and KNO_3 to enhance seed quality parameters in bell pepper. The findings align with prior research on other crops, highlighting the role of different priming agents in improving germination, seedling growth, and vigor. Among the treatments, SA priming at 75 ppm for 8 h significantly enhanced germination percentage, seedling dry weight, and vigor indices. Similar effects were observed in Arabidopsis, where SA concentrations above 50 ppm improved germination (Rajjou et al., 2006), and in rice, where SA priming in the 75-100 ppm range reduced germination time and enhanced seedling dry weight (Shatpathy et al., 2018), in Indian mustard (*Brassica juncea*), where SA priming improved germination, SVI-I and II, and early seedling establishment (Dan 2014). The observed enhancement in seed vigour can be attributed to the SA-induced activation of metabolic pathways, including the glyoxylate cycle, pentose phosphate pathway, glycolysis, and gluconeogenesis. These pathways play a crucial role in transitioning seeds from quiescence to active seedling growth by promoting enzyme activation and energy mobilization (Rajjou et al., 2006). However, seed priming with MeJA negatively affected germination, seedling growth, and vigor indices. This aligns with previous studies demonstrating that MeJA inhibits seed germination in various angiosperms (Norastehnia et al., 2007). The reduction in seedling performance may be linked to MeJA's role in activating defence mechanisms at the cost of growth-related processes, leading to energy trade-offs that negatively impact early seedling establishment (Li et al.,

Table 6. Effect of seed priming with MeJA on seed mycoflora in bell pepper

Treatments (MeJA)	Seed mycoflora (%)					
	Alternaria spp.	*Penicillium* spp.	*Fusarium* spp.	*Colletotricum* spp.	*Curvularia* spp.	Seed mycoflora (Total)
T1	1.50 (1.54)	2.75 (1.91)	0.75 (1.28)	1.75 (1.64)	0.00	6.75 (2.39)
T2	1.00 (1.39)	3.25 (2.05)	1.00 (1.39)	3.25 (2.05)	0.00	8.50 (2.59)
T3	1.25 (1.47)	3.00 (1.99)	1.50 (1.54)	2.50 (1.85)	0.00	8.25 (2.60)
T4	1.75 (1.60)	2.50 (1.86)	1.75 (1.72)	3.00 (1.99)	0.00	9.00 (2.72)
T5	2.25 (1.79)	2.00 (1.72)	1.50 (1.55)	3.25 (2.05)	0.00	9.50 (2.71)
T6	1.00 (1.39)	3.50 (2.12)	2.00 (1.72)	2.50 (1.85)	0.00	9.00 (2.69)
T7	1.75 (1.64)	1.75 (1.64)	1.25 (1.43)	2.00 (1.68)	0.00	6.75 (2.42)
T8	1.5 (1.57)	2.25 (1.79)	0.50 (1.18)	3.75 (2.17)	0.00	8.00 (2.51)
T9	2.25 (1.77)	3.75 (2.16)	1.50 (1.57)	2.50 (1.85)	0.00	10.00 (2.83)
T10	2.00 (1.72)	1.75 (1.65)	2.75 (1.93)	3.00 (1.98)	0.00	9.50 (2.78)
T11	1.25 (1.43)	2.25 (1.79)	1.00 (1.35)	2.75 (1.90)	0.00	7.25 (2.46)
T12	1.50 (1.56)	1.50 (1.57)	1.25 (1.46)	2.25 (1.80)	0.00	6.50 (2.38)
T13	0.75 (1.31)	1.25 (1.46)	0.25 (1.10)	1.00 (1.39)	0.00	3.25 (1.82)
T14	1.75 (1.62)	1.75 (1.65)	2.00 (1.72)	1.75 (1.64)	0.00	7.25 (2.50)
T15	1.50 (1.57)	1.50 (1.57)	1.75 (1.64)	1.25 (1.49)	0.00	6.00 (2.31)
T16	1.00 (1.39)	2.50 (1.87)	1.25 (1.47)	2.00 (1.72)	0.00	6.25 (2.40)
T17	4.50 (2.34)	4.75 (2.40)	4.00 (2.23)	5.00 (2.45)	3.00	21.25 (2.34)
CD (p=0.05)	(0.47)	(0.36)	(0.49)	(0.42)	NS	(0.47)

Parentheses are square root transformed values
See table 1for treatment details

2022). The effect of BABA on seed quality parameters revealed a concentration-dependent trend. At lower concentrations, BABA enhanced seed vigour parameters; however, beyond 100 ppm, a decline in seed vigour indices was observed. This phenomenon is consistent with previous reports suggesting that the direct toxic effects of BABA or the energetic costs of induced resistance can negatively impact plant growth (Silue et al., 2002). The optimal seed vigor recorded at moderate BABA concentrations suggests that BABA-induced resistance mechanisms may contribute to improved seed performance, but excessive concentrations may impose physiological stress, leading to reduced germination and seedling vigor (Cohen et al., 2016). KNO_3 priming at 1.5% for 10 h significantly improved seed germination, seedling vigor, seedling length, and seedling dry weight. Similar enhancements in seed and seedling vigor indices, and germination were reported in hot pepper (Amjad et al., 2007), tomato (Behera 2016), soybean (Ahmadvand et al., 2012) and cotton (Cokkizgin and Bolek 2015) on priming their seeds with KNO_3. Several findings in other crops support our results in which SA application significantly reduced occurrence of *Fusarium* spp. and *Cercospora* spp. in soybean (Kuchlan et al., 2017), *Alternaria alternata* incidence in pear fruits (Tian et al., 2006). Seed priming with SA has also been shown to lower Ascomycota fungi, including *Verticillium* spp. and *Fusarium* spp. (Mustafa et al., 2019). This is due to the reason that salicylic acid activates plant resistance pathway by affecting the enzymes and genes involved in ROS scavenging, prompting pathogenesis related genes (Mishraet al., 2024) and activating the defense genes like chitinase, -1,3-glucanase, peroxidase, and phenylalanine ammonia-lyase providing resistance against both necrotrophic and biotrophic pathogens (Glazebrook 2005). Seed priming with methyl jasmonate can enhance resistance to both abiotic and biotic stresses by modulating antioxidant activity and secondary metabolite production. However, its effectiveness is reduced under *in vitro* conditions due to possible phytotoxicity at higher concentrations and longer durations, and differences in uptake and signaling compared to natural growing conditions. BABA-induced resistance (BABA-IR) operates via an ABA-dependent pathway, independent of SA, JA, or ethylene (Ton & Mauch-Mani 2004). The activation of reactive oxygen species (ROS) and glycolate oxidase (GO) contributes to its antifungal effects, supporting the observed lowest mycoflora incidence in BABA-treated seeds. Likewise, potassium application in cotton reduced Fusarium wilt (Prabhu et al., 2007), and higher potassium levels

Table 7. Effect of seed priming with BABA on seed mycoflora in bell pepper

Treatments (BABA)	Seed mycoflora (%)					
	Alternaria spp.	*Penicillium* spp.	*Fusarium* spp.	*Colletotricum* spp.	*Curvularia* spp.	Seed mycoflora (Total)
T1	2.50 (1.84)	2.00 (1.68)	1.50 (1.57)	1.75 (1.64)	0.00	7.75 (2.56)
T2	2.25 (1.74)	1.25 (1.49)	0.75 (1.29)	2.25 (1.74)	0.00	6.50 (2.36)
T3	1.75 (1.65)	2.25 (1.72)	1.75 (1.64)	3.00 (1.99)	0.00	8.75 (2.69)
T4	1.75 (1.65)	1.50 (1.54)	1.00 (1.39)	2.50 (1.47)	0.00	6.75 (2.41)
T5	2.50 (1.85)	2.50 (1.85)	2.00 (1.70)	1.25 (1.29)	0.00	8.25 (2.65)
T6	2.50 (1.85)	2.00 (1.68)	2.00 (1.70)	2.50 (1.85)	0.00	9.00 (2.73)
T7	1.50 (1.54)	2.50 (1.85)	2.25 (1.79)	1.75 (1.64)	0.00	8.00 (2.59)
T8	2.00 (1.72)	1.50 (1.57)	0.75 (1.29)	2.25 (1.80)	0.00	6.50 (2.36)
T9	0.50 (1.21)	1.00 (1.39)	0.50 (1.21)	0.50 (1.21)	0.00	2.50 (1.69)
T10	1.00 (1.39)	1.50 (1.54)	1.75 (1.65)	2.00 (1.49)	0.00	6.25 (2.34)
T11	0.75 (1.29)	1.25 (1.49)	1.00 (1.39)	0.75 (1.29)	0.00	3.75 (1.94)
T12	2.00 (1.68)	1.75 (1.65)	1.50 (1.54)	1.25 (1.49)	0.00	6.50 (2.39)
T13	0.00 (1.00)	0.75 (1.29)	0.00 (1.00)	0.25 (1.10)	0.00	1.00 (1.28)
T14	1.00 (1.39)	1.50 (1.56)	1.00 (1.39)	1.25 (1.49)	0.00	4.75 (2.16)
T15	1.50 (1.54)	2.00 (1.70)	0.75 (1.29)	1.50 (1.54)	0.00	5.75 (2.26)
T16	1.00 (1.39)	2.50 (1.85)	2.75 (1.90)	2.50 (1.84)	0.00	8.75 (2.67)
T17	5.00 (2.46)	5.50 (2.55)	4.25 (2.28)	4.75 (2.40)	3.50	23.00 (4.39)
CD (p=0.05)	(0.48)	(0.45)	(0.45)	(0.45)	NS	(1.03)

Parentheses are square root transformed values
See table 1for treatment details

Table 8. Effect of seed priming with KNO_3 on mycoflora in bell pepper

Treatments (KNO_3)	Seed mycoflora (%)					
	Alternaria spp.	*Penicillium* spp.	*Fusarium* spp.	*Colletotricum* spp.	*Curvularia* spp.	Seed mycoflora (Total)
T1	1.75 (1.64)	1.75 (1.64)	2.75 (1.93)	2.25 (1.80)	0.00	8.50 (2.66)
T2	1.50 (1.57)	2.25 (1.80)	2.00 (1.72)	1.25 (1.49)	0.00	7.00 (2.45)
T3	1.50 (1.57)	2.00 (1.72)	1.75 (1.64)	1.75 (1.64)	0.00	7.00 (2.46)
T4	2.00 (1.73)	2.25 (1.79)	3.00 (1.99)	2.00 (1.72)	0.00	9.25 (2.75)
T5	1.75 (1.64)	2.75 (1.93)	2.75 (1.93)	1.50 (1.54)	0.00	8.75 (2.68)
T6	2.25 (1.79)	1.50 (1.57)	2.00 (1.72)	2.50 (1.85)	0.00	8.25 (2.63)
T7	2.50 (1.85)	2.25 (1.80)	2.25 (1.79)	2.25 (1.80)	0.00	9.25 (2.76)
T8	2.25 (1.79)	1.25 (1.47)	2.50 (1.83)	2.00 (1.72)	0.00	8.00 (2.59)
T9	1.75 (1.64)	2.00 (1.71)	1.75 (1.64)	1.25 (1.47)	0.00	6.75 (2.42)
T10	2.00 (1.72)	1.50 (1.57)	3.75 (2.15)	2.75 (1.90)	0.00	10.00 (2.82)
T11	1.50 (1.57)	1.75 (1.65)	2.25 (1.78)	1.75 (1.65)	0.00	7.25 (2.49)
T12	1.00 (1.41)	1.00 (1.39)	0.50 (1.21)	0.75 (1.31)	0.00	3.25 (1.84)
T13	1.25 (1.49)	1.25 (1.49)	0.75 (1.31)	1.00 (1.39)	0.00	4.25 (2.03)
T14	1.75 (1.65)	1.50 (1.55)	2.50 (1.85)	2.00 (1.72)	0.00	7.75 (2.56)
T15	2.00 (1.72)	2.25 (1.78)	2.25 (1.80)	1.50 (1.57)	0.00	9.00 (2.59)
T16	1.50 (1.57)	2.00 (1.72)	1.75 (1.64)	1.50 (1.57)	0.00	6.75 (2.42)
T17	4.50 (2.34)	5.00 (2.45)	4.00 (2.23)	5.25 (2.50)	3.50	22.25 (4.33)
CD (p=0.05)	(0.31)	(0.36)	(0.33)	(0.37)	NS	(1.04)

Parentheses are square root transformed values
See Table 4 for treatment details

enhanced resistance in several crops including rice, wheat, tomato, and soybean (Sweeney et al., 2000). This mechanism likely involves reduced pathogen competition for nutrients and enhanced cell wall strengthening, contributing to lower mycoflora incidence (Holzmueller et al., 2007; Mengel 2001).

CONCLUSION

This study demonstrates that seed priming with plant defence activators like SA, MeJA, BABA, and KNO_3, can significantly improve seed quality and reduce seed-borne fungal incidence in bell pepper. Among the treatments, priming with 75 ppm SA for 8 h and 1.5% KNO_3 for 10 h resulted in the highest germination rates, seedling vigor, and the lowest seed mycoflora incidence. BABA exhibited a concentration-dependent effect, enhancing seed vigor at moderate levels, while MeJA, although beneficial under certain conditions, showed a slight inhibitory effect on early seedling growth when used in higher doses and longer durations. The findings support the use of seed priming with selected defence activators as an eco-friendly alternative to chemical seed treatments, thereby promoting sustainable agriculture and healthier crop establishment. Further research is encouraged to optimize protocols and understand the mechanisms underlying these benefits.

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In vitro Bio-efficacy of *Trichoderma* Based Nano Formulation against *Pyricularia oryzae* and *Bipolaris oryzae*

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Abstract: Biological synthesis of nanoparticle is very popular for its eco-friendly and non-toxic and green nature. Plant, Bacteria, Fungi, Yeast are used in this method. Among them Fungi are now the preferred option for production of nanoparticles due to their many benefits over other microorganisms. *Trichoderma* based biocontrol is a viable option in this situation. The goal of this project was to biologically synthesise silver nanoparticles (AgNP) from *Trichoderma asperellum* and *Trichoderma harzianum*, two possible native biocontrol agents. Silver nitrate (1 mM and 2 mM) served as the precursor for AgNP formation. Fourier Transmission Infrared Scanning and a UV-Vis spectrophotometer were used to characterise the biosynthesized silver nanoparticle. A characteristic UV-Vis absorption peak was observed at 415–420 nm, confirming the presence of AgNPs. In vitro bioefficacy was done by food poison technique. Silver nanoparticles at several concentrations (10 ppm, 5 ppm, and 1 ppm) were tested for their antagonistic activity in vitro against *Pyricularia oryzae* and *Bipolaris oryzae*, two Paddy diseases.

Keywords: *Trichoderma*, Silver nanoparticles, *Pyricularia oryzae*, *Bipolaris oryzae*, Nanotechnology

Global agricultural productivity is severely hampered by plant diseases, managing those diseases one of the most important tasks for scientists. Modern agricultural practices for increasing rice production have recently led to an increase in disease severity. These practices include the use of high yielding crop varieties, excessive nitrogen fertilisation, extensive use of agrochemicals, increased plant population per unit area, and continuous rice cropping, which favours the crop's susceptibility to rice blast (Miah et al., 2017). The current plant disease management strategies have benefits and drawbacks. Although cultural practices are safer, they are not always effective in controlling disease. Chemical management comes with a number of drawbacks. Indiscriminate use of synthetic fungicide result in host toxification, which includes adverse effects on the soil microbiota, residual toxicity, fungicidal resistance, and environmental contamination (Gerhardson 2002). The overuse of pesticides has recently resulted in environmental dangers that have drawn a lot of attention and discussion. Chemical management of plant diseases degrades soil quality, pollutes water supplies, and upsets the ecosystem (Ayla and Rao 2002). In addition to the pathogen's comeback, fungicide resistance is a significant issue brought on by the harmful effects of excessive chemical use. For these reasons researchers are currently trying to develop novel, environmentally sound and more reliable methods of managing diseases. In contrast to traditional physico-chemical methods that have numerous drawbacks, the environmentally friendly method for developing nanoparticles through biological processes has garnered a lot of attention in recent years. The usage of environmentally friendly nanoparticles that don't generate harmful waste is becoming more and more necessary. Since it helps avoid an excessive buildup of chemicals that could cause residual effect in the environment, the use of nanotechnological products for pest management in agriculture is believed to be safer than the use of conventional agrochemicals. Additionally, the biocompatibility of NP usage is enhanced by these novel ecologically friendly synthesis methods (Guilger-Casagrande and Lima 2019). Nanoparticles are required in small amount to check pathogen. Nanoparticle synthesized bioagent become more essential wherever the bioagent is present or absent in a crop habitat. Nanoparticles can be isolated using a variety of techniques, including physical, chemical, and biological ones. The best of these approaches appears to be biological since it is an inexpensive, simple, and environmentally safe way to develop huge quantities of different nanoparticles. The objectives of this research work are synthesization of *Trichoderma* synthesized silver nanoparticle and evaluation of biocontrol efficacy of those nanoparticles against two pathogens infecting paddy plant.

MATERIAL AND METHODS

In this experiment silver nanoparticle was formulated by extracellular biosynthesis procedure. For the production of silver nanoparticles, two strains of *Trichoderma harzianum* (MT876632) and *Trichoderma asperellum* (MT951635) were employed. These two strains' potency against Paddy disease pathogens was evaluated.

Collection and culture of *Trichoderma harzianum* and *T. asperellum*: Two *Trichoderma* species, *Trichoderma harzianum* (MT876632) and *Trichoderma asperellum* (MT951635) were obtained from the Department of Plant Pathology's Biocontrol Lab at U.B.K.V., Pundibari, West Bengal. Throughout the trial time, these two strains' pure cultures are preserved by ongoing subculturing in potato dextrose agar slants.

Trichoderma isolates were identified using morphological analysis using the slide culture method. This procedure involved placing one glass slide within a sterilised Petri plate on top of two glass slides on blotting paper. To keep the humidity level high, a few drops of distilled water that had been sterilised were added to the blotting paper. Different sterilised Petri plate setup was employed for each isolate. Using a sterile cork borer, the mycelial disc of the *Trichoderma* isolate was put onto the glass slide. For 72 hours, plates were stored in BOD at 28±1˚C. Few drops of cotton blue were applied to the slide after the disc was later removed. The slides were examined at 10X and 40X magnifications using a microscope.

Biomass production of *Trichoderma harzianum* and *T. asperellum*: Biomass production is necessary for the manufacture of silver nanoparticles (AgNPs) utilising *Trichoderma asperellum* and *Trichoderma harzianum* culture supernatant. In potato dextrose broth (PDB), biomass was produced. Six mm disc of each bioagents incubated in PDB at 28±1˚C for 7 days. The culture supernatant was collected after seven days of incubation. following the removal of the mycelial mass (the vegetative part of fungi) from the culture broth using a sterile paper filter. The collected culture filtrate of *Trichoderma* isolates was then used to generate silver nanoparticles.

Biogenic synthesis of silver nanoparticle from *Trichoderma harzianum* and *T. asperellum*: Mycelium of

Fig. 1. Change in colour of *Trichoderma* synthesized silver nanoparticles

fungi was subjected to a metal salt for the production of metal nanoparticles by a fungus. Fungi release metabolites and enzymes with the aim to survive. This process transforms hazardous metal ions into non-toxic metallic solid nanoparticles via the catalytic action of the extracellular enzyme and secondary metabolites released by fungi. Fifty millilitres of sterile deionised water were used to create solutions of 1mM and 2 mM of silver nitrate. In a 250 ml Erlenmeyer flask, 50 ml of *Trichoderma* culture supernatant solution and 50 ml of silver nitrate solution were combined or mixed. The entire combination was continuously shaken at 200 rpm in a dark environment. The uninoculated set was used as control. Aluminium foil can be used to keep flasks in a dark condition. AgNP production is indicated by change in colour of supernatant from green to yellowish brown to brown.

Characterization of biogenic synthesized silver nanoparticles

UV-VIS spectrophotometer: Since the optical characteristics of nanoparticles are sensitive to factors like size, shape, concentration, aggregation state, and refractive index close to the nanoparticle surface, UV spectroscopy is an excellent technique for identifying, characterising, and studying nanoparticles. The fundamental requirements for the field of plasmonic are the distinct optical characteristics of materials made of specific metals, such as gold and silver, which interact strongly with particular light wavelengths when synthesised into nanoparticles. Visual observation of the solution and analysis of its UV-visible spectra were employed to regularly track the decline of silver ions by taking aliquots (2 mL) of the aqueous component on a regular basis. To ascertain the production of the biogenic silver nanoparticle resulting from the bio reduction of Ag+, an ultraviolet-visible spectrophotometer was employed. The UV-Vis Spectrophotometer of the UV-1900 series (model number: A12535780139) was utilised in our study to take measurements. The scanning range for the material was 200–800 nm. The baseline correction of the spectrophotometer was carried out using a water blank reference. The 2 ml sample was taken in a cuvette. The UV-Vis absorption spectrum of the sample was recorded, and numerical data was plotted.

Scanning in Fourier Transform Infrared Spectroscopy (FTIR): The infrared absorption spectrum that FTIR

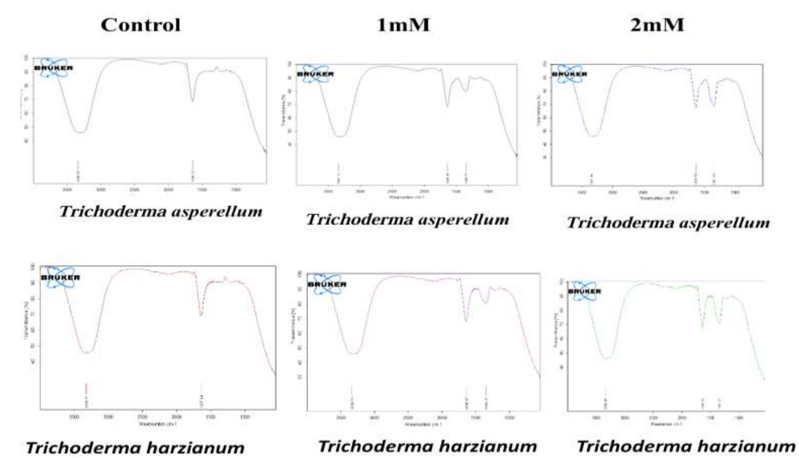


Fig. 2. UV-Vis absorption spectra obtained for silver nanoparticle

Table 1. *In vitro* biocontrol efficacy of *Trichoderma* synthesized nano particles against *Pyricularia oryzae*

Treatments	*Trichoderma harzianum* (MT876632)		*Trichoderma asperellum* (MT951635)	
	Radius of pathogen (cm)	% growth inhibition over control	Radius of pathogen	% growth inhibition over control
1mM (1ppm)	1.93	78.50	3.27	63.67
1mM (5ppm)	1.90	78.90	2.83	68.50
1mM (10ppm)	1.82	79.78	2.34	74.05
2mM (1ppm)	3.22	64.20	2.45	72.79
2mM (5ppm)	2.48	72.45	1.59	82.36
2mM (10ppm)	1.89	79.01	1.09	87.85
CD (p=0.05)	0.328	3.647	2.634	0.237

produces is similar to a molecular "fingerprint" and is a potent instrument for determining the kinds of chemical bonds present in a molecule (Senapati, 2005). This annotated spectrum illustrates how the chemical bond is characterised by the wavelength of light absorbed. FTIR can be utilised for quantitative analysis since the absorption strength is related to the concentration. This spectrometer simultaneously collects high-resolution spectral data throughout a wide spectral range. Compared to a dispersive spectrometer, which measures intensity throughout a small range of wavelengths at once, this offers a significant benefit. Infrared spectroscopy uses a sample to transmit infrared radiation. A portion of the infrared radiation is absorbed by the sample. It is transmitted in part. Since the FTIR spectra in the 1400–1700 cm–1 area reveals information on the existence of "C=O" and "N-H" groups, the measurement can also be used to investigate the presence of a protein molecule in the solution (Senapati et al., 2005). Consequently, the spectrum establishes a molecular fingerprint of the material by representing its molecular structure, transmission, and absorption. This fingerprint aids in the molecule's identification.

Isolation of *Pyricularia oryzae*: Blast affected small leaf and neck samples put in Potato Dextrose Agar (PDA) after surface sterilization with 0.1% Sodium Hypo chloride.

Identification of *Pyricularia oryzae*: Under a light microscope, morphological characteristics are used to identify *Pyricularia oryzae*.

Pathogenicity of *Pyricularia oryzae*: Healthy rice seedlings were used for the pathogenicity test. Rice grains were used to prepare pathogen inoculum. Then the pathogen from the freshly acquired lesion was viewed under microscope, confirmed and was re-isolated on potato dextrose agar media and the colony growth and morphology was evaluated. Rice plants were sprayed with pathogen inoculum at a concentration 10^6 cfu/ml after 21 days growth of pathogen.

Isolation of *Bipolaris oryzae*: *Bipolaris oryzae* was isolated under aseptic conditions in a laminar air flow chamber. Samples selected from the lesion's expanding edge; surface

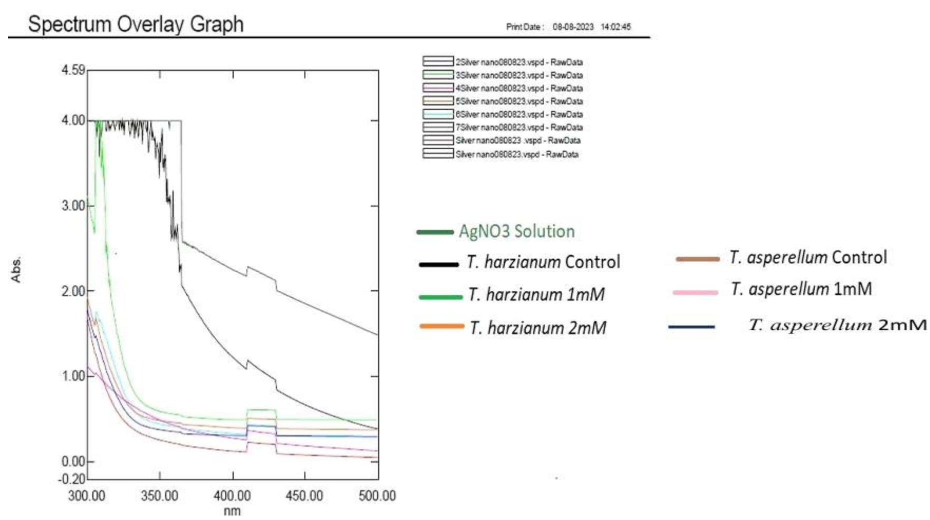


Fig. 3. Scanning in FTIR

Table 2. *In vitro* biocontrol efficacy of *Trichoderma* synthesized nano particles against *Bipolaris oryzae*

Treatments	*Trichoderma harzianum* (MT876632)		*Trichoderma asperellum* (MT951635)	
	Radius of pathogen (cm)	% growth inhibition over control	Radius of pathogen	% growth inhibition over control
1mM (1ppm)	3.93	56.38	3.81	56.05
1mM (5ppm)	3.77	58.30	3.42	62.67
1mM (10ppm)	2.93	67.54	3.01	66.51
2mM (1ppm)	3.70	58.93	3.66	60.56
2mM (5ppm)	3.45	61.74	3.27	63.63
2mM (10ppm)	3.23	64.38	3.21	64.38
CD (p=0.05)	1.945	0.175	0.317	1.455

sterilized by 1% sodium hypochlorite. After that samples put on potato dextrose agar (PDA) plate and were then incubated at 28 ± 1ºC for five to seven days.

Inoculum preparation of *Bipolaris oryzae*: Paddy grain was used to multiply of *Bipolaris oryzae.* Mycelial disc from full grown plate was transferred on autoclaved rice in a conical flask.

Identification of *Bipolaris oryzae*: *Bipolaris oryzae* identification using morphological traits. Conidia pointed at both sides are evidence of *Bipolaris oryzae*.

Pathogenicity test: Inoculum was prepared with the help of rice grain. On rice grains, pathogens doubled in size. Twenty-one days old rice seedlings were sprayed with the conidial suspension (5 x 10^6 spore/ ml) of the isolates of *Bipolaris oryzae*.

Determination of antagonistic potential of *Trichoderma* AgNPs: AgNPs' efficiency against the two infections under investigation (*Pyricularia oryzae* and *Bipolaris oryzae*) was evaluated in an in vitro experiment. Using the Poison Food Technique, the effectiveness of AgNPs was examined at concentrations of 10 ppm, 5 ppm, and 1 ppm (Kim *et al*., 2012). AgNPs were combined with sterile PDA media for this experiment prior to plating. PDA was put into a sterile Petri plate after being thoroughly mixed. After the AgNPs treated PDA solidified, a 6 mm mycelia disc of the pathogen that was actively growing with an agar plug was cut with a cork borer and kept at the centre of a Petri plate (9 cm in diameter). For ten days, each plate was incubated at 28±1˚C. When the control attained its maximum development, the colony diameter was observed. The growth inhibition rate was computed using the following formula.

Inhibition % = [(R – r) / R] * 100

where **r** is the radial growth of fungal mycelia on the plate treated with silver nanoparticles and **R** is the radial growth of fungal mycelia on the control plate.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles: The successful synthesis of silver nanoparticles (AgNP) was achieved using the extracellular synthesis approach. *T*. *harzianum* and *T*. *asperellum* culture filtrate was exposed to 1mM and 2 mM solutions of silver nitrate, it was observed that the reduction of silver ions into silver nanoparticles was followed by precipitation onto the cells. The colour shift from pale green to yellowish brown and finally dark brown, which is caused by the Surface Plasmon Resonance phenomenon, served as preliminary confirmation that silver nanoparticles were forming (Tripathi et al., 2013).

Change in the colour: When *Trichoderma* culture filtrate and silver nitrate solution were combined and stored in a shaker incubator under dark conditions, the colour progressively changed from pale yellow to yellowish brown, and after 30 days, it turned dark brown. Various workers also reported similar findings. Devi et al. (2013) observed that the shift in hue from pale yellow to dark brown showed that the *Trichoderma* isolate was clearly creating silver nanoparticles. The primary cause of the hue shift is either the deposited

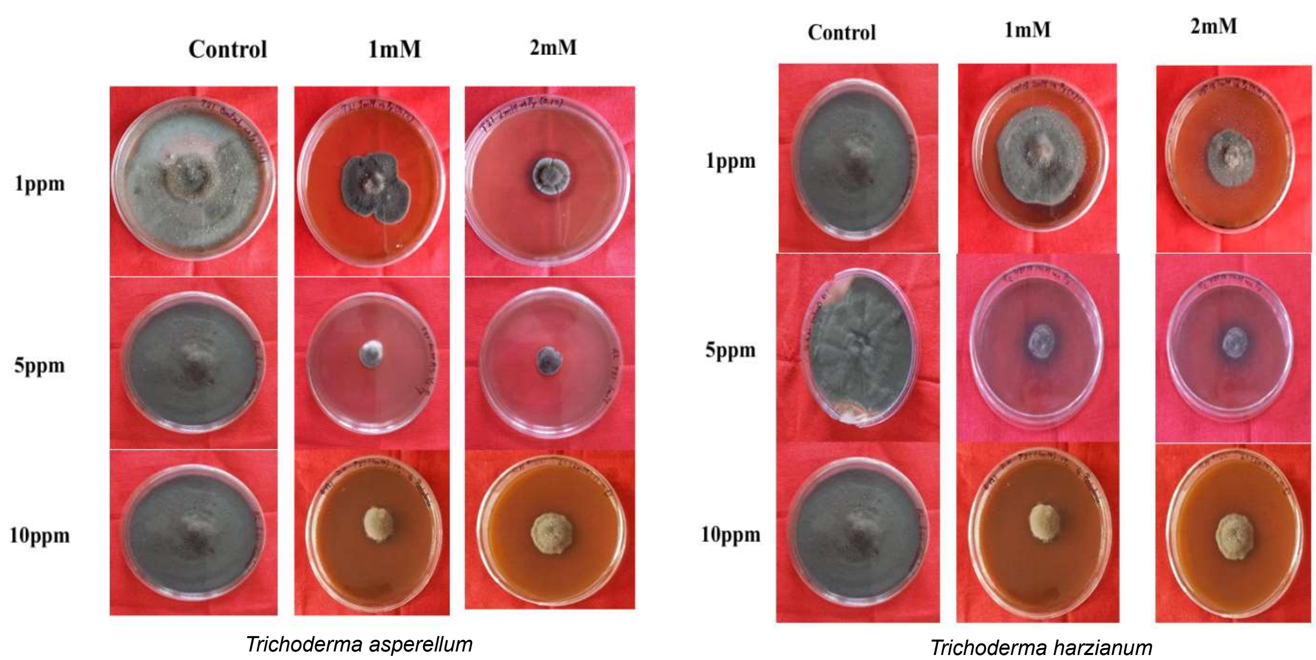
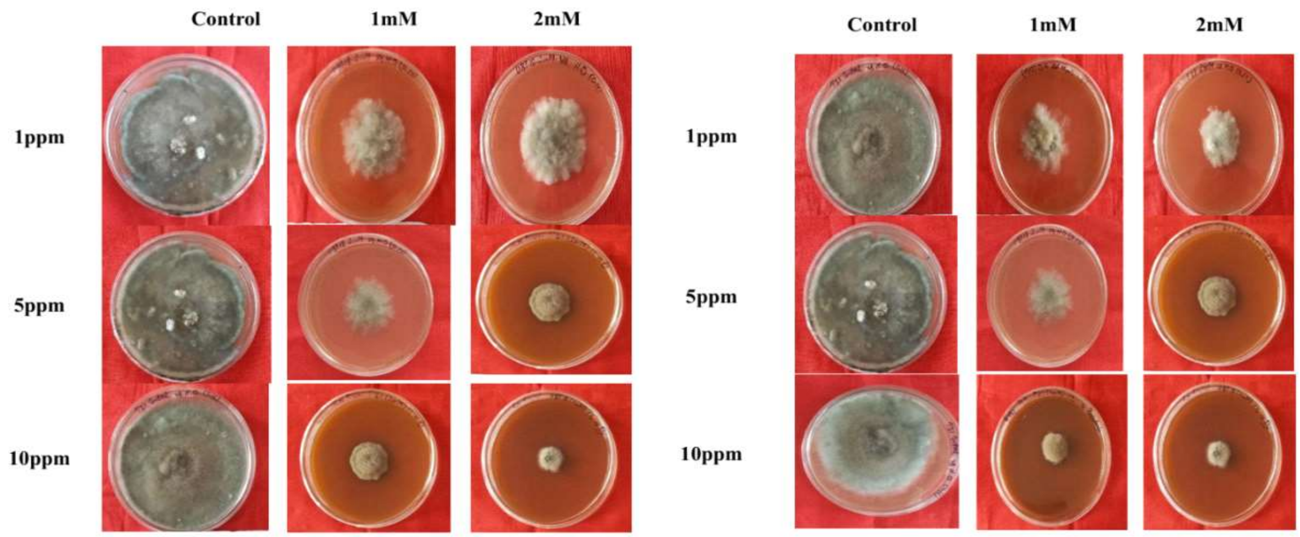


Plate 2A: In vitro efficacy of *Trichoderma harzianum* & *Trichoderma asperellum* synthesized AgNPs against Pyricularia oryzae



Trichoderma harzianum *Trichoderma asperellum*

Plate 2B. In vitro efficacy of *Trichoderma asperellum* synthesized AgNPs against *Bipolaris oryzae*:

silver nanoparticles' surface plasmon resonance or the surface electrons' collective and coherent oscillations (Link and El-Sayed 2003).

We found that there is no change in colour in case of control where silver nitrate solution was not mixed. But in case of *Trichoderma* isolate where silver nitrate solution was mixed with culture filtrate, colour had changed into dark brown. The change of colour is the confirmation or evident of producing silver nanoparticle (Devi et al., 2013).

UV-Vis spectrophotometer analysis: It is widely acknowledged that controlled nanoparticles in aqueous solutions can be investigated by applying UV-Vis spectroscopy. The surface plasmon resonance (SPR) absorption band at the crucial wavelength is provided by the free electrons in the metal nanoparticles. The simultaneous vibrating of metal nanoparticle electrons in resonance with light waves is what causes the SPR band. Silver nanoparticles exhibit an SPR peak between 410 nm and 420 nm (Devi et al., 2013). The study revealed that the SPR peak formed between 415 nm and 420 nm. The spectrum unmistakably displays the rise in silver nitrate intensity, which denotes the creation of more silver nanoparticles in the solution.

Scanning in FTIR: There were peaks I, II, and III in the FTIR spectra. Peaks II and III may have been caused by the silver ion. Peak I which was observed in both the control and *Trichoderma* synthesised silver nanoparticle cases, may have been caused by O-H bonding. Peak III originated between 1330 cm^{-1}-1420 cm^{-1}, and might likewise be the result of alcohol's O-H bonding. In contrast, *T. asperellum* synthesised silver nanoparticles produced a III peak, which might be the result of aromatic amine's C-N stretching. These III peaks could be a sign that a silver nanoparticle is forming.

***In vitro* bioefficacy of the *Trichoderma* synthesized silver nanoparticles:** *Trichoderma* synthesized silver nanoparticles in two concentrations (1mM and 2mM) were tested against two paddy pathogens namely *Pyricularia oryzae* and *Bipolaris oryzae in vitro.* Against *Pyricularia oryzae, Trichoderma harzianum* nano formulation used significant variation found in different treatment. Highest percentage inhibition (79.78%) observed in 1mM (10ppm) nano formulation which is closely followed by (79.01%) 2mM (10ppm) nano formulation. When *Trichoderma asperellum*, nano formulation used significant variation found in different treatment. Highest percentage inhibition (87.85%) recorded in 2mM (10ppm) nano formulation which is closely followed by (82.36%) 2mM (5ppm) nano formulation.

Against *Bipolaris oryzae*, *T. harzianum* nano formulation showed variation in growth inhibition (56.38 to 67.54%). Highest percentage inhibition was recorded in1mM 10 ppm nano formulation which is closely followed by 2mM 10 ppm nano. In *T. asperellum* synthesized nano formulation also showed variation in growth inhibition from 56.05%to 64.38%. Highest percentage inhibition was in 1mM 10 ppm nano formulation which is closely followed by in 2mM 10 ppm nano formulation.

CONCLUSION

Silver nanoparticles were developed using *Trichoderma* by green synthesis method without using any harmful chemical. UV-Vis Spectrophotometry, FTIR study are showed formation of silver particle. *T. harzianum* and *T.*

asperellum synthesized AgNPs exhibited antagonistic property to *Pyricularia oryzae* and *Bipolaris oryzae*. With the increase in concentration of *T*. synthesized AgNP increased, antagonism also increased.

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Efficacy of *Pseudomonas fluorescens* and *Trichoderma asperellum* against Fusarium Wilt of Cucumber in Semi-Arid Zone

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Abstract: Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), poses a significant threat to cucumber (*Cucumis sativus* L.) cultivation, particularly under continuous cropping systems. In the present study, talc-based bioformulations of *Pseudomonas fluorescens* (Pf6) (OP002308) and *Trichoderma asperellum* (Th d) (OP012701) were developed and evaluated under polyhouse conditions for the management of Fusarium wilt (FOC, OP002306) in cucumber. The combined application of seed treatment and seedling dip with Pf6 significantly reduced wilt incidence (2.5%) compared to the control (25%) and outperformed chemical fungicide (Carbendazim 50% WP). Pf6 also enhanced crop earliness and recorded the highest yield (129.98 q/ha). Rhizosphere competency studies revealed that Pf6 maintained higher colony-forming units (CFU/g) over time compared to Th d, indicating superior root colonization. The biocontrol efficacy is attributed to mechanisms such as competition, antibiosis, and induction of systemic resistance. These findings establish *P. fluorescens* Pf6 as a promising, eco-friendly alternative for managing Fusarium wilt and enhancing productivity in cucumber cultivation under protected conditions.

Keywords: *Fusarium oxysporum* f.sp. *cucumerinum* (FOC), *Pseudomonas fluorescens, Trichoderma asperellum,* Bioformulation, Rhizosphere colonization

Cucumber (*Cucumis sativus* L.), a member of the family Cucurbitaceae, is a commercially important vegetable crop cultivated worldwide, especially in tropical and subtropical regions (Hin et al., 2023). India, the primary center of origin, has cultivated cucumber for over 3000 years and exhibits considerable genetic diversity (Kaur and Dhall 2017). Despite its nutritional and economic significance, cucumber cultivation is challenged by several diseases, among which Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) is particularly damaging (Al-Tuwaijri 2015). FOC is a soil-borne pathogen that invades plant roots, colonizes the vascular tissue, and produces chlamydospores capable of surviving in soil for over a decade (Chung et al., 2008). It disrupts water transport through xylem blockage and secretes phytotoxins such as fusaric acid, leading to wilting, necrosis, and eventual plant death (Lievens et al., 2007). In continuous cucumber cropping systems, root exudates like cinnamic and *p*-hydroxybenzoic acids accumulate in the rhizosphere, increasing pathogen virulence and contributing to soil sickness (Liu et al., 2010).

Historically, control of Fusarium wilt relied on chemical fungicides and soil fumigation, including methyl bromide, which is now banned due to its environmental hazards (Zhang et al, 2008). Breeding for host resistance has had limited success due to the rapid emergence of new pathogen races (Ling et al., 2010). In this context, biological control offers a promising and sustainable alternative for managing soil-borne pathogens (Huang et al., 2012). Biocontrol agents such as *Trichoderma* spp. and *Pseudomonas* spp. have demonstrated efficacy in suppressing FOC through mechanisms including competition, mycoparasitism, production of cell wall-degrading enzymes, antibiotics, and induced systemic resistance (Cao et al., 2011). *Trichoderma* spp. are highly adaptive soil fungi that colonize the rhizosphere and antagonize pathogens through secretion of chitinases, β-glucanase, and secondary metabolites (Waghunde et al, 2016). However, their efficacy is sometimes limited by soil fungistasis and nutrient availability, necessitating improved formulation strategies (Pan et al., 2006). Similarly, *Pseudomonas* spp., particularly fluorescent strains, exhibit strong rhizosphere competence and suppress FOC through the production of compounds like 2,4-diacetylphloroglucinol (DAPG), siderophores, and hydrogen cyanide (Singh et al., 2015). The present investigation was undertaken to develop bioformulations of *Pseudomonas fluorescens* Pf 6 and *Trichoderma asperellum* Th d and to standardize their application techniques under polyhouse conditions for effective management of fusarium wilt in cucumber.

MATERIAL AND METHODS

Virulent pathogen *Fusarium oxysporum* f.sp. *cucumerinum* (FOC) (accession no. OP002306) and two most potent native biocontrol agents i.e. *Pseudomonas fluorescens* (Pf 6) (accession no. OP002308) and *Trichoderma asperellum* (Th d) (accession no. OP012701) were procured from Biocontrol Lab, Department of Plant Pathology, Punjab Agricultural University, Ludhiana and were used in the evaluation.

Field evaluation of the talc bioformulations: Talc-based bioformulations of *P. fluorescens* (Pf 6) and *T. asperellum* (Th d) were prepared individually as per Singh et al, (2024). Under polyhouse conditions, the variety used was Defender Improved F1 (Parthenocarpic cucumber). The field experiment was conducted by Randomized Block Design with six treatments and four replications (10 plants per replication) during 2020-21 at the Razapur Bet (30.955823, 75.708365), Ludhiana. The bioformulations of *P. fluorescens* (Pf 6) (6.1×10^9/g) and *T. asperellum* (Th d) (9.2×10^{11}/g) were applied as seed treatment (15g bioformulations per kg of seed) + seedling dip (15g bioformulations per litre of water for 4 hours). Th - *T. harzianum* and Tv - *T. viride* were also included in the study, which were used earlier as standard biocontrol agents in the Biocontrol Lab. A chemical fungicide, Carbendazim 50% W.P. (1.5 g per kg seed) was kept as a standard check. An untreated treatment was also maintained as a control. The per cent disease incidence, plant growth promotion parameters and cucumber yield were recorded.

Rhizosphere competency evaluation: To assess rhizosphere colonization, root-adjacent soil and small root sections (approximately 10 mm in length) were collected. These samples were suspended in 10 ml of 10 mM phosphate buffer (pH 7.2) and agitated at 150 rpm on a rotary shaker at 30°C for one hour. Following serial dilution, aliquots were spread onto nutrient agar (NA) plates, which were then incubated at 28 ± 2°C for 48 hours (Das et al, 2010). Colony counts were used to estimate the population density of *Pseudomonas fluorescens* (Pf 6) and *Trichoderma asperellum* (Th d) per gram of soil, following the methodology described by Killani et al. (2011).

$$\text{CFU per gram of soil} = \frac{\text{No. of colonies} \times \text{Dilution made} \times \text{Fresh wt. of the soil}}{\text{Oven-dry weight of the soil}}$$

Statistical Analysis: The treatment means of various parameters were separated by Duncan's Multiple Range Test (DMRT) and determined by the magnitude of the F value ($p \leq 0.05$) using statistical software SPSS version 26.0 (Kara and Arici 2019).

RESULTS AND DISCUSSION

The data in minimum wilt incidence was in the *P. fluorescens* Pf 6 (2.5%) and Tv (2.5%) as compared to the control (25%) (Table 1). Wilt incidence in Th d and chemical (Carbendazim 50% WP) was 7.5 per cent and 5 per cent, respectively. The plants treated with Pf 6 start giving yield 14 days earlier than the control plant, followed by Tv and Th d, which were at par with each other (start giving yield 8 days earlier than the control plants). The yield was highest in the plants treated with Pf 6 (129.98 q/ ha), followed by Tv (126.72q/ha). All the treatments increased the yield significantly as compared to the control (84.08 q/ha).

After the seedling dip treatment, the initial CFU/g count was 9.4×10^7 and 7.1×10^9 of *T. asperellum* (Th d) and *P. fluorescens* (Pf 6), respectively (Table 2). The population density decreased after 15 and 30 days of treatment in case of the antagonists. After 45 days, the population of *T. asperellum* decreased (7.5×10^6 CFU/g), but that of *P. fluorescens* increased (8.6×10^8 CFU/g of soil). After 75 and 90 days of treatment, the population density of *T. asperellum* increases (6.5×10^6 and 7.6×10^6 CFU/g, respectively). But the CFU count of *P. fluorescens* decreases after 75 and 90 days (5.7×10^6 and 7.2×10^5 CFU/g, respectively).

The effective management of Fusarium wilt observed in this study underlines the potential of *Trichoderma* and *Pseudomonas* spp. as practical biocontrol solutions in cucumber cultivation. Earlier studies laid the foundation for understanding the mechanisms behind this success. Liu et al. (1995) first demonstrated induced resistance in cucumber due to *Pseudomonas putida*, providing evidence of systemic defense activation as a biocontrol mechanism. Later, Yedidia et al. (2000) reported increased activity of defense enzymes such as chitinase, glucanase, cellulase, and peroxidase following *Trichoderma harzianum* colonization, further strengthening the role of induced resistance in pathogen

Table 1. Effect of seed + seedling dip treatments on disease incidence and plant growth promotion under polyhouse conditions

Treatments	Disease Incidence (%)	Yield (q/ha)	Shoot length (m)	Root length (m)	Total length (m)
Th d	07.50 (2.74)^{bc}	122.14 ± 2.59^{ab}	6.54 ± 0.44^{ab}	0.56 ± 0.02^{c}	7.10
Th	10.00 (3.16)^{cd}	114.39 ± 6.69^{c}	6.20 ± 0.85^{bc}	0.53 ± 0.06^{d}	6.73
Tv	02.50 (1.58)^{a}	126.72 ± 1.48^{a}	6.65 ± 0.59^{ab}	0.62 ± 0.07^{b}	7.28
Pf 6	02.50 (1.58)^{a}	129.98 ± 2.18^{a}	7.15 ± 0.14^{a}	0.75 ± 0.02^{a}	7.90
Carbendazim 50% WP	05.00 (2.24)^{ab}	121.37 ± 3.46^{b}	5.65 ± 0.31^{c}	0.63 ± 0.05^{b}	6.28
Control	25.00 (5.00)^{e}	84.08 ± 5.05^{d}	5.23 ± 0.57^{d}	0.55 ± 0.04^{cd}	5.79

Th: *T. harzianum*, Tv: *T. viride* (standard in lab), alphabets represent DMRT

Table 2. Rhizosphere competence of antagonists applied as seed + seedling dip

Seedling Dip Treatment (Days)	Antagonist (CFU/g of soil)*	
	Razapur Bet	
	Th d	Pf 6
0	9.4×10^7 (18.37)	7.1×10^9 (22.69)
15	9.2×10^7 (18.34)	6.8×10^9 (22.64)
30	8.7×10^6 (15.95)	5.4×10^8 (20.00)
45	7.5×10^6 (15.83)	8.6×10^8 (20.57)
60	4.1×10^5 (12.92)	6.3×10^6 (15.66)
75	6.5×10^6 (15.68)	5.7×10^6 (15.55)
90	7.6×10^6 (15.82)	7.2×10^5 (13.46)
105	8.9×10^5 (13.71)	5.9×10^5 (13.29)
120	4.7×10^5 (13.08)	2.7×10^5 (12.51)
CD (p=0.05)	Th d = 0.12, Pf 6 = 0.26, Th d * Pf 6 = 0.37	

Values in parenthesis are Log_{10} transformed values

suppression. Randhawa et al. (2007) observed improved seed germination, reduced seedling mortality, and effective management of wilt disease with *T. viride* and *T. harzianum*, establishing the field relevance of these antagonists. Gul et al. (2013) also emphasized the growth-promoting and disease-suppressing roles of bioagents under field conditions. Around the same period, Tanwar et al. (2013) recorded better nutrient uptake and biomass accumulation with *T. viride* and *P. fluorescens*, which aligns with the enhanced growth responses observed in our study. The significance of rhizosphere colonization in sustaining biocontrol effects was highlighted by Gary et al. (2004), who found consistent root association and resistance enhancement by *T. viride* and *T. harzianum*. Similarly, Zhang et al. (2013) linked increased IAA production by mutant *Trichoderma* strains with higher plant biomass and improved microbial populations in the rhizosphere, suggesting that growth promotion and disease suppression are closely connected. Arya et al. (2018) reported siderophore synthesis and inhibition of *Fusarium* by *P. fluorescens*, confirming the dual role of nutrient competition and antagonism. Lian et al. (2023) demonstrated improved fresh and dry weights and chlorophyll content in *Trichoderma*-treated plants, supporting the plant growth promotion observed in our study. Furthermore, Miftakhov et al. (2023) highlighted competition for root exudates and inhibitory compound production as critical modes of action in biocontrol, corroborating the mechanisms by which the applied bioformulations reduced wilt incidence in cucumber. Taken together, these findings indicate that the integration of *Trichoderma* and *Pseudomonas* not only reduces disease severity but also improves plant vigor through multiple mechanisms such as induced resistance, growth promotion, and rhizosphere colonization.

CONCLUSION

The present study validates and extends earlier reports by demonstrating that the application of *Pseudomonas fluorescens* Pf 6 provides consistent suppression of fusarium wilt and promotes overall plant growth under field conditions. The strain exhibited strong rhizosphere persistence and advanced crop performance, underlining its potential as a sustainable and eco-friendly alternative to chemical fungicides in cucumber cultivation.

AUTHOR'S CONTRIBUTIONS

N. Singh and D.S. Buttar were responsible for the conception and design of the study. G.S. Brar and A.K. Choudhary were responsible for the acquisition of data, performed the analysis, and wrote the original draft of the manuscript. All authors have reviewed and approved the final manuscript.

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Plant-Firefly Interactions in Kanger Valley National Park, Chhattisgarh, India

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Abstract: Fireflies are renowned for their bioluminescent capabilities and are often considered the jewels of nature. These tiny luminescent creatures play significant ecological, economic, cultural, and medical roles. This study explores the floral associations of fireflies, emphasizing their importance for conservation efforts. The research specifically examines firefly preferences between trees and smaller vegetation, as well as potential role as pollinators. Observations focused on two categories of plants: associate plants and display plants. The *Abscondita perplexa* firefly species was associated with 15 different floral families and showed preference for trees over smaller vegetation. Among the trees, the preference for Combretaceae and Dipterocarpaceae families was the highest. The fireflies exhibit a greater preference for non-flowering plants compared to flowering ones.

Keywords: *Abscondita perplexa*, Plant-firefly, Kanger Valley National Park, Combretaceae, Dipterocarpaceae

Fireflies are nocturnal insects that belong to the family Lampyridae. These fascinating insects can be bioluminescent, a phenomenon resulting from a chemical reaction in the abdominal region. In this process, luciferin, in the presence of luciferase, reacts with oxygen to create light. Approximately 2,000 species of fireflies are found globally and inhabit diverse regions across the globe, including Asia, Europe, Africa, Australia, and the Pacific islands (Lewis et al., 2020). The primary function of the light produced by fireflies is communication and plays a crucial role in deterring potential predators (Zhu et al., 2024, Novák and Jakubec 2024). Fireflies gather around certain vegetation types, such as mangroves and display trees (Jusoh et al., 2010). Male fireflies use these trees for courtship, where they come together to form a synchronized display. The diversity and structure of plant life can significantly influence the behaviour of fireflies, the arrangement and size of leaves may impact the population levels of fireflies, and the density of leaves also influences courtship behaviour (Seri et al., 2022). Additionally, chemicals released by plants can affect firefly behaviour, underscoring vegetation's crucial role in shaping firefly populations (Seri and Rahman 2024). Vegetation influences mating behaviour, as males use light signals from prominent spots on plants to attract females. Healthy, diverse vegetation supports larger firefly populations, ensuring their persistence in various ecosystems. Approximately 530 plant species are Kanger Valley National Park were reported by Kotia et al. (2013). This study focuses on firefly species *Abscondita perplexa* (Walker 1858), interactions with its floral associates in the mixed deciduous forest of Kanger Valley National Park (KVNP), Bastar, Chhattisgarh. This research will possibly aid in uncovering species of fireflies, identifying hotspot areas and understanding the plant firefly interactions within Kanger Valley National Park.

MATERIAL AND METHODS

Study area: Kanger Valley National Park (18° 51' 57.60" N, 82° 09' 53.64" E), located in the Bastar district of Chhattisgarh, covering an area of 200 square kilometres. Approximately 50 villages border Kanger Valley National Park. The targeted study sites within the study area were selected randomly based on two habitat characteristics: woodland area and wetland area. A total of 11 sites (6 wetland and 5 woodland) were selected from 5 different ranges of KVNP (Fig. 1).

Data collection: The study commenced in December 2024, and the field survey was conducted until March 30, 2025. A sweeping net was employed to collect firefly specimens, while a Naivete LED headlamp and a rechargeable LED mini torch were used for nocturnal surveys. Field observations were documented using a Nikon Coolpix DSLR camera P950, and detailed images of the firefly specimens were captured with an Adcom 12X/24X macro lens.

Collection vials were utilized to store the specimens. The fireflies were preserved in 70% ethanol. GPS waypoint app was used to track locations and save the line transect data.

Digital microscope was used to visualize the dissected genitalia of fireflies for identification purposes.

Methodology: Stratified random line transect sampling was employed to evaluate the diversity and distribution of fireflies in KVNP (Fewster et al., 2005). This method ensures that various habitat types are included, and randomization eliminates bias in the selection of sampling sites.

The study area was initially divided into distinct strata based on habitat characteristics: woodland and wetland. Three line transects were randomly placed within each stratum, each measuring 250 meters long. To ensure independent sampling units, there was a gap of 300 meters between each line transect, allowing for a practical study of the site. During the observation of each line transect survey, the species of fireflies were recorded, their floral associations, and other parameters, including latitude, longitude, altitude, temperature, humidity, soil type, and moon phase for each transect was also recorded. During the observation of floral associates of fireflies, the vegetation was categorized into two groups: associate plants (AP) and display plants (DP). For the identification of the plant species the botanical literature and guide were used (Nanda and Shaw 2008, Khanna et al., 2024).

Associate plants include plants that adult fireflies and larvae prefer for perching, resting, and laying eggs. In contrast, display Plants are the plants that male fireflies typically use for courtship to attract female fireflies. Net Sampling was conducted in two portions: the lower vegetation, which includes grass, shrubs, and the lower branches of trees, and the mid-canopy, which refers to the middle portions of the trees. The sample size for each sampling unit was 10, with 5 samples collected from the lower vegetation and 5 samples from the mid-canopy. The net sweeping method (Jaikla et al., 2020) was employed in both habitats to capture fireflies for study effectively. As a result, a diverse collection of fireflies was obtained, facilitating further analysis of their distribution and behaviour. The visual count was used to count the number of fireflies in the field based on the flashes that can be observed easily (Yiu et al., 2024, Evans et al., 2019). The time for observing, counting, and collecting fireflies was from 6 PM to 9 PM, coinciding with the emergence of fireflies between 6:20 PM and 6:40 PM, while their disappearance occurred from 7:30 PM to 9 PM.

Data analysis: R programming was used for the analysis. A Chi-square test will investigate the preferences of fireflies between different tree species and plants, contributing to a deeper understanding of their habitat selection and ecological interactions. In addition, calculating the median firefly counts will provide insights into their preferences for flowering versus non-flowering plants. Another chi-square test analysis, focusing on comparing fireflies' preferences for distinct plant categories, such as Display plants (DP) and Associated plants (AP), was performed.

RESULTS AND DISCUSSION

Fifteen plant families have been recorded in association with fireflies. The top four families include Combretaceae, Dipterocarpaceae, Asteraceae and Sapindaceae being 41.15, 14.90, 10.26, and 10.62%, respectively (Fig. 2).

The fireflies prefer trees over smaller plants, such as herbs and shrubs (Fig. 3). This can be due to wide shelter, broader leaves to hide, and tall enough so that male fireflies can perform courtship display. The mean and median values for the occurrence of fireflies in flowering and non-flowering plants indicated significant disparity between the mean and median values for both types of plants, with the median being

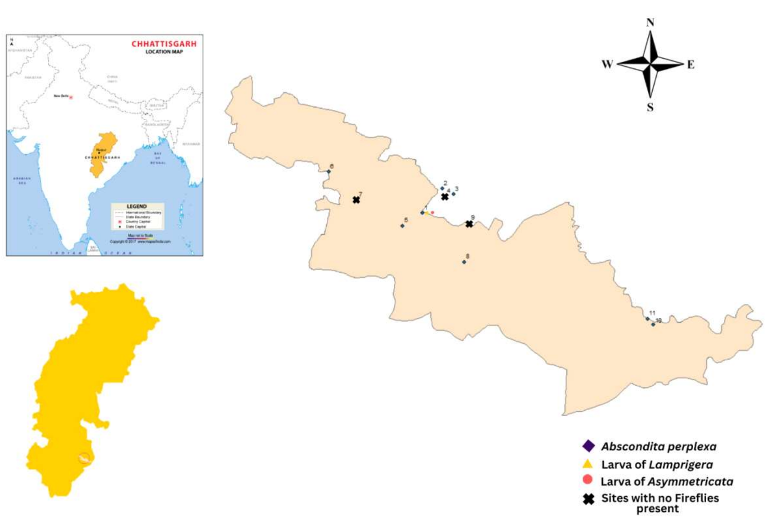

Fig. 1. Kanger Valley National Park with the study sites

lower than the mean (Fig. 4). This suggests that the data is not normally distributed and is right-skewed. Consequently, the median is a more appropriate measure for understanding firefly preferences. The median indicates that fireflies show a greater preference for non-flowering plants compared to flowering plants. To investigate whether fireflies have a preference for non-flowering plants over flowering ones, a Mann-Whitney U test was conducted. The calculated U minimum was 6, which is smaller than the critical value of 7 which indicates a significant difference in the preferences of fireflies for flowering versus non-flowering plants, leading us to reject the null hypothesis. Thus, based on the median values, can be concluded that fireflies prefer non-flowering plants over flowering ones. Chi-square test was conducted to assess the fireflies' preference for display plants (DP) versus associated plants (AP). The fireflies exhibit a stronger association with display plants compared to associated Plants. The higher number of males than females using a display plant for courtship likely stems from competition among males for the limited female population. Males often

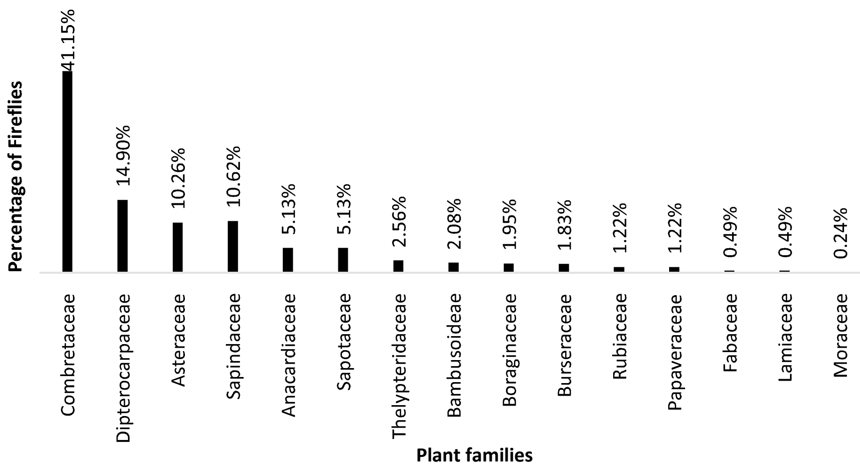

Fig. 2. Occurrence of fireflies in different floras

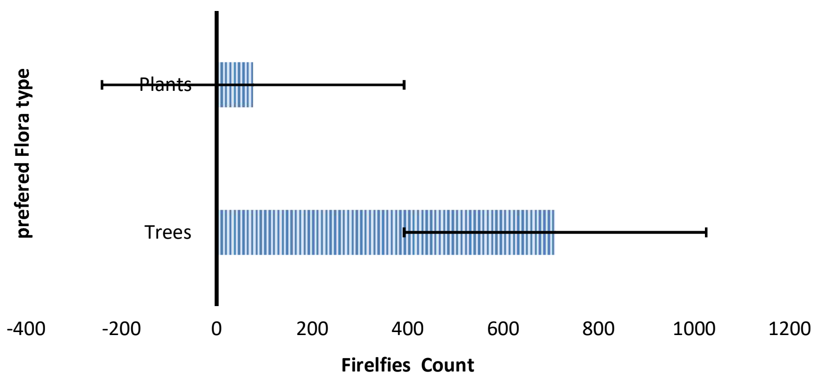

Fig. 3. Fireflies preference for trees and smaller vegetation's

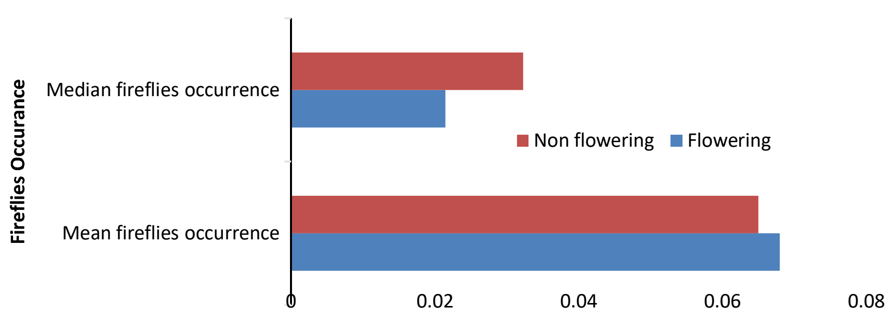

Fig. 4. Comparison of firefly preferences for flowering vs non-flowering plants

utilise these plants to showcase their traits and attract mates. This behaviour is influenced by evolutionary strategies that favour males displaying their fitness.

Additionally, environmental factors may contribute to a skewed gender ratio, further emphasizing the role of these display plants in mating dynamics. Fireflies are associated with a wide range of plants, mainly belonging to the families Combretaceae, Asteraceae, Dipterocarpaceae, and Sapindaceae, where their occurrence was highest.

When comparing flowering and non-flowering plants, fireflies favour non-flowering plants, suggesting that they are not significant pollinators. However, among flowering plants, the highest occurrence of fireflies has been observed in the Asteraceae family, which consists mainly of weeds. This indicates that fireflies may act as pollinators for these weeds. A higher number of fireflies occurs as display plants which signifies a greater number of active males. To comprehensively understand the bioecology of the fireflies in central India, future studies must incorporate a detailed analysis of abiotic environmental factors.

Table 1. GPS Location of study sites

Site ID	Range name	Latitude	Longitude
1	Nagalsar	18.864038°	81.971140°
2	Nagalsar	18.877927°	81.982376°
3	Nagalsar	18.874743°	81.988784°
4	Nagalsar	18.873435°	81.983960°
5	Darbha	18.856633°	81.959676°
6	Kotumsar	18.887568°	81.917359°
7	Kotumsar	18.871281°	81.933650°
8	Netanar	18.835859°	81.994980°
9	Netanar	18.858132°	81.997939°
10	Kolenge	18.800152°	82.103108°
11	Kolenge	18.803544°	82.099938°

Table 2. Plant families preferred as AP or DP

Plant family	AP	DP
Combretaceae	-	+
Asteraceae	+	-
Dipterocarpaceae	-	+
Sapindaceae	-	+
Anacardiaceae	-	+
Sapotaceae	-	+
Boraginaceae	-	+
Thelypteridaceae	-	+
Lamiaceae	+	-
Rubiaceae	-	+
papaveraceae	+	-
Bambusoideae	-	+
Burseraceae	-	+
Moraceae	-	+
Fabaceae	-	+

CONCLUSION

This study examines the interaction between plants and fireflies within Kanger Valley National Park. It reveals a clear preference among fireflies (*Abscondita perplexa*) for trees over smaller vegetation and for non-flowering plants over flowering ones. Among the diverse flora preferred by fireflies, the families Combretaceae and Dipterocarpaceae stand out with the highest preference. The findings also indicate that while fireflies are not significant pollinators, they do assist in the pollination of certain weed species, notably within the Asteraceae family. These insights strongly emphasize the importance of conserving habitats rich in flora for the protection of firefly populations.

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Indian Odonata: Diversity, Ecology, and Conservation Challenges

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Abstract: Odonata is one of the most ancient groups of flying insects, with fossil records dating back to the Permian era (299-251 million years ago). Classified into three suborders based on morphology: the Anisozygoptera, Zygoptera, and Anisoptera, they are amphibious hemi-metabolic insects having the aquatic egg and larval stages, while the adults are terrestrial. Among the Anisozygoptera, *Epiophlebia laidlawi* Tillyard 1921, one of only four known species, is found in Darjeeling. This review summarizes literature of last 30 years and reveals: (i) the total diversity of species encountered remains very small- 508, considering country's large area as well as the multitude of habitat kinds; (ii) only 12 species of dragon flies and 14 species of damselflies find mention as rare, endangered, and threatened species (RET), while a very significant number (179) have been categorized as data deficient or not evaluated. The limited recorded diversity and conservation status of Indian odonates reflect a lack of comprehensive research, even in terms of basic inventorization. Most studies focus solely on species inventorization, overlooking ecological aspects. This paper highlights the urgent need for in-depth studies addressing habitat types and environmental changes. Addressing these gaps is crucial to safeguard these bioindicators and ensure the sustainability of freshwater ecosystems.

Keywords: Anisoptera, Anisozygoptera, Damselflies, Dragonflies, Odonata, Specialized species, Zygoptera

Nearly 1 million of the 1.9 million species documented globally are insects. Taxonomists estimate that the total number of species worldwide ranges between three million and hundred million. These diversity estimates, derived from taxonomic studies differ from those based on macro ecological patterns and biodiversity ratios (Masih and Pathak 2022). Among the insects that hover over forests, farms, meadows, ponds, and rivers most frequently are odonates, which include dragonflies and damselflies. Globally, 6,442 species of Odonata have been identified (Paulson et al., 2025), with 508 species reported from India, and 137 species from the State of Uttarakhand. The order Odonata is divided into three suborders based on morphology- Anisozygoptera, Zygoptera, and Anisoptera: Anisoptera (dragonflies), Anisozygoptera, and Zygoptera (damselflies), each comprising several superfamilies, families, genera, and species that reflect the rich taxonomic diversity of this insect group.

Globally, there are only four known species of Anisozygoptera- *Epiophlebia superstes* (Selys 1889), found exclusively in Japan; *E. laidlawi* Tillyard 1921 is restricted to the Himalayan region (Dawn 2021); while *E. sinensis* Li and Nel 2012 and *E. diana* Carle 2012 were both, reported recently from China (Blanke et al., 2013, Büsse and Ware 2022), respectively. Dragonflies and damselflies are easily distinguishable in their natural habitats (Masih and Pathak 2022). Dragonflies are generally more robust insects compared to damselflies, and while resting, dragonflies hold their wings outstretched, whereas damselflies fold their wings along their bodies. Despite these morphological differences, both groups share similar overall life cycles, and more or less, similar habitat conditions (Masih and Pathak 2022). Within Anisoptera, the superfamily Aeshnoidea includes the family Aeshnidae, which consists of 13 genera and 52 species known for their large size and powerful flight. The superfamily Gomphoidea is represented by the family Gomphidae, with 31 genera and 89 species characterized by their clubbed tails. The Cordulegastroidea superfamily comprises two families: Chlorogomphidae and Cordulegasteridae, which together contain 6 genera and 17 species, typically inhabiting stream environments. The largest diversity within Anisoptera is found in the superfamily Libelluloidea, which includes families such as Libellulidae (43 genera, 97 species), Macromiidae (2 genera, 17 species), and Corduliidae (2 genera, 2 species), alongside 2 genera and 15 species of uncertain familial placement (incertae sedis) (Table 1). The family Libellulidae is particularly diverse and widespread, commonly encountered in a variety of freshwater habitats. The small suborder Anisozygoptera includes only one family, Epiophlebiidae, represented by a lone species- *Epiophlebia laidlawi* Tillyard 1921, reflecting its ancient and relict status (Table 1).

In contrast, the suborder Zygoptera displays substantial diversity across multiple superfamilies. For instance, Lestoidea contains the families Lestidae and Synlestidae, with a combined 6 genera and 27 species (Table 1). The

Platystictoidea superfamily, represented by the family Platystictidae, includes 3 genera and 23 species mostly found in shaded, forested streams. The Calopterygoidea superfamily is home to several families, including Calopterygidae (6 genera, 9 species), Chlorocyphidae (8 genera, 22 species), Euphaeidae (5 genera, 20 species), and Philogangidae (2 genera, 2 species), all notable for their bright colors and iridescence (Table 1). Lastly, the superfamily Coenagrionoidea consists mainly of the families Coenagrionidae and Platycnemididae, which together account for 26 genera and 115 species, making them the most speciose among the damselflies (Table 1). Overall, this classification underscores the extensive taxonomic richness and ecological diversity of odonates, emphasizing their importance in freshwater ecosystems and their value as indicators of environmental health.

Due to their wide range of ecological roles and their ability to serve as bioindicators for assessing aquatic habitats, odonates are increasingly being utilized in both basic and applied research (Masih and Pathak 2022) as model organism for testing key hypotheses related to sexual selection, complex life cycles, the evolution of flight, and community ecology (Córdoba-Aguilar 2008); and being sensitive to environmental changes- both biotic as well as abiotic, they are invariably associated with the studies related to conservation management, and assessment of aquatic environmental health (Córdoba-Aguilar 2008). Further, studies conducted on odonate spectrum extension analyses by latitude, have lately been suggested that odonates may provide valuable insights about climate change, or its effects vis-à-vis changes in population dynamics of odonates (Masih and Pathak 2022). Given their ecological importance, aesthetic value, and potential as environmental indicators, particularly as relates to understanding the relationship between Odonata diversity and the quality of their preferred habitats, odonates offer immense scope for advancing biodiversity research and conservation efforts. In this review, attempt is made to categorize Indian odonata in terms of their distribution across different states into generalized and specialized groups. Further, by consolidating existing records, this study seeks to provide a comprehensive understanding of the current status of odonata diversity in India, identify key research gaps, and propose strategies for their conservation and sustainable management amidst increasing anthropogenic and climatic pressures.

RESULTS AND DISCUSSION

Detailed literature review conducted for the last 35 years (1989 to 2025) brings out the following facts: Anisoptera (dragonflies) is distributed across 4 super families, 8 families,

Table 1. Distribution of the species across families and sub-orders

Suborders	Super family	Family	Genera	Species
Anisoptera	Aeshnoidea	Aeshnidae	13	52
	Gomphoidea	Gomphidae	31	89
	Cordulegastroidea	Chlorogomphidae	03	08
		Cordulegasteridae	03	09
	Libelluloidea	Corduliidae	02	02
		Libellulidae	43	97
		Macromiidae	02	17
		Genera incertae sedis	02	15
Anisozygoptera	Epiophlebioidea	Epiophlebiidae	01	01
Zygoptera	Lestoidea	Lestidae	05	21
		Synlestidae	01	06
	Platystictoidea	Platystictidae	03	23
	Calopterygoidea	Calopterygidae	06	09
		Chlorocyphidae	08	22
		Euphaeidae	05	20
		Philogangidae	02	02
	Coenagrionoidea	Coenagrionidae	11	60
		Platycnemididae	15	55
		Total	156	508

99 genera, and altogether 289 species, while Zygoptera is represented by 4 super families, 9 families, 56 genera, and 218 species. The rare order Anisozygoptera is represented by a monotypic species- *Epiophlebia laidlawi* Tillyard 1921 (Fig. 1). Out of the total 192 references consulted, as relates to distribution and habitats, 36 studies have been conducted on a landscape level, followed by water habitats (e.g., lakes, rivers, etc)- 29, those confined to the protected areas (PAs) constitute 27. Other studies are more or less, equitably conducted in other habitat types, such as forests (16), campus and educational institutes (14), agro-fields (11), and urban ecosystems (07).

A significant number of studies could not be categorized, and thus have been relegated as 'Not specified' (52), i.e., constitutes just inventorisation of the species. Those, related to ecological analyses vis-à-vis habitat alteration/changes, or those incorporating the distribution or abundance of the odonates over a temporal or spatial scale, constitute just 38. In brief, current survey, only strengthens the views of Aghade et al. (2022) and (Masih and Pathak 2022) that studies on odonates lack both in expanse (i.e., lack of studies, that explores more habitats/sites), and on ecological aspects- i.e., studies, that explores the distribution and abundance of odonates across environmental gradient- habitats, temporal and spatial distribution, effect of changed climatic conditions, habitat alterations, and the like. Nonetheless, the wide-ranging habitats types reflect the ecological significance of odonates and the necessity of habitat-specific conservation efforts. Further, despite being one of the largest countries in the world, with wide range of habitats, not to speak of greater number of water bodies- types as well as numbers, research on odonates in India remains limited and geographically skewed. Studies are confined to only a few states, and within these states, research is often restricted to specific localities, rather than being comprehensive.

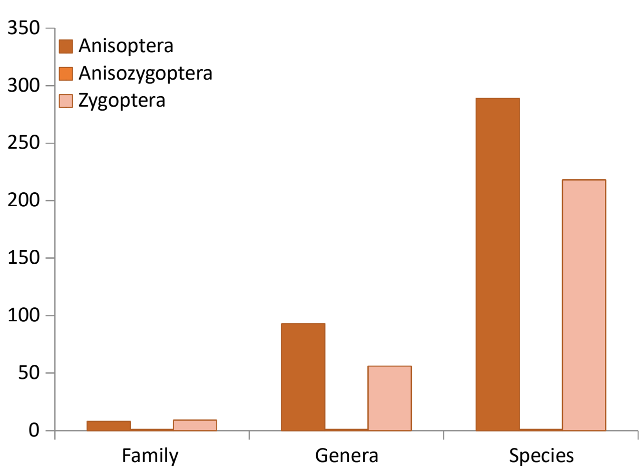

Fig. 1. Distribution of the odonates across the three sub-orders

In terms of studies conducted across the different states-most studies are reported from the state of Maharashtra (14), followed by, Kerala (12), Assam, Madhya Pradesh, West Bengal, and Odisha (10 each), Tamil Nadu and Rajasthan (9 each), Himachal Pradesh, Karnataka and Gujarat (7 each), followed by other states, where the number of studies numbers just a few (between 1-5). Table 2 reflects upon the Odonata diversity within each state. Overall, the total diversity exhibits an increase by 4 species, i.e., 508, instead of the earlier 504. The states which show an increase in Odonata diversity are the following- Andhra Pradesh (78 against 72), Chhattisgarh (88 against 87), Gujarat (74 against 63), Himachal Pradesh (136 against 122), Haryana (48 against 26), Jammu & Kashmir (94 against 67), Kerala (191 against 178), Maharashtra (143 against 139), Odisha (118 against 114), Punjab (70 against 41), Rajasthan (99 against 51), Sikkim (97 against 82), Uttar Pradesh (77 against 70), Uttarakhand (137 against 127), and West Bengal (241 against 240) (Table 2).

The present review improves upon the last data carried out by Subramanian and Babu 2024, regarding the distribution of odonates across the States. The total diversity of odonates within the country has improved by 4 species- (i) *Melanoneura agasthyamalaica* sp. nov, (ii) *Phylloneura rupestris* (Chandran, Chandran and Jose 2024), (iii) *Protosticta sexcolorata* sp. nov, and (iv) *Euphaea wayanadensis* Anooj, Susanth and Sadasivan 2025. Similarly, for the State of Uttarakhand, the total number of odonates have improved from 127 to 137, including 3

Fig. 2. Three new records being reported for the first time from the State of Uttarakhand. (1) *Ophiogomphus reductus* Calvert 1898 (2) *Calicnemia nipalica* Kimmins 1958 and (3) *Ceriagrion olivaceum* (Laidlaw 1914)

species- (i) *Ophiogomphus reductus* Calvert 1898, (ii) *Calicnemia nipalica* Kimmins 1958 and (iii) *Ceriagrion olivaceum* (Laidlaw 1914), which are being reported for the first time (Fig. 2).

Altogether, 508 species are reported from the country (Table 1), out of which 12 species from Anisoptera, and 15 species from Zygoptera, and the lone species from Anisozygoptera, are included in the IUCN Red Data list (IUCN 2024, Table 3). The Rare, Endangered, and Threatened (RET) species (28) could further be categorized, in terms of the status, as the following- Critically endangered (01), endangered (03), vulnerable species (11), and the near threatened (13) (Table 3). These classifications emphasize the urgency of conservation efforts to protect these species and their habitats from ongoing threats such as habitat destruction, pollution, and climate change.

Table 2. Distribution of Odonata diversity across India

States	Species diversity		Reference
	Subramanian and Babu (2024)	Current study	
Andhra Pradesh	72	78	Prasad 2007, Amaravathi et al., 2018
Arunachal Pradesh	110	95	Ram and Prasad 1999, Payra et al., 2017,
Assam	173	117	Baruah and Saikai 2015, Choudhury et al., 2020, Thakuria and Kalita 2021, Das et al., 2022, Chetia and Das, 2022
Chhattisgarh	87	88	Dawn and Chandra 2014, Sahu and Rai 2019
Gujarat	63	74	Rohmare et al., 2016, Mokaria and Jethva 2019, Sharma and Kumar 2020, Rathod et al., 2021
Goa	87	79	Rangnekar et al., 2010, Rangnekar and Naik 2014, D'Souza and Pal 2019
Himachal Pradesh	122	136	Sharma 2019, Singh et al., 2021, Raj et al., 2024
Haryana	26	48	Sharma and Joshi 2007
Jammu and Kashmir	67	94	Paray and Mir 2023, Hussain et al., 2024
Jharkhand	72	57	Saha and Hembrom 2020, Devidas et al., 2023
Karnataka	141	101	Kumar and Shetty 2019, Thampuran et al., 2021, Rani et al., 2023
Kerala	178	191	Nair et al., 2021, Chandran et al., 2021, 2023, 2024, Bhatia and Kumari 2024, Davisian et al., 2024
Madhya Pradesh	88	84	Mishra et al., 2019, Chourasia et al., 2020, Dubey et al., 2021, Tiple et al., 2022
Maharashtra	139	143	Koparde et al., 2014, Tiple and Koparde 2015, Gajbe 2020, Supanekar et al., 2021, Bharthi and Koparde 2022, Sawant et al., 2022, 2023
Manipur	92	56	Takhelmayum and Gupta 2014, Singh et al., 2016
Meghalaya	160	72	Bora 2019, Bora et al., 2020
Mizoram	65	28	Laltanpuli et al., 2013
Nagaland	107	90	Joshi and Kunte 2014
Odisha	114	118	Pandey and Mohapatra 2017, Debata et al., 2017, Payra et al., 2019, 2020
Punjab	41	70	Singh 2022
Rajasthan	51	99	Singh et al., 2017, Singh and Hermans 2019, Bishnoi and Dang 2019, Johari and Jain 2021
Sikkim	82	97	Mitra 2004, Payra and Bhutia 2017
Tamil Nadu	147	113	Veeramani et al., 2018, Pavithran et al., 2020, Manikandan et al., 2021, Manikandan et al., 2023
Telangana	57	35	Rehman et al., 2015
Tripura	76	75	Majumder et al., 2014, Datta et al., 2023
Uttar Pradesh	70	77	Kanaujia et al., 2015, Dubey and Dubey 2019
Uttarakhand	127	137	Dayakrishna and Arya 2015, Uniyal et al., 2019, De et al., 2021
West Bengal	240	241	Goswami et al., 2018, Payra and Tiple 2019, Ghosh 2023, Samanta et al., 2023

Table 3. RET species as per IUCN 2024

Sub-order	Species	Status
Zygoptera (01)	*Protosticta myristicaensis* Joshi and Kunte 2020	CR
Anisoptera (02)	*Idionyx galeata* Fraser 1924, *Orthetrum andamanicum* Bedjanič, Kalkman and Subramanian 2020	EN
Zygoptera (01)	*Libellago balus* Hämäläinen 2002	
Anisoptera (03)	*Chlorogomphus xanthoptera* Fraser 1919, *Cyclogomphus ypsilon* Selys 1854, and *Chloropetalia selysi* Fraser 1929	VU
Zygoptera (08)	*Disparoneura apicalis* Fraser 1924, *Calicnemia nipalica* Kimmins 1958, *Coeliccia fraseri* Laidlaw 1932, *Anisopleura vallei* St. Quentin 1937, *Libellago blanda* Hagen in Selys 1853, *Libellago andamanensis* Fraser 1924, *Protosticta sanguinostigma* Fraser 1922, *Indosticta deccanensis* Laidlaw 1915	
Anisoptera (07)	*Planaeschna intersedans* Martin 1909, *Anormogomphus kiritschenkoi* Bartenef 1913, *Asiagomphus personatus* Selys 1873, *Heliogomphus promelas* Selys 1873, *Neallogaster ornata* Asahina 1982, *Idionyx optata* Selys 1878, and *Megalogomphus hannyngtoni* Fraser 1923	NT
Anisozygoptera (01)	*Epiophlebia laidlawi* Tillyard 1921	
Zygoptera (05)	*Indolestes indicus* Fraser 1922, *Indocypha vittata* Selys 1891, *Elattoneura atkinsonii* Selys 1886, *Melanoneura bilineata* Fraser 1922, and *Phylloneura westermanni* Selys 1860	

CR- Critically Endangered, EN- Endangered, V- Vulnerable, NT- Near threatened

Table 4. List of highly specialized species restricted to just a few sites/ or reported from 1-2 states only

Species	State	Reference
Anisoptera		
Gynacantha pallampurica (Lahiri, Sandhu and Walia 2007)	Himachal Pradesh	Lahiri et al., 2007, Kalkman et al., 2020
Gynacantha andamanae (Yeh and Veenakumari 2000)	Andaman	Yeh and Veenakumari, 2000, Kalkman et al., 2020
Gynacantha odoneli (Fraser 1922)	West Bengal	Kalkman et al., 2020, Dawn 2021
Gomphidia williamsoni (Fraser 1923)	West Bengal	Kalkman et al., 2020, Dawn 2021
Orthetrum andamanicum Bedjanič, (Kalkman and Subramanian 2020)	Andaman	Bedjanic et al., 2020, Kalkman et al., 2020
Orthetrum erythronigrum (Subramanian, Babu and Kalkman 2020)	Andaman	Subramanian et al., 2020
Orthetrum martensi (Asahina 1978)	Andaman	Kalkman et al., 2020
Rhyothemis phyllis (Sulzer 1776)	Andaman	Raja et al., 2000, Kalkman et al., 2020
Idionyx stevensi (Fraser 1924)	West Bengal	Dawn 2021
Idionyx gomantakensis (Subramanian, Rangnaker and Nayak 2013)	Kerala	Nair et al., 2022
Idionyx intricata (Fraser 1926)	Kolkata	Kalkman et al., 2020
Zygoptera		
Lestes barbarus (Fabricius 1798)	West Bengal	Subramanian and Babu 2017, Dawn 2021
L. garoensis (Lahiri 1987)	West Bengal	Subramanian and Babu 2017, Dawn 2021, Samanta et al., 2023
Ceriagrion pratermissum (Lieftinck 1929)	West Bengal	Subramanian and Babu 2017, Kalkman et al., 2020, Dawn 2021
C. olivaceum (Laidlaw 1914)	West Bengal, Rajasthan, Uttarakhand.	Dawn 2021, Singh 2022, own record (unpublished, Figure 2)
Coenagrion exclamationis (Fraser 1919)	West Bengal	Subramanian and Babu 2017, Kalkman et al., 2020, Dawn 2021
Calicnemia nipalica (Kimmins 1958)	West Bengal, Uttarakhand, Sikkim	Subramanian and Babu 2017, Kalkman et al., 2020, Dawn 2021, own record (unpublished, Figure 2)
Coeliccia svihleri (Asahina 1970)	Kolkata	Subramanian and Babu 2017, Kalkman et al., 2020, Dawn 2021
C. prakritiae (Lahiri 1985)	Kolkata	Subramanian and Babu 2017, Kalkman et al., 2020, Dawn 2021
Melanoneura agasthyamalaica sp. nov	Kerela	Chandran et al., 2024
Phylloneura rupestris (Chandran, Chandran and Jose 2024)	Thiruvananthapuram	Chandran et al., 2024
Protosticta sexcolorata sp. nov	Wayanad	Chandran et al., 2023
Euphaea wayanadensis Anooj, Susanth and Sadasivan 2025	Wayanad	Anooj et al., 2025

More significantly, 179 species- 113 and 66 species from Anisoptera and Zygoptera, respectively, are categorized as 'Data deficient' or 'Not evaluated'. Further, two species- *Nihonogomphus pulcherrimus* (Fraser 1927) from the family Gomphidae and *Lyriothemis flava* (Oguma 1915), from the family Libellulidae, remain unaccounted for; since they are just mentioned in the check list by (Subramanian and Babu 2024), however, find no mention, elsewhere. Among Anisoptera, around 11 species, distributed across 8 Genera are highly specialized, while in Zygoptera, 12 species across 9 genera are highly specialized, i.e., are reported from a few sites, only (Table 4). These species would require more intensive studies to ascertain their precise status in the wild, so that conservation efforts could be undertaken.

CONCLUSION

The number of Odonata species reported across the states of India is relatively low, considering the country's vast area, multitude of water bodies and diversity of habitat types, climate regimes, diversity of vegetation profiles, etc. Most of the extensive studies are again, confined to few states, such as Assam, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, and West Bengal; and even within these states, studies are mostly confined to the 'inventorization' of odonates, rather than those addressing the ecological aspects of its distribution, both on spatial and temporal scales, as well as effects on its abundance (primarily) vis-à-vis changes in habitat conditions, and like. The lack of studies is reflected in relatively meagre number of species designated as RET species (28), while those designated as 'Data deficient' and 'Not evaluated', number a huge 179. Further, an additional 23 species have been reported from a few sites, or 1-2 states only. Even though, over the years, 40 new species of odonates, totalling 508, have been reported from the country, which do reflect upon the resurgence in research on odonates, there is a need for more intensive and extensive study of odonates that incorporates the vulnerability aspects, and not just confined to the habitat studies alone. This is more so, since odonates remain an excellent bioindicators, reflecting upon the health of the ecosystems. Any decline in their abundance, reflects upon the deterioration of their habitats. In other words, preserving their habitats is essential to maintain stable populations and diversity of these insects.

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Biological and Productivity Linked Parameters of Lac Insect, *Kerria lacca* (Kerr.) on *Flemingia macrophylla* and *Flemingia semialata* in Punjab

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Abstract: The effect of the leguminous host plants, *Flemingia macrophylla* and *Flemingia semialata* was studied on the biological and productivity linked parameters of the lac insect, *Kerria lacca* during *Baisakhi* and *Katki* crops. In *Baisakhi* crop, the mean initial settlement density was 77.20 and 82.20 crawlers/cm^2 in *F. macrophylla* and *F. semialata*, respectively. During *Katki* crop, the respective density was 76.33 and 79.73 crawlers/cm^2. The mean initial mortality was 9.38 and 9.62 per cent in *F. macrophylla* and *F. semialata*, respectively during *Baisakhi* crop and in *Katki* crop, the mean mortality was 10.92 and 8.48 per cent, respectively. The total life period of lac females was 234.33 and 232.00 days in *Baisakhi* crop on *F. macrophylla* and *F. semialata*, respectively and in *Katki* crop was 106.67 and 108.33 days, respectively. The mean weight of female cells of lac insect was 8.66 and 8.94 mg on *F. macrophylla* and *F. semialata*, respectively during *Baisakhi* crop. Similarly, in *Katki* crop the mean weight of female cells was 7.48 and 7.65mg, respectively. The mean resin output during *Baisakhi* crop was 5.95 and 6.10mg per female cell on *F. macrophylla* and *F. semialata*, respectively, while the mean weight of resin output was 5.36 and 5.50mg on *F. macrophylla* and *F. semialata*, respectively in *Katki* crop. It is concluded that both *F. semialata* and *F. macrophylla* could be promising hosts for lac cultivation under Punjab conditions.

Keywords: Biology, *Kerria lacca, Flemingia macrophylla*, *Flemingia semialata*, Productivity

Lac resin, a natural substance of animal origin, is secreted by the lac insect, *Kerria lacca* (Hemiptera: Tachardiidae). The resin along with other lac products has an economic importance which finds its use in many industries. All the stages of lac insect except neonates are sedentary in nature and suck the plant phloem sap throughout their life from certain lac host plants (Ahmad et al., 2012). The neonates crawl, settling at specific parts of these plants, providing feed for the resting stages of the insects. Lac cultivation is practiced in many South Asian countries like India, Thailand China, Bangladesh and Indonesia. India is the largest producer of lac in the world wherein it is commercially produced in states like Chhattisgarh, Jharkhand, Madhya Pradesh, Orissa and West Bengal (Yogi et al., 2015). More than 400 plants are reported to serve as host for the survival and development of the lac insect. Certain host species as well as varieties thereof have been reported to support more specifically to lac insect (Sharma et al., 1997). The lac insect has been reported to thrive well on the tender shoots of various natural hosts like ber (*Ziziphus mauritiana*), palas (*Butea monosperma*) and kusum (*Schleichera oleosa*) in these states (Sharma 2017). However, the commercial production of lac is declining due to non-availability of its natural hosts owing to depletion of the forest covers. There is need to explore alternate host plants, showing promising results on the growth and development of lac insect. Leguminous plants, *Flemingia macrophylla* (Willd.) Merr. and *Flemingia semialata Roxb*. ex W. T. Aiton reported as potential alternative to lac insects' natural hosts (Jaiswal and Singh 2012; Kumar *et al*. 2017). These plants are quick growing and are suitable for lac cultivation. Both *F. macrophylla* and *F. semialata* are suitable for *Kusmi* as well as *Rangeeni* strains of the lac insect, *K. lacca*. Growing of various horticultural crops like aonla (*Phyllanthus emblica*), ber (*Ziziphus maurutiana*), guava (*Psidium guajava*), lime (*Citrus aurantifolia*) and seasonal vegetables in vertical hierarchy pattern was compatible with *F. semialata* (Singhal et al., 2014). Being the phloem feeders, the quantity as well as quality of food may determine the growth and survival of lac insect. Various host plant species have been reported to alter lac insect biological attributes (Sandeep et al., 2020). Apart from abiotic factors, lac production is affected by various biological and productivity linked parameters like initial density of settlement, mortality, sex-ratio, survival at maturity, food quality, etc (Mohanasundaram et al., 2014, Mohanta 2014). Thus keeping in mind the importance of host plant characteristics affecting lac insect growth and development, the present study was conducted to determine the effect of host plants *F. macrophylla* and *F. semialata* on biology and productivity linked parameters of *K. lacca* under Punjab conditions.

MATERIAL AND METHODS

Raising of the host plants: The initial seed culture of *F. macrophylla* and *F. semialata* was procured from ICAR-National Institute of Secondary Agriculture (NISA), Ranchi. The nursery plants were raised in the polythene bags (20 x 10 cm). A sandy loam soil + FYM mixture was prepared for raising nursery plants. Holes were made at the bottom of the polythene bags for proper drainage. The nursery was sown in the last week of April. The polythene bags were kept under partial shade conditions. Utmost care was taken for the irrigation requirements of the germinated plants, due to hot weather conditions during the summer months. The nursery plants were transplanted in the well drained field during the last week of June in a paired row system, wherein plant to plant distance of 1m was maintained. The row to row spacing of 0.5 m was maintained. The nutritional requirements of the plants were met with application of manures, taking into consideration the soil type, as well as the leguminous nature of the host plants. These plants were raised and maintained for the whole year and were used for further inoculations.

Brood lac inoculation: The brood lac from the previously maintained *Rangeeni* strain of the *K. lacca* was used for the inoculation. For this brood lac sticks (5 cm each), having mature female (brood) cells, were tied to the plants of *F. macrophylla* and *F. semialata* separately. The nymphs were allowed to emerge from mature females for about two weeks and the crawlers were allowed to settle. Thereafter, the *Phunki* lac (left over after the emergence of nymphs) was removed from the host plants. For *Katki* crop, broodlac stick was inoculated during July and inoculated shoots were monitored for the emergence of crawlers. Thereafter, various biological and productivity linked parameters were recorded till October before the harvesting of the crop. The brood lac sticks were inoculated during November for *Baisakhi* crop and the data on biological and productivity linked parameters were recorded till June next year. Once the crawlers emerged from brood cells, the following observations were recorded for different parameters:

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 of the plant infested with lac insect was selected on three sites (upper, lower, middle) on same plant and lac insect larvae settled were counted.

Initial mortality (%): The above process was repeated at 21 days after the inoculation of brood lac and initial mortality was calculated using following formula:

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

Sex-ratio (% of male insects): After the differentiation of male and female cells, male cells were counted from three sites (upper, middle, lower) from the same plant and percentage male insects were calculated

Duration of pre-sexual stages: The time elapsed between date of inoculation to male and female cell differentiation was recorded.

Male emergence initiation (days): Time elapsed between date of inoculation and initiation of male emergence from cells was recorded.

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.

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.

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).

Weight (in mg) of the female cell: Weight of individual female lac insect was recorded after larval emergence has completed.

Resin output: 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/replicates) were collected for this purpose.

Statistical analysis: The data were subjected to appropriate statistical tools and mean standard error was worked out using functions of Windows MS Excel.

RESULTS AND DISCUSSION

Pre-harvest Biological Parameters on *F. macrophylla*

***Baisakhi* crop:** In *Baisakhi* crop, the mean initial density of settlement (no./cm^2) was 77.20 crawlers/ cm^2. The initial percentage mortality ranges from 4.12 to 12.57 per cent, while the mean initial mortality was 9.38per cent. The data presented in the table 1 revealed that the mean duration of female and male cell differentiation i.e. duration of pre-sexual stages was 116.67days. The male emergence was initiated at 155th day and it was recorded up to 159th day. The mean male emergence initiation was at 157.67±0.67 days. The sex-ratio (% male insects) ranged from 6.90 to 13.89 per cent, and the mean sex-ratio was 11.85. The mean density of surviving female lac insects was recorded as 4.80cm^2. The longevity of female cell ranged between 210 days to 240 days. The mean longevity of female cell was 234.33days.

Maximum crawlers emerged from a single female cell were 150, with a minimum of 99 crawlers per female cell. The mean number of crawlers per female cell was 121.12. The total life cycle of lac insect (*Rangeeni* strain) in *Baisakhi* crop was 210 to 240 days (Fig. 1). Swami et al. (2021) also recorded productivity linked parameters of *Rangeeni* strain of lac insect in *Baisakhi* season and initial settlement density on *F. macrophylla* was90.70/cm^2 with mean fecundity of 380 crawlers per female cell and mean initial mortality of 11.40 per cent.

***Katki* crop:** During *Katki* crop, the initial lac insect settlement density ranged from 59-95 crawlers/cm^2 and the mean initial settlement density was 76.33insects/ cm^2. The mean mortality was 10.92 per cent, while the initial mortality ranged from 8.53-17.06 per cent (Table 1). The mean duration of pre-sexual stages was 19.80days, ranging between 17-22 days. The mean lac insect males' emergence initiation was 44.00 days, with a range of 40-45 days. The mean sex-ratio (% male insects) was at 12.15 per cent and the sex-ratio ranged between 9.09-14.93 per cent. The surviving density of female lac insects recorded with a mean of 4.67, and ranged from 3-5/cm^2. Similarly, the mean longevity of female cell was 106.67days and the range of female cell longevity was between 99-110 days. The total life cycle of lac insect (*Rangeeni* strain) in *Katki* crop was 99 to110 days (Fig. 1). Maximum crawlers emerged from a single female cell were 120, while the minimum were 98 crawlers per female cell. The mean number of was 107.74 crawlers per female. Meena et al. (2019) observed mean initial density in the range of 70.67-116.00 crawlers per cm^2 of *K. lacca* on *F. macrophylla* during *Katki* crop. The mean mortality varied from 16.05 to 36.42 per cent, while the mean fecundity ranged between 285.40 to 644.60 crawlers per female.

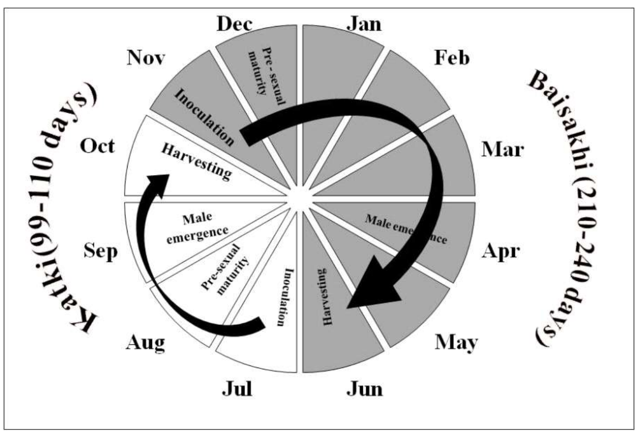

Fig. 1. Life cycle of lac insect (*Rangeeni* strain) on *Flemingia macrophylla*

Table 1. Biological and productivity linked parameters of lac insect (*Rangeeni* strain; *Baisakhi* and *Katki* crops) on *Flemingia macrophylla*

Parameters	*Baisakhi* crop	*Katki* crop
Initial density of settlement (no./cm^2)	77.20±1.17	76.33±1.33
Mortality (%)	9.38±1.33	10.92±2.10
Sex-ratio (% male insects)	11.85±1.18	12.15±0.93
Survival at maturity (no./cm^2)	4.80±0.23	4.67±0.21
Duration of pre-sexual stages (days)	116.67±1.20	19.80±1.11
Initiation of male emergence (days)	157.67±0.67	44.00±0.58
Longevity of female cell (days)	234.33±2.85	106.67±0.88
Fecundity/female (no.)	121.12±2.93	107.74±1.75
Female cell weight (mg)	8.66±0.25	7.48±0.18
Resin output (mg/female cell)	5.95±0.27	5.36±0.73

Means (±SE)

Post-harvest Productivity Parameters on *F. macrophylla*

Baisakhi* crop*: The mean weight of female cells of lac insect was 8.66±mg and ranged from 6.30-10.70 mg (Table 1). The mean weight of resin output by an individual female cell was 5.95mg, while the resin output ranged between 4.50-7.40 mg per female cell.

Katki* crop:* The female cell weight ranged from 6.50 to 8.90 mg. The mean weight of female cells of lac insect on *F. macrophylla* was 7.48±mg. The weight of resin output ranged between 4.00 to 6.70 mg and the mean resin output was 5.3673 mg from an individual female cell. Meena et al. (2019) also reported female cell weight ranging from 10.60 to 16.20 mg and mean resin output of 2.62-3.28 mg during on post-harvest productivity parameters *K. lacca* on *F. macrophylla*.

Pre-harvest Biological Parameters on *F. semialata*

Baisakhi* crop*: The initial density of lac insect settlement (no./cm^2) ranged from 65-97, with mean density of 82.20±insects/ cm^2. The mean initial percentage mortality was 9.62with a range of 7.73-13.58 per cent (Table 2). The mean duration of female and male cell differentiation i.e. duration of pre-sexual stages was 113.67days. The duration of pre-sexual stages ranged from 110 to 120 days. The male emergence was initiated after 154 to 159 days, with mean male emergence initiation at 158.00 days. The mean sex-ratio (% male insects) was 12.85ranging from 8.33 to 15.58 per cent. The mean density of surviving female lac insects (after initial mortality and emergence of male lac insects) was observed to be 4.93/cm^2, and ranged from 3.00 to 7.00/cm^2. The longevity of female cell ranged between 216 d to 239 days. The mean longevity of female cell was 232.00 days. The maximum crawlers emerged from a single female cell on *F. semialata* were 154, while minimum crawlers per female cell were 101. The mean fecundity of 128.92 crawlers was recorded from single female cell. The total life cycle of lac insect (*Rangeeni* strain) in *Baisakhi* crop was 216 to 239 days

(Fig. 2). Swami et al. (2021) recorded initial settlement density on *F. semialata* (64.87/cm^2) in the *Baisakhi* crop on *F. semialata.* The mean fecundity of 368 crawlers per female cell and initial mortality of 15.64 per cent was also reported.

***Katki* crop:** The mean initial settlement density (no. of crawlers/cm^2) of lac insect was 79.73insects/ cm^{2}and ranged from 59-95 crawlers/cm^2. The initial percentage mortality ranged from 5.12-12.36 per cent, while the mean per cent mortality was 8.48 (Table 2). The mean duration of pre-sexual stages was 20.20days, ranging between 18-22 days. The initiation of the mean male emergence was 42.33days. The sex-ratio (% male insects) ranged between 7.69-15.15 per cent with mean of 11.91per cent. The mean density of surviving female lac insects was 4.73±0.39. The mean longevity of female cell was 108.33days. The longevity of female cell ranged between 98-112 days. The mean fecundity per female was 110.14crawlers, with maximum crawlers with range of 98- 129 crawlers per female cell. The total life cycle of lac insect (*Rangeeni* strain) in *Katki* crop varied from 98 to112 days (Fig.2). Mishra *et al.* (1999), also observed fecundity in the range of 253-565 crawlers per female cell on *F. semialata* during investigating intra-specific variation in host-plant affecting productivity of the lac insect, *K. lacca*.

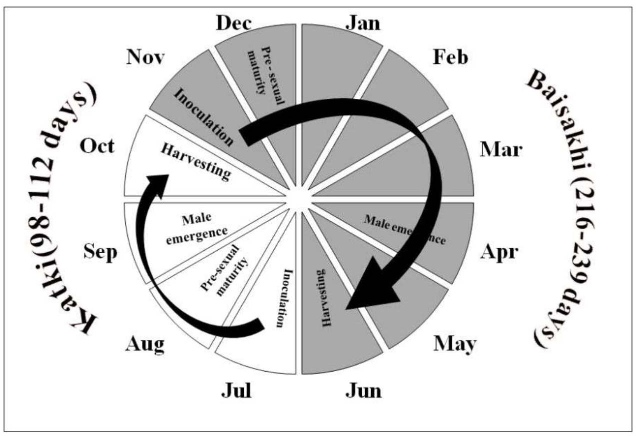

Fig. 2. Life cycle of lac insect (*Rangeeni* strain) on *Flemingia semialata*

Table 2. Biological and productivity linked parameters of lac insect (*Rangeeni* strain; *Baisakhi* and *Katki* crops) on *Flemingia semialata*

Parameters	*Baisakhi* crop	*Katki* crop
Initial density of settlement (no./cm^2)	82.20±3.52	79.73±3.74
Mortality (%)	9.62±1.41	8.48±1.95
Sex-ratio (% male insects)	12.85±1.21	11.91±1.28
Survival at maturity (no./cm^2)	4.93±0.29	4.73±0.39
Duration of pre-sexual stages (days)	113.67±1.86	20.20±0.95
Initiation of male emergence (days)	158.00±0.58	42.33±1.20
Longevity of female cell (days)	232.00±4.04	108.33±3.18
Fecundity/female (no.)	128.92±4.42	110.14±2.27
Female cell weight (mg)	8.94±0.22	7.65±0.18
Resin output (mg/female cell)	6.10±0.19	5.50±0.27

Means (±SE)

Post-harvest Productivity Parameters on *F. semialata*

***Baisakhi* crop:** The maximum female cell weight during *Baisakhi* crop was 11.00 mg and minimum was 7.04 mg (Table 2). The mean weight of female cells of lac insect on *F. semialata* was 8.94±0.22mg. The mean weight of resin output was be 6.10mg and ranged between 4.98-6.80 mg during *Baisakhi* crop.

***Katki* crop:** During Katki crop, the mean weight of female cells of lac insect was 7.65mg and ranged from 6.5-8.6 mg of the female cell weight (Table 2). Resin output in the range of 4.30-7.0 mg while the mean weight of resin output was 5.50 mg. Mishra *et al.* (1999) recorded female cell weight in the range of 8.00 to 19.00 mg on *F. semialata*.

CONCLUSION

Both *F. semialata* and *F. macrophylla* were found to be promising for lac cultivation under Punjab conditions as *Rangeeni* strain of lac insect thrives well on these hosts both during *Katki* and *Baisakhi* crops. Moreover, these two leguminous plants may be utilized to develop integrated model for lac cultivation in potential agricultural and horticultural farming systems.

AUTHORS CONTRIBUTION

Conceptualization was done by PSS and KSS; methodology by PSS and SS; experimentation by AT and HM; data analysis by PSS and RK. All authors have read the manuscript.

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Avian Species Richness and Feeding Guild Patterns in Urban Lakes of Mysore, Southern India

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Abstract: This study investigates the avian diversity in two freshwater lakes, Hinkal Lake and Bogadi Lake, located in Mysore city of Karnataka, India. Both lakes are subjected to pollution from domestic waste and have distinct hydrological and vegetative characteristics. Data were collected from November 2024 to February 2025 through bi-monthly surveys, by total count method. A total of 2042 individual birds, representing 89 species across 17 orders and 41 families, were recorded. Bogadi Lake exhibited higher species diversity and evenness compared to Hinkal Lake, with notable species from families like Ardeidae, Accipitridae, and Sturnidae. The study identified eight feeding guilds, with carnivorous birds being dominant. Temporal changes in bird populations were linked to fluctuating water levels and increasing temperatures. The results highlight the importance of these urban lakes as critical habitats for migratory and resident birds, particularly in the winter season, and emphasize the role of vegetation and wetland features in shaping avian diversity.

Keywords: Bird diversity, Feeding guilds, Relative diversity index, Urbanization

Birds are an essential part of ecosystems, playing a significant role in the food chain and serving as key seed dispersers, pollinators, pest controllers, and bio-indicators of ecosystem health (Neelgund and Kadadevaru 2020). Presently, bird population diversity is on the decline due to a range of environmental disturbances and human actions. These include habitat loss caused by domestic and industrial effluents, agricultural runoff, wetland degradation, expansion of agriculture, overgrazing in grasslands, and urbanization (Grimmet et al., 2016). Avifaunal diversity is a crucial ecological indicator used to assess habitat quality (Ashwini and Vijaya Kumara 2023). Conserving species diversity is one of the key global priorities. As such, bird surveys are essential in formulating strategies for protecting endangered species.

Urbanization is one of the three main factors driving species extinction (Ordóñez-Delgado et al., 2022). The growing human population living in and around urban areas now makes up more than 50% of the global population (Ritchie et al., 2024). The increasing demand to house the growing human population in cities is accelerating the rate and extent of land conversion into urban areas (Seto et al., 2012). This trend has major implications for biodiversity and the survival of species in the remaining natural habitats (Tali et al., 2022). Studies on urban bird communities frequently explore the effects of natural environmental factors, including the characteristics of small-scale vegetation and the size and spatial distribution of natural habitat patches (Lawal and Iwajumo 2020). Urban bird communities are typically marked by low species richness and high overall density or biomass when compared to surrounding natural areas (Barth et al., 2015). Intermediate levels of suburban development are often linked to a peak in bird diversity, as both urban-avoiding native species and urban-adapted exotic species thrive in these areas, where abundant food and low predation pressure contribute to higher reproductive success and density (Patankar et al., 2021). Exploring the relationship between urban environments and avian habitats has significantly expanded our understanding, playing a crucial role in biodiversity conservation and ecosystem health monitoring (Tali et al., 2022). The present study was undertaken on species richness and diversity of various bird species of two urban wetlands of Mysore city to highlight the importance of urban water bodies in sustaining the bird population, despite rapid urbanization.

MATERIAL AND METHODS

Study area: The avian diversity was investigated in two freshwater lakes in the Mysore city of Karnataka state (Fig. 1). The Hinkal Lake (Lake 1) lies between 12˚31'73''N longitude '' and 76˚59'95''E with a water-spread area of 11.5 acres bordered by dump yard and crematorium. The lake water is replenished by rainfall, surface runoff and sewage. However, it completely dries during summer season. The Bogadi Lake (Lake 2) lies between 12˚30'98''N longitude'' and 76˚60'25''E latitude with a water-spread area of 19.9 acres. The lake is fed by direct rainfall, surface flow and sewage, which tend to dry moderately during the summer. It has a fair portion of submerged and floating vegetation (water hyacinth, water lettuce) that attracts plenteous migratory birds during post monsoon season. Both the lakes were polluted with domestic waste and garbage. The predominant vegetation surrounding the lakes includes common lantana *(Lantana camara),* yellow trumpetbush *(Tecoma stans),* crown flower *(Calotropis gigantean),* peepal *(Ficus religiosa),* oil cake tree *(Albizzia amara)*, *Ficus* sp., and *Acacia* sp.

Bird survey: Data was collected from November 2024 to February 2025. Observation was taken twice a month. Birds were counted by total count method (Bibby et al., 2012) using Binocular (Olympus 8X40 and Pentax SP 8X40), which involves searching a designated area for a specific duration and recording the number of birds observed and heard. Calls of unsighted birds were recorded using Cornell Lab's Merlin Bird ID (Cornell University (Version 2.1.5)) and the checklist was uploaded on eBird app. The conservation status of the birds was derived from the IUCN Red List of Threatened Species (IUCN 2024). The recorded birds were classified into different feeding guilds according to their ecological food preferences, such as carnivore, insectivore, omnivore, granivore, frugivore, mixed guild, nectivore, and herbivore (Basnet et al., 2016, Grimmett et al., 2016, Jangral and Vashishat 2022).

Data analysis: The Paleontological Statistics (PAST) 4.03 educational software (Hammer et al., 2001) was used to measure various α-diversity indices such as Fisher's-α diversity, Shannon-Weiner index, and Evenness index for the summarised data for each habitat type. The relative diversity (RD_i) of each bird family was estimated (Torre-Cuadros et al., 2007).

$$RD_i = \frac{\text{Number of species in the family}}{\text{Total number of species}} \times 100$$

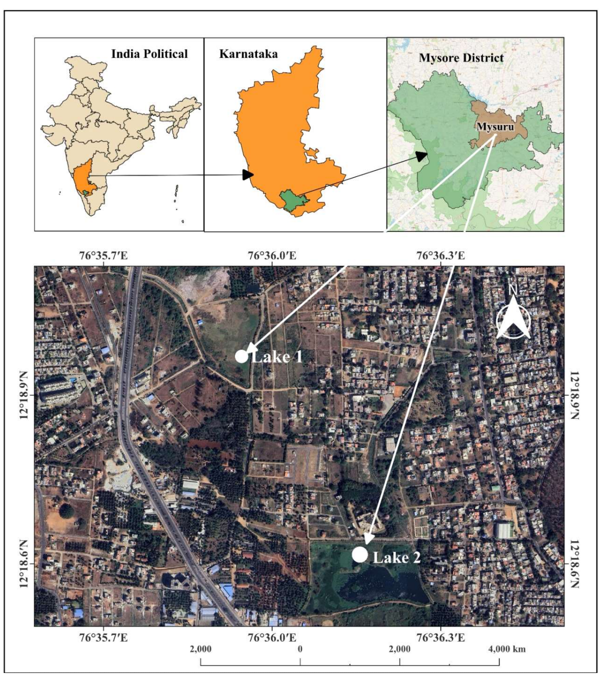

Fig. 1. Study area (Lake 1-Hinkal Lake, Lake 2- Bogadi Lake)

Sorensen's similarity index was performed to measure the similarity between the two water bodies (Magurran 2004):

$$C_s = \frac{2c}{a + b}$$

where 'a' is the number of species of birds found in Hinkal Lake, 'b' is the number of species found in Bogadi Lake and 'c' is the number of species found in both sites.

RESULTS AND DISCUSSION

Species diversity: In present study, 2042 individual birds, including 89 species, 17 orders, and 41 families, were recorded in the study area (Hinkal Lake - 981; Bogadi Lake - 1061) (Table 1, Fig. 2 & 3). The order Passeriformes is represented by the highest number of species (N = 35) and lowest was in the orders Strigiformes, Psittaciformes, and Podicipediformes, with a single species each. Among all recorded avian species, only one was in the vulnerable category, the Tawny Eagle (*Aquila rapax*); the rest were the least concerned species. The two areas had similar species composition, with a Sorenson index of 66% (Cs = 0.66) reflecting a very high similarity, which indicates that the species diversity is fairly similar across the habitats. In general, overall bird species diversity was higher in Bogadi Lake (H' = 3.243) than Hinkal Lake (H' = 2.88). Similarly, highest evenness index (J = 0.35) and Fisher's α-diversity index (17.79) was observed in Bogadi Lake as compared to Hinkal Lake (J = 0.30) and Fisher's α-diversity index (13.79). The relative diversity (RDi) among avian families revealed that three families Ardeidae, Accipitridae and Sturnidae had maximum relative diversity (4 species, RDi =6.78) in Hinkal Lake (Fig. 4) while Ardeidae family (6 species, RDi =8.11) followed by Rallidae, Threskiornithidae, and Accipitridae (4 species, RDi =5.41) recorded highest relative diversity index in Bogadi Lake (Fig. 5) representing dominant group. There was a gradual decline in the abundance of the bird population during different months of the study period (Fig. 6), which can be attributed to the receding water levels and an increase in temperature over time. The trophic composition of birds in this study showed that carnivorous birds dominated the feeding guild, followed by insectivorous, omnivorous, granivorous, frugivorous, nectivorous, herbivorous, and mixed guilds across both lakes (Fig. 7). Panda et al. (2021) also in their guild study, attributed that feeding guilds with a higher number of species also had a greater number of individuals. The studies on bird feeding guilds provide valuable insights into species ecology and are especially useful in identifying the specific ecological factors driving community changes (Jangral and Vashishat 2022). The identification of eight feeding guilds across both lakes suggests a clear trophic segregation within the community,

Table 1. Checklist of bird species recorded during the study period, with their common name, scientific name, order, family, IUCN status, and feeding guild

Order/Family	Common name	Scientific name	IUCN status	Food habit
1. Order – Galliformes				
Phasianidae	Gray Francolin	*Ortygornis pondicerianus* (Gmelin, 1789)	LC	I
Phasianidae	Indian Peafowl	*Pavo cristatus* (Linnaeus, 1758)	LC	O
2. Order – Anseriformes				
Anatidae	Indian Spot-billed Duck	*Anas poecilorhyncha* (Foster, 1781)	LC	H
Anatidae	Northern Shoveler	*Spatula clypeata* (Linnaeus, 1758)	LC	O
Anatidae	Garganey	*Spatula querquedula* (Linnaeus, 1758)	LC	O
3. Order – Apodiformes				
Apodidae	Alpine Swift	*Tachymarptis melba* (Linnaeus, 1758)	LC	I
Apodidae	Asian Palm Swift	*Cypsiurus balasiensis* (J.E. Gray, 1829)	LC	I
4. Order – Cuculiformes				
Cuculidae	Greater Coucal	*Centropus sinensis* (Stephens, 1851)	LC	C
Cuculidae	Asian Koel	*Eudynamys scolopaceus* (Linnaeus, 1758)	LC	I
Cuculidae	Blue-faced Malkoha	*Phaenicophaeus viridirostris* (Jerdon, 1840)	LC	I
5. Order – Columbiformes				
Columbidae	Rock Pigeon (Feral Pigeon)	*Columba livia* (Gmelin, 1789)	LC	O
Columbidae	Spotted Dove	*Spilopelia chinensis* (Scopoli, 1786)	LC	G
Columbidae	Eurasian Collared-Dove	*Streptopelia decaocto* (Frivaldszky,1838)	LC	G
6. Order – Gruiformes				
Rallidae	Eurasian Coot	*Fulica atra* (Linnaeus, 1758)	LC	O
Rallidae	Eurasian Moorhen	*Gallinula chloropus* (Linnaeus, 1758)	LC	O
Rallidae	Gray-headed Swamphen	*Porphyrio poliocephalus* (Latham, 1801)	LC	O
Rallidae	White-breasted Waterhen	*Amaurornis phoenicurus* (Pennant, 1769)	LC	O
7. Order – Podicipediformes				
Podicipedidae	Little Grebe	*Tachybaptus ruficollis* (Pallas, 1764)	LC	O
8. Order – Charadriiformes				
Charadriidae	Little Ringed Plover	*Charadrius dubius* (Scopoli, 1786)	LC	I
Charadriidae	Red-wattled Lapwing	*Vanellus indicus* (Boddaert, 1783)	LC	MG
Charadriidae	Yellow-wattled Lapwing	*Vanellus malabaricus* (Boddaert, 1783)	LC	I
Recurvirostridae	Black-winged Stilt	*Himantopus himantopus* (Linnaeus, 1758)	LC	C
Jacanidae	Bronze-winged Jacana	*Metopidius indicus* (Latham, 1790)	LC	C
Scolopacidae	Common Sandpiper	*Actitis hypoleucos* (Linnaeus, 1758)	LC	C
Scolopacidae	Wood Sandpiper	*Tringa glareola* (Linnaeus, 1758)	LC	C
Scolopacidae	Green Sandpiper	*Tringa ochropus* (Linnaeus, 1758)	LC	C
9. Order – Ciconiiformes				
Ciconiidae	Painted Stork	*Mycteria leucocephala* (Pennant, 1769)	LC	C
10. Order – Suliformes				
Phalacrocoracidae	Indian Cormorant	*Phalacrocorax fuscicollis* (Stephens, 1826)	LC	C
Phalacrocoracidae	Little Cormorant	*Microcarbo niger* (Vieillot, 1817)	LC	C
Anhingidae	Oriental Darter	*Anhinga melanogaster* (Pennant, 1769	LC	C
11. Order – Pelecaniformes				
Ardeidae	Indian Pond-Heron	*Ardeola grayii* (Sykes, 1832)	LC	C

Cont...

Table 1. Checklist of bird species recorded during the study period, with their common name, scientific name, order, family, IUCN status, and feeding guild

Order/Family	Common name	Scientific name	IUCN status	Food habit
Ardeidae	Little Egret	*Egretta garzetta* (Linnaeus, 1766)	LC	C
Ardeidae	Medium Egret	*Ardea intermedia* (Wagler, 1829)	LC	C
Ardeidae	Eastern Cattle-Egret	*Ardea coromanda* (Boddaert, 1783)	LC	C
Ardeidae	Gray Heron	*Ardea cinerea* (Linnaeus, 1758)	LC	C
Ardeidae	Purple Heron	*Ardea purpurea* (Linnaeus, 1766)	LC	C
Threskiornithidae	Red-naped Ibis	*Pseudibis papillosa* (Temminck, 1824)	LC	C
Threskiornithidae	Black-headed Ibis	*Threskiornis melanocephalus* (Latham, 1790)	LC	C
Threskiornithidae	Eurasian Spoonbill	Platalea leucorodia (Linnaeus, 1758)	LC	C
Threskiornithidae	Glossy Ibis	Plegadis falcinellus (Linnaeus, 1766)	LC	C
12. Order – Accipitriformes				
Accipitridae	Black Kite	*Milvus migrans* (Boddaert, 1783)	LC	C
Accipitridae	Brahminy Kite	*Haliastur indus* (Boddaert, 1783)	LC	C
Accipitridae	Oriental Honey-buzzard	*Pernis ptilorhynchus* (Temminck, 1821)	LC	C
Accipitridae	Tawny Eagle	*Aquila rapax* (Temminck, 1828)	VU	C
Accipitridae	Shikra	*Tachyspiza badia* (Gmelin,1788)	LC	C
13. Order – Strigiformes				
Strigidae	Brown boobook	*Ninox scutulata* (Raffles, 1822)	LC	C
14. Order – Coraciiformes				
Alcedinidae	White-throated Kingfisher	*Halcyon smyrnensis* (Linnaeus, 1758)	LC	C
Alcedinidae	Common Kingfisher	*Alcedo atthis* (Linnaeus, 1758)	LC	C
Meropidae	Green Bee-eater	*Merops orientalis* (Latham, 1801)	LC	I
Meropidae	Blue-tailed Bee-eater	*Merops philippinus* (Latham, 1767	LC	I
15. Order – Piciformes				
Megalaimidae	White-cheeked Barbet	*Psilopogon viridis* (Boddaert, 1783)	LC	F
Megalaimidae	Coppersmith Barbet	*Psilopogon haemacephalus* (Müller, 1776)	LC	F
Picidae	Black-rumped Flameback	*Dinopium benghalense* (Linnaeus, 1758)	LC	I
16. Order – Psittaciformes				
Psittaculidae	Rose-ringed Parakeet	*Psittacula krameri* (Scopoli, 1769)	LC	F
17. Order – Passeriformes				
Cisticolidae	Ashy Prinia	*Prinia socialis* (Sykes, 1832)	LC	I
Cisticolidae	Common Tailorbird	*Orthotomus sutorius* (Pennant, 1769)	LC	I
Cisticolidae	Zitting Cisticola	*Cisticola juncidis* (Rafinesque, 1810)	LC	I
Paridae	Cinereous Tit	*Parus cinereus* (Vieillot, 1818)	LC	I
Acrocephalidae	Booted Warbler	*Iduna caligata* (Lichtenstein, 1823)	LC	I
Corvidae	House Crow	*Corvus splendens* (Vieillot, 1817)	LC	O
Dicaeidae	Pale-billed Flowerpecker	*Dicaeum erythrorhynchos* (Latham, 1790	LC	N
Dicruridae	Black Drongo	*Dicrurus macrocercus* (Vieillot, 1817	LC	I
Hirundinidae	Red-rumped Swallow	*Cecropis daurica* (Laxmann, 1769)	LC	I
Hirundinidae	Barn Swallow	*Hirundo rustica* (Linnaeus, 1758)	LC	I
Motacillidae	Grey Wagtail	*Motacilla cinerea* (Tunstall, 1771)	LC	I
Motacillidae	Paddyfield Pipit	*Anthus rufulus* (Vieillot, 1818)	LC	I
Motacillidae	White-browed Wagtail	*Motacilla maderaspatensis* (Gmelin, 1789)	LC	I

Cont...

Table 1. Checklist of bird species recorded during the study period, with their common name, scientific name, order, family, IUCN status, and feeding guild

Order/Family	Common name	Scientific name	IUCN status	Food habit
Motacillidae	Western Yellow Wagtail	*Motacilla flava* (Linnaeus, 1758)	LC	I
Muscicapidae	Indian Robin	*Copsychus fulicatus* (Linnaeus, 1766)	LC	I
Muscicapidae	Oriental Magpie-Robin	*Copsychus saularis* (Linnaeus, 1758)	LC	I
Muscicapidae	Pied Bushchat	*Saxicola caprata* (Linnaeus, 1766)	LC	I
Nectariniidae	Purple Sunbird	*Cinnyris asiaticus* (Latham, 1790)	LC	N
Nectariniidae	Purple-rumped Sunbird	*Leptocoma zeylonica* (Linnaeus, 1766)	LC	N
Laniidae	Brown Shrike	*Lanius cristatus* (Linnaeus, 1758)	LC	C
Laniidae	Long-tailed Shrike	*Lanius schach* (Linnaeus, 1758)	LC	C
Alaudidae	Jerdon's Bushlark	*Plocealauda affinis* (Blyth, 1845)	LC	I
Leiothrichidae	Yellow-billed Babbler	*Argya affinis* (Jerdon, 1845)	LC	O
Leiothrichidae	Large Gray Babbler	*Argya malcolmi* (Sykes,1832)	LC	O
Passeridae	House Sparrow	*Passer domesticus* (Linnaeus, 1758)	LC	G
Pycnonotidae	Red-vented Bulbul	*Pycnonotus cafer* (Linnaeus, 1766)	LC	O
Pycnonotidae	Red-whiskered Bulbul	*Pycnonotus jocosus* (Linnaeus, 1758)	LC	O
Pycnonotida	White-browed Bulbul	*Pycnonotus luteolus* (Lesson, 1841)	LC	O
Estrildidae	White-rumped Munia	*Lonchura striata* (Linnaeus, 1766)	LC	G
Estrildidae	Scaly-breasted Munia	*Lonchura punctulate* (Linnaeus, 1758)	LC	G
Rhipiduridae	Spot-breasted Fantail	*Rhipidura albogularis* (Lesson, 1831)	LC	I
Sturnidae	Chestnut-tailed Starling	*Sturnia malabarica* (Gmelin, 1789)	LC	O
Sturnidae	Rosy Starling	*Pastor roseus* (Linnaeus, 1758)	LC	O
Sturnidae	Common Myna	*Acridotheres tristis* (Linnaeus, 1766)	LC	O
Sturnidae	Jungle Myna	*Acridotheres fuscus* (Wagler, 1872)	LC	O

Abbreviations: IUCN – International Union for Conservation of Nature Status, LC – Least Concern, VU – Vulnerable. Key: C (Carnivore), F (Frugivore), G (Granivore), I (Insectivore), MG (Mixed Guild), N (Nectarivore), O (Omnivore), H (Herbivore)

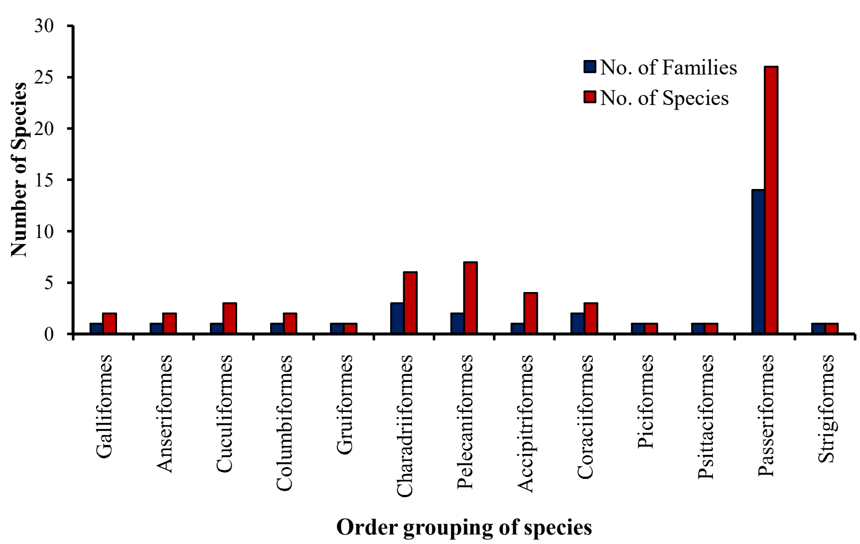

Fig. 2. Bird orders and number of species in Hinkal Lake

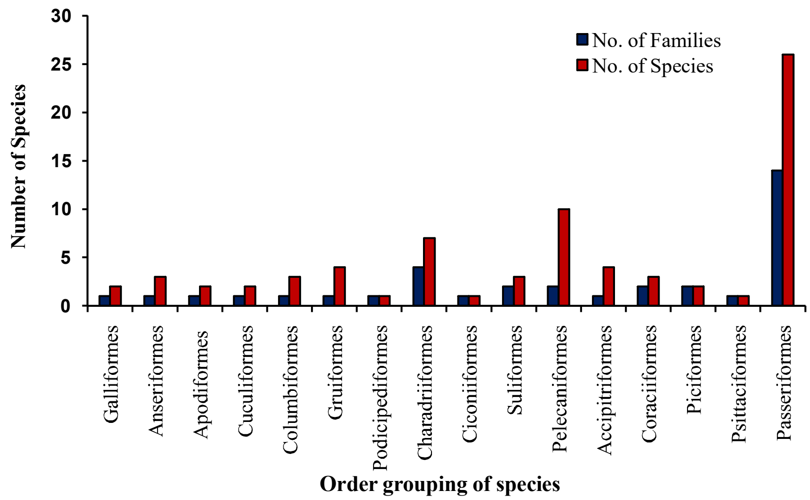

Fig. 3. Bird orders and number of species in Bogadi Lake

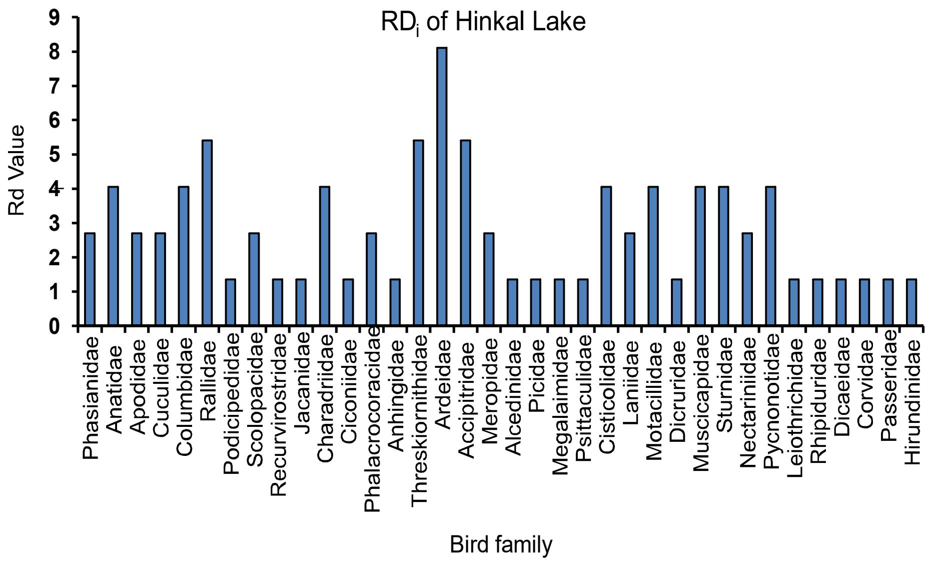

Fig. 4. Relative diversity (RD$_i$) of various bird families recorded from Hinkal Lake, Mysore, India

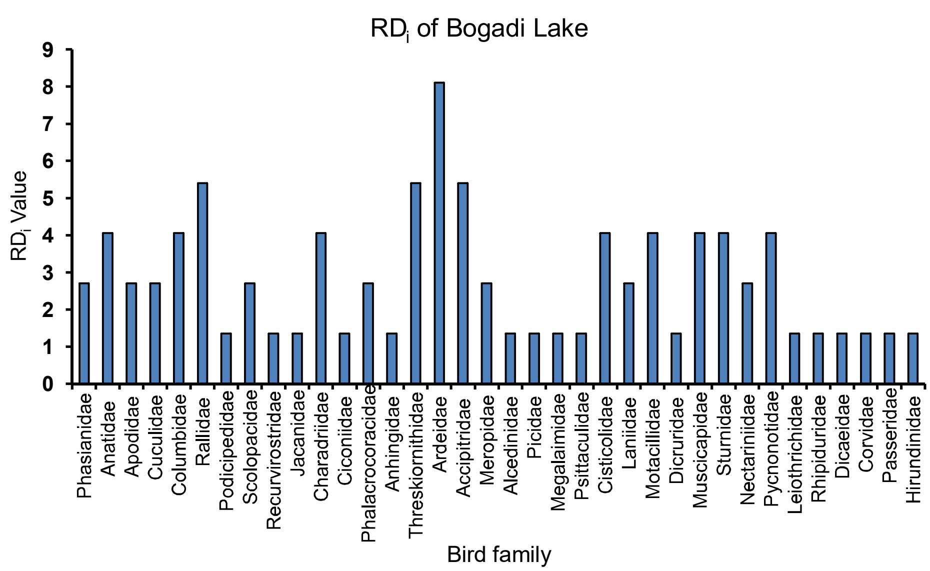

Fig. 5. Relative diversity (RD$_i$) of various bird families recorded from Bogadi Lake, Mysore, India

with species utilizing a range of food sources through various behavioural strategies and morphological adaptations (Rajpar et al., 2018).

The diversity and spatio-temporal distribution of bird species are influenced by the type of vegetation and the characteristics of the wetlands such as water depth, water quality and habitat structure (Malik and Joshi 2013, Choudaj and Wankhade 2023). Furthermore, species composition is shaped by the arrival of migratory species and the presence of resident species; migratory species tend to maintain more specialized niches in their wintering habitats and may be less tolerant of human disturbance and habitat changes in urban environments (Leveau et al., 2021).

Wetland birds were observed to choose aquatic habitats based on their needs for feeding, nesting, hiding, and breeding (Jamakhandi and Kadadevaru 2024). Birds from the order Anseriformes, such as the Indian Spot-billed Duck, Northern Shoveler, and Garganey, favoured open, deep-water zones with submerged vegetation. These species steered clear of dense emergent vegetation due to limited movement and decreased foraging efficiency in those areas

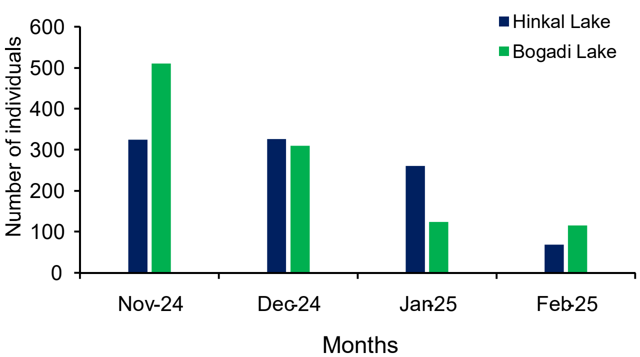

Fig. 6. Monthly variation in number of individuals observed during the study period

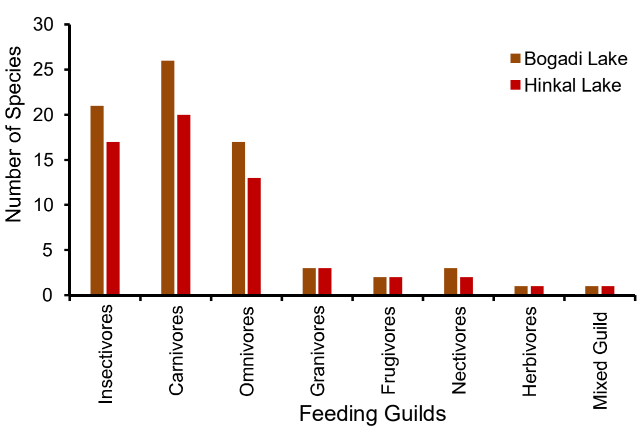

Fig. 7. Feeding guilds of bird species in Hinkal Lake (Lake 1) and Bogadi Lake (Lake 2)

Fig. 8. Black-winged Stilt (*Himantopus himantopus)*

Fig. 9. Red-wattled Lapwing (*Vanellus indicus)*

(Ramamurthy and Rajakumar, 2014, Sekhon et al., 2023). Wading birds like herons, egrets, painted storks, ibises, and spoonbills were restricted to shallow waters and marshes, as deep water has been shown to reduce the availability and accessibility of invertebrates for feeding (Kumar 2021). Shorebirds such as lapwings, stilts, jacanas, plovers, sandpipers, and wagtails were restricted to the edges of the ponds. Interestingly, we recorded a high abundance of Black-winged Stilt (Fig. 8) and Red-wattled Lapwing (Fig. 9) in both the water bodies, suggesting their rapid adaptation to the urban conditions (Muralidhar and Barve 2013). The spotted multiple breeding pairs of Red-wattled Lapwings, along with their chicks, through the shores of both water bodies was also observed. Numerous studies have emphasized that the shallow shores of lakes, particularly those with islands, provide ideal breeding sites for Lapwings (Muralidhar and Barve 2013, Elhassan et al., 2021). The dominance of the order Passeriformes could be attributed to the diverse vegetation. The shrubs and trees provide a variety of flowers and fruits, attracting a broad range of insects, which, along with the berries, serve as the primary food sources for these birds (Kukreti 2021). Additionally, the vegetation provided shelter from predators and harsh weather, as well as suitable nesting spots (Rajpar and Zakaria 2011,Sharma and Tripathi,2023).

In general, lakes in urban areas play a crucial role as feeding and breeding grounds for birds. The presence of water birds can be an indicator of the health of water ecosystems, as their numbers often reflect the quality of wetland habitats (Green and Elmberg, 2014). Other studies have reported similar findings (Bachheti et al., 2023, Awash and Tekalign 2023, Mishra et al., 2024). This research highlighted that the studied habitats provide suitable feeding and roosting areas for migrating and resident birds, particularly during the winter season.

CONCLUSION

Hinkal Lake and Bogadi Lake are highly productive avian habitats, supporting a wide range of bird species. A total of 89 bird species were recorded during the winter season across both lakes. Although, the lakes currently support various bird populations, anthropogenic activities in the vicinity are reducing the available habitat for birds, which could significantly impact their abundance and the long-term survival of the water bodies in the area. The greater diversity of birds observed in this study in close proximity to human settlements implies that land-use patterns can be optimized in urban landscape planning to support resident bird species while maintaining the richness of local indigenous species.

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Distribution and Habitat Use of Spotted Owlet *Athene brama* (Temminck,1821) (Aves: Strigiformes: Strigidae) in Navsari Agricultural University, Gujarat, India

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Abstract: Present study assessed the habitat use and distribution of the spotted owlet, *Athene brama* (Temminck 1821) in the Navsari Agricultural University campus. Data were collected from February to April 2024 through surveys conducted across 24 grids, covering a total distance of 85 kilometres. These surveys resulted in the encounter of 38 Spotted Owlets, yielding a naive occupancy rate of 0.56 and a mean encounter rate of 0.65 owlets per kilometre. The proportion of agricultural land significantly influences habitat use, exhibiting a curvilinear relationship. Encounter rates increased with the proportion of agricultural land up to 50-60%, followed by a decline at higher proportions (r^2=0.31). Additionally, the presence of orchards negatively affected habitat uses, as indicated by polynomial regression analysis (r^2=0.13). Built-up areas, water bodies, and roads did not show significant effects on the owlet's habitat use.

Keywords: Spotted Owlet , *Athene brama*, Habitat use, Occupancy

Raptors, or birds of prey, are a diverse group of carnivorous birds known for their keen eyesight, sharp talons, and hooked beaks used for hunting and feeding on others. Among these, owlets, members of the Order Strigiformes, include around two hundred species of mainly solitary and nocturnal birds. They are characterized by binocular vision, binaural hearing, sharp talons, and specialized feathers that enable silent flight (Shanker et al., 2018). They range in size from small owlets to large owls and can rotate their heads and necks up to 270 degrees. Owls are divided into two families, the true owls (Strigidae) and the Barn Owls (Tytonidae) (Shanker 2018). The spotted owlet (*Athene brama*, Strigidae) is a common raptor found throughout India. They measure about 21 cm in length (Grimmett et al., 2019), and weigh between range 235–245 grams (Yosef et al., 2011). They are highly adaptable and prefer open habitats such as farmland and urban areas. Their ecological adaptability allows them to persist in both rural and urban settings (Gaba and Vashishat 2019). In India, there are three subspecies of spotted owlet: *Athene brama brama, A. brama indica, and A. brama ultra* (Santhanakrishnan 2011). The Spotted Owlet is commonly found in agricultural areas and human habitations, where it primarily preys on insects and rodents, making them important for maintaining ecological balance (Sunitha et al., 2022). In agricultural areas, they act as biocontrol agents by controlling pest populations, which significantly benefits farmers (Sunitha et al., 2022). Spotted Owlets usually nest in natural tree hollows, holes in dilapidated walls, and spaces between the ceiling and roof of both abandoned and occupied buildings(Pande et al., 2007). They often roost and nest near human dwellings, utilizing trees, buildings, and electric poles as their sites (Sunitha et al., 2022).

Although the spotted owlet is classified as a species of Least Concern by the International Union for Conservation of Nature (IUCN), superstitions pose a significant threat to Spotted Owlets well-being. In Indian, these superstitions often lead to illegal hunting and persecution of owls, stemming from misunderstandings of their behavior (Pande et al., 2007). Navsari Agricultural University campus provides an excellent setting to study spotted owlets within an agricultural landscape. The variety in vegetation, from dense tree canopies to open fields, offers ample nesting and roosting sites, as well as a rich prey base. Previous studies on spotted owlets have primarily focused on diet and nesting habits (Santhanakrishnan 2011, Sunitha 2022), but limited research exists on their distribution in agricultural landscapes. This study aims to bridge this gap by assessing the distribution and habitat use of spotted owlets on the NAU campus.

MATERIAL AND METHODS

Study area: Navsari Agricultural University (NAU) is located in Navsari, Gujarat, India (20.9248°N, 72.9079°E) (Figure 1) and spans an area of 400 hectares, encompassing diverse habitat types. The university is situated at an elevation of 20 m above sea level, with a predominantly flat topography interspersed with slight undulations. The major habitats within the campus include agricultural fields, orchards, experimental plots, and patches of natural vegetation. The soil composition varies, with alluvial soil being predominant, along with patches of sandy and loamy soils. Agricultural lands form a significant portion of the campus, primarily used for crop cultivation and research. The orchards mainly consist of fruit-bearing trees such as mango (*Mangifera indica*), sapota (*Manilkara zapota*), and banana (*Musa paradisiaca*). Pockets of natural vegetation are preserved across the campus, comprising native tree species such as neem (*Azadirachta indica*), banyan (*Ficus benghalensis*), and tamarind (*Tamarindus indica*). Additionally, the campus features small ponds and irrigation channels that support both agriculture and local wildlife.

Data collection: The study was conducted from 16 February 2024 to 10 April 2024, using systematic field surveys to document the presence of spotted owlets (*Athene brama*). Navsari Agricultural University (NAU) campus was divided into 24 square grids using Google Earth, each covering approximately 16.67 hectares (Fig. 1). Each grid was surveyed five times over a three-month period to record Spotted Owlet occurrences using a spot light. Surveys were conducted during night time and early morning hours to assess presence across different time periods. During each survey, grid number, occasion (day or night), date, number of individuals observed, latitude, longitude, habitat type, tree species or man-made structures used, and roosting height was recorded. The *Locus Map* application was used to record the distance travelled during each survey. Land use classification was conducted using Google Earth, where polygons were created and color-coded to represent different land use categories: agricultural land, horticultural land, human-built areas, water bodies, and roads. The percentage distribution of each land use category within the grids was calculated, and these values were later used as predictor variables to analyse spotted owlet habitat use.

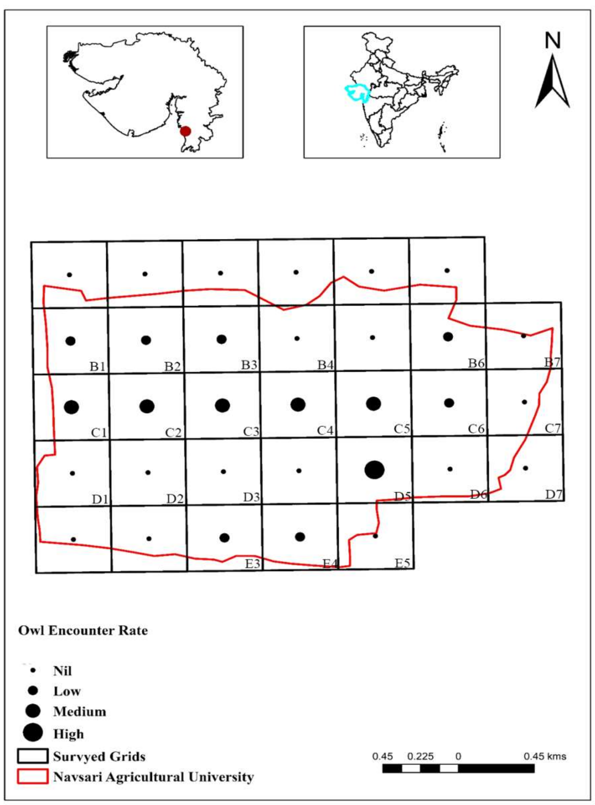

Fig. 1. Map of the study area along with grid-wise owl encounter rate across the Navsari Agricultural University campus. Grid IDs (B1 to E5)

Data analysis: To assess the distribution and status of spotted owlets, the number of grids with spotted owlets presence was divided by the total number of grids to determine the occupancy of spotted owlets. To evaluate the population status encounter rate index was used, calculated by dividing the total number of individuals encountered by the total area surveyed in each grid, along with overall encounter rates (MacKenzie et al., 2002). The relative encounter rates were mapped using GIS to visualize distribution patterns. Polynomial regression models was usded to analyse habitat uses, with encounter rates as the dependent variable and habitat variables as independent variables (Zar, 1999). All statistical analyses were performed using R Studio 2023.12.1 (R Core Team, 2023).

RESULTS AND DISCUSSION

A total of 22 grids were surveyed between February 2024 and April 2024, resulted in an effort of 85 km and yielding 38 encounters of spotted owlets. Owlets were recorded in 14 of the 22 grids, indicating a naive occupancy of 0.56 and suggesting a broader distribution across the Navsari Agricultural University campus. The mean±S.E. encounter rate of spotted owlet was 0.65 ± 0.007 individuals per km, with spatial variation across grids. Eight grids exhibited higher-than-average encounter rates, while another eight recorded no detections (Fig. 1).

Among the five habitat variables assessed, agricultural land was the only significant predictor of Spotted Owlet encounter rates, following the relationship: -0.10 + 0.04*(agriculture proportion) - 0.0003*(agriculture proportion) ^2 ($R^2 = 0.31$) ($p < 0.05$). The linear coefficient was significant indicating a positive association with increasing agricultural land proportion, while the quadratic term suggested a threshold effect, with peak encounters occurring at 50-60% agricultural cover before declining (Fig. 2a). Khan et al. (2023) reported the species adaptability to modified habitats, moderate agriculture provides suitable foraging conditions, while intensive practices such as

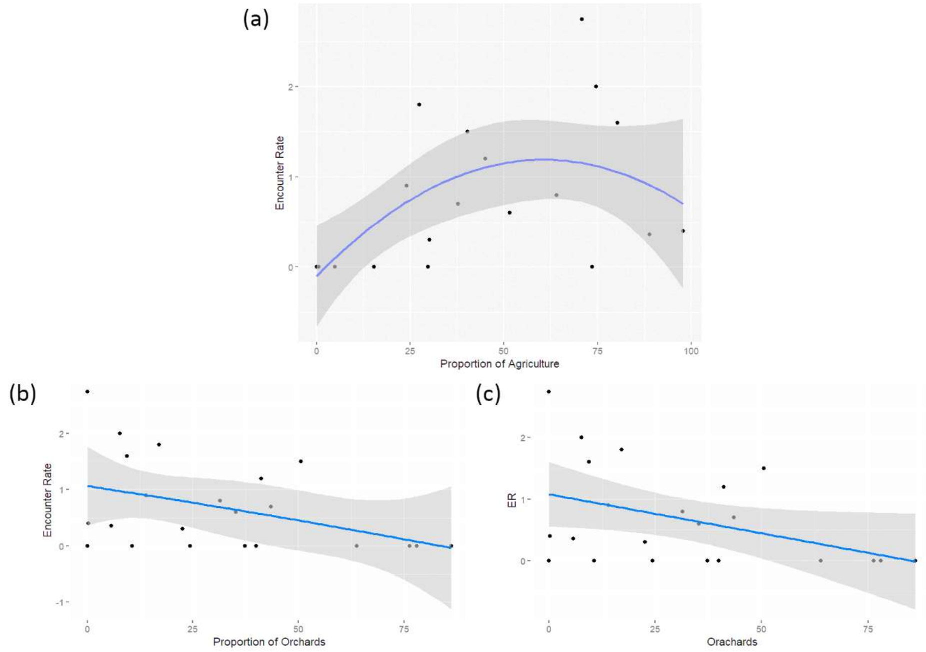

Fig. 2. a. shows a positive relationship between the proportion of agricultural land and encounter rate, b. indicates a non-significant negative association between the proportion of orchards and the encounter rate, while, c. reflects a negative relationship between orchards and encounter rate

Table 1. Proportional representation (%) of different land use types across sampling grids in the study area

Grids	Agriculture land	Horticulture land	Human built up	Water body	Roads
B1	64.02	31.47	1.81	1.91	0.77
B2	37.76	43.54	0.75	5.53	1.47
B3	51.56	35.23	3.03	4.8	2.2
B4	73.56	0.00	15.82	7.82	2.78
B6	30.16	22.58	35.14	0.00	0.52
B7	0.00	78.06	5.64	0.00	1.06
C1	80.4	9.43	2.86	0.00	1.5
C2	45.01	41.16	4.71	0.00	1.94
C3	40.38	50.59	6.55	0.00	1.65
C4	74.68	7.69	14.07	2.05	1.49
C5	27.48	17.06	27.49	4.28	2.39
C6	23.98	13.93	50.25	2.15	2.17
C7	0.00	39.95	45.09	1.31	3.36
D1	29.8	63.89	2.5	0.00	0.2
D2	4.96	76.38	9.26	1.59	1.89
D3	0.52	86.19	6.94	0.89	1.14
D5	70.92	0.00	12.21	0.00	2.84
D6	0.00	10.68	84.28	0.00	5.03
D7	0.00	24.34	56.13	0.00	1.97
E3	88.92	5.70	0.9	1.08	2.26
E4	97.93	0.23	3.61	0.00	3.05
E5	15.3	37.34	10.98	3.91	2.6

Grids represent fixed-area spatial units overlaid on the landscape, as shown in Figure 1.
Values were derived from satellite imagery and verified through field surveys

mechanization and pesticide use up to a threshold, reduce habitat quality and prey availability.

Orchard proportion did not exhibited a significant effect on spotted owlet encounters (Fig. 2b), 1.06 - 0.002*(orchards proportion) - 0.000005*(orchards proportion) ^2 (R^2 = 0.08). Regression analysis (y = 0.20 - 0.01*proportion of orchards, R^2 = 0.13) ($p<0.05$) indicated a negative association between orchard cover and habitat use (Fig. 2c). At NAU, orchards are intensively managed, involving regular pruning, pesticide application, and frequent human activity, which is influencing spotted owlets occurrence. Intensive orchard management reduces structural complexity and foraging opportunities, negatively impacting avian habitat use (Myczko et al., 2013). Bouvier et al. (2011) found that insectivorous birds are more affected by intensive pest control strategies, due to reduced prey availability.

Other habitat variables, including built-up areas, water bodies and roads (Fig. 3a,b,c) did not significantly influence habitat use by spotted owlet, indicating a weak or no correlation between these features and owlet presence, Pande et al. (2011) and Gaba and Vashishat (2013) observed that the species tolerates urbanization and frequently roosts in old buildings, roadside trees, and fragmented green spaces. Recent studies conducted in rural and semi-urban landscapes of India have demonstrated that avian diversity and distribution

Plate 1. Photographic records of spotted owlets from Navsari Agricultural University

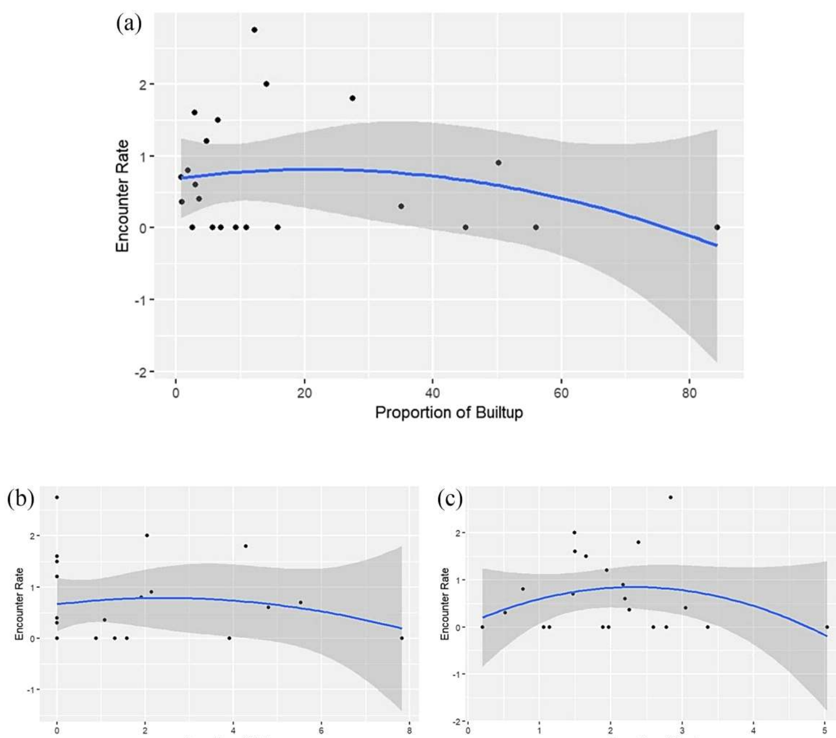

Fig. 3. a. shows the relationship with the proportion of human built-up areas, b. represents the relationship with the proportion of water bodies, and c. shows the relationship with the proportion of roads

are strongly shaped by land-use heterogeneity (Sharma and Tripathi, 2023, Brraich et al., 2023). These studies included the spotted owlet in their avifaunal checklists, further supporting its association with human-modified habitats such as agricultural and semi-urban areas.The weak relationship with built-up areas, water bodies, and roads suggests that spotted owlets can persist in altered landscapes as long as suitable nesting and foraging conditions are available.

CONCLUSION

This study confirms that spotted owlets can persist in moderately disturbed agricultural landscapes, with encounter rates peaking at 50-60% agricultural land cover. The orchards negatively influence their habitat use, built-up areas, water bodies, and roads have no significant impact, reinforcing their adaptability to human-modified environments. These findings highlight the importance of maintaining habitat heterogeneity to support Spotted Owlet populations.

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Avifaunal Diversity in Different Habitats of Semi-Arid Region of Karnataka, India

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***Abstract*:** This study investigates avifaunal diversity in four sites of in and around Raichur city using the Short-Strip Transect Count (SSCT) method and point count method. The total of 52 bird species, belonging to 35 families and 16 orders, were recorded. Among these, 67.03% were resident species, while 32.69% comprised local, partially migratory, and highly migratory species. The family *Ardeidae* had the highest number of species occurrences, while the order *Passeriformes* was the most represented, with 17 species. Insectivorous birds dominated the foraging guilds, followed by carnivorous species. According to the IUCN Red List, 50 species were classified as least concern, while the spot-billed pelican and the black-headed ibis were listed as near threatened. Species abundance was highest at Manchalapur Lake and Maliyabad Lake, moderate in the Krishna River, and lowest at Raichur University, where only a few rare species were observed.

Keywords: Birds, Biodiversity, Raichur city, Migratory birds, Insectivores, foraging pattern

Biodiversity plays a crucial role in maintaining ecological balance, and biodiversity conservation is an important factor in protecting the ecological environment (Mi et al., 2021). India is one of the mega-diverse countries of the world and home to many of the world's most biodiverse ecosystems, including deserts, terrestrial, marine, and freshwater ecosystems; tropical and temperate forests, swamplands, plains; grasslands; riverine habitats; and islands (Rajagopal et al., 2022). Wetlands are habitats that are in the intermediate position between terrestrial and aquatic bodies, where flora, fauna, and birds are one of the essential biotic factors of wetlands and play the highest role in the food chain (Krishna et al., 2018). Avian diversity and abundance are heralds of healthy functioning of ecosystems, vegetation, and food that determine the diversity of species in a habitat (Walker and Shochat 2010; Tanalgo et al., 2015). Due to the destruction of natural habitat and anthropogenic activity, avifaunal populations have been reduced. Avifaunal populations have been declining due to the disruption of natural habitat and human activity. They have important functions as consumers, pollinators, predators, and indicators, among other things. Wetland migratory birds are significant components of global diversity. Birds are test animals for studying environmental issues. Of the recorded 310 wetland species found in India, half of the species visit India from China, Russia, and Central Asia (Manohara et al., 2016).

The amount of natural and semi-natural vegetation cover in a landscape influences how bird communities respond to agricultural intensification. The difference in bird species composition between small- and large-scale farms increases as vegetation cover increases, which further promotes the heterogeneity of bird communities at the landscape level (Marcacci et al., 2020, 2022; Gremion et al., 2022). Recently, with the increased consciousness for biodiversity survey and monitoring, many new species were discovered or re-described. The present investigation is focused on diversity and occurrence status.

MATERIAL AND METHODS

Study area: Raichur district is located in northeastern part of Karnataka state and lies between the two major rivers, Krishna and Tungabhadra. The Krishna River flows on the Northern part and Tungabhadra river flows on the southern part of the district which lies on the geographical Coordinates (16.2160ºN and 77.3566ºE) on Deccan plateau. It has an average elevation of 407m (1,335ft). The temperature around Raichur varies from 29ºC to 43ºC.Rainfall for the whole district is 728.2 mm, annually. Overall, the region is warm and humid most of the year. The abundance of bird species was studied in 4 different areas of Raichur, namely the habitats/sites Raichur University, Maliyabad Lake, the Krishna River, and Manchlapur Lake (Table 1, Fig. 1).

Data collection: The observations were recorded from July to December 2023 twice a week through visual encounter method two time in a day, morning from 6:00 to 9:30 AM and in the evening from 4 to 7 PM. The detailed survey of birds was conducted by the direct count method and the short-strip transects count method (SSCT) (Colin et al., 1993) and Point count method (Verner et al., 1985). The birds were sighted with the help of binoculars (Celestron Up-Close 10×40). Spot identification was done by using guide (Ali and Ripley 1968-74). Data collected through using digital cameras [Canon EOS 5D] and [Nikon D5600] through photography.

RESULTS AND DISCUSSION

Diversity based on abundance: Total of 52 species of birds belonging to 16 orders of 35 families (Table 2). The majority of birds were resident (67.3%) and others migratory (32.69%). Both the Krishna River and the Raichur University campus have 47 species. whereas, in Manchlapur Lake and Malyabad, 49 species were observed (Table 3). The documentation includes 15 aquatic and 37 terrestrial birds. The study conducted indicated significant variation in diversity and abundance in different seasons that might be due to migration patterns, habitat changes, and climatic conditions (Aynalem and Bakele 2008). The 90% of species show similarity of species in each site and might be due to interspersed similar plants, and many contain similar species of birds that share the same habitat (Tsegaye and Godisa 2016, Daselgnetal 2021, Dasalegnm and Fasika 2022). Common birds were maximum in Manchalapur Lake, followed by Krishna river, Raichur University campus, and Maliyabad, (Table 3). Similarly abundant species were high in Manchalapur and Maliyabad followed by Raichur University and Krishna river. Rare birds were high in Raichur University, followed by Krishna river, Malyabad lake and Machalapur lake. The higher bird abundance observed may be attributed to intact and moderate levels of taxonomic diversity, species richness, and overall bird abundance (Gashe et al., 2018). Mosisa et al. (2023) reported that seasonal and habitat variations influenced the presence of bird species, with 48.32% categorized as abundant, 17% as uncommon, and 1% as rare particularly among forest-adapted species. Similar findings were reported by Simamora et al. (2021). Mukhopadhyay and Mazumdar (2017) observed that bird diversity is closely regulated by the quality and quantity of available food resources. Factors such as abundance, habitat types, seasonal fluctuations in food supply, and breeding behaviors also play a crucial role in shaping bird communities (Aynalem and Bakele 2008, Girma et al., 2017). The high presence of common bird species was along the Krishna River, likely due to the diverse flora and fauna in the river basin, which provides suitable habitats for a wide range of bird species (Tamenut and Fasik 2022). Furthermore, the presence of trees along riverbanks has been found to significantly influence bird abundance and species richness, as supported by Mistry et al. (2015) and Bideberi et al. (2013).

Diversity based on IUCN status: Based on the IUCN status,

Table 1. Site coordinates

Sites	Coordinates Latitude (N)	Longitude (E)
Krishna river	16.382712° N	77.356433° E
Manchlapur lake	16.200829º N	77.362289º E
Raichur University	16.0984º N	77.4129º E
Maliyabad Lake	16.0296º N	76.6846º E

Fig. 1. Four different sites of study area

Table 2. Birds from the study area

Birds name	Scientific name	Order name	IUCN (Status)	Status	Food
Laughing dove	*Spilopelia senegalensis*	*Columbiformes*	LC	R	G, H
Eurasian collared dove	*Streptopelia decaocto*	*Columbiformes*	LC	R	G, F
Indian roller	*Coracias benghalensis*	*Coraciiformes*	LC	R	O
White-throated Kingfisher	*Halcyon smyrnensis*	*Coraciiformes*	LC	R	C,O
Asian green bee-eater	*Merops orientalis*	*Coraciiformes*	LC	R	I, C
Black-winged stilt	*Himantopus himantopus*	*Coraciiformes*	LC	M	C,I
Pied Kingfisher	*Ceryle rudis*	*Coraciiformes*	LC	R	C,P
Large grey babbler	*Argya malcolmi*	*Passeriformes*	LC	R	G
Brahminy starling	*Sturnia pagodarum*	*Passeriformes*	LC	R	F,I,O
House crow	*Corvus splendens*	*Passeriformes*	LC	R	O,I,F
Baya weaver	*Ploceus philippinus*	*Passeriformes*	LC	R	O,G
Barn Swallow	*Hirundo rustica*	*Passeriformes*	LC	M	I
Indian Silverbill	*Euodice malabarica*	*Passeriformes*	LC	M	G, H
Ashy prinia	*Prinia socialis*	*Passeriformes*	LC	R	I
Purple-rumped sunbird	*Leptocoma zeylonica*	*Passeriformes*	LC	R	I, H
Common tailor bird	*Orthotomus sutorius*	*Passeriformes*	LC	R	C,I
Scaly-breasted munia	*Lonchura punctulata*	*Passeriformes*	LC	R	G,F,I
White-browed wagtail	*Motacilla maderaspatensis*	*Passeriformes*	LC	R	I
Pied bushchat	*Saxicola caprata*	*Passeriformes*	LC	R	I
Indian robin	*Copsychus fulicatus*	*Passeriformes*	LC	R	O
Indian bushlark	*Mirafra erythroptera*	*Passeriformes*	LC	WM	C
Long-tailed shrike	*Lanius schach*	*Passeriformes*	LC	R, PM	I, C
Jungle babbler	*Argya striata*	*Passeriformes*	LC	R	I, G
Red-vented bulbul	*Pycnonotus cafer*	*Passeriformes*	LC	R	O
Eastern great egret	*Ardea modesta*	*Pelecaniformes*	LC	PM	C,P
Cattle egret	*Bubulcus*	*Pelecaniformes*	LC	PM	I
Eurasian Spoonbill	*Platalea leucorodia*	*Pelecaniformes*	LC	PM	C,I
Glossy ibis	*Plegadis falcinellus*	*Pelecaniformes*	LC	M	C,P,I
Black-headed ibis	*Threskiornis melanocephalus*	*Pelecaniformes*	NT	M	C,P
Spot billed pelican	*Pelecanus philippensis*	*Pelecaniformes*	NT	WM	O
Little egret	*Egretta garzetta*	*Pelecaniformes*	LC	HM	C
Malayan night heron	*Gorsachius melanolophus*	*Pelecaniformes*	LC	M	C,P
Indian pond heron	*Ardeola grayii*	*Pelecaniformes*	LC	R	C
Red-naped ibis	*Pseudibis papillosa*	*Pelecaniformes*	LC	R	O
Grey heron	*Ardea cinerea*	*Pelecaniformes*	LC	R	C
Black-winged kite	*Elanus axillaris*	*Accipitriformes*	LC	R	C
Shikra	*Accipiter badius*	*Accipitriformes*	LC	R	C
Plumbeous kite	*Ictinia plumbea*	*Accipitriformes*	LC	M	I
Greater coucal	*Centropus sinensis*	*Cuculiformes*	LC	R	I
Asian Koel	*Eudynamys scolopaceus*	*Cuculiformes*	LC	PM,R	F,I,O
Red-wattled lapwing	*Vanellus indicus*	*Charadriiformes*	LC	R	I
Common sandpiper	*Actitis hypoleucos*	*Charadriiformes*	LC	M	C
Yellow-wattled Lapwing	*Vanellus malabaricus*	*Charadriiformes*	LC	R	C
Indian cormorant	*Phalacrocorax fuscicollis*	*Suliformes*	LC	R	C
Indian spot-billed duck	*Anas poecilorhyncha*	*Anseriformes*	LC	R	O
Peacock	*Pavo cristatus*	*Galliformes*	LC	R	O
Swamp hen	*Porphyrio porphyrio*	*Gruiformes*	LC	M	O
Black-rumped flameback	*Dinopium javanense*	*Piciformes*	LC	R	F,O
Painted stork	*Mycteria leucocephala*	*Ciconiiformes*	LC	R	C,P
Indian grey hornbill	*Ocyceros birostris*	*Bucerotiformes*	LC	R	O,F,I
Spotted owlet	*Athene brama*	*Strigiformes*	LC	R	I, C
Rose-ringed parakeet	*Psittacula krameri*	*Psittaciformes*	LC	R	G,F,H

Status: R-Resident, M-Migratory, PM-Partially Migratory, HM-Highly Migratory, WM-Winter Migratory
Food: H-Herbivorous, C-Carnivorous, I-Insectivorous, G-Granivorous, P-Piscivorous, O-Omnivorous, F-Frugivorous
IUCN Status: LC- Least concern, NT- Near Threatened

Table 3. Abundance of bird species in different habitat

Birds name	Scientific name	Krishna river	Manchlapur lake	Raichur University	Maliyabad
Laughing dove	*Spilopelia senegalensis*	+++	+++	+++	+++
Eurassian collared dove	*Streptopelia decaocto*	+++	+++	+++	+++
Indian roller	*Coracias benghalensis*	++	++	+++	++
White-throated Kingfisher	*Halcyon smyrnensis*	+++	+++	++	+++
Asian green bee-eater	*Merops orientalis*	+++	+++	+++	+++
Black-winged stilt	*Himantopus himantopus*	+++	+++	+	++
Pied Kingfisher	*Ceryle rudis*	++	++	-	+
Large grey babbler	*Argya malcolmi*	+++	+++	+++	+++
Brahminy starling	*Sturnia pagodarum*	++	++	+++	++
House crow	*Corvus splendens*	++	++	+++	+++
Baya weaver	*Ploceus philippinus*	+++	+++	+	++
Barn Swallow	*Hirundo rustica*	+++	+++	+	++
Indian Silverbill	*Euodice malabarica*	+++	+++	+++	+++
Ashy prinia	*Prinia socialis*	++	+++	+++	+++
Purple-rumped sunbird	*Leptocoma zeylonica*	++	++	+++	+++
Common tailor bird	*Orthotomus sutorius*	+	++	++	++
Scaly-breasted munia	*Lonchura punctulata*	+++	+++	++	+++
White-browed wagtail	*Motacilla maderaspatensis*	+++	+++	+++	+++
Pied bushchat	*Saxicola caprata*	++	++	+++	++
Indian robin	*Copsychus fulicatus*	++	+++	+++	+++
Indian bushlark	*Mirafra erythroptera*	-	+	++	++
Long-tailed shrike	*Lanius schach*	+	++	+++	+++
Jungle babbler	*Argya striata*	-	++	++	+++
Red-vented bulbul	*Pycnonotus cafer*	++	+++	+++	+++
Eastern great egret	*Ardea modesta*	++	+++	++	++
Cattle egret	*Bubulcus*	+++	+++	+++	+++
Eurassian Spoonbill	*Platalea leucorodia*	++	+++	-	+
Glossy ibis	*Plegadis falcinellus*	++	+++	+	++
Black-headed ibis	*Threskiornis melanocephalus*	+++	+++	+	++
Spot billed pelican	*Pelecanus philippensis*	-	+++	-	-
Little egret	*Egretta garzetta*	+++	+++	++	+++
Malayan night heron	*Gorsachius melanolophus*	-	+	-	-
Indian pondheron	*Ardeola grayii*	+++	+++	+++	+++
Red-naped ibis	*Pseudibis papillosa*	++	+++	++	+++
Grey heron	*Ardea cinerea*	+++	+++	++	+++
Black-winged kite	*Elanus axillaris*	++	++	++	++
Shikra	*Accipiter badius*	++	++	+++	+++
Plumbeous kite	*Ictinia plumbea*	+	-	-	-
Greater coucal	*Centropus sinensis*	++	++	+++	+++
Asian Koel	*Eudynamys scolopaceus*	++	++	+++	++
Red-wattled lapwing	*Vanellus indicus*	+++	+++	+++	+++
Common sandpiper	*Actitis hypoleucos*	+++	+++	+	++
Yellow-wattled Lapwing	*Vanellus malabaricus*	++	++	+++	+++
Indian cormorant	*Phalacrocorax fuscicollis*	+++	+++	++	+++
Indian spot-billed duck	*Anas poecilorhyncha*	+++	+++	+	++
Peacock	*Pavo cristatus*	++	++	+++	+++
Swamp hen	*Porphyrio porphyrio*	++	+++	+	++
Black-rumped flameback	*Dinopium javanense*	+	-	++	++
Painted stork	*Mycteria leucocephala*	+++	+++	++	+++
Indian grey hornbill	*Ocyceros birostris*	-	-	++	+
Spotted owlet	*Athene brama*	+	++	++	+++
Rose-ringed parakeet	*Psittacula krameri*	++	++	+++	+++
Total- 52	--------	47	49	47	49

Rare + Common ++ Abundance

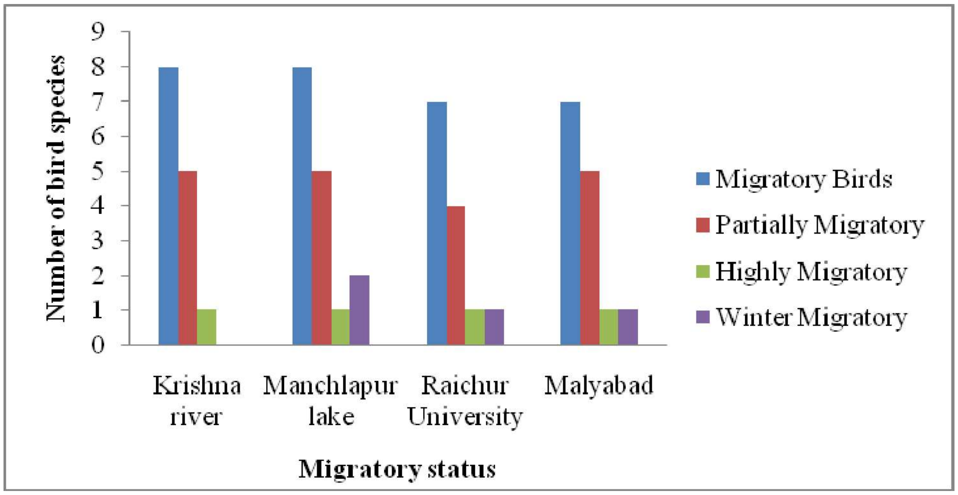

Fig. 2. Conservation/ (IUCN) status of bird species

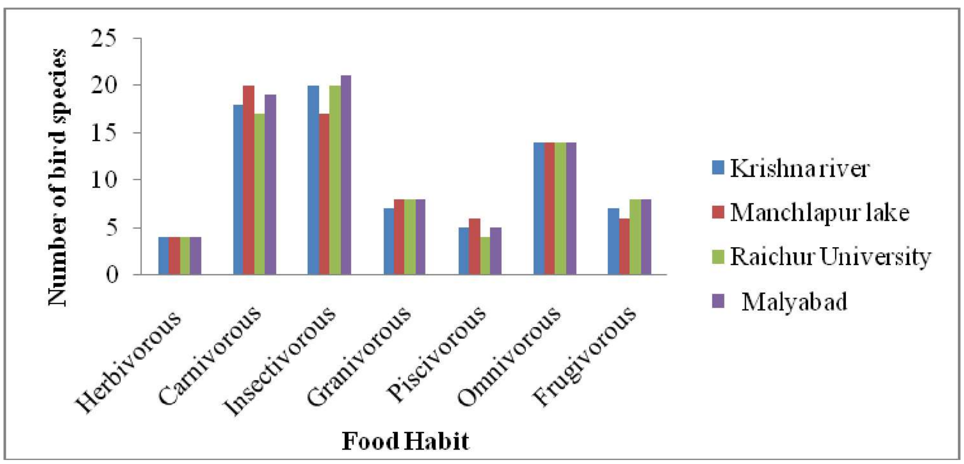

Fig. 4. Different feeding habits of birds

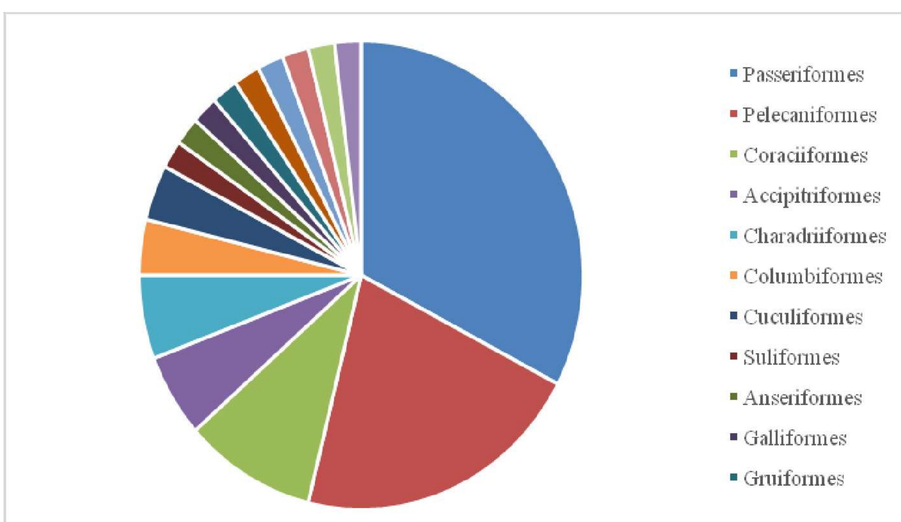

Fig. 5. Percent occurrence of birds species of different orders

the observed birds were categorized as the least concerned species highest observed in Malyabad, Manchalapur, Raichur University and lowest Krishna river, where maximum (37) resident birds were in Malayabad followed by Raichur University, Manchalapur and Krishna river (Fig. 3). Ther near threatened were highest in Manchalapur followed by Krishna river, Malyabad and Raichur University. Black-headed ibis and spot-billed pelican are near-threatened species as per IUCN status, which are almost rare species but found abundantly in Krishna River and Manchlapur Lake sites which might be due to preferred habitat and foraging area (Barik et al 2021). Highest percentage was of Passeriformes (32.69%) and followed by Pelicaniformes, Coraciiformes, Accipitriformes, Columbiformes .

Diversity based on migratory status: Migratory birds accounted for 32% of the total avifauna, while resident species comprised 67.3%. The highest number of migratory birds was at the Krishna River and Manchalapur Lake, followed by Raichur and Malyabad. (Fig. 4) Similar trend was observed for partially migratory species, except at Raichur University. The highly migratory species displayed a consistent distribution pattern across all sites. In contrast, winter migrants were most abundant at Manchalapur Lake, followed by the other two locations, with the exception of the Krishna River. Biswas et al. (2025) recorded 78.57% resident birds and 21.43% migratory birds, suggesting that study area offers favorable environmental conditions and potential to attract more migratory species.

Diversity based on feeding habit: Based on the food/feeding, the avifauna is dominated by carnivorous followed by insectivorous, omnivorous, and frugivorous birds (Fig. 5). Herbivorous shown similar trend in all sites. However, the insectivorous bird community was dominant in Maliyabad. This might be due to semi-arid open grasslands and man-made water bodies Biswas et al., (2025) observed that 41% of bird species were omnivores, 32% carnivores, and 16% insectivores. Similarly, More and Patil (2024) reported that 40% of the total bird species were insectivores, likely due to their high adaptability to human-modified agricultural landscapes. 52 species of birds belonging to 16 orders of 35 families (Fig. 6) Passeriformes were the highest followed by Pelicaniformes, coracciformes.

CONCLUSION

The total of 52 bird species were recorded, with Passeriformes and Pelecaniformes being the most numerous. Manchalapur Lake exhibited the highest species diversity, while Maliyabad Lake showed the greatest bird abundance. Most of the recorded species were resident and classified as of least concern. However, near threatened species were observed at each site, with Manchalapur having the highest number. The migratory bird presence was most prominent at the Krishna and Manchalapur sites. In terms of feeding behavior, carnivorous and insectivorous species were the most dominant. Despite being a semiarid region, the area supports significant avian diversity that must be preserved and safeguarded.

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Impact of Short-Term Ultraviolet (UV) Radiation Exposure on *Paramecium* Behavior, Viability, Proliferation and Implications for Freshwater Ecosystem Health

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Abstract: *Paramecium caudatum*, a well-established freshwater protozoan model, was employed to assess the sub-lethal and lethal effects of ultraviolet (UV) radiation exposure at 254 nm. *Paramecia* were exposed to varying durations of UV light ranging from 1 to 5 minutes, and observed for behavioral and physiological changes over a period of 96 hours. Short-term exposure (1-3 minutes) resulted in no mortality and retained normal motility and morphology. However, 4-minute exposure induced noticeable stress responses, including reduced swimming speed, disrupted locomotion patterns, and partial loss of cellular activity, with approximately 50% of individuals becoming inactive. The most severe effects were recorded following 5-minute exposure, where paramecia exhibited pronounced morphological deformities such as swelling, elongation, and loss of symmetry, along with pigmentation changes such as darkening and spotting of cytoplasm, loss of shape and structure, impaired coordination, and complete mortality by 24 hours post-exposure. Due to its ecological importance and sensitivity to UV radiation *Paramecium* serves as a valuable bioindicator for assessing the environmental impacts of increasing UV radiation associated with climate change. Additionally, it contributes to SDG 14 (Life below Water) by emphasizing that the UV sensitivity of freshwater protozoa reflects broader risks to aquatic microbial communities.

Keywords: *Paramecium caudatum*, Ultraviolet (UV) radiation, Environmental stress, Climate change

As global temperatures continue to rise, the impacts of climate change and ozone depletion pose significant threats to ecosystems worldwide, particularly freshwater environments. In this context, *P. caudatum*, a freshwater ciliate and established model organism, serves as a valuable bioindicator for assessing environmental stressors. Its sensitivity to ultraviolet (UV) radiation intensified by ozone layer depletion makes it especially vulnerable to DNA damage and membrane disruption, often resulting in elevated rates of cell mortality (Campbell and Romero 2009). Additionally, climate change can exacerbate the bioavailability and toxicity of pollutants such as pesticides and heavy metals, further endangering aquatic organisms. *P. caudatum* has been extensively employed in toxicological studies to evaluate the sub-lethal effects of these environmental contaminants, including drugs and natural food dyes, as well as to develop behavioral and physiological biomarkers (Venkateswara Rao et al., 2012, Hussain et al., 2008, Houneida et al., 2012, Pokhrel 2015). Its structural simplicity, short generation time (4-8 hours), ease of cultivation, and large cell size make it a practical and cost-effective model for laboratory research and bioassays (Mansano et al., 2016, Zhang 2022). Ubiquitous in stagnant and flowing freshwater systems, Paramecium plays a critical ecological role by influencing trophic dynamics and maintaining ecosystem balance (Potekhin et al., 2020). These qualities make it a reliable sentinel species in freshwater ecology, particularly as environmental stressors intensify due to climate change.

Paramecium has a long history as a model organism in cellular and genetic research, offering insights into intracellular differentiation, senescence, inheritance, and nucleocytoplasmic interactions (Houneida et al., 2012). Its ability to undergo autogamy and its interactions with symbiotic bacteria, viruses, and algae provide avenues for investigating host-microbe relationships (Sbartai Ibtissem et al., 2009). Studies examining its responses to environmental pollutants have revealed significant changes in motility, chemotaxis, phagocytosis, viability, and population dynamics. Notably, exposure to pesticides can lead to a concentration-dependent cascade of cellular abnormalities, including blistered and ruptured membranes, cell lysis, protoplasmic disintegration, and protein coagulation (Venkateswara Rao et al., 2006, Vijayakumar and Amanchi 2025). As climate change continues to amplify ecological stress, the role of *P. caudatum* in monitoring environmental health becomes increasingly crucial. The objective of this study is to investigate the effects of ultraviolet (UV) light exposure on *P. caudatum* by assessing its behavioral responses, survival rate, and reproductive capacity. Together, these assessments will provide a comprehensive understanding of how UV light affects the physiology and ecology of *P. caudatum* (Valentine et al., 2021).

MATERIAL AND METHODS

Freshwater samples were collected from the vicinity of Osmania University (GPS coordinates are 17.413333° N, 78.528611° E), Hyderabad, for the isolation, identification, and cultivation of *P. caudatum* for experimental analysis. A droplet from these water samples was extracted and examined under a microscope to confirm the presence of *Paramecium*. For culturing, a standard hay infusion medium was prepared using dried hay segments (1–8 cm in length), following the protocol described by Sonneborn (1950). The medium was prepared by boiling 6 grams of dried hay leaves in one liter of distilled water, followed by cooling, filtration, and autoclaving at 15 psi for 15 minutes. After sterilization, the medium was diluted with distilled water in a 1:1 ratio and transferred into 150 ml conical flasks for culturing and sub-culturing purposes (Bick 1972). To enhance bacterial growth, which serves as a primary food source for *Paramecium* cooked lady's finger (Okra) was added to the medium. Regular cultivation of multiple isolation lines and monitoring of growth rates were carried out to ensure reliable culture conditions (Sonneborn 1950). Sub-culturing was performed every sixth day to maintain healthy and log phage culture (Amanchi and Archana 2021).

Experimental details: About 25 *Paramecium* cells were isolated from a log-phase culture and distributed into five separate cavity blocks for experimental analysis. Each block was labeled according to exposure time intervals 1, 2, 3, 4, and 5 minutes and placed inside a laminar air flow chamber under a UV light source. The blocks were positioned at 65 cm from the UV lamp emitting light at a wavelength of 254 nm (Campbell and Romero 2009). Following UV exposure, immediate observations were conducted for first four hours at 30-minute intervals, and subsequent assessments were made at 24, 48, 72, and 96 hours post-exposure. During these observation periods, both behavioral responses and cellular proliferation were recorded. Key characteristics evaluated included body shape, structure, size, color, motility, swimming pattern, speed, general movement, and mortality; all assessed manually (Venkateswara Rao et al., 2006).

RESULTS AND DISCUSSION

Paramecia exposed to UV light for 1 to 3 minutes exhibited normal activity with no signs of mortality during continuous observation over a 4-hour period, recorded at 30-minute intervals. However, significant behavioral and physiological changes were observed with longer exposure durations. Morphological deformities in body shape and structure were assessed following UV exposure, with common abnormalities including swelling, elongation along the anterior–posterior axis, and loss of overall body symmetry. Visual determination of body size revealed a consistent reduction in length accompanied by an increase in body width, suggestive of structural stress induced by UV radiation. Under normal conditions, the paramecium exhibits a transparent body; however, post-exposure individuals displayed marked changes in pigmentation, including loss of transparency, darkening, and the appearance of unusual spots, suggesting cellular damage. Behavioral responses were also significantly affected. Initially, organisms exhibited erratic movements, followed by migration toward the corners of the cavity block, presumably as an avoidance response to UV radiation. Notable alterations in swimming behavior included uncoordinated motion, circling, sinking, variations in swimming speed, and overall loss of locomotor control. In 4-minute UV exposure, the movement of paramecia became noticeably slow, with reduced spinning behavior and instances of the organisms rotating around their own axis. Approximately 50% of the observed cells became highly inactive, displaying signs of severe stress and nearing death. The most profound effects were observed following 5 minutes of UV exposure. *Paramecia* subjected to this duration showed pronounced morphological changes, including distortion of body shape, narrowing of the anterior end, and an overall loss of their typical body structure. These individuals also exhibited a drastic reduction in swimming speed, loss of orientation and coordination, and impaired movement. In 24 hours post-exposure, all paramecia subjected to the 5-minute treatment had died, indicating complete lethality at this level of UV stress.

Extensive research has demonstrated that environmental stressors, particularly ultraviolet (UV) radiation, exert profound effects on the physiology, behavior, and cellular integrity of aquatic microorganisms such as *P. caudatum* (Barbara Kammerlander, et al., 2018). UV radiation, especially at 254 nm, induces DNA damage and disrupts critical cellular structures, leading to mutations and physiological impairments that compromise essential biological functions including motility, reproduction, and survival (Kammerlander et al., 2018). Short-term UV exposure has been shown to elicit marked behavioral alterations in *Paramecium*, characterized by decreased swimming speed, disrupted locomotion patterns, loss of orientation, and diminished responsiveness to environmental stimuli such as light and food sources (Niculite et al., 2018, Arlinghaus et al., 2019). These behavioral disruptions primarily arise from UV-induced damage to the cilia, the organelles responsible for propulsion and environmental sensing, thereby impairing the organism's capacity to navigate and adapt to its surroundings effectively

Table 1. Behavioral and morphological characteristics observation in *Paramecium caudatum* following UV (254nm) exposure with different time periods

UV (254nm) exposure duration (Minutes)	Motility (Before)	Motility (After)	Movement (Before)	Movement (After)	No of paramecia (Before)	No of paramecia (After)	Body shape (Before)	Body shape (After)	Colour (Before)	Colour (After)	Observation
1	Yes	Yes	Good	Good	20±5	20±5	Slipper	Slipper	Transparent	Transparent	No Effect
2	Yes	Yes	Good	Good	20±5	20±5	Slipper	Slipper	Transparent	Transparent	No effect
3	Yes	Yes	Good	Good	20±5	20±5	Slipper	Slipper	Transparent	Transparent	No Effect
4	Yes	UV avoidance noticed	Good	Erratic and reached to corners of cavity block	20±5	20±5	Slipper	Elongated, Narrowed at anterior	Transparent	Pigmentation, Blackening of cytoplasm	Significant Behavioral changes noticed
5	Yes	UV avoidance noticed	Good	Erratic movement and loss of coordination	20±5	20±5	Slipper	Loss of symmetry, Bulged	Transparent	Spotting in cytoplasm, Blackening of cytoplasm	Significant Behavioral changes noticed

Table 2. Growth Inhibition in *Paramecium caudatum* following exposure to 254 nm UV Radiation at relative to control (n = 5)

Exposure distance	Duration (Minutes)	No. of. organisms	No. of. organisms (Growth proliferation)				No. of. organisms (% growth inhibition)			
			Control group (hrs)				Experimental group (hrs)			
			24	48	72	96	24	48	72	96
65cm	1 min	20±5	783.6 (100)	1022.8 (100)	1284.6 (100)	1558.0 (100)	438 (44.06)	342 (66.56)	159 (87.62)	13 (99.16)
65cm	2 min	20±5					352 (55.07)	345 (66.26)	97 (92.44)	12 (99.22)
65cm	3 min	20±5					317 (59.54)	308 (69.88)	125 (90.26)	15 (99.03)
65cm	4 min	20±5					247 (68.47)	151 (85.13)	82 (93.61)	08 (99.49)
65cm	5 min	20±5					Nil (100)	Nil (100)	Nil (100)	Nil (100)

(Kammerlander et al., 2018, Arlinghaus et al., 2019). Prolonged UV exposure exacerbates these deleterious effects, resulting in pronounced morphological deformities, including aberrations in body shape and loss of structural integrity, ultimately culminating in cell death (Gopi Krishna et al., 2022).

Paramecium's pivotal ecological role in freshwater systems regulating microbial populations and contributing to nutrient cycling such impairments can disrupt ecosystem function and stability (Ruben et al., 2001, Goodwin et al., 2018, Nichols et al., 2018). The sensitivity of *Paramecium* to UV-induced stress underscores its utility as a bioindicator for environmental monitoring climate change. These findings highlight that UV radiation exposure at 254 nm for 4 to 5 minutes significantly compromises *Paramecium caudatum*'s motility, viability, and reproductive capacity, with broader implications for microbial community dynamics and freshwater ecosystem health (Kammerlander et al., 2018, Niculite et al., 2018, Arlinghaus et al., 2019, Gopi Krishna et al., 2022). This study supports SDG 13 (Climate action) by demonstrating the ecological impacts of increased UV radiation, a consequence of climate change. The use of *P. caudatum* as a bioindicator highlights the potential for early detection of climate-induced stress in aquatic ecosystems. Additionally, it contributes to SDG 14 (Life below Water) by emphasizing that the UV sensitivity of freshwater protozoa reflects broader risks to aquatic microbial communities. These findings underscore the need for monitoring micro-scale biological responses to environmental change as part of global adaptation and ecosystem protection efforts.

CONCLUSION

The present study demonstrates that ultraviolet (UV) radiation at 254 nm induces significant morphological and behavioral alterations in *Paramecium caudatum*, with the severity of effects directly related to exposure duration. Short-term exposures (1–3 minutes) had negligible effects, whereas longer exposures (4–5 minutes) resulted in marked deformities, impaired motility, and ultimately, complete mortality within 24 hours. Key physiological changes included swelling, elongation, body asymmetry, pigmentation loss, and ciliary dysfunction, which collectively compromised the organism's locomotion and orientation. These effects reflect underlying cellular damage likely due to UV-induced DNA disruption and structural impairment. Furthermore, the sensitivity of *P. caudatum* to UV radiation highlights its potential as a biological marker for assessing and monitoring the health of freshwater ecosystems. These findings underscore the broader ecological consequences of increased UV exposure associated with climate change and align with Sustainable Development Goal 14 by emphasizing the vulnerability of aquatic microbial communities and the importance of early detection and adaptive ecosystem protection strategies.

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Activity Pattern of the Malabar Giant Squirrel (*Ratufa indica maxima*) in Karikulam Forest Range of Ranny Division of Kerala, India

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Abstract: The Malabar giant squirrel (*Ratufa indica maxima* (Schreber 1784) is an arboreal species, endemic to the forests of the Western Ghats, India. The present study was undertaken with the objective of systematically examining the activity patterns and feeding behaviour of the Malabar giant squirrel in the Karikulam forest of Kerala from March to November, 2021. The research employed the focal animal sampling method, to systematically collect data on the activity patterns and feeding behavior. Total of 13,590 minutes of focal sampling was conducted. Feeding and resting behaviors, accounted for approximately 86% of the squirrels' total observed activity. The squirrel exhibited significant feeding activity primarily during the early morning and late afternoon. The marked increase in resting behaviors was observed during the midday period. In terms of dietary preferences, the study indicate that the most frequently utilized species include *Xylia xylocarpa, Tamarindus indica, Terminalia paniculata, Terminalia crenulate*. The squirrels utilize 16 distinct tree species for nesting purposes. The trees predominantly selected for nesting include, *Xylia xylocarpa, Terminalia paniculata, Artocarpus hirsutus, Terminalia crenulate*. These findings highlight the species' dependence on specific tree species for both food and nesting, emphasizing the need to conserve these key resources to ensure the survival of *R. indica maxima* populations in the Western Ghats.

Keywords: Malabar giant squirrel, *Ratufa indica maxima*, Karikulam Forest

Malabar giant squirrel (*Ratufa indica maxima*) is widely distributed in the Indian peninsula (Agrawal and Chakraborty 1979, Jathana et al., 2008) and is predominantly found across a range of natural forests from moist deciduous to dry deciduous forests types, old mature teak forests (Ramachandran 1988), riparian forests (Baskaran et al., 2011), and teak-mixed forests (Kumara and Singh 2006). It is known for its striking appearance, with a colorful pelage and large, bushy tail. As an arboreal mammal, the giant squirrel exhibits a range of fascinating behaviors and adaptations that enable it to thrive in its complex forest habitat. Much of its activity is mainly concentrated in the top to mid-canopy (Kumara and Singh 2006) as they prefer higher taller trees (> 15 m) for feeding and nesting (Baskaran et al 2011). The nesting behaviour and tree selection observed in different landscapes, showing a strong preference for large, contiguous canopy trees for nesting (Mudappa and Raman 2007). Being an integral species to the forest ecosystems of Kerala, exhibits distinct activity patterns critical for its survival and ecological role. Despite ecological importance, detailed studies on the activity patterns of the Malabar giant squirrel are sparse. Understanding these patterns is vital for conservation, as they reveal critical insights into the squirrel's behavioral ecology. This study aims to document and analyze the activity patterns of the Malabar giant squirrel in Kerala Forest ecosystems, Karikulam forest range, which is a fragmented forest near to Perumthenaruvi Waterfalls, falling under the Ranny Forest division, Kerala.

MATERIAL AND METHODS

Site location: The study was conducted in the Karikulam forest station in the Ranni forest range, Kerala, India. The area lies between 9°23'42"N and 76°49'42"E, covering approximately 136.2 square kilometers (Fig. 1). This research area has evergreen, semi-evergreen, and deciduous types of forests.

Methodology: The study was conducted from March to November, 2021. Data on activity patterns and feeding were recorded through direct observation using the focal animal sampling method (Altman 1974). Focal animals were followed, and observations were recorded on CANNON Digital camera. Observations were made for 3 days per week (6 hours per day: 6:00 am-8:00 am, 10:00 am -12:00 pm and 4:00 pm -6:00 pm) per month. Focal sampling was made at 15 minutes intervals (10 min observations and 5-minute break). The activities of the giant squirrels were grouped in the following seven major categories: 1. Feeding, which includes the foraging and consumption of food sources; 2. Resting, involving periods of relaxation and repose; 3. Travel, encompassing movement from one location to another; 4. Intraspecific activity, referring to interactions and behaviors within the squirrel population; 5. Grooming involves the cleaning and maintenance of fur and body (cleaning, licking, biting through the hairs and scratching); 6. Community behaviour, encompassing social interactions and engagements within the squirrel community; 7. Dray-related activities, include behaviors associated with the construction and use of drays, such as nesting and sheltering activities. The remaining activities, namely chasing, defecation, urination, calling, nest building, and mating, are collectively classified as 'others (Borges 1989).

RESULTS AND DISCUSSION

Activity pattern: Total of 13,590 minutes of observation were conducted using the focal animal sampling method to analyze the activity budget of the Malabar giant squirrel. The collective duration allocated to feeding (46.8%) and resting (39.1%) accounts for 85.9% of the daily activity of the squirrels. Squirrels spend most of their time feeding, which accounts for the largest portion of their daily activities. Movement makes up 9.2% of time, while other activities such as grooming, searching for food, chasing, and nest building collectively makeup 4.9%. Specifically, grooming accounts for 0.7%, food searching for 2.3%, chasing and mating for 0.3%, and drey-related activities for 1.6% of their time (Table 1, Fig. 2). Similar findings were reported in earlier studies, where feeding and resting dominated their daily schedules (Bhaskaran et al., 2011)

Feeding behavior: The squirrels frequently ascend to the extremities of branches to procure food items using its mouth while foraging. It subsequently relocates to sturdier, horizontal branches, where it assumes a seated position for feeding. It secures the food items primarily within its mouth, occasionally utilizing its forelimbs for support on these robust branches. In the current investigation, movements occurring over short distances within the same tree during feeding, with food material in the mouth or forelimb, were categorized as

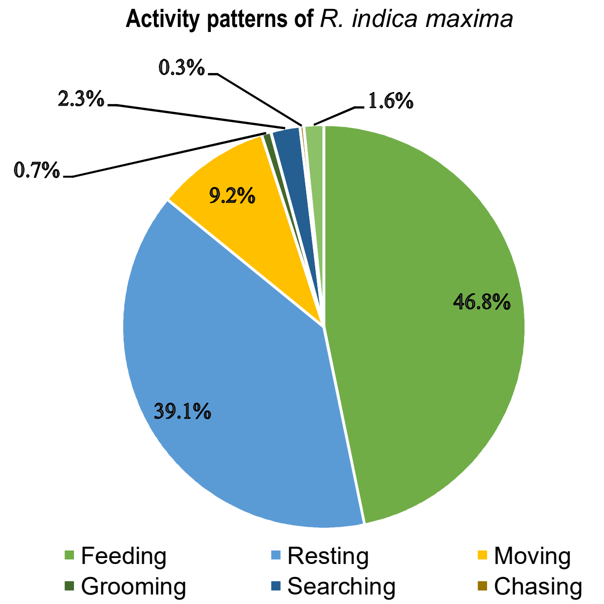

Fig. 2. Percentage of time spent on different activities

Table 1. Activity pattern and time spentfor each activity by the Malabar giant squirrel

Activity	Time spent (minutes)
Feeding	6356
Resting	5314
Moving	1241
Grooming	104
Searching	317
Chasing	42
Nestbuilding	216

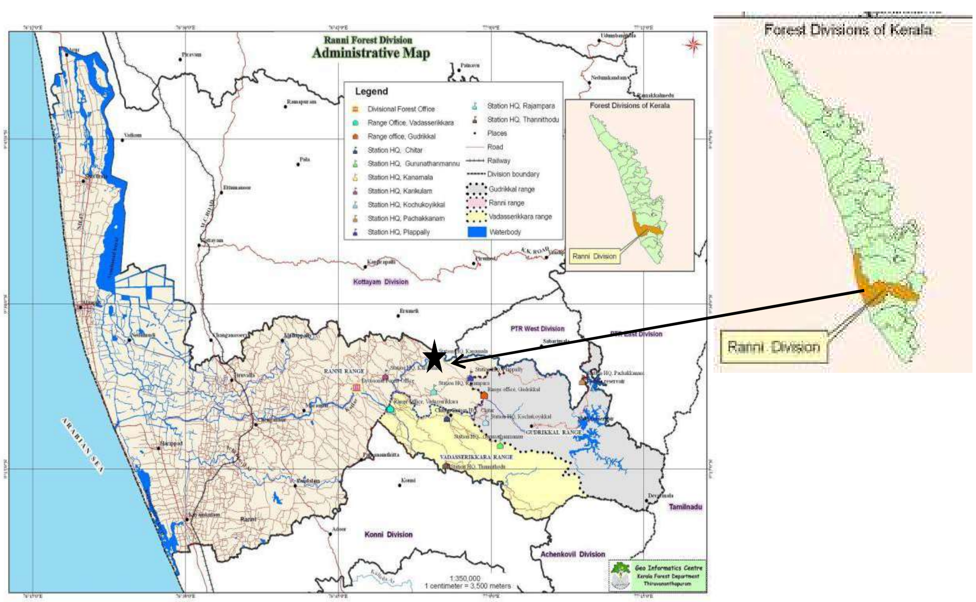

Fig. 1. Study area - Karikulam forest under the Ranny Forest division

part of the feeding activity. The allocation of time to various activities and the feeding behavior of different plant species were determined based on observations made over five months.

Food: *Xylia xylocarpa*, serves as a significant food source for the squirrels (Table 2). This tree bears flat seed pods, with its seeds being particularly favored during December through March. Giant squirrels play a crucial ecological role by dispersing seeds through consumption and defecation (Menon 2014). Conversely, the leaves of this tree are not preferred. Squirrels feed on the bark of seven plant species and consume termites. Their feeding behavior involves removing bark from trees to access termites. Among the tree species observed, *Xylia xylocarpa, Tamarindus indica, Terminalia paniculata,* and *Terminalia crenulata* are preferred for feeding by the squirrels. Nayak and Patra (2015) also observed that Malabar Giant Squirrels consume seeds, bark, and leaves, with seeds being the most preferred.

Feeding and other activities: The feeding behavior of the Malabar giant squirrels is predominantly observed during the morning and evening hours. This species is strictly diurnal, confining its activities to daylight hours. Upon daybreak, the squirrels emerge from their drey and proceed to the food tree to commence their feeding routine. The duration of feeding and resting activities was meticulously quantified. It was observed that the squirrels commenced feeding immediately upon emerging from their nests. A zenith in feeding behavior was observed in the morning, spanning from 6:00 am to 6:30 am, and in the late afternoon from 5:00 pm to 5:30 pm (Table 3). Feeding came to a complete halt after 11:30 am, during which time the squirrels engaged in complete resting (Table 3). In the morning, the squirrels journeyed away from their dreys, whereas in the evening, they gravitated towards their nests. The percentage of resting is higher during the mid-day and the animals are less active in this period (from 11.00 am-12.00 pm). From the heat map it is evident that the two activities of the Malabar giant squirrel namely feeding and moving are positively correlated and are clustered together (Fig. 3). However, the time interval 6:00 am-6:30 am and 5:00 pm to 5:30, the animal shows less resting activity and is

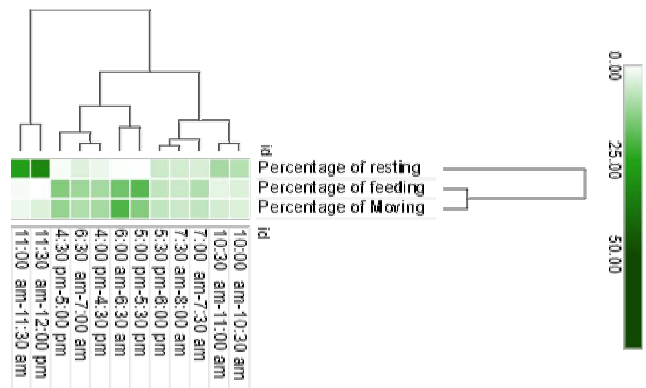

Fig. 3. Activity patterns of *Ratufa indica maxima* represented by a Heatmap (%)

Table 3. Time spent for various activity

Time	Feeding (%)	Resting (%)	Moving (%)
6:00 am-6:30 am	16.00	0.35	19.98
6:30 am-7:00 am	11.00	3.85	9.02
7:00 am-7:30 am	9.00	4.45	7.01
7:30 am-8:00 am	5.99	5.21	5.96
10:00 am-10:30 am	4.00	8.42	4.03
10:30 am-11:00 am	3.01	10.12	5.00
11:00 am-11:30 am	0.99	25.86	2.01
11:30 am-12:00 pm	0.00	32.91	3.95
4:00 pm-4:30 pm	10.01	2.15	9.99
4:30 pm-5:00 pm	14.00	0.85	12.01
5:00 pm-5:30 pm	19.01	0.05	14.02
5:30 pm-6:00 pm	7.00	5.78	7.01

Table 2. Parts of different plant species consumed by the giant squirrel

Parts of plants	Plant species
Young fruit	*Xylia xylocarpa*
Sprouting leaves	*Zizyphus oenoplia*, *Bombax ceiba*, *Albiziaodoratissima*
Fruits	*Bombax ceiba*, *Terminalia crenulata*, *Lagerstromiamicrocarpa*, *Acacia auriculiformis*, *Xylia xylocarpa*, *Mangifera indica*, *Grewia tilifolia*, *Pongamia pinnata*, *Terminalia bellirica*, *Careya arborea*
Leaf petiole	*Mangifera indica*, *Pongamia pinnata*
Bark	*Terminalia bellirica*, *Acacia auriculiformis*, *Terminalia crenulata*, *Xylia xylocarpa*, *Albizia odoratissima*, *Mangifera indica*
Leaves	*Albizia odoratissima*, *Mangifera indica*, *Terminalia crenulata*, *Xylia xylocarpa*
Twigs	*Artocarpus heterophyllus*, *Terminalia crenulata*
Flower	*Xylia xylocarpa*, *Terminalia crenulata*, *Lagerstromiamicrocarpa*, *Acacia auriculiformis*, *Mangifera indica*, *Xylia xylocarpa*
Seed	*Xylia xylocarpa*, *Terminalia crenulata*, *Albizia odoratissima*, *Careya arborea*, *Scheichera oleosa*, *Artocarpus hirsutus*

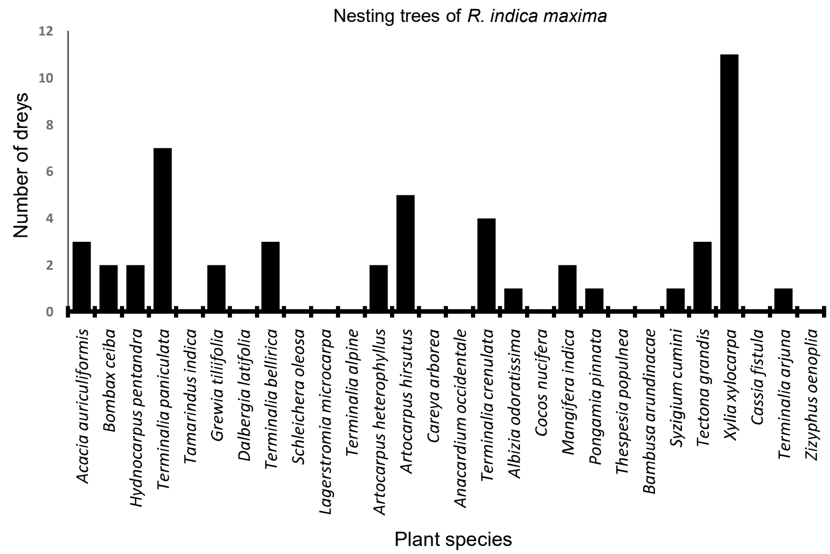

Fig. 4. Preference of nesting trees by *Ratufa indica maxima* based on drey count

contrary to the percentage of feeding and moving, which is more percentage in this particular time interval.

Nesting behaviour: The Malabar giant squirrels construct multiple large nests, known as dreys, using leaves and twigs within their natural habitat. A total of 49 nests were observed. Among the 28 tree species documented, the squirrels preferred only 16 species when building their nests. *Xylia xylocarpa* emerged as the most favored tree species for drey construction. The branches of the specific tree housed a total of 11 dreys. *Terminalia paniculata* holds the position of the second most favored tree species in this particular locale (Fig. 4). *Xylia xylocarpa* is a food tree and also used as a nesting tree. During the nesting period, squirrels bring small twigs of *Xylia xylocarpa* to build their nests. *Terminalia paniculata* is the second most frequently chosen tree utilized for nesting, although it is not the preferred food source for squirrels. The Malabar giant squirrels utilized building materials obtained from plant species such as *Pongamia pinnata, Xylia xylocarpa*, and *Terminalia crenulata* for the construction of their nests. These nests were meticulously erected in trees with broad, vertical spreads, systematically positioned at the lower extremities of the canopy and distanced from the primary trunk. The giant squirrel prefers the higher canopy positions for nesting as compared to the middle part of the tree (Samson 2020). Observations from the study revealed two instances of nest construction, with both efforts requiring a three-day timeframe for completion. The nesting behavior involved the procurement of materials such as twigs and leaves. Significantly, the placement of the nests was deliberate, as they were consistently situated at the apices of the trees, affording superior concealment and maximal security. The high preference for these trees could offer better protection and escape from predators. Squirrels prefer trees that are tall and have a large number of branches for nest building (Mohan et al., 2023). The nesting trees were significantly larger in all aspects than the non-nesting trees. The selection of this type of tree could facilitate easy movement to and from the nest in all directions, a major advantage to escape from predators and to move to other parts of the home range for foraging and other activities.

Malabar giant squirrels were most active during early morning and evening, leading to more sightings during these times. The nest trees of the Malabar giant squirrel were predominantly from a selection of tree species, including *Acacia auriculiformis*, *Bombax ceiba*, *Hydnocarpus pentandra, Grewia tiliifolia, Terminalia paniculata, Terminalia bellirica, Artocarpus heterophyllus, Artocarpus hirsutus, Terminalia crenulata, Albizia odoratissima, Mangifera indica, Pongamia pinnata, Xylia xylocarpa, Terminalia arjuna, Syzygium cumini, and Tectona grandis*. The results of the nesting tree study indicate that squirrels tend to prefer the largest available trees and choose the highest locations within their home range to build their nests. However, their selection is significantly influenced by the species of the trees and their physical characteristics, including canopy contiguity (Pradhan et al., 2012, Pradhan et al., 2017).

CONCLUSION

The activity pattern of Malabar giant squirrel (*Ratufa indica maxima*) is characterized by nearly equal periods of feeding and resting. The availability and abundance of food resources play a vital role in determining the survival of the animal species. It exhibits a strong preference for nesting in taller trees with contiguous canopies, which likely provide enhanced protection from predators and environmental stressors. It is recommended that conservation initiatives focus on preserving and restoring tall, continuous canopy forests, as these habitats are critical for sustaining viable populations and mitigating potential threats from habitat fragmentation and human encroachment.

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Biometric Analysis and Length Weight Relationship of *Carassius Carassius* (Linneaus 1758) in Dal Lake, Kashmir

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Abstract: This study was aimed to describe the morphometrics, meristic and length weight relationship of *C. carassius* in Dal Lake, Kashmir based on 180 specimens for a period of six months. The total length ranged from 88.62 mm to 177.45 mm with the corresponding weight of 8 gm and 86.5 gm respectively. Thirteen morphometric measurements were observed and the coefficient of determination (R^2) value was highest between total length and standard length (0.946) and least between head length and snout length (0.503) indicating high degree of relationship between the characters compared. The fin formula of the fish was D(I/18-19); A(I/7); P_1(11-13); P_2(8-9); C(19-22); LL(30-33): LLP(13-20). The coefficient of determination (R^2) for length-weight relationship was 0.872 for females, 0.855 for males and 0.868. The value of "b" for male fish was 3.016 and for females was 3.002. The 'b' for pooled data was 3.054 that showed almost isometric growth in the fish.

Keywords: Biometric analysis, *Carassius carassius,* Length-weight relationship, Dal Lake

Carassius carassius Linneaus (crucian carp) is a medium sized fish belonging to family Cyprinidae. Its habitat includes lakes, ponds, and slow-moving rivers and is native to England. They are deep bodied and laterally compressed fish with terminal and strongly curved mouth and thick lips having no barbels. Color is grey on back, lighter on sides with whitish underside and dusky fins, with 31-33 Scales in a lateral series, lateral line scales tubed only on anterior 12-25 scales, branched dorsal fin rays 18-20, branched anal fin rays 6-7, gill rakers about 26-27 and the third dorsal and anal fin rays are strong and posteriorly serrated. These are broadly described as having a body of golden-green shining color, young fish are golden – bronze but darken with maturity until they gain a dark green back, deep bronze upper flanks, and gold on the lower flanks and belly and reddish or orange fins (Lintermans 2023), *C. carassius* in Kashmir is known as "Crucian carp" and was introduced in Kashmir during 1956-1958 in Dal lake (Shafi 2012). It is among one of the common cyprinids found in Dal lake Kashmir and is well adapted to wide range of habitats to enable tolerance with varied environmental factors. It spawns in dense submerged vegetation (Kottelat and Freyhof 2007). Maqbool (2017) reported *C. carassius* contributes 32.09% in catch composition and 20.5% by biomass in Dal Lake. This study aimed to understand the changes in morphometric and meristic characters in *C. carassius* which can be an important aspect of adaptation and evolution happening in the region and can provide a basis for population differentiation.

MATERIAL AND METHODS

A total of 180 specimens of *Carassius carassius* were observed for a period of six months (December to May). Thirteen morphometric measurements were studied using the standard procedures described by earlier researchers (Lagler et al., 1962, Laevastu 1965, Appa Rao 1966, Dwivedi and Menezes 1974, Grant and Spain 1977).Meristic characters have definite number and count, they were analyzed following the conventional method as described by Hubbs and Lagler (1958). During the present study, seven meristic characters were counted. Relationships between the various body measurements to the total length and head length were calculated. Range, mean, median, standard deviations and correlation of coefficient were estimated for the characters under study.

Morphometric characters were plotted using Scattergram. The relationship between the various morphometric measurements was determined by linear regression formula: Y = a + b X Where, 'Y' is the dependent variable, 'X' is the independent variable, 'a' is a constant (intercept) and 'b' the regression coefficient (slope). The length-weight relationship was estimated from the allometric separately for both sexes and significant differences, if any, in the slopes of the regression lines for males and females were ascertained (Le-Cren 1951).

$W = aL^b$ or $\text{Log } W = \log a + b \log L$

Where, W is the total body weight in grams, L is the total length in mm, a and b are the coefficients of the functional regression between W and L.

RESULTS AND DISCUSSION

The total length ranged from 88.62 (January) to 177.45 mm (April) with the corresponding weight of 13.5 and 86.5

gm respectively. The standard length was maximum in April (142.75 mm) and minimum in January (71.03 mm). Total length showed a maximum coefficient of variation (0.40%) while standard length, pre-orbital length, and post orbital length showed minimum variation (0.12%) (Table 1). The coefficient of determination (R^2) was highest between total length and standard length (0.946) that was followed by total length and pre-anal and pre-pelvic length. The least was between head length and snout length (0.503) followed by head length and eye diameter (0.544) indicating high degree of relationship between the characters compared (Fig. 1, 2). Variety of morphological, physiological, behavioral and biochemical characteristics are used in the identification of fishes. The 'b' values obtained among various morphometric characters showed highest correlation (0.834) between total length and standard length, while as lowest 'b' value (0.083) was between total length and post orbital length. Bhat et al. (2010) studied the morphometric characteristics of *Schizothorax* species in the River Lidder of Kashmir and reported maximum growth in standard length (0.9080) and least in maximum body depth (0.1730) with respect to the total length of the fish. Shah et al. (2011) in farmed rainbow trout in Kashmir reported high level of interdependence between the fourteen morphometric characters. Qadri et al. (2017) also reported high relation in total length and standard length and lowest between total length and head length of S. curvifrons in River Jhelum, Kashmir. Similar inferences as

Table 1. Weight (gms) and various morphometric characters (mm) of *Carrassius carassius* Linneaus

Statistical estimates	Range (mm)		Mean (mm)	Coefficient of variation (%)
	Min	Max		
Total length	88.62	177.4	129.55	0.40
Weight(gm)	8.0	86.5	37.73	0.40
Standard length	71.03	142.75	105.52	0.12
Pre-dorsal length	34.71	75.25	52.97	0.13
Pre-pectoral length	16.59	46.19	31.15	0.15
Pre- pelvic length	33.05	76.01	54.09	0.13
Body depth	20.58	54.9	40.15	0.14
Pre-anal length	53.14	111.28	81.26	0.13
Head length	15.03	33.45	25.54	0.18
Snout length	4.45	12.85	8.75	0.20
Eye diameter	4.32	9.56	7.08	0.13
Caudal fin length	18.46	35.03	27.14	0.13
Pre-orbital length	6.16	13.87	10.22	0.12
Post-orbital length	9.04	16.97	13.30	0.12

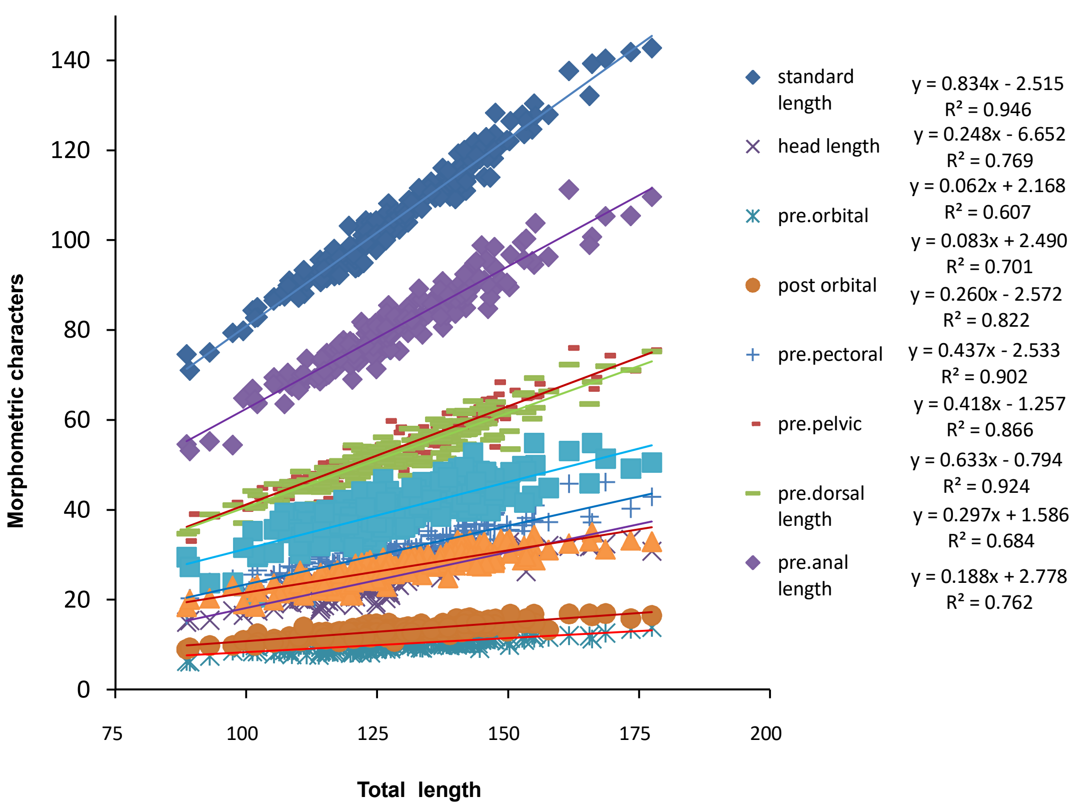

Fig. 1. Logarithmic relationship of different morphometric characters with total length of *Carassius carassius* Linneaus

well as high positive correlation among morphometric characters were reported in earlier studies (Balai et al., 2017, Arafat and Bakhtiyar 2020, Sidiq et al., 2021, Khan et al., 2022, Zakiya et al., 2024).

Meristic characters: Meristic characters have definite number and are countable, repetitive structures that help in fish classification; sometimes they vary and fall under some specific range. (Langer et al., 2013, Jan and Ahmed 2020) Seven meristic characters i.e., number of lateral line scales, dorsal fin rays, pectoral fin rays, pelvic or ventral fin rays, caudal fin rays, anal fin rays and lateral line pores of *C. carassius* were recorded in current study, out of which lateral line scales ranged from 30-33, dorsal fin, 18-1, 19, pectoral fin 11-13, pelvic fin 8-9, caudal fin 19-22, anal fin 1, 7 and lateral line pores ranged from 13-20 in length groups. The number of spines measured in dorsal fin and anal fin was one spine in all length groups (Table 2). Findings show that some counts of *C. carassius* showed similarities, while some counts showed variation in all length groups. Fin formula was:

D (I/18-19); A (I/7); P_1 (11-13); P_2 (8-9); C (19-22); LL (30-33); LLP (13-20)

Kottelat and Freyhof (2007) and Froese and Pauly (2011) reported Dorsal spines: 3 - 4; Dorsal soft rays: 13-22; Anal spines: 2 - 3; Anal soft rays: 5 - 7; V; lateral line with 31-36 scales; Caudal fin with 18-20 rays and no barbells in *C. carassius.* FAO (2017) reported 27-30 scales in lateral line, dorsal fin ray: 3, 15-19; pectoral fin ray: 1, 16-17; ventral fin ray: 1, 8; anal fin ray: 3, 5 in *C. carassius* showing certain variations from present study. Variations in meristic characters were reported in many fishes such as *Nematalosa nasus* (Al Hassan 1987), *Pterophyllum scalare* (Bibi et al., 2008) and *Crossochelius latius* (Brraich and Akhter (2015). During present study, meristic counts are dependent of body size and there is change in meristic counts with increase in body length. The lateral line counts observed during the present study varied from the earlier studies, the difference indicates that different locations and environment have considerable impact on meristic characters. The differences can be due to geography, ecology and human activities (Lawson 2010) and considerable impact of abiotic factors and biotic factors like depth, turbidity, and temperature of water population size, fish growth (Ezeafulukwe et al., 2015, Hasan et al., 2021).

Length-weight relationship: The length range of 88.62 mm to 177.45 mm and weight range of 8g to 86.5 g comprising of 91 males and 89 females were analyzed for length weight relationship. The relationships for males, females and combined were established as, Log W = -4.830 + 3.016 Log L; Log W = -4.778 + 3.002 Log L and Log W = -4.889 + 3.054 Log L respectively (Fig. 3, 4 and 5).The coefficient of determination (R^2) for length-weight relationship was 0.872 for females, 0.855 for males and 0.868 for pooled data. The value of “b” for male fish was 3.016 and for females it was found to be 3.002. The 'b' (3.054) for pooled data showed isometric growth in the fish which show fish grows with equal proportions in all dimensions. Zargar *et al*. (2012) recorded length weight relationship of *C. carassius* in three water bodies from Kashmir that included Anchar Lake, Dal Lake and Manasbal Lake and reported different values of 'b' showing inter Lake Variation. The fish showed positive allometric growth b>3.0; heavy group in Manasbal Lake and Anchar Lake and isometric growth of equal increment of both parameters of length and weight in the Dal Lake showing similarity with the results from the present study. Bhat et al. (2010) reported the value of 'b' between 2.9467 to 3.0997 in *Schizothorax* species showing almost ideal growth pattern of the three species. Shafi and Yousuf (2012) observed exponential value (b) indicating the isometric growth for the fish of *Schizothorax niger* from Dal Lake. Farooq et al. (2017) and Qadri et al. (2017) observed negative allometric growth in *S. labiatus* and *S.curvifrons.* Shah et al. (2013) and Wali et al. (2019) reported isometric growth for rainbow trout from Kashmir indicating fish growth with equal proportions in all dimensions. Zakiya et al. (2024) reported b value of 3.152, which indicated positive allometric growth in *S. progastus* in Ladakh region.

Table 2. Meristic characters of *C. carassius* Linneaus in different length groups

Meristic characters	Length groups (mm)									
	85-95	96-105	106-115	116-125	126-135	136-145	146-155	156-165	166-175	176-185
Lateral line scales	30	31	31	31	31	32	32	32	33	33
Dorsal fin	1,18	1,19	1,19	1,19	1,19	1,19	1,19	1,19	1,19	1,19
Pectoral fin	11	11	12	12	12	12	12	13	13	13
Pelvic fin	8	8	8	8	8	8	8	9	9	9
Caudal fin	19	19	20	20	20	20	21	21	21	22
Anal fin	1,7	1,7	1,7	1,7	1,7	1,7	1,7	1,7	1,7	1,7
Lateral line pores	13	13	14	14	14	16	17	18	20	20

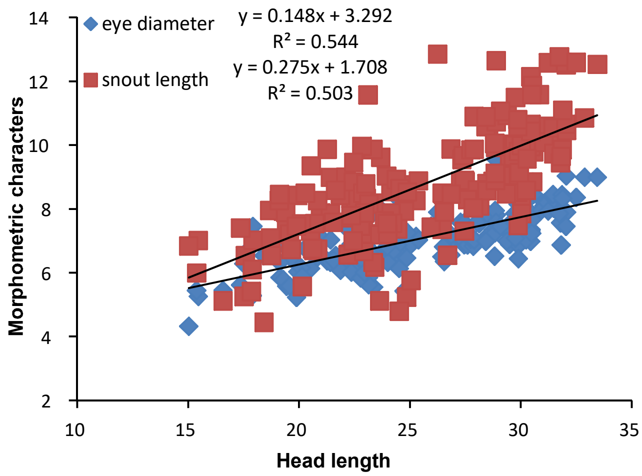

Fig. 2. Logarithmic relationship of different snout length and eye diameter with head length of *Carassius carassius* Linneaus

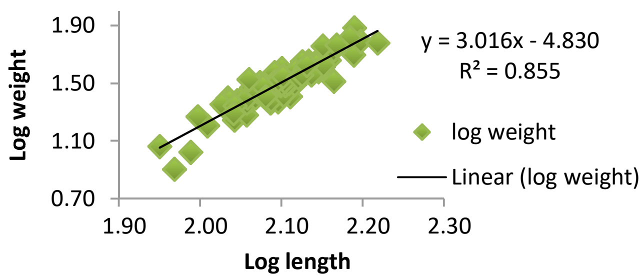

Fig. 3. Logarithmic relationship between length and weight in *Carassius Carassius* Linneaus (Male)

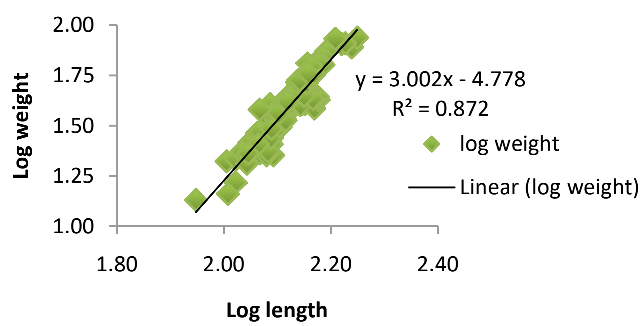

Fig. 4. Logarithmic relationship between length and weight in *Carassius Carassius* Linneaus (Female)

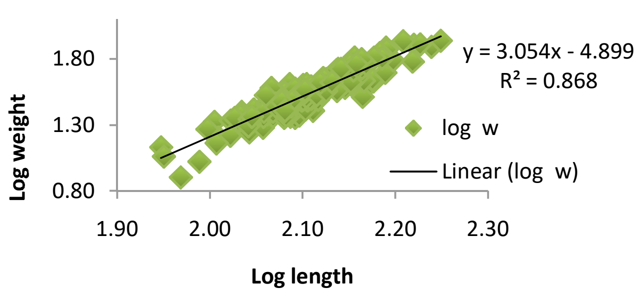

Fig. 5. Logarithmic relationship between length and weight in *Carassius carassius* Linneaus (Combined)

CONCLUSION

This study provides the valuable data on the biometric analysis and length-weight relationship and different morphometric measurements and meristic counts can also be used to differentiate the external changes, ontogenic associations and recognition, stock structure of the species and explain the effect of various environmental factors on growth in fish. *Carassius carassius* showed an isometric growth that is relatively suitable for the growth of this species in this region and making the data valuable for further biological studies. Moreover, comprehensive studies are required for a better understanding of the population dynamics of the species and the status of fish population in different habitats.

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Performance Assessment of Disc-Type Furrow Openers in Residue-Retentive Soils under Conservation Tillage

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Abstract: Effective residue management is essential for successful conservation tillage, especially in rice–wheat systems where combine harvesting leaves substantial straw on the surface. This study aimed to compare the performance of two disc-type furrow openers—single disc (SD) and double disc (DD)—in terms of straw-cutting efficiency and draft force under controlled soil bin conditions. Trials were conducted using loamy soil prepared to match field moisture content and compaction. Freshly harvested rice straw was spread uniformly at three densities (1, 2, and 3 t/ha), and the openers were tested at forward speeds of 1.5, 2.0, and 2.5 km/h, maintaining a working depth of 5 cm. The double disc opener consistently outperformed the single disc in cutting efficiency, achieving a maximum of 81.36% at 1.5 km/h forward speed. However, this came with a higher draft requirement, averaging 368.5 N under optimal conditions, the single disc required less draft force but showed reduced cutting performance, particularly at higher speeds and residue levels. Statistical analysis confirmed that furrow opener type, speed, and residue density significantly affected both draft force and straw-cutting efficiency. Overall, the double disc furrow opener was more effective for high-residue conservation tillage, offering a better balance between cutting performance and manageable draft force.

Keywords: Conservation tillage, Furrow opener, Double disc, Straw cutting efficiency, Draft force, Residue management

Conservation tillage is increasingly recognized as a sustainable agricultural practice aimed at minimizing soil disturbance, enhancing organic matter retention, and improving water conservation (Kolesnikov 2020, Francaviglia et al., 2023). It offers several agronomic and environmental advantages, such as reduced erosion, improved soil structure, and better moisture infiltration (Busari et al., 2015, Boincean et al., 2019). However, the retention of crop residues on the soil surface, a cornerstone of conservation tillage, poses operational challenges during sowing, especially in the rice–wheat cropping systems predominant in the Indo-Gangetic Plains (Singh et al., 2022, Leharwan et al., 2023). In these systems, combine harvesters are widely used, leaving a substantial layer of straw residues behind. This surface residue can obstruct furrow formation, cause seed placement issues, and increase the risk of "hair pinning," where straw is pushed into the furrow instead of being cut cleanly (Evans et al., 2009, Kumar et al., 2024). As a result, effective residue management becomes a prerequisite for successful seeding in conservation tillage.

Furrow openers play a central role in addressing this challenge. They are responsible for cutting through surface residue, creating a uniform furrow, and facilitating accurate seed placement—all with minimal soil disruption (Barr et al., 2016, Aikins et al., 2018, Madhusudan and Preetham 2020). Among various designs available, disc-type furrow openers are particularly suitable for conservation tillage because of their rolling action and capacity to slice through residue with less soil inversion. Of these, the single disc (SD) and double disc (DD) openers are the most used (Ahmad et al., 2017, Madhusudan et al., 2024). The SD opener, consisting of a single angled cutting disc, is known for its low draft requirements and narrow furrow profile. It is often used in dry and moderately compacted soils where minimal disturbance is desired. DD opener utilizes two angled discs to create a V-shaped furrow (Ahmad et al., 2017, Rathod et al., 2024). Its design tends to generate greater downward and lateral cutting forces, making it more effective in managing surface straw, especially under high-residue conditions. However, it also typically requires more draft power than the SD opener (Aikins et al., 2018). While both designs are widely implemented in the field, few studies have conducted a detailed mechanical comparison under controlled conditions. Most evaluations are carried out under open-field settings, where factors like uneven residue distribution, soil heterogeneity, and inconsistent moisture make it difficult to isolate the effects of tool geometry and operating parameters. soil bin environment offers a unique advantage in this context, allowing for the controlled study of furrow opener performance across variables such as forward speed and straw density. This study aims to assess and compare the relative performance of SD and DD furrow openers under simulated soil bin conditions.

MATERIAL AND METHODS

Experimental site and soil bin setup: The study was conducted during 2023-24 in the Soil Dynamics Laboratory of the Division of Agricultural Engineering at ICAR–Indian Agricultural Research Institute (IARI), New Delhi. The experiments were performed in a soil bin with dimensions of 25 m in length, 1.8 m in width, and 1 m in depth. The soil type in the bin was sandy loam with a composition of 80% sand, 10% silt, and 10% clay. Before each experimental run, the bin was tilled, leveled, and compacted using a 1015 kg cylindrical roller to standardize soil conditions across trials. The moisture content, bulk density, and cone penetration resistance were adjusted and maintained at 12% (dry basis), 1.5 g/cm³, and 1.45 MPa, respectively, to simulate post-harvest field conditions in Indo-Gangetic Plains. These parameters were validated by oven-drying soil samples at 105°C and using a digital cone penetrometer (Gilson HM-559A) with a 60° cone angle.

Furrow openers and frame setup: Two disc-type furrow openers were selected for the study: the Single Disc (SD) and the Double Disc (DD) openers (Fig. 1). The SD opener consisted of a high-carbon steel disc of 350 mm diameter set at a 12° tilt to the vertical to reduce soil disturbance while cutting through surface residue. The DD opener comprised two plain rolling discs of the same size, angled to form a V-shaped furrow. Both openers were mounted on a steel frame equipped with a depth adjustment mechanism and fixed at a working depth of 5 cm.

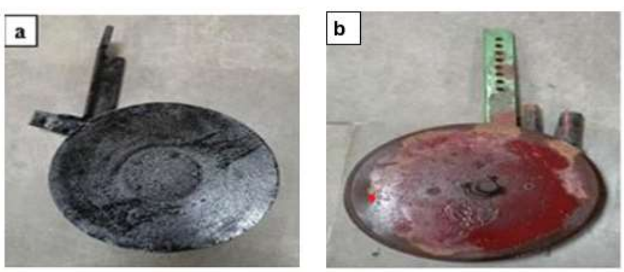

Fig. 1. Various furrow openers used for experimentation: **(a)** single disc (SD) furrow opener, **(b)** double disc (DD) furrow opener

Straw preparation and experimental conditions: Rice straw, collected post-harvest from a combine-operated paddy field, was uniformly spread over the soil surface in the bin at three different densities: 1, 2, and 3 t/ha (Fig. 2). The straw moisture content was approximately 18% (wet basis), and spreading was done manually to achieve uniform coverage. Each furrow opener was tested at three forward speeds (1.5, 2.0, and 2.5 km/h) using a motorized trolley mounted on rails alongside the soil bin. This speed range represents typical operational speeds for seed drills and no-till planters in conservation tillage.

Draft force measurement: Draft force was measured using a calibrated S-type load cell (GUANG CE YZC-516C, 1960 N capacity) placed between the furrow opener frame and the drawbar of the trolley (Fig. 3). The force signal was captured in real-time using an Arduino-based data acquisition system and visualized using serial oscilloscope software. The peak steady-state force during the 2-meter test section was recorded as the draft requirement.

Straw cutting efficiency: After each test run, the soil surface was carefully examined, and the straw was collected and separated into cut and uncut fractions. The uncut straw was weighed using a precision digital scale, and the cutting efficiency was calculated (Dong et al., 2013).

$$\text{Straw cutting efficiency (\%)} = 100 - \frac{\text{Weight of uncut straw}}{\text{Total straw weight}} \times 100$$

Three replications were performed for each combination of furrow opener type, speed, and straw density.

Statistical analysis: The collected data on draft force and straw-cutting efficiency were analyzed using two-way ANOVA to determine the main and interaction effects of furrow opener type and forward speed (or straw density). For multiple comparisons among group means, Tukey's Honest Significant Difference (HSD) test was used at a 5% significance level. Statistical analyses were performed using SPSS v25 and R software.

Fig. 2. Soil bin with straw cover

RESULTS AND DISCUSSION

The comparative assessment of single disc (SD) and double disc (DD) furrow openers revealed distinct performance trends under varying forward speeds and straw residue densities. Both furrow opener type and forward speed had a statistically significant influence on draft force while their interaction was not significant (Table 2). The draft force increased with increasing both forward speed and straw density for both furrow openers. At 1.5 km/h, the SD furrow opener recorded an average draft force of approximately 275 N, which rose to about 390 N at 2.5 km/h. Similarly, the DD furrow opener required more draft throughout the speed range, starting from 325 N and increasing to around 435 N at the highest speed (Fig. 4). The higher draft associated with the DD furrow opener is attributable to its dual-disc configuration, which engages more soil and residue compared to the SD furrow opener design. Sahu and Raheman (2006) also reported a similar relationship between operational speed and draft force, largely due to increased soil resistance and tool acceleration.

Straw cutting efficiency results followed a different pattern. The furrow opener type was the dominant factor influencing this parameter, with the DD furrow opener demonstrating significantly higher straw-cutting efficiency across all conditions (Table 2). At 1.5 km/h, the DD furrow opener achieved straw cutting efficiencies exceeding 80%, while the SD furrow opener managed around 65%. However, as forward speed increased, cutting performance declined

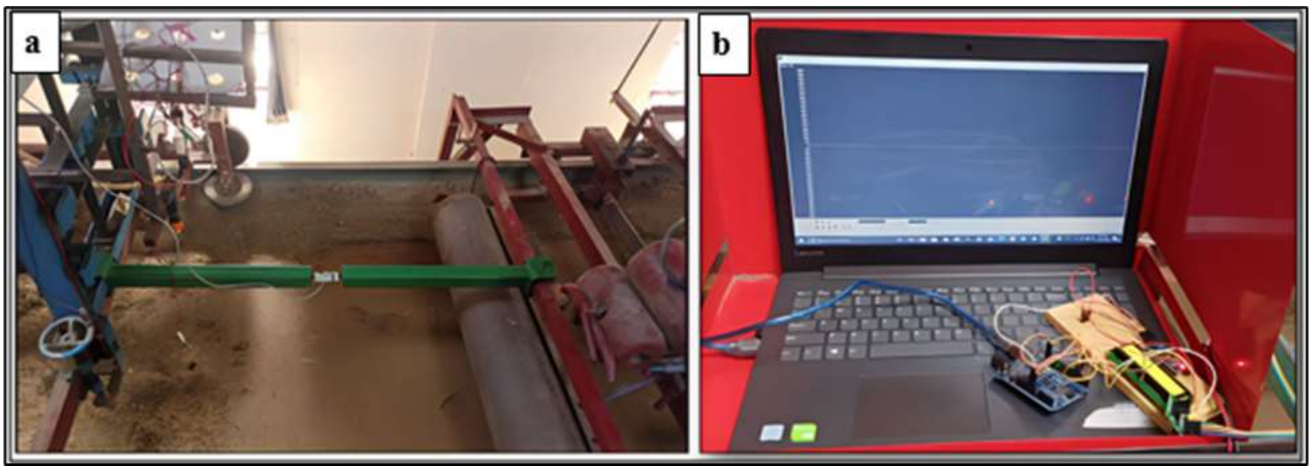

Fig. 3. Data acquisition setup (a) Load cell (b) Serial oscilloscope software

Table 1. Experimental plan for soil bin testing of furrow openers

Independent parameters	Levels	Dependent parameters
Furrow openers	2 i. Single disc furrow opener ii. Double disc type furrow opener	Draft force (N) Straw cutting efficiency (%)
Forward speed (km/h)	2 (1.5, 2 and 2.5)	
Straw density (t/ha)	2 (1, 2, and 3)	
Replications	2	

Table 2. Regression model for draft force requirement and straw cutting efficiency

Source of variation	df	Draft force				Straw cutting efficiency			
		SS	MS	F-value	p-value	SS	MS	F-value	p-value
Furrow Opener Type (A)	1	24570.5	24570.5	92.38	<0.001	4237.2	4237.2	116.96	<0.001**
Forward Speed (B)	2	48291.3	24145.7	90.83	<0.001	378.3	189.1	5.23	0.009*
Residue Density (C)	2	16852.7	8426.3	31.72	<0.001	281.5	140.75	3.88	0.029*
A × B Interaction	2	428.9	214.45	0.81	0.455	47.6	23.8	0.66	0.521
Error	36	9567.8	265.8			1302.7	36.2		
Total	43	99867.2				6247.3			

**, * indicate 1% and 5% level of significance, respectively for both furrow openers more for the SD furrow opener, whose efficiency dropped below 50% at 2.5 km/h (Fig. 2). These observations suggest that increased speed reduces the contact time between the opener and the straw, making it harder to sever residue, particularly for the single disc configuration which relies on a single point cutting action.

Similarly, straw residue density also had a clear effect on both draft force and straw cutting efficiency. The increasing the straw density from 1 to 3 t/ha led to a steady increase in draft requirements for both furrow openers (Fig. 5). The DD furrow opener exhibited a sharper increase in draft force with increase in residue density, indicating greater interaction between the furrow opener and the crop residue density. This reinforces the importance of considering residue load in equipment selection, particularly in conservation tillage systems where surface residue load is intentionally retained. In contrast, increasing the straw density reduces the straw cutting efficiency for both the furrow openers. The DD furrow opener demonstrated the highest cutting efficiency, exceeding 80%, while the SD furrow opener managed around 65%. The superior straw-cutting efficiency observed in the DD furrow opener is attributed to the result of applying a tensile force on both the straw and the soil (Ahmad et al., 2015, Xu et al., 2024). Additionally, the sticky nature of sandy loam soil in the soil bin led to a reduced occurrence of the hair pinning phenomenon in the DD furrow opener (Ahmad et al., 2015, Xu et al., 2024). Previous studies have also reported the superior performance of DD furrow openers in cutting deposited straw by employing a simple shearing and rolling action, with the soil acting as a counter knife (Aikins et al., 2018, Leharwan et al., 2023).

The interaction between forward speed and furrow

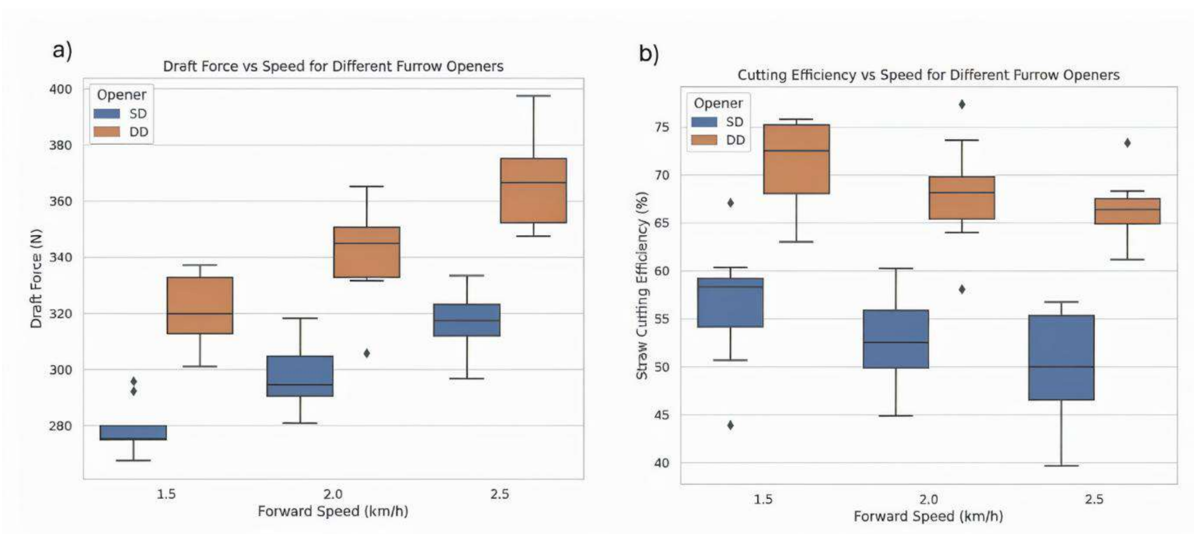

Fig. 4. Effect of forward speed and straw density on draft force for different furrow openers

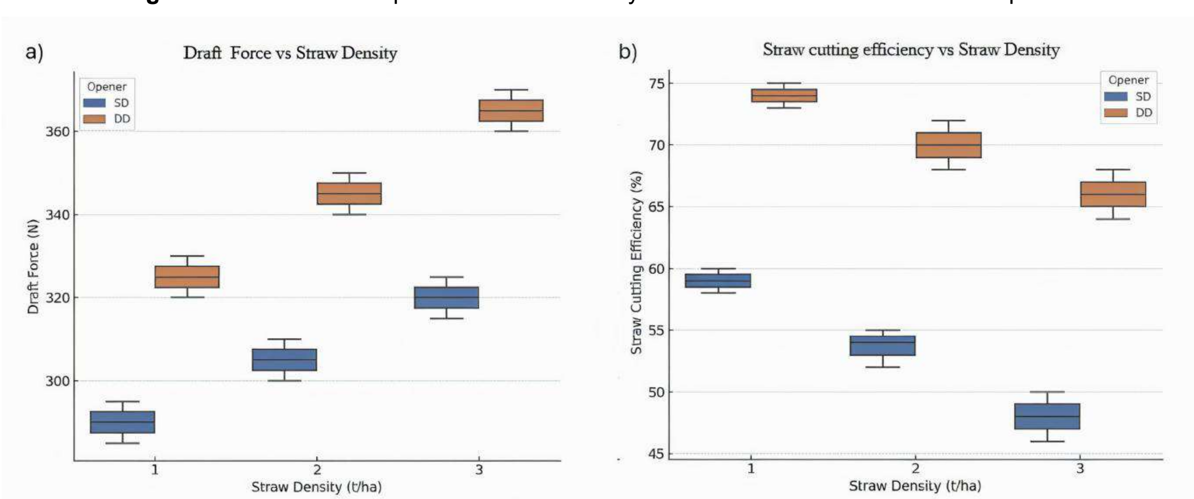

Fig. 5. Effect of forward speed and straw density straw cutting efficiency for different furrow openers

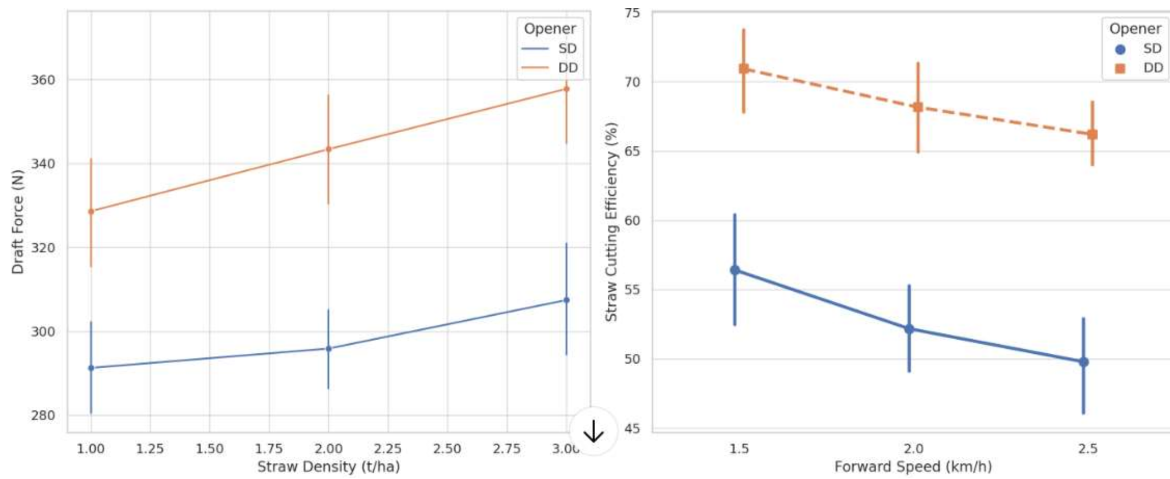

Fig. 6. Interactive effect of forward speed and furrow opener type on Draft force and straw cutting efficiency

opener type, though not statistically significant, revealed useful trends. the straw cutting efficiency of the SD furrow opener declined more steeply with forward speed than the DD furrow opener (Figure 6). This can be attributed to the DD furrow opener's symmetrical disc layout, which provides shearing force from both sides, improving its ability to slice through surface residue even at higher operational speeds. The SD opener, in contrast, lacks lateral engagement and tends to push straw into the furrow (hair pinning) at higher speeds. Aikins et al. (2018) and Leharwan et al. (2023,) also reported enhanced straw cutting and residue management with double disc openers in similar conservation tillage setups.

The results point to a clear trade-off between draft force and cutting efficiency. The SD furrow opener offers lower draft requirements, which could benefit small-scale or low-power tractors. However, it falls short in high-residue conditions and at higher speeds, where residue handling is critical. The DD furrow opener, while requiring more draft force, delivers superior straw cutting performance and maintains its efficiency more consistently across operational conditions. Based on these findings, the double disc furrow opener is better suited for conservation tillage applications, particularly where straw density exceeds 2 t/ha. For optimal performance, forward speeds should be maintained between 1.5 and 2.0 km/h to balance draft demand with effective residue cutting. These insights contribute to more informed machinery selection and operation strategies, ultimately supporting the wider adoption of sustainable residue-retentive farming practices.

CONCLUSION

This study provides a detailed comparison of single disc (SD) and double disc (DD) furrow openers under simulated conservation tillage conditions, focusing on their performance across different forward speeds and surface straw densities. both forward speed and straw residue density significantly affected draft force and straw-cutting efficiency. The DD furrow opener consistently achieved higher cutting efficiency, exceeding 80% at low speeds and residue levels, due to its symmetrical disc configuration and effective shearing action. However, this came with a higher draft requirement compared to the SD opener. The SD opener required less draft force, making it suitable for low-powered tractors, but its cutting efficiency declined significantly at higher speeds and straw densities. The interaction between forward speed and furrow opener type, while statistically non-significant, revealed that the DD opener maintained better efficiency across conditions, whereas the SD opener's performance deteriorated. The double disc furrow opener offered better cutting efficiency in high-residue conditions (>2 t/ha) but required higher draft force. Operating it at 1.5–2.0 km/h achieved an effective balance between residue management and energy use, supporting sustainable conservation tillage practices.

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Phenotypic Trends and Evaluation of Economic Traits in Surti Buffaloes under Organized Farm

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Abstract: The study evaluated the effect of non-genetic factors, including age, season, period, and parity, on economic traits in Surti buffaloes. Data from 391 buffaloes under the Network Project on Buffalo Improvement (Surti) at LRS, Vallabhnagar, Udaipur (RAJUVAS, Bikaner), were analysed over a 30-year period (1993–2023), encompassing 868 lactation records. Age had a non-significant effect on TMY, TMY_305, LL, CI, DP, and ADMY_LL but significantly influenced LPY and ADMY_CI. Season significantly affected all economic traits, with the highest production observed during winter (November to February). The period of calving had a significant influence on all economic traits, with the highest performance recorded during the first period (1993–1997). Parity significantly affected LPY and ADMY_LL. A declining trend in economic traits was observed up to the sixth period (2018–2023), indicating a need for improved management strategies to sustain productivity.

Keywords: Economic traits, Non-genetic factors, Surti buffaloes, Phenotypic trend

India, with the world's largest buffalo population, plays a crucial role in milk and meat production, significantly contributing to socio-economic development in both rural and urban areas. Of the 204 million buffaloes worldwide, 97% are in Asia, with India alone hosting 109.85 million, representing 57% of the global population (20th Livestock Census 2019). Buffaloes are the highest milk-producing species in India and have a higher fat percentage compared to cattle, making them more valuable for dairy production. The average daily milk yield of buffaloes in India (6.9 kg/animal) is higher than that of indigenous cattle (3.9 kg/animal), reinforcing their economic importance in the dairy industry (BAHS 2024).

The productivity of a dairy herd is determined by economic traits such as total milk yield (TMY), 305-day total milk yield (TMY_305), lactation peak yield (LPY), lactation length (LL), calving interval (CI), dry period (DP), average daily milk yield per lactation length (ADMY_LL), and average daily milk yield per calving interval (ADMY_CI). These traits vary across breeds and individual animals due to genetic differences, management practices, environmental conditions, feeding regimes, and geographical distribution (Chitra et al., 2018).

To enhance the productivity of dairy animals, understanding the influence of non-genetic factors on economic traits is essential. Factors such as season, period of calving, parity, and management practices significantly impact milk production, reproductive efficiency, and overall performance. Identifying these factors is critical for optimizing management strategies and implementing effective selection programs to improve productivity and ensure sustainable genetic progress in future generations. Therefore, the present study aims to evaluate the impact of non-genetic factors on economic traits in Surti buffaloes, and estimate the least squares means of these traits.

MATERIAL AND METHODS

The present study was conducted using data from the Network Project on Buffalo Improvement (Surti) at the Livestock Research Station, Vallabhnagar, Udaipur (RAJUVAS, Bikaner), covering records spanning from 1993 to 2023. The dataset included animals with well-documented pedigrees and detailed economic trait records to ensure accuracy and reliability in the analysis. Outliers were systematically excluded to maintain data integrity. Records with abnormalities such as abortions, stillbirths, lactation lengths of less than 100 days, total milk yields below 500 kg, or any other pathological conditions affecting economic traits were excluded. Additionally, sires with fewer than three progeny were not considered in the analysis to improve the robustness of genetic evaluations.

The study analyzed economic traits, including total milk yield (TMY), 305-day total milk yield (TMY_305), peak yield (PY), lactation length (LL), calving interval (CI), dry period (DP), average daily milk yield per lactation length (ADMY_LL), and average daily milk yield per calving interval (ADMY_CI). These traits were evaluated in relation to age, season, period of calving, and dam's parity.

The data were analyzed to assess the influence of non-genetic factors using the Mixed Model Least-Squares and

Maximum Likelihood method (Harvey 1990). The statistical mixed model applied was as follows:

$$Y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + e_{ijkl}$$

Where,

Y_{ijklmn} = performance record of the n^{th} progeny of i^{th} sire belonging to j^{th} period of calving, k^{th} season of calving, l^{th} parity of animal, m^{th} age group of each calving.

μ = Population mean common to all observations

A_i = Random effect of i^{th} sire

B_j = Fixed effect of j^{th} period of calving (j = 1, 2, 3, 4, 5 and 6)

C_k = Fixed effect of k^{th} season of calving (= 1, 2 and 3)

D_l = Fixed effect of l^{th} parity (l= 1, 2, 3, 4, 5, 6 and above)

E_m = Fixed effect of m^{th} age group on each calving

e_{ijklmn} = Residual random error associated with Y_{ijklmn} and assumed to be identically and independently distributed with mean zero and constant variance.

Estimation of phenotypic trends: Phenotypic trends were estimated by analysing the changes in the observed phenotypic performance of the traits over time. After standardization of data, the phenotypic trend was calculated by taking regression of time mean performance of the population on the time as:

$$P = b_{P.T}$$

The standard error of the phenotypic trend was calculated as:

$$\text{SE of } (\Delta P) = \left[\frac{\sum P^2 - b_{P.T}(\sum PT)}{\left(\sum T^2\right)(N-2)}\right]^{1/2}$$

The regression analysis was performed using the lm function in R to explore the relationship between the average phenotypic performance of the trait on the time of birth.

Statistical analysis: Duncan's Multiple Range Test (DMRT), as modified by Kramer (1957), was used to make pairwise comparisons among the least-squares means. function using agricolae package in R software. The difference between the means of treatment *i* (Xi) and treatment *j* (Xj) was considered significant at the 5% level (P ≤ 0.05) if it satisfied the following condition:

$$(X_i - X_j)\sqrt{\frac{2}{(C_{ii} + C_{jj} + 2C_{ij})}} > \sigma e Z p n_2$$

RESULTS AND DISCUSSION

General performance: The overall least-squares means for total milk yield, total milk yield at 305 days, lactation peak yield, lactation length, calving interval, dry period, average daily milk yield per lactation length, and average daily milk yield per calving interval were 1466.59 kg, 1605.97 kg, 9.46 kg, 290.42 days, 503.10 days, 206.02 days, 5.08 kg, and 3.14 kg, respectively (Table 1, 2). Total milk yield, was in consistent with findings by Rathod et al. (2018) and Pawar et al. (2018). However, Kumar, (2018) reported lower estimates, potentially due to differences in environmentalconditions and feeding management). Similarly, total milk yield at 305 days was in accordance with earlier findings by Tailor and Singh (2014) and Rathod et al. (2018), suggesting the breed's potential for improved milk production under optimal management. Lactation peak yield, indicating the highest daily milk yield during lactation, was 9.46 kg, aligning with results from Kumar (2018) and Brar et al. (2022) in Surti and Murrah buffaloes, respectively. The higher LPY reflects better genetic potential and management conditions (Kaur and Narang, 2021). In contrast, lactation length (LL) was 290.42 days, which is comparable to findings from Shashikant (2022) and Chakraborty and Dhaka (2023), but slightly lower than previous reports (Pawar et al., 2018, Kumar 2018). Variability in lactation length across studies may be due to differences in milking frequency, feed availability, and environmental stressors. Calving interval, a crucial reproductive trait affecting milk production efficiency, was estimated at 503.10 days. These results support previous, emphasizing the need for genetic and environmental improvements to shorten CI studies (Rathod et al., 2018, Vyas et al., 2021). Similarly, dry period, representing the non-lactating phase between successive calvings, was recorded as 206.02 days, aligning with earlier findings in Surti buffaloes (Kumar 2018, Shashikant 2022). Shorter dry periods contribute to higher lifetime milk production and improved herd efficiency.

Among production efficiency indicators, average daily milk yield per lactation length was 5.08 kg, which aligns with earlier studies, indicating stable productivity over lactation (Jakhar et al., 2017, Kaur and Narang 2021). Meanwhile, average daily milk yield per calving interval was 3.14 kg, consistent with results from Thiruvenkadan (2011) and Kumar (2018). Higher ADMY_CI which indicate improved productivity efficiency across lactations.

Effect of Various Factors on Economic Traits

Random effect of sire: The sire effect was significant for most economic traits, indicating genetic variability among Surti buffaloes. Significant sire effects on total milk yield, 305-day total milk yield, peak yield, lactation length, calving interval, dry period, and average daily milk yield traits were reported in several studies (Nagda 2005, Shashikant 2022). Highly significant sire effect was observed for TMY and LPY, consistent with findings in Surti buffaloes (Bharat et al., 2004) and Murrah buffaloes (Verma et al., 2017, Brar et al., 2022). However, non-significant sire effects on some traits were noted in Surti buffaloes (Jatolia 2008) and Murrah buffaloes (Jakhar et al., 2017). The genetic variation among sires influences milk production efficiency, reinforcing the need for sire selection based on breeding values and progeny testing.

Age: The effect of age on most traits was non-significant, although an increasing trend in milk yield and lactation performance was observed as animals matured. Buffaloes aged 110-124 months produced the highest TMY (1636.32 kg), TMY_305 (1760.65 kg), and LPY (10.49 kg/day). Similar trend was observed in Mehsana buffaloes (Singh, 1992) and Murrah buffaloes (Prakash et al., 1988) and Nili-Ravi buffaloes (Singh et al., 2011). Younger buffaloes (<50 months) had lower yields, likely due to incomplete physiological maturity and suboptimal udder development (Kumar, 2018). Lactation length and calving interval also increased with age, with older buffaloes showing longer LL (306.24 days) and shorter CI (464.54 days), consistent with findings in Surti buffaloes (Vyas et al., 2021) and Murrah buffaloes (Thiruvenkadan et al., 2014).

Period of calving: The period of calving had a highly significant effect on all economic traits, reflecting long-term genetic trends, environmental changes, and management practices. The highest TMY (1759.10 kg) was in buffaloes calving during 1993-1997, whereas the lowest (1368.14 kg) in 2018-2023, marking a 22.2% decline. Similar declining trends in TMY have been reported in Surti buffaloes (Kumar, 2018; Shashikant, 2022) and Pandharpuri buffaloes (Gaikwad et al., 2022).

Table 1. Production traits in Surti buffaloes (Least-squares mean and SE)

Effect	TMY	TMY_305	LPY	LL
N	868	350	868	868
μ±S.E.	1466.59	1605.97	9.46	290.42
Age (Month)	NS	NS	***	NS
1 (35-49)	1409.02(97)	1578.41(36)	8.59^{a} (97)	284.25(97)
2 (50-64)	1478.34(161)	1585.19(75)	8.88^{a} (161)	296.79(161)
3 (65-79)	1487.17(154)	1626.31(64)	9.22^{b} (154)	281.77(154)
4 (80-94)	1541.02(135)	1629.34(52)	9.70^{c} (135)	291.17(135)
5 (95-109)	1570.73(113)	1644.29(38)	9.93^{c} (113)	294.62(113)
6 (110-124)	1636.32(85)	1760.65(33)	10.49^{d} (85)	298.97(85)
7 (125 and above)	1607.42(123)	1722.29(52)	9.94^{c} (123)	306.24(123)
Season	***	**	***	*
Summer (S_1)	1509.39^{a} (106)	1592.76^{a} (40)	9.40^{a} (106)	292.41^{ab} (106)
Rainy (S_2)	1466.83^{a} (473)	1634.04^{a} (174)	9.32^{a} (473)	286.74^{a} (473)
Winter (S_3)	1622.36^{b} (289)	1721.73^{b} (136)	9.90^{b} (289)	301.06^{b} (289)
Period	**	**	***	*
(1993-1997) P_1	1759.10^{d} (22)	1821.43^{d} (13)	9.51^{b} (22)	310.85^{bc} (22)
(1998-2002) P_2	1637.36^{c} (159)	1723.37^{c} (80)	9.64^{b} (159)	309.02^{b} (159)
(2003-2007) P_3	1508.70^{b} (246)	1678.74^{c} (79)	10.37^{c} (246)	280.28^{a} (246)
(2008-2012) P_4	1426.86^{ab} (158)	1531.04^{a} (56)	9.55^{b} (158)	287.63^{a} (158)
(2013-2017) P_5	1496.99^{b} (130)	1647.5^{c} (57)	9.47^{b} (130)	282.15^{a} (130)
(2018-2023) P_6	1368.14^{a} (153)	1494.90^{a} (65)	8.69^{a} (153)	290.48^{a} (153)
Parity	NS	NS	**	NS
1	1403.82(254)	1508.27(108)	9.00^{a} (254)	296.74 (254)
2	1548.07(183)	1625.49(78)	9.665^{bc} (183)	305.02 (183)
3	1553.08(143)	1717.69(51)	9.785^{c} (143)	290.44 (143)
4	1576.19(104)	1704.08(43)	9.62^{bc} (104)	295.77 (104)
5	1591.84(76)	1658.01(33)	9.74^{bc} (76)	292.76(76)
6 and above	1524.16 (108)	1683.45(37)	9.41^{b} (108)	279.67 (108)
Sire	*	NS	***	*

NS= non-significant, *p<0.05= significant, **p<0.01= highly significant, ***p<0.001= highly significant.
Figures in parenthesis indicate number of observations

The decline in milk yield traits over time suggests possible genetic dilution, environmental stressors, and nutritional variations. Studies on Murrah buffaloes also observed decreasing milk yields over decades due to climate change and shifts in feeding systems (Gandhi et al., 2009, Jakhar et al., 2017). Lactation length also declined significantly over time, from 310.85 days (1993-1997) to 280.28 days (2003-2007), indicating the impact of changing lactation management practices (Patel et al., 1998, Thiruvenkadan et al., 2014). Calving interval increased by 22.8% over different calving periods, similar to findings in Surti buffaloes (Nagda 2005, Vyas et al., 2021). The increasing CI reflects declining reproductive efficiency and suboptimal fertility management over time (Kumar 2018).

Season of calving on economic traits: Season had a highly significant effect on TMY, TMY_305, LPY, LL, and reproductive traits. The highest TMY was in winter (1622.36 kg), whereas the lowest in the rainy season (1466.83 kg), indicating a 10.6% decrease. Similar results have been reported in Surti buffaloes (Kumar 2008, Shashikant 2022) and Murrah buffaloes (Thiruvenkadan et al., 2010, 2011, 2014).

Winter-calving buffaloes had longer lactation lengths (301.06 days) and shorter calving intervals (473.12 days), suggesting better reproductive efficiency in colder months. Higher milk yield in winter can be attributed to optimal temperatures, better feed availability, and reduced heat stress, whereas rainy-season calvings coincide with

Table 2. Reproductive and efficiency traits in Surti buffaloes

Effect	CI	DP	ADMY_CI	ADMY_LL
N	661	661	661	868
μ±S.E.	503.10	206.02	3.14	5.08
Age (Month)	NS	NS	**	NS
1 (35-49)	554.78 (73)	255.86 (73)	2.65^{a} (73)	5.07 (97)
2 (50-64)	540.46 (130)	229.91 (130)	2.91^{a} (130)	5.09 (161)
3 (65-79)	505.04 (120)	202.58 (120)	3.22^{c} (120)	5.34 (154)
4 (80-94)	503.47 (111)	204.80 (111)	3.21^{c} (111)	5.32 (135)
5 (95-109)	464.54 (87)	169.32 (87)	3.54^{d} (87)	5.33 (113)
6 (110-124)	494.28 (68)	197.74 (68)	3.46^{d} (68)	5.46 (85)
7 (125 and above)	480.09 (72)	165.72 (72)	3.59^{d} (72)	5.28 (123)
Season	***	**	*	***
Summer (S_1)	533.41^{c} (71)	219.40^{b} (71)	3.06^{a} (71)	5.25^{a} (106)
Rainy (S_2)	473.12^{a} (365)	181.66^{a} (365)	3.28^{b} (365)	5.14^{a} (473)
Winter (S_3)	511.75^{b} (225)	210.05^{b} (225)	3.34^{b} (225)	5.42^{b} (289)
Period	*	*	**	***
(1993-1997) P_1	446.15^{a} (22)	133.98^{a} (22)	3.86^{c} (22)	5.62^{c} (22)
(1998-2002) P_2	548.08^{c} (139)	229.03^{b} (139)	3.16^{ab} (139)	5.28^{b} (159)
(2003-2007) P_3	510.20 (156)	208.92^{b} (156)	3.25^{b} (156)	5.41^{b} (246)
(2008-2012) P_4	502.56^{b} (126)	207.91^{b} (126)	2.95^{a} (126)	5.03^{a} (158)
(2013-2017) P_5	512.29^{bc} (110)	223.96^{b} (110)	3.17^{ab} (110)	5.4^{b} (130)
(2018-2023) P_6	517.28^{bc} (108)	218.44^{b} (108)	2.97^{a} (108)	4.86^{d} (153)
Parity	NS	NS	NS	**
1	514.27 (193)	217.02 (193)	2.95 (193)	4.71^{a} (254)
2	509.62 (147)	207.19 (147)	3.15 (147)	5.10^{b} (183)
3	510.38 (115)	211.69 (115)	3.24 (115)	5.41^{c} (143)
4	524.59 (81)	214.31 (81)	3.26 (81)	5.39^{c} (104)
5	509.04 (56)	198.00 (56)	3.39 (56)	5.52^{c} (76)
6 and above	468.66 (69)	174.02 (69)	3.39 (69)	5.48^{c} (108)
Sire	NS	NS	**	***

NS= non-significant, *p<0.05= significant, **p<0.01= highly significant, ***p<0.001= highly significant
Figures in parenthesis indicate number of observations

increased humidity, poor nutritional status, and higher disease susceptibility (Verma et al., 2017, Brar et al., 2022). The dry period was also significantly affected by season, with the longest DP in summer (219.40 days) and the shortest in the rainy season (181.66 days). Similar seasonal patterns in DP was observed in Murrah buffaloes (Thiruvenkadan et al., 2014) and Bhadawari buffaloes (Kushwaha et al., 2013).

Parity: Parity did not have a significant effect on most traits, but a general increasing trend in milk yield was observed until the fifth parity, after which a decline was noted. The highest TMY (1591.84 kg), TMY_305 (1717.69 kg), and LPY (9.74 kg/day) were in buffaloes of the fifth parity, aligning with findings in Murrah buffaloes (Pawar et al., 2012, Brar et al., 2022) and Mehsana buffaloes (Galsar et al., 2016). Reproductive traits showed a decreasing trend in calving interval and dry period with increasing parity, indicating improved reproductive efficiency in older buffaloes (Kumar 2018, Vyas et al., 2021). However, the average daily milk yield traits (ADMY_LL, ADMY_CI) were highest in third to fifth-parity buffaloes, after which a decline was noted due to age-related metabolic constraints (Thiruvenkadan et al., 2014).

Phenotypic trends in surtax buffaloes: Phenotypic trends for key economic traits in Surti buffaloes were analyzed across multiple two-year intervals, the total milk yield exhibited yield exhibited statistically significant negative trend of -21.47 kg per period, indicating a consistent decline in overall milk production across years (Table 3). This downward trajectory may reflect the combined impact of environmental stressors, suboptimal management, and possible dilution of genetic potential over time. Similar declining trends in milk yield were reported in Murrah buffaloes suggesting a broader concern across Indian dairy breeds (Chander 2002, Godara et al., 2015).

The 305-day Milk Yield (TMY_305) also showed a significant negative phenotypic trend of -18.04 kg per period, reaffirming the reduction in standardized lactation output. These findings are consistent with earlier observations in

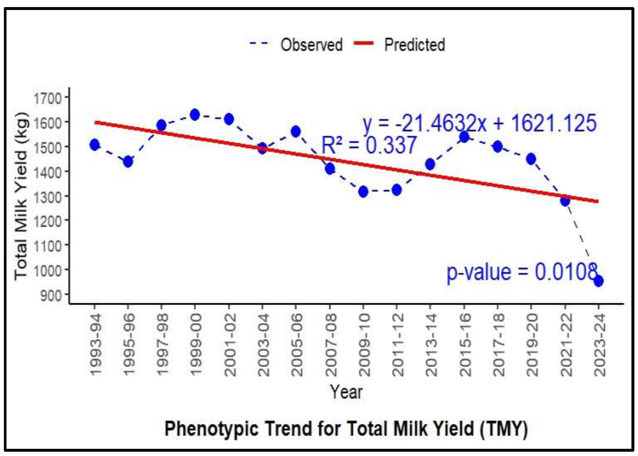

Fig. a. Phenotypic trends for total milk yield

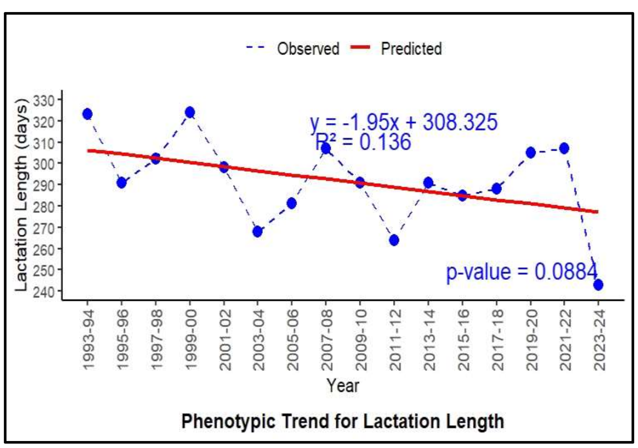

Fig. b. Phenotypic trends for lactation length

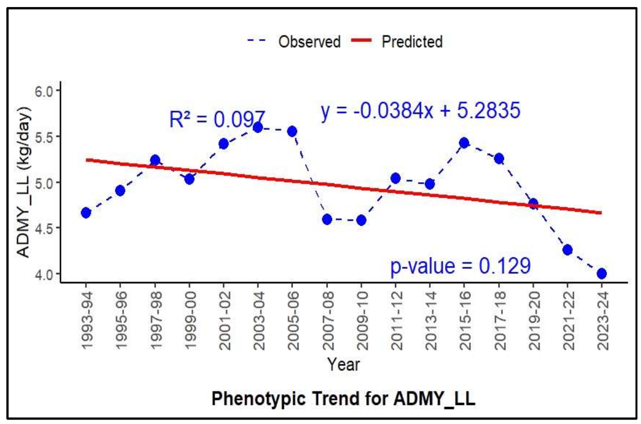

Fig. c. Phenotypic trends for average daily milk yield per lactation length

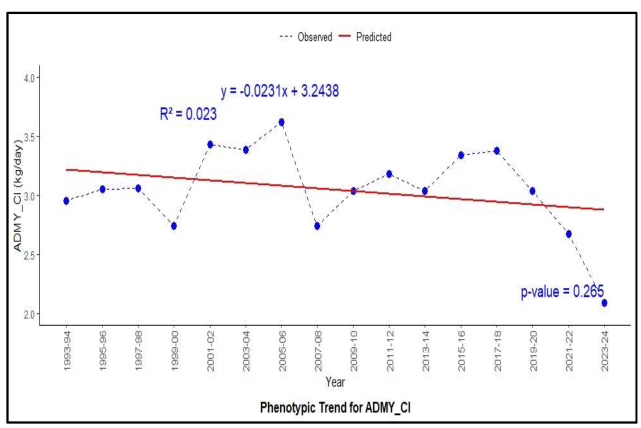

Fig. d. Phenotypic trends for average daily milk yield per calving interval

Table 3. Phenotypic trends of economic traits

Traits	Phenotypic	P-Value	R^2
TMY	-21.47	0.01	0.337
TMY_305	-18.04	0.05	0.181
LPY	-0.05	0.38	-0.013
LL	-1.96	0.08	0.136
CI	-2.92	0.278	0.019
DP	-1.45	0.506	-0.037
ADMY_LL	-0.04	0.129	0.097
ADMY_CI	-0.02	0.265	0.023

Hariana and Sahiwal cattle emphasizing the need for renewed genetic selection and improved nutrition strategies (Singh et al., 2002, Raja et al., 2009). For lactation peak yield, a small but negative trend of -0.05 kg per period was recorded. Although not statistically significant, this slight reduction in peak daily milk yield suggests that genetic improvement programs may have overlooked peak yield while focusing predominantly on total yield. Comparable pattern was recently observed by Sharma et al. (2024) in Murrah buffaloes, underlining the importance of including peak yield in selection indices. The lactation length displayed a non-significant but negative phenotypic trend of -1.96 days per period, indicating a gradual shortening of productive periods over the years and may result from changes in environmental conditions, such as heat stress or fluctuating feed availability, which are known to affect lactation duration. Although subtle, this trend warrants attention as it may ultimately reduce lifetime productivity.

The negative phenotypic trend of -2.92 days per period was observed for the calving interval, indicating an apparent improvement in reproductive efficiency through shortened intervals between successive calvings. This decline was not statistically significant but is favorable development likely attributed to better reproductive management and health practices. These findings are supported by studies in Murrah and Sahiwal cattle by Dev et al. (2017) and Dash et al. (2021), respectively.

The dry period also demonstrated a non-significant negative trend of -1.45 days per period, suggesting a marginal reduction in non-lactating periods. Shorter dry periods contribute to better annual milk yield efficiency and reflect improved herd health and management. Similar beneficial trends have was observed by Singh et al. (2011) and Sharma et al. (2024).

In terms of milk yield efficiency, both Average Daily Milk Yield during Lactation Length and Average daily milk yield during calving interval presented negative phenotypic trends of -0.04 kg and -0.02 kg per period, respectively. Although these trends were not statistically significant, but signal a slow decline in daily milk productivity. This may stem from cumulative environmental stresses, inconsistent genetic selection, or inadequate adaptation of breeding programs to changing climatic conditions.

Overall, the phenotypic trends observed in Surti buffaloes point to a concerning decline in milk production traits, with only marginal improvements in reproductive efficiency. These findings underscore the need for targeted interventions, including enhanced genetic selection, nutritional improvements, and adaptive management strategies, to reverse these unfavorable trends and sustain productivity in the breed.

CONCLUSION

This study demonstrates the significant influence of sire, age, period of calving, season of calving, and parity on key economic traits in Surti buffaloes. The sire effect emphasizes genetic variability, highlighting the importance of progeny testing for genetic improvement. The declining trend in total milk yield over periods emphasizes the need for enhanced management and breeding strategies. Winter-calving buffaloes performed better across most traits, reinforcing seasonal adaptation in herd management. Improved nutrition, reproductive management, and genetic selection are essential to sustain productivity. These findings contribute to scientific breeding programs and productivity enhancement in Surti buffaloes.

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